Dhananjaya Pratap Singh
Harikesh Bahadur Singh
Ratna Prabha *Editors*

Plant-Microbe Interactions in Agro-Ecological Perspectives

Volume 1: Fundamental Mechanisms, Methods and Functions

Springer

Plant-Microbe Interactions in Agro-Ecological Perspectives

Dhananjaya Pratap Singh
Harikesh Bahadur Singh • Ratna Prabha
Editors

Plant-Microbe Interactions in Agro-Ecological Perspectives

Volume 1: Fundamental Mechanisms, Methods and Functions

Springer

Editors
Dhananjaya Pratap Singh
ICAR-National Bureau of Agriculturally Important Microorganisms
Maunath Bhanjan, Uttar Pradesh, India

Ratna Prabha
Chhattisgarh Swami Vivekanand Technical University
Durg, Chhattisgarh, India

Harikesh Bahadur Singh
Department of Mycology & Plant Pathology, Institute of Agricultural Sciences
Banaras Hindu University
Varanasi, Uttar Pradesh, India

ISBN 978-981-10-5812-7 ISBN 978-981-10-5813-4 (eBook)
DOI 10.1007/978-981-10-5813-4

Library of Congress Control Number: 2017953933

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword

त्रिलोचन महापात्र, पीएच.डी.
एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
सचिव एवं महानिदेशक
TRILOCHAN MOHAPATRA, Ph.D.
FNA, FNASc, FNAAS
SECRETARY & DIRECTOR GENERAL

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली 110 001
GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION
AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE AND FARMERS WELFARE
KRISHI BHAVAN, NEW DELHI 110 001
Tel.: 23382629; 23386711 Fax: 91-11-23384773
E-mail: dg.icar@nic.in

Interactions of diverse microbial communities with plants and soils have been an integral part of our agro-ecosystem. Plants and soils recruit their own microbiome that interact with them and their abiotic environment through a cross-talk mechanism, which have remained central to the idea of studying the basis of microbial interactions. Such studies, after long efforts, have paved the way for the understanding of intrinsic biochemical, molecular and genetic mechanisms of plant microbe interactions and deciphering the ultimate benefits to plants and soils. Research efforts on plant-microbe interactions have further been facilitated with the developments in isolation and characterization of microbial communities, studies on the biology of community structure and functions, chemistry and biology of root rhizosphere, epiphytic and endophytic microbial associations, identification and behavior of phytopathogens and beneficial impacts of microbial interactions on plants and soils. Such studies have strengthened the prospect of manipulating plant and soil biology and root rhizosphere with beneficial microbial population at a greater pace.

The book, *Plant-Microbe Interactions in Agro-ecological Perspectives: Volume I – Fundamental Mechanisms, Methods and Functions*, presents a detailed account of principles and mechanisms of microbial communities, methods used to decipher such interactions and functional mutual benefits to plants, microbes and soils. In this well-compiled volume, the authors have presented widened views on microbial interactions taking into account various plant-microbe association systems, emphasizing on various mechanisms, different tools involved to decipher results and

evaluating functional benefits out of such interactions. I am very sure that this compilation will attract a wide readership of researchers, students, scholars, agricultural professionals and all those who are interested in this area of research and development.

New Delhi
10th April, 2017

T. Mohapatra

Preface

Agriculture is a live, dynamic, and ecologically sustained system based on key constituents like plants, soils, biological diversity, and the environment. The ecological dynamics and sustainability of this system can be witnessed in terms of multipronged interactions among its constituents. Microorganisms (microfauna and microflora) constitute numerous small- to micro-scale stakeholders of interactions, and their interactions among themselves and with plants, soils, and the environment make the whole agroecological system so vital and live that even at a time scale of microseconds, multifarious biological, biochemical, physiological, and molecular events are organized, disintegrated, and reorganized at the cellular level of all the living cells that interact. The total output of these interactive events can be calculated in terms of plant health and development, soil health, and ecological balance of the whole system toward sustainability. This is why the importance of multiphasic plant-microbe interactions and its impact on native soils, microbial communities, and the plant itself have been recognized in the past few decades. This realization has yielded numerous work from all corners of the world on various plant-microbe systems on which in-depth data has been generated to decipher the mode of interactions; direct and indirect impacts on plants, microbes, other communities, and soil health; assays at cellular, ultrastructural, physiological, biochemical, and enzymatic levels; mechanisms at genetic, genomic, transcriptomic, proteomic, metabolomic, and phenomic levels in both plants and microbes; and benefits to both the partners (plant and microbes) due to environmental adversities. The research reflected that the benefits arising due to tripartite interactions among plants, microbes, and the environment (soil conditions, drought, temperature, etc.) can be helpful in obtaining better yield, better crops, and better environment at the field level. This directly transferable benefit of results at laboratory scale to the field level is the actual practical relevance of this subject area having precise, proven, and impactful benefit transfer to the farms. The book *Plant-Microbe Interactions in Agro-ecological Perspectives* is dedicated to the real work of researchers all across the world who, by their continuous efforts, made this area as dynamic and live as it remains in the fields. In a series of two volumes, the first volume "Fundamental Mechanisms, Methods, and Functions" shares with its readership the work that has been conducted to decipher plant-microbe interactions, the methodology to obtain genuine results, and the functions related to the interactive partnering in soils and plants. This volume presents pertinent topics on soil-plant-microbe interactions and

their impact on plant and soil health; dynamics of rhizosphere microbial communities; molecular tools to study communities and community functions (metagenomics); microbe-root interactions in the rhizosphere; belowground microbial crosstalk and rhizosphere (root-associated) microbial communities; genomics at plant-virus interface; microbiome in interactive mode in conventional vs. organic production system; symbiotic and pathogenic associations; plant-fungi interactions; endophytic and epiphytic interactions and benefits; microbial functions in the hotspot, i.e., rhizosphere; molecular signaling determinants in rhizosphere; quorum sensing in plant-microbe interactions; arbuscular mycorrhizal interactions with roots; genetically modified crop-mycorrhizal symbiosis; microbial interactions to improve soil structure and function; nutrient mobilization and soil fertility benefits due to interactions in climate change era; microbial interactions and induced resistance in plants; pathogenic interactions and disease suppression due to biological control; interaction of entomophagous fungi for soilborne pest control; and interaction competence of bioinoculants in the field. We believe that this volume will attract a wide readership because of its integrated and holistic endeavor of describing microbial communities, their interactions with plants and soils, and the functional role of microbial interactions with plants for crop benefits. The views of the authors are authoritative, thorough, well-thought, and based on their long experiences while working over the subject area. We hope that this volume will benefit a wide readership of researchers, academicians, students, and those who are looking for practically sound and workable solutions to the heavy chemicalization of present-day agricultural systems.

ICAR-NBAIM, Mau, India — Dhananjaya P. Singh
BHU, Varanasi, India — Harikesh B. Singh
CSVTU, Bhilai, Chhattisgarh — Ratna Prabha

Contents

Contributors

Beáta Ábrahám Faculty of Economics and Socio-Human Sciences and Engineering, Department of Bioengineering, Sapientia Hungarian University of Transylvania, Miercurea-Ciuc, Romania

Hussein Hasan Abulreesh Department of Biology, Faculty of Sciences, Umm Al-Qura University, Makkah, Kingdom of Saudi Arabia

Iqbal Ahmad Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India

Muhammad Sohail Akram Department of Botany, Government College University, Faisalabad, Pakistan

Mohd. Musheer Altaf Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India

Gautam Anand Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi, India

Galdino Andrade Laboratory of Microbial Ecology, Department of Microbiology, State University of Londrina, Londrina, Paraná, Brazil

Khalid Anwar School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

Hafiz Naeem Asghar Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Rahul Bakade ICAR-Research Complex for Eastern Region (ICAR-RCER), Patna, Bihar, India

André R. Barazetti Laboratory of Microbial Ecology, Department of Microbiology, State University of Londrina, Londrina, Paraná, Brazil

Purnima Bhandari Department of Botany, Panjab University, Chandigarh, India

Prachi Bhargava Institute of Biosciences and Technology, Shri Ramswaroop Memorial University, Lucknow, Uttar Pradesh, India

Brijmohan Singh Bhau Plant Genomic Laboratory, Medicinal Aromatic & Economic Plants (MAEP) Group, Biological Sciences & Technology Division (BSTD), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Academy of Scientific and Innovative Research (AcSIR), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Virendra S. Bisaria Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi, India

Tapas Biswas Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Utkarsh M. Bitla School of Edaphic Stress Management, ICAR-National Institute of Abiotic Stress Management, Pune, India

Bitupon Borah Plant Genomic Laboratory, Medicinal Aromatic & Economic Plants (MAEP) Group, Biological Sciences & Technology Division (BSTD), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Academy of Scientific and Innovative Research (AcSIR), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

G.P. Brahmaprakash Department of Agricultural Microbiology, University of Agricultural Sciences, Bangaluru, India

L. Canfora CREA-Research Center Agriculture and Environment, Rome, Italy

Martha V.T. Cely Institute of Agrarian and Environmental Sciences, Federal University of Mato Grosso, Sinop, Mato Grosso, Brazil

M.M. Chapala Rice Tec, Alvin, TX, USA

Kanika Chowdhary Centre for Rural Development and Technology, Indian Institute of Technology-Delhi, New Delhi, India

Andreas L. Chryssafidis Laboratory of Veterinary Toxicology, Department of Preventive Veterinary Medicine, State University of Londrina, Londrina, Paraná, Brazil

Biplab Dash Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Khondoker M.G. Dastogeer Plant Biotechnology Research Group, Western Australian State Agricultural Biotechnology Centre (SABC), Murdoch University, Perth, Western Australia, Australia

Bangladesh Agricultural University, Mymensingh, Bangladesh

Seth DeBolt Department of Horticulture, University of Kentucky, Lexington, KY, USA

Igor M.O. dos Santos Laboratory of Microbial Ecology, Department of Microbiology, State University of Londrina, Londrina, Paraná, Brazil

J.S. Escobar Ortega Unit of Agricultural and Environmental Microbiology, Department of Applied Biology and Foods, Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina

A.N. Ganeshamurthy ICAR-Indian Institute of Horticultural Research, Bengaluru, India

Vijaykumar K. Gangaraddi Department of Agricultural Microbiology, University of Agricultural Sciences, Bangaluru, India

I.E. García de Salamone Unit of Agricultural and Environmental Microbiology, Department of Applied Biology and Foods, Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina

Neera Garg Department of Botany, Panjab University, Chandigarh, India

R.K. Gaur Department of Biosciences, College of Arts, Science and Humanities, Mody University, Sikar, Rajasthan, India

Paushali Ghosh School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, India

Reeta Goel Department of Microbiology, College of Basic Sciences & Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Sunil Kumar Gunri Department of Agronomy, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Amrita Gupta ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, Uttar Pradesh, India

Éva György Faculty of Economics and Socio-Human Sciences and Engineering, Department of Food Science, Sapientia Hungarian University of Transylvania, Miercurea Ciuc, Romania

Muhammad Baqir Hussain Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Muhammad Ijaz College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Layyah, Pakistan

Shruti Jain Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Allahabad, Uttar Pradesh, India

K. Jaya Chandra Acharya N. G. Ranga Agricultural University, Regional Agricultural Research Station, Anakapalle, Andhra Pradesh, India

Babita Joshi Plant Genomic Laboratory, Medicinal Aromatic & Economic Plants (MAEP) Group, Biological Sciences & Technology Division (BSTD), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Academy of Scientific and Innovative Research (AcSIR), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Chandandeep Kaur ICAR-Indian Institute of Horticultural Research, Bengaluru, India

Mohd. Sajjad Ahmad Khan Department of Biology, College of Medicine, Imam Abdulrahman Bin-Faisal University, Dammam, Kingdom of Saudi Arabia

Muhammad Yahya Khan University of Agriculture, Vehari, Pakistan

Ashok Kumar School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, India

Jay Kumar School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, India

Prahalad Kumar Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Vinay Kumar ICAR-National Institute of Biotic Stress Management, Baronda farm, Raipur, Chhattisgarh, India

Vivek Kumar Amity Institute of Microbial Technology, AMITY University, Noida, India

School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, India

B. Łabanowska Research Institute of Horticulture, Skierniewice, Poland

Nita Lakra School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

Éva Laslo Faculty of Economics and Socio-Human Sciences and Engineering, Department of Bioengineering, Sapientia Hungarian University of Transylvania, Miercurea Ciuc, Romania

G. Lavanya Department of Agricultural Microbiology, University of Agricultural Sciences, Bangaluru, India

Hai-Bi Li Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning, China

Yang-Rui Li Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning, China

Guangxi Key Laboratory of Sugarcane Biotechnology and Genetic Improvement, Ministry of Agriculture, Sugarcane Research Center, Chinese Academy of Agricultural Sciences; Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, China

Ying Ma Centre for Functional Ecology, Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal

Saquib Mahmood School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

E. Malusá Research Institute of Horticulture, Skierniewice, Poland

Gyöngyvér Mara Faculty of Economics and Socio-Human Sciences and Engineering, Department of Bioengineering, Sapientia Hungarian University of Transylvania, Miercurea Ciuc, Romania

Avinash Marwal Department of Biosciences, College of Arts, Science and Humanities, Mody University, Sikar, Rajasthan, India

Kamlesh K. Meena School of Edaphic Stress Management, ICAR-National Institute of Abiotic Stress Management, Pune, India

Faisal Mehmood Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Mitali Mishra Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Allahabad, Uttar Pradesh, India

Rohit Mishra Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Allahabad, Uttar Pradesh, India

D. Mohandass Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India

Dhiman Mukherjee Bidhan Chandra Krishi Viswavidyalaya, Directorate of Research, Kalyani, West Bengal, India

T. Muthukumar Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India

Sneha S. Nair Department of Agricultural Microbiology, University of Agricultural Sciences, Bangaluru, India

Miguel O.P. Navarro Laboratory of Microbial Ecology, Department of Microbiology, State University of Londrina, Londrina, Paraná, Brazil

Kiran Pavuluri Sirius Minerals Plc, Scarborough, UK

F. Pinzari CREA-Research Center Agriculture and Environment, Rome, Italy

V. Rajesh Kannan Rhizosphere Biology Laboratory, Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Mohammad Radhi Sahib Department of Horticulture, University of Kentucky, Lexington, KY, USA

Pramod Kumar Sahu ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, Uttar Pradesh, India

Abhineet Sain Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi, India

Andrea Sanchez-Barrios Department of Horticulture, University of Kentucky, Lexington, KY, USA

Debojit Kumar Sarmah Plant Genomic Laboratory, Medicinal Aromatic & Economic Plants (MAEP) Group, Biological Sciences & Technology Division (BSTD), CSIR-North East Institute of Science and Technology, Jorhat, Assam, India

Sh. M. Selim Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

G. Selvakumar ICAR-Indian Institute of Horticultural Research, Bengaluru, India

Amrita Sengupta Department of Agronomy, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Muhammad Shahid Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

Satyawati Sharma Centre for Rural Development and Technology, Indian Institute of Technology-Delhi, New Delhi, India

Shilpi Sharma Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi, India

Shivesh Sharma Department of Biotechnology, Motilal Nehru National Institute of Technology (MANNIT) Allahabad, Allahabad, Uttar Pradesh, India

Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Uttar Pradesh, India

Ane S. Simionato Laboratory of Microbial Ecology, Department of Microbiology, State University of Londrina, Londrina, Paraná, Brazil

Anjali Singh School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, India

Ankit K. Singh Institute of Biosciences and Technology, Shri Ramswaroop Memorial University, Lucknow, Uttar Pradesh, India

Dhananjaya P. Singh ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, Uttar Pradesh, India

Divya Singh School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, India

Jaspreet Singh Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad, Uttar Pradesh, India

Narendra P. Singh School of Edaphic Stress Management, ICAR-National Institute of Abiotic Stress Management, Pune, India

Pratiksha Singh Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning, China

Rajesh Kumar Singh Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning, China

Ravindra Soni Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Ajay M. Sorty School of Edaphic Stress Management, ICAR-National Institute of Abiotic Stress Management, Pune, India

N.M. Sudheep Department of Plant Science, School of Biological Sciences, RST Campus, Central University of Kerala, Kasaragod, Kerala, India

C.S. Sumathi PG and Research Department of Microbiology, K. S. Rangasamy College of Arts and Science, Tiruchengode, Tamil Nadu, India

Deep Kumar Suyal Department of Microbiology, College of Basic Sciences & Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Muhammad Tahir Department of Environmental Sciences, COMSATS Institute of Information Technology, Islamabad, Pakistan

M. Tartanus Research Institute of Horticulture, Skierniewice, Poland

Durgesh Kumar Tripathi Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Allahabad, Uttar Pradesh, India

Madhu Bala Tyagi Botany Department, MMV, Banaras Hindu University, Varanasi, India

Sana Ullah Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Neha Upadhyay Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad, Uttar Pradesh, India

S. Uppala Texas A&M AgriLife Research Center, Beaumont, TX, USA

P. Kishore Varma Acharya N. G. Ranga Agricultural University, Regional Agricultural Research Station, Anakapalle, Andhra Pradesh, India

Pankaj Verma Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad, Uttar Pradesh, India

Rishi Verma Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad, Uttar Pradesh, India

K. Vijay Krishna Kumar Acharya N. G. Ranga Agricultural University, Regional Agricultural Research Station, Anakapalle, Andhra Pradesh, India

Kanchan Vishwakarma Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad, Uttar Pradesh, India

Stephen J. Wylie Plant Biotechnology Research Group, Western Australian State Agricultural Biotechnology Centre (SABC), Murdoch University, Perth, Western Australia, Australia

Li-Tao Yang Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning, China

Mona S. Zayed Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

About the Editors

Dhananjaya Pratap Singh is presently Principal Scientist in Biotechnology at ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, India. He did his master's degree from G. B. Pant University of Agriculture and Technology, Pantnagar, and Ph.D. in Biotechnology from Banaras Hindu University, Varanasi. His research interests include plant-microbe interactions, bioprospecting of metabolites of microbial and plant origin, microbe-mediated stress management in plants, metabolomics-driven search for small molecules, and bioinformatics in microbial research. He was involved in the development of a supercomputation infrastructure facility for agricultural bioinformatics in microbial domain at ICAR-NBAIM under the National Agricultural Bioinformatics Grid (NABG) program of ICAR. He has been awarded with various prestigious awards including the Dr. A. P. J. Abdul Kalam Awards for Scientific Excellence in 2016 from Marina Labs. Currently he has published more than 134 publications including 73 research papers, 16 scientific reviews, 25 book chapters, 20 magazine articles, several workshop manuals/training modules, 3 edited books, and one Indian patent.

Harikesh Bahadur Singh is presently Professor and Head of the Department of Mycology and Plant Pathology at the Institute of Agricultural Sciences, Banaras Hindu University. He served the State Agriculture University, Central University, and CSIR institutes in teaching, research, and extension roles. His major research focus is on bioinoculants, biological control of plant pathogens, and nanobiotechnology. In recognition of his scientific contributions and leadership in the field of plant pathology, he has been honored with several prestigious awards, notably being the CSIR Technology Prize for Biological Sciences by the Honorable Prime Minister of India, M. S. Swaminathan Award by the Society for Plant Research, Vigyan Bharti Award, Prof. V. P. Bhide Memorial Award by the Society for Plant Research, Scientist of Excellence Awards, BRSI Industrial Medal Award, Jyoti Sangam Award, Akshyavat Samman Award, Distinguished Scientist Award by the Society for Research Development in Agriculture, Prof. Panchanan Maheshwari Medal by the Indian Botanical Society, Rashtriya Gaurav Award by IIFS, Plant Pathology Leader Award by IPS, CSIR Award for S&T Innovation for Rural Development (CAIRD), Environment Conservation Award, and Vigyan Ratna Award by the UP Council of Science and Technology. Dr. Singh has been a fellow

of the National Academy of Agricultural Sciences. Currently, he is also serving as an associate/academic/board editor in journals of international repute. Professor Singh has published more than 300 publications, several training modules/manuals, 17 edited books, and 20 patents (USA, Canada, PCT).

Ratna Prabha obtained her master's degree in Bioinformatics from Banasthali Vidyapeeth and Ph.D. degree in Biotechnology from Mewar University, India. She has been awarded with the SERB-National Postdoctoral Fellowship of the Department of Science and Technology (DST), Government of India, and is presently affiliated with Chhattisgarh Swami Vivekanand Technical University, Bhilai. She has been engaged in developing various digital databases on plants and microbes and has published two edited books, many book chapters, and various research papers and review articles in journals of international repute. Her current research interest lies in microbe-mediated stress management in plants, database development, comparative microbial genome analysis, phylogenomics and pangenome analysis of prokaryotic genomes, and metagenomics data analysis. She has completed several bioinformatics demonstration tasks at different national training programs on bioinformatics and computational biology. She has been awarded Young Scientist Awards at G. B. Pant University of Agriculture and Technology; S&T SIRI, Telangana; and CGCOST, Chhattisgarh.

Microbial Interactions and Plant Growth

1

Sh.M. Selim and Mona S. Zayed

Abstract

Microbial interactions in soil are considered as one of the most important activities that occur in the terrestrial ecosystem. They affect all the dynamic processes of plants and other living organisms that live near from them either directly or indirectly. There are two types of microbial interaction that occur in soil. The interactions that occur between individuals within the same species are called intraspecific interaction, and those that occur between organisms of different species either two microbial populations or microbial population and plants or animals are called interspecific interactions. Each microorganism could perform more than one type of interaction depending on the sounding environmental conditions, its partner in the interaction. Microbial interactions are very essential for plant growth and health.

Keywords

Microbial interactions • Intraspecific interaction • Interspecific interactions and plant growth

1.1 Introduction

Soil is the biggest active terrestrial ecosystem, and this activity is determined by the numerous and diverse interactions among its physical, chemical, and biological components, which are controlled by the environmental conditions (Barea et al. 2005;

S.M. Selim • M.S. Zayed (✉)
Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
e-mail: monaszayed@agr.asu.edu.eg

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_1

Buscot and Varma 2005). Through these interactions, varieties of relationships occur between different microorganisms either between themselves or with plants.

The rhizosphere's content regularly contains thousands of interactions between its different constituents. These processes include exudation, water uptake, nutrient mobilization, organic matter decomposition, and respiration (DeAngelis 2013; van der Heijden and Hartmann 2016). By comparing the properties of both root-associated soil and root-free soil, there were major differences in their biological, physical, and chemical properties, which are responsible for changing in microbial diversity, numbers, and activity (Barea et al. 2005).

This chapter is concerning about illustrating different microbial interactions that occur in the soil especially the rhizosphere and linking these relations with their effects on plant growth and performance.

Symbiosis in Biology

The term symbiosis is taken from the Greek sym which means "with" and biosis that means "living" at which is defined as "living together," which is usually defined as long coexistence of two organisms. The term was first coined in 1879 by the German mycologist, Heinrich Anton de Bary, as "the living together of unlike organisms" (Das and Varma 2009; Martin and Schwab 2012, 2013). He believed that this terminology should include parasitic, communalistic, and mutualistic relationships between different species of microorganisms.

This terminology faced a lot of confusion and variation for over 130 years since Anton de Bary (1879) coined the word (Martin and Schwab 2012, 2013; Paracer and Ahmadjian 2000). For example, some biologists believed that mutualism is considered common restrictive definition of symbiosis. Furthermore, Pianka (2000) reformed the definition of symbiosis to comprise the interactions at which no species is harmed (i.e., mutualism, commensalism, and neutralism) (Martin and Schwab 2012). Therefore, in this chapter we decided not to discuss this term or insert it into the types of microbial interactions because of the confusions that face it.

1.2 Microbial Interactions in Soil

Soil microorganisms perform a number of interactions during their presence in the soil that comprise interaction with plant roots in the rhizosphere, interaction with soil constituents, as well as the interaction with other microbial communities that inhabit the rhizosphere (Barea et al. 2005; Bowen and Rovira 1999; Kennedy 1998). Microbial interactions regularly improve the sustainable development of agroecosystem, plant growth, and health.

The microbial community that existed in the rhizosphere is different forms that could be found in the bulk soil, as it was affected by root exudates that lead to high availability of nutrients and microbial biomass, which change the environmental conditions in the rhizosphere as a consequence of interactions between microorganisms as well as microbial interactions with higher plants and animals (Barea et al. 2005).

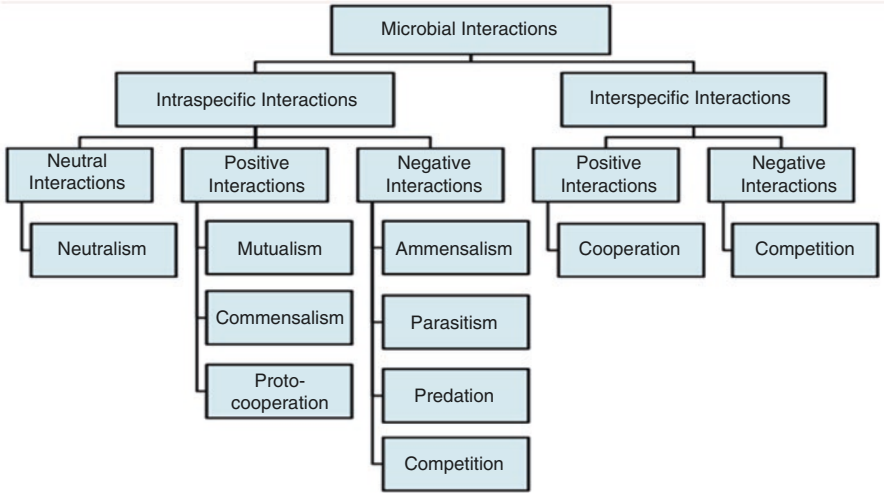


Fig. 1.1 Simplified scheme of microbial interactions

There are two types of microbial interactions that transpire in the rhizosphere: intraspecific interactions and interspecific interactions (Fig. 1.1). Intraspecific interactions occur between organisms of the similar species, while interspecific interactions occur between organisms of dissimilar species either two microbial populations or microbial population and plants or animals.

1.3 Intraspecific Interactions

It could be defined as interactions among individuals of single microbial population. This could be classified into two types:

1.3.1 Positive Interaction

This type of interaction is called cooperation or intraspecific cooperation as it improves the growth of the microbial population (Tarnita 2017). It appears in different types such as:

- Extended lag phase if small inoculum is used (less than 10% inoculum used) to avoid failure to grow
- Adherence of microcolonies to normal habitats by the minimum infectious dose
- Motile bacteria that remain in colonies during the growth by making synchronized immigration (mass movement) to appear in the form of colony
- Attaching of the cells to the matrix during biofilm formation

- Cooperation of the cells in degrading insoluble substrates such as lignin and cellulose by production of suitable enzymes
- Genetic exchange between members of the same population through transformation, transduction, and conjugation to acquire resistance to different abiotic stress

1.3.2 Negative Interaction

Intraspecific competition occurs as a negative interaction between the individuals of the same population (competition within population). It is considered as a very important factor that regulates population size and density. Also it is responsible for the equal distribution of individuals within population in the ecosystem (Atlas and Bartha 1986). It appears in different types such as:

- Low concentrations of available nutrients in natural habitats since all the cells use the same substrates and occupy the same ecological niche
- High microbial densities in natural habitat that lead to accumulation of some toxic products

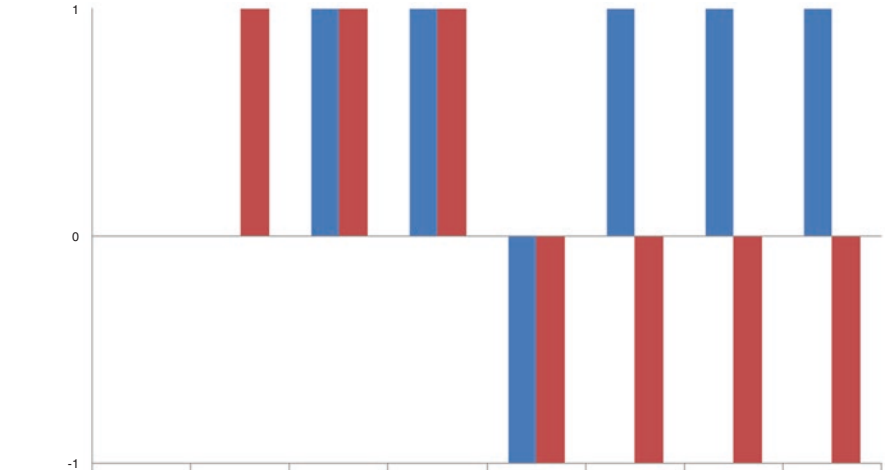
1.4 Interspecific Interactions

It occurs among diverse microbial population that exhibit many different types of interactions. When it leads to increase the growth rate, it is called positive interaction, while it is referred to as negative interaction when it leads to decrease the growth rate, while some interactions are indifferent or neutral. In accordance to Burkholder (1952), different researchers illustrated different microbial interactions by using his famous symbols +, −, and 0 for any pair of interacting species at which + = beneficial effect, − = harmful effect, and 0 = neutral effect (Martin and Schwab 2012, 2013). Most microbial interactions are illustrated in Fig. 1.2, by considering that interaction occurs between to different populations at which one of them is (A) and the other is (b), and the type of interaction is symbolized as + 1 = beneficial effect, − 1 = harmful effect, and 0 = neutral effect.

1.4.1 Neutral Interactions (Neutralism)

It is a neutral association between dissimilar microorganisms inhabiting the same environment without impacting each other (the two members neither losing nor achieving anything from the relationship). Such association mostly is not a prevalent form of interaction (it is rare) as it is always transitory since environmental conditions always change.

This relationship occurs if the populations are living in culture with distinctive characteristics (Freilich et al. 2011; Weiner et al. 2012), such as:



	Neutralism	Commensalism	Proto-cooperation	Mutualism	Competition	Predation	Parasitism	Ammensalism
	Neutral Interactions	Positive Interactions			Negative Interactions			
Population A	0	0	1	1	-1	1	1	1
Population B	0	1	1	1	-1	-1	-1	-1

Fig. 1.2 Microbial interactions, basic characteristics of neutral, and positive and negative interactions that occur between different microorganisms (+ 1 = beneficial effect, − 1 = harmful effect, and 0 = neutral effect)

1. Separated by vast distance
2. Having dissimilar nutrient requirement
3. Living in oligotrophic lakes or marine habitats
4. Living in environment that does not authorize microbial growth like frozen products, polar ice, and frozen habitats

1.4.2 Positive Interactions

Such interactions consist of different relationships between different populations at which one population at least is benefited while the other is either benefited or not affected.

1.4.2.1 Mutualism

It is an obligatory or highly specific interaction between two populations in which both of them benefit from each other. It usually required close physical connection in which both partners may act as if they are one. When they exist separately, the physical tolerance and metabolic activities will be different for each single symbiont. Theoretically, mutualism could lead occasionally to the assembling of a new species (Freilich et al. 2011; Leung and Poulin 2008; Weiner et al. 2012).

1.4.2.1.1 Types of Mutualism

Mutualism could be classified into different types according to partner's selection and function or purposes of the relationship.

1.4.2.1.2 Types of Mutualism According to Interaction Patterns

According to the partner's selection, mutualism could occur in two forms:

- *Obligate Mutualism*: It occurs when both microorganisms live together in close proximity, and both species cannot survive without its mutualistic partner.
- *Facultative Mutualism*: It occurs when one of the two partners can survive without its mutualistic partner by itself in some conditions.

1.4.2.1.3 Types of Mutualism According to Interaction Purposes

Mutualistic relationships between different populations have three main purposes**:**

Trophic Mutualism It is also called *resource-resource interactions*. It is a type of mutualistic association, which comprises the exchange of nutrients between two species. Also, it is called "*syntrophism*" (Greek meaning: syn = mutual and trophe = nutrition)**.**

Defensive Mutualism It is also called *service-resource relationships*. It appears when one organism provides shelter or protection from predators or pathogens, while the other provides food.

Service-Service Mutualism It appears when one species receives service from its partner in return for transporting another service to the other organism. This type of mutualism is not common between microorganisms in the soil.

1.4.2.1.4 Some Examples of Mutualism

No clear or sharp type of mutualism could be detected between microorganisms in the soil since two or more types could be integrated together in nature.

a. Lichen

The most common example for mutualism is the lichen, which is an association between fungus (ascomycetes) and algae (green algae) or cyanobacteria (blue green algae), since most types of mutualisms occur between the two symbionts. Fungal partner surrounds the algal partner's cells within fungal tissues that are exclusive to lichen associations.

In this type of association, algae get benefits through protection afforded to it by fungal hyphae from environmental biotic and abiotic stresses and excess light intensity as well as it is provided with water and minerals that help it to grow while fungal partner obtains nutrients and oxygen from alga. When the blue green algae are the partners in the lichen association, the fungus gets benefit also from the fixed nitrogen.

Although lichen association improves the range of ecological survival for both partners, nonetheless, this relationship is not permanently necessary for their growth and reproduction in natural environments especially algae, since many of the algal symbionts can live independently (Aislabie et al. 2013; Lutzoni et al. 2001; Nash 1996).

b. Mycorrhizae

It is a mutualistic association among mycorrhizal fungi and plant roots, in which plants provide fungus with carbohydrates and offer it protection. In turn the fungus increases the surface area of plant roots for absorbing water, nitrogenous compounds, phosphorus, and other inorganic nutrients (e.g., phosphate) from the surrounding soil and delivers them to the plant which improves plant growth and health (Zayed et al. 2013). Also, mycorrhizal fungi shelter plant roots from invasion by soilborne root-infecting pathogens.

Endomycorrhizal symbiosis increases plant performance through improving their tolerance to different environmental stresses, which may be biotic, e.g., pathogen attack, or abiotic (e.g., drought, salinity, heavy metal toxicity, or presence of organic pollutants (Manaf and Zayed 2015) and also enhancing soil structure through formation of hydro-stable aggregates essential for good soil structure (Barea and Pozo 2013).

c. Symbiotic N_2 Fixation

The nitrogen-fixing bacteria provide the plants with nitrogenous compounds, while in return the plants provide the nitrogen-fixing bacteria with carbohydrates. This mutualistic association improves plant growth and health, and it has different types which include *Rhizobium* spp. with root nodules of legume plants and *Frankia* which is an actinomycete (nodule-forming filamentous bacteria) with the roots of *Alnus* and *Casuarina* trees which are "nonlegumes" (Selim et al. 2003).

1.4.2.2 Commensalism

It is a relationship at which one population benefits, while the other population is unaffected (neither harmed nor benefited). It is a very common relationship between different microbial populations. It is usually unidirectional, not obligatory relationship and occurs when the unaffected population adapts the habitat in such a way that the other population benefits.

1.4.2.2.1 Examples of Commensalism

(a) During the alteration of complex molecules by one population into other substrates in soil, the degraded products are regularly used by numerous other fungi and bacteria which cannot utilize complex molecules in the soil, like conversion of cellulose and lignin by fungi through production of extracellular enzymes**.** This process improves the nutritional properties of the soil which in

return improve the activities of microbial communities in soil and improve plant growth and health.

(b) During the growth of facultative anaerobes and obligate anaerobes in the same site, the facultative anaerobes consume the oxygen from the environment which helps the obligate anaerobes to grow. This process occurs commonly in soil (Atlas and Bartha 1986).

1.4.2.3 Protocooperation (Synergism)

Synergism (protocooperation) is a relationship that occurs between two or more populations at which both or all of them benefit. In this relationship microbial populations perform a function which may not be performed individually or produce a new product that neither each population can produce alone.

This relationship is different from mutualism because as it is not an obligatory interaction, none of the species depend on the relationship for existence, as each member can live and produce its own food individually. It is also called loose relationship since one member can be replaced by another microorganism (Atlas and Bartha 1986).

1.4.2.3.1 Types of Protocooperation

There are different types of protocooperation relationship that could be found in the terrestrial ecosystem which is considered very useful in agriculture:

- *Nutritional protocooperation*: It is the most popular relationship between terrestrial populations at which the populations exchange nutrients between each other. Such a cooperation is also called syntrophism protocooperation.
- *Metabolism of toxic end products*: In this type of association one organism embellishes its associate by eliminating toxic substances from the habitation versus obtaining carbon products made by the other associate partner.
- *Production of derivative enzymes*: *Arthrobacter* and *Streptomyces* (soil flora) produce enzymes which collectively degrade diazinon which is an organophosphate pesticide (useful in the degradation of xenobiotics or recalcitrant compounds).

1.4.2.3.2 Examples of Protocooperation

(a) *Thiobacillus* spp. is an autotrophic bacterium which is aerobic, acidophilic, carbon dioxide fixer as well as sulfur and iron oxidizer, while *Beijerinckia* spp. is a heterotrophic bacterium which is an aerobic nitrogen fixer and slow grower. These two organisms could be grown together since *Thiobacillus* spp. fix carbon dioxide for itself and *Beijerinckia* spp., while *Beijerinckia* spp. fix nitrogen to satisfy the need from nitrogenous compounds for itself and *Thiobacillus* spp. in medium devoid of carbon and nitrogen sources. Also, the association of *T. ferrooxidans* with *Beijerinckia lacticogenes* enhanced the ratio and amount of Cu-Ni sulfide concentrate leaking in the medium (Barbosa et al. 2000; Trivedi and Tsuchiya 1975; Tsuchiya et al. 1974). This relationship in the terrestrial ecosystem improves the carbon and nitrogen content in the soil as well as

mineral contents which in turn improve the growth and nutritional contents of plants in soil.

(b) Protocooperation also occurs between higher plants growing in the soil and bacteria or fungi living in the rhizosphere. Neither each of them is dependent on this association, since bacteria and fungi get benefits from the exudates of plant roots and interact with each other to form the essential nutrients necessary for plant's growth such as decomposed organic materials, production of phytohormones, minerals, water, vitamins, and amino acids which in return improve soil fertility as well as the plant health and growth (Seneviratne et al. 2008).

1.4.3 Negative Interactions

It consists of different relationships between different populations either two or more, at which one population at least is harmed while the other is either harmed, benefited, or not affected.

1.4.3.1 Ammensalism (Antagonism)

It is the most common negative relationship in nature at which one microbial population suppresses or adversely influences the growth or the activities of the other population in the same environment by producing inhibitory substances either directly or indirectly.

The population that produces the inhibitors is not affected by them and therefore gains the antagonistic edge. These inhibitors may be antibiotics, toxins, organic acids, alcohols, or other allelochemicals, lytic enzymes, as well as harmful gases like methane, ethylene, HCN, nitrite, or sulfides or other volatile sulfur compounds. The population that adversely affects the other is called antagonistic species, and it constantly has great practical importance.

1.4.3.1.1 Types of Antagonism

There are diverse types of antagonism according to the nature of substances that is used in the antagonism.

- *Antagonism by Antibiosis*: This process is called antibiosis in which the antibiotics or other allelochemical metabolites are produced by one organism to inhibit another organism (Ahmad et al. 2008; de Souza et al. 2003).
- *Antagonism by Lytic Enzymes*: Many soil microorganisms, like myxobacteria and *Streptomyces*, could antagonize disease-causing agents by the production of some lytic enzymes which destroy other cells by digesting their cell wall or other protective surface layers such as glucanase, protease, cellulase, and chitinase enzymes (Dunne et al. 1997). Such enzymes have the ability to devastate the oospores of phytopathogenic fungi (El-Tarabily 2006), affect germination of spore and germ-tube elongation of phytopathogenic fungi (Frankowski et al. 2001), as well as degrade bacterial cell wall (El-Tarabily 2006).

1.4.3.1.2 Compound Antagonism

It is clear that diffident mechanisms in antagonism could be appearing in nature between different microorganisms. One organism could use different types of antagonisms to fight the pathogens.

1.4.3.1.3 For example:

(a) *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptomyces* spp. were described as producers of antibiotics (antibacterial or antifungal) that inhibit various pathogens and suppress different plant diseases (de Vasconcellos and Cardoso 2009), like *F. oxysporum* (Kumar 1999) and *Rhizoctonia solani* (Asaka and Shoda 1996), *Verticillium albo-atrum*, *Alternaria solani*, *Pseudomonas solanacearum* (El-Abyad et al. 1993), *Alternaria brassicicola*, *Colletotrichum gloeosporioides*, *Penicillium digitatum*, and *Sclerotium rolfsii* (Khamna et al. 2009), which are considered as fundamental agents of different plant diseases (Bouizgarne 2013).

(b) In lichens, algae produce O_2 which prevents the growth of anaerobic bacteria on it, while the fungi produce cyanide in concentrations toxic to other microorganisms.

(c) *Thiobacillus* spp. reduces the soil pH to reach values as low as 2 through the oxidation of sulfide to sulfate. This low pH inhibits the growth of any pH-sensitive microorganism in soil.

1.4.3.1.4 Parasitism

It is a relationship between two dissimilar organisms that is called host-parasite relationship in which one of them (parasite) lives in or on the other organism (host). The parasite lives in close contact with the host and forms metabolic association with the host and feeds on their cells, tissues, or fluids in which the parasite is profited, while the host is adversely affected. Sometimes the relation between the host and parasite could be diverged from parasitic relationship to a pathogenic relationship.

This relationship is widely spread in soil communities and characterized by its long period of contact and the specialization between parasite and host. Also, parasite is usually smaller than the host (in most cases). This relationship has two sides, one is useful while the other harmful. If the parasitism is accomplished on bacteria that are considered pathogenic to plants, it is considered as a useful relationship for plant growth and health. While if the parasitism is accomplished on bacteria that are considered profitable to plants, it is considered as a harmful relationship for plant growth and health (Compant et al. 2005; Wu et al. 2009).

1.4.3.1.5 Types of Parasitism

Parasitism could be classified according to its nature of parasitism and its infection type.

1.4.3.1.6 Types of Parasitism According to Parasitism Patterns

- *Obligate parasitism*: Occurs when the parasite cannot live without its host.

- *Facultative parasitism*: Occurs when the parasite can survive by itself without its host cells in some conditions.

1.4.3.1.7 Types of Parasitism According to Infection Form

- *Ectoparasitism*: The parasite remains outside the host cells.
- *Endoparasitism*: The parasite penetrates the host cells.

1.4.3.1.8 Examples of Parasitism

(a) Viruses which attack bacteria (bacteriophages), fungi, algae, or plants are strict endoparasites (intracellular parasites) as they obligate parasite and cannot be cultivated on the media as free-living forms.
(b) Chytrid fungi parasitize on algae as well as other fungi by penetration into the host.

1.4.3.2 Predation

Predation is the most dramatic relationship among microorganisms in nature, at which predator organism directly attacks a prey organism and feeds on it. This relationship has short duration, at which predators may or may not kill their prey prior to feeding on them, but the normal result is generally absorption of the prey's tissue through ingestion and subsequently the death of prey. Prey may be larger or smaller than predator.

1.4.3.2.1 Important of Predation

The predators have the capability to mineralize the organic compounds that are produced by autotrophs before it reaches the higher consumers; this process increases the rate of nutrient cycling, in addition to returning the nutrients to the primary producers, which stimulate their activities that leads to improve the nutritional content of the soil. Also the predators protect the environment from the prey by ingesting it, which is usually plant pathogen.

1.4.3.2.2 Examples of Predation

The following predatory bacteria have been observed and characterized in soil:

(a) *Bdellovibrio bacteriovorus* is a predatory bacterium, which penetrates the cell wall and multiplies between the wall and the plasma membrane, which causes lysis of the prey and releases its progeny. It attacks and consumes different bacterial strains, including *Escherichia coli* and *Aquaspirillum serpens*, *Salmonella typhimurium*, and *Helicobacter pylori* (Dwidar et al. 2012).
(b) *Vampirococcus* spp. adheres to the surface of phototrophic bacteria *Chromatium* spp. (purple sulfur bacterium). It does not penetrate its prey's cells as it remains attached to the cell wall by specific attachment structures, and it destroys its prey (Dwidar et al. 2012).
(c) *Daptobacter* spp. penetrates and degrades the cytoplasm of several genera of *Chromatiaceae*. It grows and propagates in the cytoplasm (Guerrero et al. 1986).

1.4.3.3 Competition

It is a relation that occurs between different populations in the soil which use the same limiting resources that are insufficient to support all the individuals. These resources include raw materials important for life such as water, light, nutrients, oxygen, and space for occupying or any other resources, which is essential for survival and reproduction. In this relation, the superior adapted microorganism will dominate and/or eliminate the others, which are relying on the same inadequate nutrient substances. Also, organisms which have the capability to grow faster are considered good competitors (Hibbing et al. 2010; Wu et al. 2009).

1.4.3.3.1 Types of Competition

There are two ways in which microorganisms compete:

Resource Competition It occurs when the growth rates of both populations are limited by the same resource and one population has the ability to diminish the availability of that resource for the other populations. It is also called indirect competition, passive competition, and exploitative competition.

Interference competition It occurs between two populations in which one of them damages the other population's habitat either physically or chemically and excludes it from the habitation. This relationship is also called direct competition or active competition.

Few Examples The chlamydospores of *Fusarium*, oospores of *Aphanomyces*, as well as conidia of *Verticillium dahliae* need exogenous nutrients to germinate in soil, while some other fungi bacteria that inhabit the soil have the ability to deplete these important nutrients that are required for spore germination and thereby delay its germination which results in decrease in the population of these plant pathogens in the soil (Hibbing et al. 2010; Wu et al. 2009).

1.5 Conclusion

Soil harbors great diversity of microorganisms; this diversity is responsible for biological equilibrium created by the associations and interactions of all individuals found in the community. Plants are the main responsible for most of these interactions due to their root exudates. These interactions perform significant roles on plant growth and health and the ecological fitness and resistance of plants to different biotic and abiotic stresses in soils.

There is no single type of microbial interaction that could be found for each microorganism since one organism could have different types of interactions with other populations and these interactions could be diverged in accordance to diverging of ecological factors, plant type, and microbial diversity in its terrestrial ecosystem. For example, *Trichoderma* spp. could be considered as the most essential biological control agent in that soil because it utilizes different mechanisms to fight

various disease-causing agents including parasitism, which directly attack the pathogens especially fungi; competition, as it has the ability to colonize the soil or to compete for nutrients which is causing relegation of pathogen from plant rhizosphere; antagonism (antibiosis), as it is able to produce secondary metabolites which have a lethal or depressant effect on the plant pathogen as well as it has ability to make a repressive environment by diverse relations in the soil community to create unfavorable ecological conditions that limit the development or multiplication of pathogenic populations; and the secretion of numerous compounds that induce the mechanisms of plant resistance to combat pathogens attack.

References

Ahmad F, Ahmad I, Khan M (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163(2):173–181

Aislabie J, Deslippe JR, Dymond J (2013) Soil microbes and their contribution to soil services. Ecosystem services in New Zealand: conditions and trends. Manaaki Whenua Press: Lincoln, New Zealand, pp 143–161

Asaka O, Shoda M (1996) Biocontrol of Rhizoctonia solani damping-off of tomato with Bacillus subtilis RB14. Appl Environ Microbiol 62(11):4081–4085

Atlas RM, Bartha R (1986) Interactions among microbial populations. In: Brady IB, Lisa D (eds) Microbial ecology: fundamentals and applications. The Benjamin/Cummings Publishing Company, Inc., California, pp 60–98

Barbosa HR, Thuler DS, Shirakawa MA, Miyasaka NR (2000) Beijerinckia derxii stimulates the viability of non-N2-fixing bacteria in nitrogen-free media. Braz J Microbiol 31(3):167–172

Barea J, Pozo M (2013) Arbuscular Mycorrhizas and their significance in promoting soil-plant systems sustainability against environmental stresses. In: Rodelas B, González-López J (eds) Beneficial plant-microbial interactions: ecology and applications. Beneficial Plant-Microbial Interactions: Ecology and Applications. CRC Press, Boca Raton, pp 353–387

Barea J-M, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56(417):1761–1778

Bouizgarne B (2013) Bacteria for plant growth promotion and disease management. In: Maheshwari DK (ed) Bacteria in agrobiology: disease management. Springer, Berlin

Bowen G, Rovira A (1999) The rhizosphere and its management to improve plant growth. Adv Agron 66:1–102

Burkholder PR (1952) Cooperation and conflict among primitive organisms. Am Sci 40(4):600–631

Buscot F, Varma A (2005) Microorganisms in soils: roles in genesis and functions. Springer, Berlin

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959

Das A, Varma A (2009). In: Symbiotic fungi. Springer, pp 1–28

DeAngelis KM (2013) Rhizosphere microbial communication in soil nutrient acquisition. Mol Microb Ecol Rhizosphere 1 & 2:823–832

de Souza JT, Arnould C, Deulvot C, Lemanceau P, Gianinazzi-Pearson V, Raaijmakers JM (2003) Effect of 2, 4-diacetylphloroglucinol on Pythium: cellular responses and variation in sensitivity among propagules and species. Phytopathology 93(8):966–975

de Vasconcellos RLF, Cardoso EJBN (2009) Rhizospheric streptomycetes as potential biocontrol agents of Fusarium and Armillaria pine rot and as PGPR for *Pinus taeda*. BioControl 54(6):807–816

Dunne C, Crowley JJ, Moënne-Loccoz Y, Dowling DN, O'Gara F (1997) Biological control of *Pythium ultimum* by Stenotrophomonas maltophilia W81 is mediated by an extracellular proteolytic activity. Microbiology 143(12):3921–3931

Dwidar M, Monnappa AK, Mitchell RJ (2012) The dual probiotic and antibiotic nature of Bdellovibrio bacteriovorus. BMB Rep 45(2):71–78
El-Abyad M, El-Sayed M, El-Shanshoury A, El-Sabbagh SM (1993) Towards the biological control of fungal and bacterial diseases of tomato using antagonistic Streptomyces spp. Plant Soil 149(2):185–195
El-Tarabily KA (2006) Rhizosphere-competent isolates of streptomycete and non-streptomycete actinomycetes capable of producing cell-wall-degrading enzymes to control *Pythium aphanidermatum* damping-off disease of cucumber. Botany 84(2):211–222
Frankowski J, Lorito M, Scala F, Schmid R, Berg G, Bahl H (2001) Purification and properties of two chitinolytic enzymes of Serratia plymuthica HRO-C48. Arch Microbiol 176(6):421–426
Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E (2011) Competitive and cooperative metabolic interactions in bacterial communities. Nat Commun 2:589
Guerrero R, Pedrós-Alió C, Esteve I, Mas J, Chase D, Margulis L (1986) Predatory prokaryotes: predation and primary consumption evolved in bacteria. Proc Natl Acad Sci 83(7):2138–2142
Hibbing ME, Fuqua C, Parsek MR, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8(1):15–25
Kennedy A (1998) The rhizosphere and spermosphere. Principles and applications of soil microbiology. Prentice Hall, Upper Saddle River, pp 389–407
Khamna S, Yokota A, Lumyong S (2009) Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World J Microbiol Biotechnol 25(4):649–655
Kumar BD (1999) Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chick pea growing in soils infested with Fusarium oxysporum f. sp. ciceris. Biol Fertil Soils 29(1):87–91
Leung T, Poulin R (2008) Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie Milieu 58(2):107
Lutzoni F, Pagel M, Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411(6840):937–940
Manaf HH, Zayed MS (2015) Productivity of cowpea as affected by salt stress in presence of endomycorrhizae and *Pseudomonas fluorescens*. Ann Agric Sci 60(2):219–226
Martin BD, Schwab E (2012) Symbiosis: "Living together" in chaos. Stud Hist Biol 4(4):7–25
Martin BD, Schwab E (2013) Current usage of symbiosis and associated terminology. Int J Biol 5(1):32
Nash T III (1996) Nitrogen, its metabolism and potential contribution to ecosystems. Lichen biology. Cambridge University Press, Cambridge, pp 121–135
Paracer S, Ahmadjian V (2000) Symbiosis: an introduction to biological associations. Oxford University Press, New York
Pianka ER (2000) Evolutionary ecology, 6th edn. Benjamin/Cummings, San Francisco, 512 p. Pirozynski K.A. Book reviews//Lichenologist. 1987, 19:439–442
Selim SM, Eweda W, Zayed M (2003) Prospects for evaluation of Frankia-Casuarina association under Egyptian conditions. 2.-interacting effects of Frankia with Casuarina species, soil types and va mycorrhizal inoculation. Arab Universities Journal of Agricultural Sciences (Egypt)
Seneviratne G, Kecskés ML, Kennedy IR (2008) Biofilmed biofertilisers: novel inoculants for efficient nutrient use in plants. In: ACIAR Proc. pp 126–130
Tarnita CE (2017) The ecology and evolution of social behavior in microbes. J Exp Biol 220(1):18–24
Trivedi N, Tsuchiya H (1975) Microbial mutualism in leaching of Cu− Ni sulfide concentrate. Int J Miner Process 2(1):1–14
Tsuchiya H, Trivedi N, Schuler M (1974) Microbial mutualism in ore leaching. Biotechnol Bioeng 16(7):991–995
van der Heijden MG, Hartmann M (2016) Networking in the plant microbiome. PLoS Biol 14(2):e1002378

Weiner A, Schopf S, Wanner G, Probst A, Wirth R (2012) Positive, neutral and negative interactions in cocultures between Pyrococcus furiosus and different methanogenic archaea. Microbiol Insights 5:1

Wu CH, Bernard SM, Andersen GL, Chen W (2009) Developing microbe–plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. Microb Biotechnol 2(4):428–440

Zayed MS, Hassanein M, Esa NH, Abdallah M (2013) Productivity of pepper crop (*Capsicum annuum* L.) as affected by organic fertilizer, soil solarization, and endomycorrhizae. Ann Agric Sci 58(2):131–137

Dynamics of Rhizosphere Microbial Communities of Cover Crops Dried with Glyphosate

2

J.S. Escobar Ortega and I.E. García de Salamone

Abstract

The use of cover crops (CC) may be associated with other management practices recommended to achieve high yields and collaborate to use available resources more efficiently. Glyphosate is a nonselective systemic herbicide, which is commonly used for drying CC. Here we included a review of the related topics and showed the effects of drying oats and rye with glyphosate, inoculation with two plant growth-promoting rhizobacteria, and nitrogen fertilization on rhizosphere microbial communities at field conditions in the western Pampas of Argentina. Rhizosphere samples were obtained at three times: before drying the CC, a month after this, and at harvest time of soybean which was grown after each CC. Counts of viable cells and physiology of rhizosphere microbial communities were analyzed. The inclusion of CC dried with glyphosate modifies their associated rhizosphere microbial communities. Their numbers significantly decreased or increased. For some microorganisms, these changes were temporary because their amounts at soybean harvest time did not differ from those obtained when the sampling was done before drying CC with glyphosate application. Besides, our results indicate that the drying time must be chosen taking into account CC types and their phenology. This scientific information is evidence of changes on rhizosphere microbial communities due to the management of CC with glyphosate in combination with or without both inoculation and fertilization of CC. These data are agronomic and environmentally relevant because they have shown that the type of management would impact on the quality and health of the soil and therefore in agroecosystem sustainability.

J.S. Escobar Ortega • I.E. García de Salamone (✉)
Unit of Agricultural and Environmental Microbiology, Department of Applied Biology and Foods, Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina
e-mail: igarcia@agro.uba.ar

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_2

Keywords
Rhizosphere microorganisms • Soybean • Rye • Oats • *Pseudomonas fluorescens* • *Azospirillum*

2.1 Introduction

In recent years, agriculture has evolved to long agricultural cycles and in some cases to continuous agriculture (Ruffo and Parsons 2004) combined with non-tillage and the use of agrochemicals (Pound 1998; García 1999). This situation led to intensive land use, driven by the expansion of the agricultural frontier over areas not suitable for agriculture, where soils are much more fragile and more susceptible to water and wind erosion (Pound 1998; Pengue 2009; Sasal et al. 2006; Salsal 2012, 2013). The most relevant characteristics of this phenomenon called "agriculturization" are, on the one hand, the increase of the annual summer crops at the expense of the stagnation of the winter crops and, on the other hand, the exponential growth of the soybean (*Glycine max* L. Merrill) in comparison with the rest of the species (Carreño and Viglizzo 2011; ACSOJA 2015). In each region, other crops and livestock were replaced by this legume (Ruffo and Parsons 2004). Currently, soybean monoculture, the intensive use of agrochemicals, and the low replenishment of carbon and nutrients are common (Scianca et al. 2009). Due to their high profitability, the extensive crop farmers focused exclusively on cultivating transgenic soybeans with resistance to the herbicide glyphosate. Thus, this crop went from occupying almost 5 million hectares in 1990 to 20.6 million hectares for the summer season 2015–2016, only in Argentina. Grain production, for its part, increased from 10 to 57.6 million tons for the same period of time (MinAgri 2016). In this way, Argentina became the first country to export soybean oils and flour and the third exporter of soybean grains (ACSOJA 2015). Similar situation was developed in Brazil.

On the other hand, the limited contributions of soybean stubble, even under non-tillage, have shown to affect the contents of organic matter (Andriulo et al. 1999; Satorre 2003) with a consequent negative carbon balance in the soil favoring degradation processes (Alvarez et al. 2006). This is worrying, because approximately 0.1% of organic matter is lost per centimeter of degraded soil (Casas 2013). In addition, Salsal (2013) indicates that the monoculture does not provide ecological and agronomic benefits, as it leads to a decrease in the biodiversity of both the quality and the amount of organic matter available in the soil. In this environment, microorganisms degrade organic matter and directly impact soil properties (Ferreras et al. 2009).

Thus, due to the great economic importance of soybean cultivation worldwide and the low input of residues with a low C/N ratio (Studdert and Echeverría 2000), a negative N balance is also established, which contributes to soil impoverishment (Zotarelli et al. 2002; Cheng et al. 2003). In addition, since its residue decomposes rapidly and leaves the soil exposed to erosive action (Salsal 2013), the need arises to incorporate tools to favor the sustainability of the system.

2.2 Use of Cover Crops as a Management Alternative

One of the alternatives is to include cover crops (CC) in the crop sequence in order to increase the soil carbon input through their residues. Thus, the quality of the soil is improved (Alvarez et al. 2006; Martinez et al. 2013), and in the medium term, the negative carbon balance suffered by the extensive agricultural systems is mitigated (Scianca et al. 2011). The productive capacity of soils is directly associated with their organic matter content, which is the main reserve of organic carbon and the main source of nutrients for the crops (Urquiaga et al. 2004). For this reason, CC represents a technological alternative that balances the carbon of the soil, contributing a significant improvement for the physical and chemical properties of it (Cordone and Hansen 1986; Altieri 1994; Ruffo and Parsons 2004; Carfagno et al. 2008). However, very little is known about the dynamics of the microbiological properties associated with this system.

CC can be defined as those crops that grow specifically to keep the soil covered, protecting it from erosion, avoiding the loss of nutrients by washing and runoff. In addition, they are used to reduce compaction, minimize residual nitrate leaching, increase carbon content, improve plant nutrition, lower soil temperature, increase water use efficiency, contribute to water table depression in very humid periods, as well as reduce the level of weeds and the use of agrochemicals (Ruffo and Parsons 2003, 2004, Scianca et al. 2011; Fernández et al. 2012). Therefore, the use of CC and zero tillage is an effective measurement to conserve and maintain productive potential of the soil (Altieri 1994).

CC are species with desirable characteristics to include in the crop sequence with the commercial crops (Espindola et al. 2005) such as soybean. CC are different from pasture because they do not produce a direct rent due to the reason that they are not harvested. They grow out between two commercial crops and are not incorporated into the soil and are not grazed unlike the green manures (Ruffo and Parsons 2003, 2004; Restovich et al. 2012). The residues of CC remain on the surface, releasing nutrients contained in the aerial and radical biomass of the plants, providing energy to the microbial and mesofauna communities, and thus improving soil fertility (Ruffo and Parsons 2004; Álvarez et al. 2008). In some cases, CC are legume species that can receive N inputs through biological fixation, while other CC act by limiting the leaching of nutrients, especially N, to the underground aquifer (Parkin et al. 2006). On the other hand, CC residues inhibit weed growth, creating conditions similar to those that can be found at greater depth, i.e., less light and low daily thermal amplitude (Pérez and Scianca 2009). In addition, these residues sometimes release phytotoxic substances resulting from their degradation processes (Teasdale 1996; Teasdale et al. 2007). These characteristics of CC residues would be related to the amount of biomass they produce (Liebman and Davis 2000). The biomass produced can vary according to the species, so it is very important to consider the rate of decomposition of the residues, water contribution to the soil profile, types of crops of the sequence and the nutritional requirements of the next crop in the sequence (Carfagno et al. 2007).

The most used grasses are oats (*Avena sativa* L.), black oats (*Avena strigosa* L.), yellow oats (*Avena byzantine* L.), rye (*Secale cereale* L.), and Italian ryegrass (*Lolium multiflorum* L.), which are used as winter-rainfed crops to suppress weeds and reduce erosion in the season prior to the sowing of maize or soybean (FAO 1994; Amigone and Tomaso 2006; Restovich et al. 2012). In the Pampa region of Argentina, the grasses most commonly used are rye and oats (Pérez and Scianca 2009). Rye is widely used because it contributes large volumes of plant residues that decompose slowly, compared to other winter grasses. Once their decomposition begins, they release harmful substances such as phenols, terpenes or alkaloids, which affect the germination of weed seeds (Ruffo and Parsons 2004; Pérez and Scianca 2009; Carfagno et al. 2013). In addition, rye is considered one of the most tolerant crops to cold and water stress. Oats are used as CC for the wide availability of varieties which are adapted to different areas of the Pampa region (Ruffo and Parsons 2004) and for the production of high volumes of vegetal biomass added to the soil (Cordone and Hansen 1986). However, oat cultivars are generally not resistant to very low temperatures. For that reason, this CC is used in temperate zones (Ruffo and Parsons 2004). Although oats can grow in any types of soil, it is important that they do not have moisture retention problems, because this CC has high water consumption due to its high transpiratory coefficient (Ruffo and Parsons 2004; Infoagro 2015). Some authors consider that, in addition to rye and oats, the most commonly used species in the Pampas region are raigrass and triticale (Melo et al. 1993; Garza et al. 2007; Carfagno et al. 2013). In the case of legumes, the most adapted to this region are the *Vicia sativa*, *Vicia villosa*, and the clovers. In the north of Argentina, species of *Crotalaria*, *Vigna*, lupines, and soft clover are also used, with very promising results. These CC are sown without soil remotion, generally once the soybean has been harvested (Melo et al. 1993; Carfagno et al. 2007; Garza et al. 2007).

2.3 Use of Plant Growth-Promoting Rhizobacteria (PGPR) for Cover Crops

Furthermore, the inclusion of CC can be combined with other technological alternatives, such as the use of plant growth-promoting rhizobacteria or PGPR. These have a significant effect on agroecosystem sustainability (Antoun and Prevost 2006), since PGPR inoculation contributes to the implantation, development, biomass, and grain production of crops, such as rice, wheat, and maize (Lucy et al. 2004; Siddiqui 2006; García de Salamone 2012). However, very little information is available about its effects on forage plants that are used as CC. It is necessary to know the microbial interactions that can occur in the CC's rhizosphere under field conditions, in order to evaluate the overall impact of CC inoculation technology on this type of agroecosystem for achieving maximum efficiency. PGPR are particularly important in the soil-plant relationship and are responsible for the increase of nutrient supply as well as for the production of growth factors or phytohormones. Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Azotobacter*, and *Arthrobacter* and

Bacillus subtilis stand out because of their potential as PGPR biofertilizers and they have a significant impact on crop yield and quality (Glick 1995; Bashan and Holguin 1997; Dobbelaere et al. 2003; García de Salamone 2012). Studies with microorganisms of the genus *Azospirillum* and *Azotobacter* have demonstrated that these bacteria besides fixing nitrogen in nonsymbiotic associations with plants also segregate growth-promoting substances such as auxins, gibberellins, and cytokinins, which directly benefit the plant (Bashan et al. 2004; Halda-Alija 2003; Pedraza et al. 2010). The genus *Azospirillum* stands out because, besides being a supplier of phytohormones, it can fix nitrogen under microaerobiosis conditions (García de Salamone et al. 1996; Okon 1994). Higher levels of nitrogen, phosphorus, potassium, and various micronutrients in plants inoculated with *Azospirillum* has been reported (Caballero-Mellado 2004; García de Salamone et al. 1996; Pedraza et al. 2010). In addition, significant effects have been observed on the development and production of wheat (Caballero-Mellado 2004; Naiman et al. 2009; Bashan et al. 1990), maize (García de Salamone 2012) and rice (Baldani and Baldani 2005; Garcia de Salamone et al. 2010, 2012). On the other hand, there are reports which showed experiments carried out including PGPR of the genus *Pseudomonas*, which can solubilize phosphorus (P) and thus supply the soluble P to plants through several mechanisms (De Freitas et al. 1997; Rodriguez et al. 2006). In addition, some strains of *P. fluorescens* are capable of producing cytokinins (García de Salamone et al. 2001, 2006). However, the greater amount of information on the activity of *Pseudomonas* strains is associated with the indirect effects that they produce, through the control of pathogenic microorganisms (Siddiqui 2006). This can reduce the incidence of diseases through a number of mechanisms, including increases in available nutrients, production of antibiotics, and induction of siderophores as a mechanism of control of phytopathogenic agents (Dowling and O'Gara 1994). In addition, PGPR can increase crop performance and shorten their cycles, as well as reduce both the use of chemical fertilizers and in consequence the environmental pollution (Park et al. 2005).

Thus, the inoculation with PGPR, based on two microorganisms such as *Azospirillum brasilense*, which can provide nitrogen via biological fixation and promotes a greater root and vegetative development, and *Pseudomonas fluorescens* which stimulates growth because it can facilitate phosphorus solubilization and provide phytosanitary protection and cytokinin supply, could be associated with other recommended management practices to achieve high yields or collaborate to use the available resources more efficiently (García de Salamone et al. 2001, 2012; García de Salamone and Monzón de Asconegui 2008). In this sense, the biological fixation of N_2 by *A. brasilense* acquires relevance and can be incorporated through the plant-PGPR association to contribute N to the agroecosystem (García de Salamone et al. 1996; Urquiaga et al. 2004), where the soybean crop leaves a negative balance of N. This constitutes an economic and ecological alternative to increase plant production (Cassan and García de Salamone 2008). It is recognized that the use of these PGPR would bring about an improvement of sustainability, contributing to the recovery of soil fertility while preserving the environment (García de Salamone et al. 2012; Lara Mantilla et al. 2011).

On the other hand, it should be taken into account that CC should not compete with profitable crops or affect their yield. Because of that, suppression of their growth is necessary to avoid excessive consumption of water. The date of planting and the type of CC should be taken into account to manage the time of growth interruption. That moment should be prior to the maximum demand of the plants, which is flowering for both legumes and grasses (Casas 2007). The achievement of the greatest coverage and the contribution of carbon to the soil will depend on the number of days of CC growth, and this in turn is strongly determined by the environmental conditions of the site under study (Álvarez et al. 2005; Caviglia et al. 2013). Therefore, the available water and the carbon input that CC leaves in the soil for the next summer crop can be modified by managing the time of their growth interruption (Alvarez et al. 2005; Carfagno et al. 2013).

2.4 Impact of the Use of Glyphosate to Stop the Growth of CC

The time of interruption of CC growth should be adjusted to the conditions of each region to ensure the recharge of the soil profile with spring precipitation (Carfagno et al. 2008). The herbicide glyphosate (N phosphonomethylglycine, C3H8NO5P) is usually used to stop the growth or drying of CC. The molecule belongs to the class of organophosphates. It is a nonselective, broad-spectrum, postemergent herbicide that is mainly used for the removal of undesirable grasses and shrubs, in agricultural areas (Gómez et al. 2008), forests, and landscape environments (Busse et al. 2001; Nivia 2001). This herbicide exerts its action through inhibition of the 5-enolpyruvy l-shikimate-3-phosphate synthetase (EPSPS) enzyme, thus preventing plants from making three essential aromatic amino acids, namely, tryptophan, phenylalanine, and tyrosine, which are important for growth and survival of plants (Jaworski 1972; Steinrücken and Amrhein 1980; Duke et al. 2003; Gómez et al. 2008). This herbicide is absorbed by the leaves and stems and then translocated to the roots and vegetative underground organs causing the death of nonresistant plants (Villalba 2009).

Because the metabolic pathway of shikimic acid does not exist in animals, the acute toxicity of glyphosate is considered low (Levesque and Rahe 1992). However, this herbicide may interfere with some enzymatic functions in animals, but the symptoms of poisoning only occur at very high doses. Commercial products of this herbicide contain other compounds which may be highly toxic (Nivia 2001), such as different surfactants or adjuvants that serve to achieve herbicide penetration into plant tissues. Therefore, the toxicological characteristics of the market products are different from those of glyphosate (Cox 2004). Many authors emphasize the need to study the toxic effects of the glyphosate blend plus the surfactant or adjuvant used in the field rather than studying only the individual components (Monosson 2005; Cox and Surgan 2006; Mesnage et al. 2010). Several studies have reported the emergence of resistant weeds (Mueller et al. 2003; Papa 2009; Villalba 2009; Papa et al. 2012; Papa and Tuesca 2014) and a higher incidence of diseases (Levesque et al.

1993; Johal and Huber 2009). Despite the information on glyphosate and its commercial formulations, their use has been intensified due to the good results that have been obtained after application. However, the available information on the long-term effect of continuous herbicide use is scarce (Gómez et al. 2008).

On the other hand, while it is stated that glyphosate has a very short half-life, it can be maintained in the environment for long periods, mainly because it adheres to soil minerals and sediments (Andréa et al. 2003). Some authors have pointed out that it has a moderate persistence in the soil, approximately 47 days (Tejada 2009). However, this cannot be generalized since other authors have pointed out that glyphosate can be very mobile in soil and slowly degraded (Piccolo et al. 1994). In this regard, it has been noted that when this herbicide is bound to other compounds, it cannot be degraded. Moreover, when it binds to soil minerals, it can be released and dispersed again after long periods of time after application (Pessagno and dos Santos Afonso 2006). Thus, the availability of glyphosate depends mainly on two factors: the rate of degradation by soil microorganisms and the degree of adsorption to soil particles that immobilize and temporarily inactivate it (Zabaloy and Gómez 2005; Zabaloy et al. 2008). Once the glyphosate begins to be degraded by the microorganisms, carbonated and phosphatized components are released to the soil, which can also be used by soil microorganisms (Shushkova et al. 2009). Thus, glyphosate can affect the functioning of the terrestrial ecosystem, which depends heavily on soil microbial activity (Paul and Clark 1996; Doran and Zeiss 2000). This is because microorganisms actively participate in the degradation of organic matter and consequently in all biogeochemical cycles (Schlesinger 1997; León et al. 2008).

Glyphosate can affect microbial activity (Tejada 2009) by the mentioned intervention in the metabolic cycle of shikimic acid that is present in the majority of the microorganisms (Jaworski 1972; Bode et al. 1986; Bentley 1990). This herbicide may be considered to interfere with the decomposition of organic matter (Abdel-Maller et al. 1994; Alef and Nannipieri 1995), and thus the nutrients would be retained and their availability to plants would be reduced (De Baets et al. 2011). In addition, the soil physical characteristics would be affected, as it would reduce the release of microbial products, which participate in particle aggregation and in consequence in soil structure (Paul and Clark 1996). Therefore, the potential degradation of glyphosate depends on the ability of the microorganisms to adapt to the new environmental conditions, and this needs to be analyzed in detail for each system under study (Zucchi et al. 2003). However, most of the trials to evaluate the effects of glyphosate on soil microbial communities have been carried out under controlled conditions and not over the rhizosphere under field conditions. Therefore, in the case of CC in succession with soybean, it was necessary to carry out field studies to know the possible effects that would be producing on the native microbial communities, growth interruption or drying of the CC with glyphosate at doses used year after year by farmers. This is because no studies were found in relations with the influence of this herbicide on the microbial communities associated to CC. For this purpose, we performed a series of field experiments in the west of Buenos Aires Province, Argentina, to study the effect of CC dried with glyphosate in sequence in soybean crops. Thus, we could observe that the amount of the glyphosate degrader

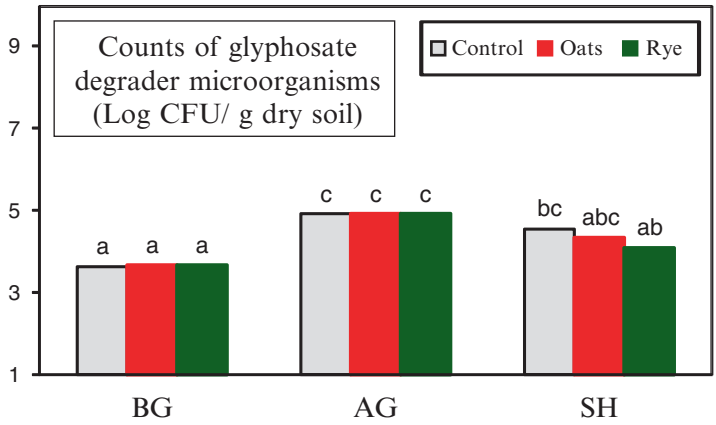


Fig. 2.1 Counts of glyphosate degrader microorganisms in the rhizosphere for the interaction between three coverage treatments and three sampling times in average of two drying times. *BG* Before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest, *Log CFU* Logarithm of colony forming units. *Bars* with different letters indicated differences between coverage treatments by Tukey's test ($p \leq 0{,}05$)

microorganisms increased a month after glyphosate application and their numbers stayed higher at the end of the agricultural cycle at soybean harvest (Fig. 2.1). We also observed that the number of fungi in the rhizosphere of three coverage treatments did not change due to glyphosate application but at soybean harvest time the amounts of fungi decreased significantly but the rhizosphere of oats showed the highest numbers of this type of soil microorganisms (Fig. 2.2). It was depicted that this herbicide increased the presence of certain species of fungi and decreased others. In addition, some authors observed that this herbicide decreased respiration and decomposition rates of organic matter (Abdel-Maller et al. 1994). The influence of glyphosate on the arbuscular mycorrhizal fungus *Glomus intraradices* in carrot roots (Wan et al. 1998) and transgenic soybean (Powell et al. 2009) was also identified under and showed to be contradictory in controlled condition experiments. This effect should be analyzed under conditions that allow evaluating its ecological relevance, since most plants grow poorly without this symbiotic relationship and there is evidence that could be affected by fumigations with glyphosate, but there is no information about what happens at field conditions. At this regard, we did not observe glyphosate effects on native *Mycorrhiza* However, we did observe that oats had higher percentages of root fungal colonization, arbúsculos and vesicles than rye, but there were no effects of fertilization and inoculation on native *Mycorrhiza* of these crops (Table 2.1). This demonstrates that certain management decisions imposed to the systems provoked significant changes in certain microbial communities with their particularities.

On the other hand, in plants of transgenic soybean with resistance to glyphosate, it was found that the bacterium *Bradyrhizobium japonicum*, which fixes nitrogen in the roots of this plant, possesses a glyphosate sensitive enzyme and that when it is

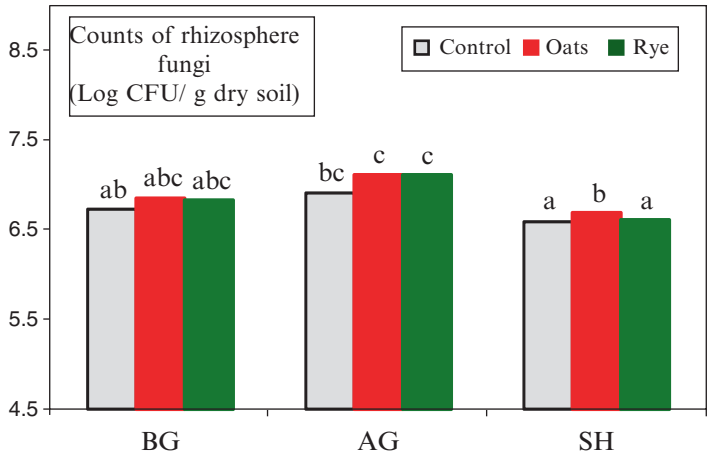


Fig. 2.2 Counts of rhizosphere fungi for the interaction between sampling times after glyphosate application and coverage treatments *BG* before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest, *Log CFU* Logarithm of colony forming units. *Bars* with different letters indicated differences between coverage treatments by Tukey's test ($p \leq 0{,}05$)

Table 2.1 Fungal colonization and structures of native arbuscular mycorhiza of oats and rye at tillering stage growing at field conditions

Cover crops	Root fungal colonization (%)	Arbuscules (%)	Vesícles (%)	Spores (%)
Oats	50.9 b	34.4 b	14.8 B	8.7 A
Rye	33.2 a	19.3 a	6.8 A	5.3 A

Different lower and capital letters indicate significant differences by Tukey's test ($P \leq 0.05$) and Kruskal Wallis's test ($P \leq 0.05$), respectively, for each variable

exposed to this herbicide accumulates shikimic acid and hydroxybenzoic acids, which cause inhibition of growth and even death of the bacteria when high concentrations of the acids mentioned are present. It was also found that glyphosate accumulates in nodules of soybean roots (Zablotowicz and Reddy 2004). In this regard, in the sequence of field experiments that we performed to study the effect of cover crops dried with glyphosate, it could be detected that the amount of rhizosphere native nitrogen fixers associated with both oats and rye were decreased after glyphosate application (Fig. 2.3). It can be assumed that glyphosate can affect the growth of all leguminous plants and overall soil health, as this herbicide would be affecting the nitrogen cycle in the agroecosystem. It was also possible to determine, in contrast to some short-term research reports, effects on soil microorganisms that depended on the concentration of glyphosate used. Roslycky (1982) conducted an experiment under controlled conditions where the soil was mixed with different concentrations of glyphosate and sampled during the 214 days later. Thus, concentrations of 1, 10, 50, and 100 μg g^{-1} of glyphosate soil had no effect on bacterial, fungal, and actinomycete populations, whereas concentrations of 500 and 1000 μg g^{-1} of soil of this herbicide increased initially the number of bacteria, fungi, and

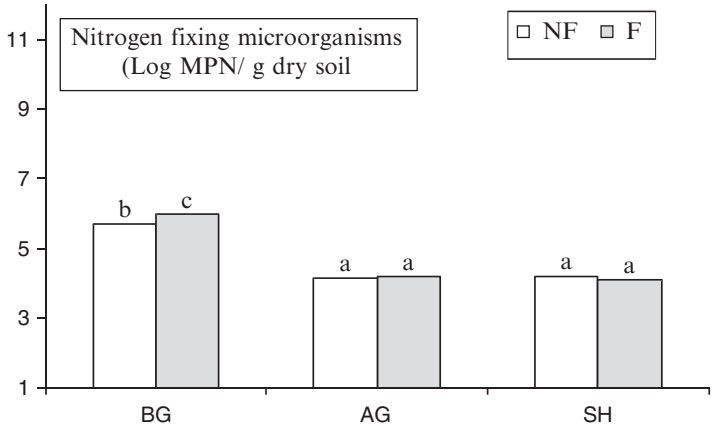


Fig. 2.3 Counts of Most Probable Number (MPN) of nitrogen fixers microorganisms associated with oats and rye for the interaction between sampling times with respect to the glyphosate application and urea application *BG* Before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest, *NF* Non Fertilized, *F* Fertilized. *Bars* with different letters indicated differences between fertilization treatments by Tukey's test ($p \leq 0{,}05$)

actinomycetes, followed by a decrease and then an increase but not as marked as initially observed. Other authors also observed under controlled conditions increases in respiration and enzymatic activity of the soil as a consequence of the application of different concentrations of glyphosate (Gianfreda et al. 1995; Haney et al. 2000). In the field experiments performed by our group, interesting information was obtained in relation with the rhizosphere community of actinomycetes because this taxonomic group has shown to have high sensitivity to glyphosate application (Fig. 2.4). The interaction between the phenological stage of CC when glyphosate was applied to drying them, sampling time and CC displayed differences between both tillering and jointing stages. In the latter, no effects due to the coverage treatments were observed, but in the former, it was possible to detect significant differences among treatments and sampling time. This microbial community is highly sensitive in the case of the control without CC, and it could be differentiated to oat and rye rhizospheres. Thus, actinomycetes associated with rye were less affected than those in oats' rhizosphere by glyphosate application.

On the other hand, there is very little information about the influence of management practices on the structure and functioning of the microorganisms, due to the inoculation of CC with PGPR such as *A. brasilense* and *P. fluorescens*. Therefore, in attending this need, we have studied the effects of the CC oats and rye and their inoculation on rhizosphere microbial communities at field conditions (Fig. 2.5). We observed that there were not effect of the CC but the application of glyphosate produced a permanent impact on the community level physiological profiles analysis using the technique described by Di Salvo and García de Salamone (2012) because there were significant differences between the PC of microbial communities of the sampling time before glyphosate application at jointing phenological stage of the

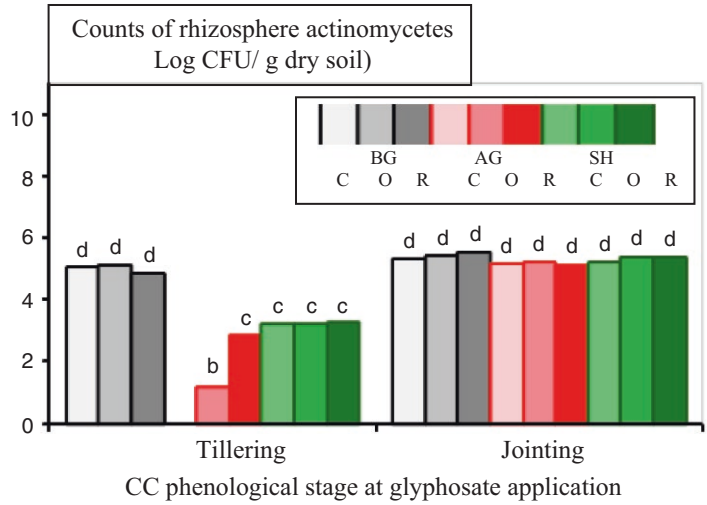


Fig. 2.4 Counts of rhizosphere actinomycetes for the interaction between phenological stages of glyphosate application at Tillering and Jointing, sampling times *BG* before glyphosate application, *AG* a month later glyphosate application, *SH* Soybean harvest and coverage treatments C control without CC, *O* oats, *R* rye, *Log CFU* Logarithm of colony forming units. *Bars* with different letters indicated differences among means performed with Tukey's test ($p \leq 0.05$)

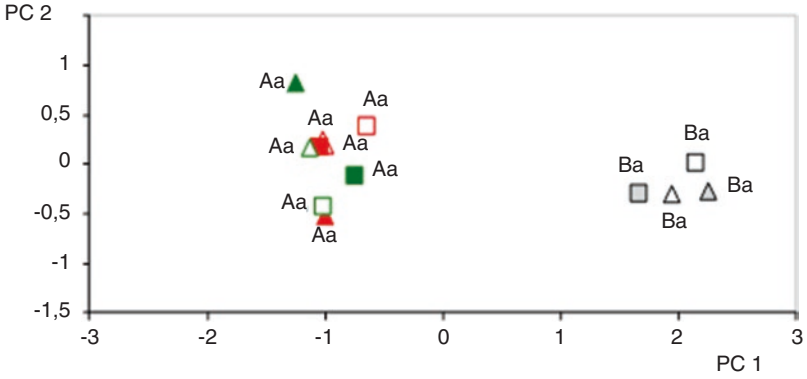


Fig. 2.5 Principal components (PC) multivariate analysis of the rhizosphere microbial communities for the interaction between sampling time, inoculation and two cover crops, rye (*triángules*) and oat (*squares*) grown at field conditions. Sampling times are before glyphosate application (*grey*) at jointing phenological stage, *red*: a month later glyphosate application and *green*: at soybean harvest grown after cover crops. Empty and full symbols are without and with PGPR inoculation on the seeds. *Capital letters* indicate significant differences for PC 1 and lower letters indicate significant differences for PC 2 obtained through mean comparison with Tukey's test ($p \leq 0{,}05$). *Numbers in parenthesis* indicate the percentages of the explained variance by each PC

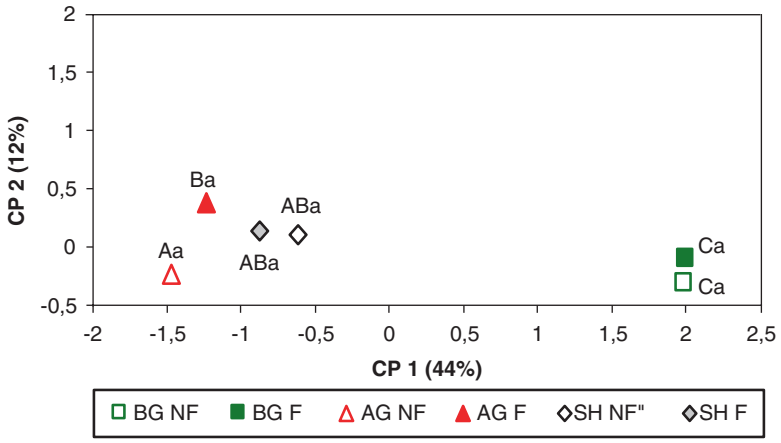


Fig. 2.6 Principal components (PC) multivariate analysis of the rhizosphere microbial communities for the interaction between sampling time and fertilization. *BG* Before application of Glyphosate, *AG* After Application of Glyphosate, *SH* At Soybean Harvest, *NF* Non Fertilized, *F* Fertilized. *Capital letters* indicate significant differences for PC 1 and lower letters indicate significant differences for PC 2 obtained through mean comparison with Tukey's test ($p \leq 0{,}05$). *Numbers in parenthesis* indicate the percentages of the explained variance by each PC

CC with respect to the other two sampling time whose physiological profiles were not different. It was also observed that fertilizer addition at sowing of CC had impact of the physiological profiles of the microbial communities a month later of the application of glyphosate (Fig. 2.6).

2.5 Conclusion

In relation to what has been expressed so far, we observed significant changes produced by the management of the CC oats and rye on the dynamics of their rhizosphere microbial communities. It has generated information capable of connecting processes that occur in the aerial portion of the system with processes taking place in the underground portion. This has been described as one of the challenges of agroecological research (Wardle 2002).

Acknowledgments The works reported in this chapter had been partially supported by the following grants coordinated by IEGS, PICT1864-FONCYT 2008 from the MINCyT, UBACyT projects 20020090100255 and 20020130100716 of the Universidad de Buenos Aires (UBA) in Argentina. JSEO had a scholarship of the National Council of Scientific and Technical Research.

We would like to dedicate this work in memory of Dr. Katia RS Teixeira, Brazilian researcher of the EMBRAPA, Rio de Janeiro, Brazil, who always will be in our hearts.

References

Abdel-Maller AY, Abdel Kader MIA, Shonkeir AMA (1994) Effect of glyphosate on fungal population, respiration and the decay of some organic matters in Egyptian soil. Microbiol Res 149(1):69–73

ACSOJA (2015) La importancia Económica de la Soja. Available via: http://www.francomanopicardi.com.ar/news/004_abril2008/04_21al25/03_agricultura_ACSOJA_ImportanciaEconomica.htm. Accessed 22 Nov 2016

Alef K, Nannipieri P (1995) Methods in applied soil microbiology and biochemistry. Academic, London, pp 130–132

Altieri MA (1994) Bases agroecológicas para una producción agrícola sustentable. Agric Técnica 54(4):371–386

Álvarez C, Barraco M, Díaz Zorita M et al (2005) Influencia de cultivos de cobertura en el aporte de residuos, balance de agua y contenido de nitratos. Boletín de divulgación técnica N° 87. Aspectos del manejo de los suelos en sistemas mixtos de las regiones semiárida y subhúmeda Pampeana. Ediciones INTA. p 31

Álvarez C, Scianca C, Barraco, M et al (2006) Inclusión de los cultivos de cobertura en rotaciones con base soja. Aporte de carbono e Influencia sobre propiedades edáficas. Ediciones INTA. p 21–23

Álvarez C, Scianca C, Barraco, M et al (2008) Cultivos de cobertura en un argiudol típico del Noroeste Bonaerense. EEA INTA General Villega. Memoria Técnica 2007–2008. p 15–18

Amigone MA, Tomaso JC (2006) Principales características de especies y cultivares de verdeos invernales. Informe para Extensión. 103 pp

Andréa MMD, Peres TB, Luchini LC et al (2003) Influence of repeated applications of glyphosate on its persistence and soil bioactivity. Pesq Agrop Brasileira 38(11):1329–1335

Andriulo A, Mary B, Guérif J (1999) Modeling soil carbon dynamics with various cropping sequences on the rolling pampas. Agronomie 19:365–377

Antoun H, Prevost D (2006) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Baldani JI, Baldani VL (2005) History on the biological nitrogen fixation research in graminaceous plants: special emphasis on the Brazilian experience. An Acad Bras Cienc 77(3):549–579

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiology advances (1990–1996). Can J Microbiol 43:103–121

Bashan Y, Harrison SK, Whitmoyer RE (1990) Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. Appl Environ Microbiol 56:769–775

Bashan Y, Holguin G, de-Bashan LE (2004) *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances (1997–2003). Can J Microbiol 50:521–577

Bentley RE (1990) The shikimate pathway – a metabolic tree with many branches. Crit Rev Biochem Mol Biol Boca Raton 25(5):307–384

Bode R, Schauer F, Birnbaum D (1986) Comparative studies on the enzymological basis for growth inhibition by glyphosate in some yeast species. Biochem Physiol Pflanzer 181:39–46

Busse MD, Ratcliff AW, Shestak CJ et al (2001) Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol Biochem 33:1777–1789

Caballero-Mellado J (2004) Uso de Azospirillum como alternativa tecnológica viable para cultivos de cereales. In: Monzón de Asconegui MA, García de Salamone IE, Miyazaki SS (eds) Biología del Suelo, Transformaciones de la materia orgánica, usos y biodiversidad de los organismos edáficos. Editorial FAUBA, Universidad de Buenos Aires, p 45–49. ISBN 950-29-0790-6

Carfagno P, Eiza MJ, Michelena R (2007) Inclusión de los cultivos de cobertura bajo agricultura de secano en la región semiárida pampeana. Jornada de cultivos de cobertura. 28–29 de Septiembre. Resultados parciales de la red de ensayos de cultivos de cobertura. General Villegas y General Pico

Carfagno PF, Eiza MJ, Quiroga A et al (2008) Cultivos de cobertura: Efecto sobre la dinámica del agua en el suelo. Paper presented in XXI Congreso Argentino de la Ciencia del Suelo. Salta, Argentina

Carfagno PF, Eiza MJ, Quiroga A et al (2013) Agua disponible en monocultivo de soja con cultivos de cobertura y barbechos reducidos en la Región Semiárida y Subhúmeda Pampeana. Cienc Suelo 31(1):67–81

Carreño L, Viglizzo E (2011) Provisión de los servicios ecológicos y gestión de los ambientes rurales en Argentina. Proyecto del área estratégica de gestión ambiental. INTA, Buenos Aires

Casas R (2007) Cultivos de Cobertura: una agricultura sustentable Suplemento Campo. La Nación. 24 de febrero

Casas R (2013) Nota: Se pierde un 0,1 por ciento de materia orgánica por cada centímetro de suelo degradado. Rev Investig Agropecuarias RIA 39(2):123

Cassan FD, García de Salamone IE (eds) (2008) *Azospirillum* sp.: cell physiology, plant interactions and agronomic research in Argentina. Asociación Argentina de Microbiología, Buenos Aires. http://www.aam.org.ar/src/img_up/08052014.7.pdf

Caviglia OP, Novelli L, Gregorutti VC, et al (2013) Cultivos de cobertura invernales: una alternativa de intensificación sustentable en el centro-oeste de Entre Ríos. En: Contribuciones de los cultivos de cobertura a la sostenibilidad de los sistemas de producción. Ediciones INTA, p 148–157

Cheng W, Jonson DW, Shenglei F (2003) Rhizosphere effects on decomposition: control of plant species, phenology and fertilization. Soil Sci Soc Am J 67:1418–1427

Cordone G, Hansen O (1986) Efecto de distintas especies invernales utilizadas como abonos verdes o cultivos de cobertura en la producción de soja. Carpeta de Producción Vegetal, Tomo VIII, serie Soja, Información N° 73. EERA INTA Pergamino

Cox C (2004) Herbicide factsheet glyphosate. J Pestic Reform 24(4):10–15

Cox C, Surgan M (2006) Ingredientes inertes no identificados en los pesticidas: implicaciones para la salud humana y del medio ambiente. Environ Health Perspect 1803–1806

De Baets S, Poesen J, Meersmans J et al (2011) Cover crop and their erosion-reducing effects during concentrated flow erosion. Elsevier B V Catena 85:237–244

De Freitas JR, Banerjee MR, Germida JJ (1997) Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.) Biol Fertil Soils 24:358–364

Di Salvo LP, García de Salamone IE (2012) Laboratory standardization of an economical and reliable technique to evaluate physiological profiles of soil-microbial communities (CLPP). Ecol Austral 22:129–136

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22(2):107–149

Doran JW, Zeiss MR (2000) Soil health and sustainability: managing the biotic component of soil quality. Appl Soil Ecol 15:3–11

Dowling DN, O'Gara F (1994) Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends Biotechnol 12(4):133–141

Duke SO, Baerson SR, Rimando AM (2003) Herbicides: glyphosate. In: Plimmer JR, Gammon DW, Ragsdale NN (eds) Encyclopedia of agrochemicals. Wiley, New York. http://www.mrw.interscience.wiley.com/eoa/articles/agr119/frame.html

Espindola JA, Guerra JGM, De-Polli H et al (2005) Adubação verde com leguminosas. Embrapa Informação Tecnológica, Brasilia

FAO (1994) Organización de las naciones unidad para la agricultura y la alimentación. Departamento de Agricultura y protección del consumidor. Agricultura de la conservación

Fernandez R, Quiroga A, Noellemeyer E (2012) Cultivos de cobertura, ¿una alternativa viable para la region semiarida pampeana? Cienc Suelo 30(2):137–150. Available in: http://www.scielo.org.ar/pdf/cds/v30n2/v30n2a01.pdf

Ferreras L, Toresani S, Bonel B et al (2009) Parámetros químicos y biológicos como Indicadores de la calidad del suelo en diferentes manejos. Cienc Suelo 27(1):103–114

García F (1999) Aspectos principales de siembra directa y los cultivos de soja y maíz en Argentina. In: Conferencia Anual da Revista Plantio Direto. IV p 21–32

García de Salamone IE (2012) Use of soil microorganisms to improve plant growth and ecosystem sustainability. 233–258. The molecular basis of plant genetic diversity. Mahmut Caliskan. 978-953-51-0157-4. open access: http://www.intechopen.com/articles/show/title/use-of-soil-microorganisms-to-improve-plant-growth-and-ecosystem-sustainability, INTECH, Rijeka

García de Salamone IE, Monzón de Asconegui MA (2008) Ecofisiología de la respuesta a la inoculación con *Azospirillum* en cultivos de cereales. In: *Azospirillum* sp.: cell physiology, plant interactions and agronomic research in Argentina. Asociación Argentina de Microbiología, Buenos Aires 14: 209–226. ISBN: 978-987-98475-8-9. http://www.aam.org.ar/src/img_up/08052014.7.pdf

García de Salamone IE, Dobereiner J, Urquiaga S et al (1996) Biological nitrogen fixation in Azospirillum strain-maize genotype associations as evaluated by the ^{15}N isotope dilution technique. Biol Fertil Soils 23:249–256

García de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47:404–411

García de Salamone IE, Hynes RK, Nelson LM (2006) Role of cytokinins in plant growth promotion by rhizosphere bacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 173–195

García de Salamone IE, Di Salvo LP, Escobar Ortega JS et al (2010) Field response of rice paddy crop to inoculation with Azospirillum: physiology of rhizosphere bacterial communities and the genetic diversity of endophytic bacteria in different parts of the plants. Plant Soil 336:351–362

García de Salamone IE, Funes JM, Di Salvo LP et al (2012) Inoculation of paddy rice with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact of plant genotypes on the rhizosphere microbial communities and field crop production. Appl Soil Ecol 61:196–204

Garza HN, Pérez Olvera MA, Castillo González F (2007) Evaluación de cinco especies vegetales como cultivos de cobertura en valles altos de México. Rev Fitotec Mex 30(2):151–157. ISSN: 0187-7380. Disponible en: http://www.redalyc.org/pdf/610/61030206.pdf

Gianfreda L, Sannino F, Violanea A (1995) Pesticidal effects on the activity of free, inmobilized and soil invertase. Soil Biol Biochem 27:1201–1208

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Gómez E, Ferreras L, Lovotti L (2008) Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina. Eur J Soil Biol 45:163–167

Halda-Alija L (2003) Identification of indole-3-acetic acid producing freshwater wetland rhizosphere bacteria associated with *Juncus effusus* L. Can J Microbiol 49(12):781–787

Haney RL, Senseman SA, Hons FM (2000) Effect of glyphosate on soil microbial activity and biomass. Weed Sci 48:89–93

Infoagro (2015) Cereales. Available in: http://www.infoagro.com/herbaceos/cereales/avena.htm

Jaworski EG (1972) Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. J Agric Food Chem 20:1195–1198

Johal GS, Huber DM (2009) Glyphosate effects on diseases of plants. Eur J Agron 31:144–152

Lara Mantilla C, Oviendo L, Betancur C (2011) Bacterias nativas con potencial en la producción de ácido indolacético para mejorar los pastos. Zootec Trop 29(2):187–194

León JD, Díez MC, Castellanos J et al (2008) Grupos funcionales de microorganismos en suelos degradados por minería de aluvión plantados con *Acacia mangium*. Suelos Ecuatoriales 38:75–80

Levesque CA, Rahe JE (1992) Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu Rev Phytopathol 30:579–602

Lévesque CA, Rahe JE, Eaves DM (1993) Fungal colonization of glyphosate-treated seedlings using a new root plating technique. Mycol Res 97:299–306

Liebman M, Davis AS (2000) Integration of soil, crop, and weed management in low-external-input farning systems. Weed Res 40:27–47

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Anton Leeuw 86:1–25

Martínez JP, Barbieri PA, Sainz Rozas HR et al (2013) Incorporación de cultivos de cobertura previos a soja en el sudeste bonaerense. In: Informaciones Agronómicas de Hispanoamérica. p 21–25. Available in: http://www.ipni.net/publication/ialacs.nsf/0/49D614D9BBB83C2E85257B83005A566F/$FILE/IAH%2010%20-%20JUN%202013.pdf

Melo FB, Cardoso MJ, Italiano EC et al (1993) Manejo do solo com cobertura verde em sistemas isolado e consorciado como o milho. 1. Encontro Latino Americano sobre Plantio Direto na Pequena Propriedade Ponta Grossa (Brasil). 22–26 Nov. Anais., Ponta Grossa (Brasil). Instituto Agronômico do Paraná. p 123–129

Mesnage R, Clair E, Séralini GE (2010) Roundup en modificados genéticamentencultivos: Regulación y toxicidad en los mamíferos. Theor Ökol 16:31–33

MinAgri (2016) Informe diario del mercado de granos. Ministerio de Agricultura, Ganadería y Pesca. Presidencia de la Nación. p 1–7. Available in: http://www.minagri.gob.ar/dimeagro/Informe_diario/2014/infogra_2014-07-11.pdf

Monosson E (2005) Mezclas químicas: Teniendo en cuenta la evolución de la toxicología y la evaluación química. Environ Health Perspect 113:383–390

Mueller TC, Massey JH, Hayes RM et al (2003) Shikimate accumulates in both glyphosate-sensitive and glyphosate-resistant horseweed (*Conyza canadensis* L. Cronq.) J Agric Food Chem 51:680–684

Naiman AD, Latronico AE, García de Salamone IE (2009) Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact on the production and rhizospheric microflora. Eur J Soil Biol 45:44–51

Nivia E (2001) Las fumigaciones aéreas sobre cultivos ilícitos si son peligrosas – Algunas aproximaciones. en: Conferencia "Las Guerras en Colombia: Drogas, Armas y Petróleo" "The Wars in Colombia: Drugs, Guns and Oil" Instituto Hemisférico de las Américas. Universidad de California, Davis, p 17–19

Okon Y (1994) *Azospirillum*/plant association. CRC Press, Boca Ratón. Florida USA

Papa JC (2009) Problemas actuales de malezas que pueden afectar al cultivo de soja. En: Para mejorar la producción. Ediciones INTA, EEA Oliveros 42: 97–105

Papa JC, Tuesca D (2014) Los problemas actuales de malezas en la región sojera núcleo argentina: origen y alternativas de manejo. En: Para mejorar la producción. Ediciones INTA EEA Oliveros 52: 151–165

Papa JC, Tuesca D, Ponsa JC et al (2012) Confirmación de la Resistencia a Glifosato en un Biotipo de Raigrás Anual (*Lolium multiflorum* Lam.) del Noreste de la Provincia de Buenos Aires. XIV Jornadas Fitosanitarias Argentinas. En: Red de conocimiento en malezas resistentes (REM). Disponible en: http://www.aapresid.org.ar/wp-content/uploads/sites/3/2013/04/Papa-JC.-et-al.-Raigras-resistente-a-glifosato.pdf

Park M, Chungwoo K, Yanga J et al (2005) Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiol Res 160:127–133

Parkin TB, Kaspar TC, Singer JW (2006) Cover crop effects on the fate of N following soil application of swine manure. Plant Soil 289:141–152

Paul EA, Clark FE (1996) Soil microbiology and biochemistry. Academic, San Diego, p 273

Pedraza RO, Teixeira KRS, Scavino AF et al (2010) Microorganismos que mejoran el crecimiento de las plantas y la calidad de los suelos. Rev Corpoica Cienc Tecnología Agropecuaria 11(2):155–164. Colombia

Pengue W (2009) El desarrollo rural sostenible y los procesos de agriculturización, ganaderización y pampeanización en la llanura Chaco-Pampeana. En: Morello J, Rodríguez A (eds) El Chaco sin Bosques: La pampa o el desierto del futuro. p 111–146

Pérez M, Scianca C (2009) Efecto de los cultivos de cobertura sobre las poblaciones de male-zas en un hapludol thapto árgico del N.O. Bonaerense. Memoria Técnica 2008–2009 p 22–24

Pessagno RC, dos Santos Afonso MT (2006) Estudio comparativo del impacto ambiental de tres herbicidas de uso común en cultivos de soja y trigo. En: Gallardo Lancho JF (ed)

Medio ambiente en Iberoamérica. Visión desde la Física y la Química en los albores del siglo XXI. Tomo III. p 345–352
Piccolo A, Celano G, Arienzo M, Mirabella A (1994) Adsorption and desortion of glyphosate in some Eupean soils. J. of Environ Sc. Health. Part B Pesticides, Food Contaminants and Agricultural Wastes. 29: 6. 1105–1115. En: Cox, C. 1995. Glyphosate Part 2: Human exposure and ecological effects. Journal of Pesticide Reform
Pound B (1998) Cultivos de Cobertura para la Agricultura Sostenible en América. Conferencia electrónica de la FAO sobre agroforestería para la producción animal en Latinoamérica. p 24
Powell JR, Campbell RG, Dunfield KE et al (2009) Effect of glyphosate on the tripartite symbiosis formed by *Glomus intraradices*, *Bradyrhizobium japonicum*, and genetically modified soybean. Appl Soil Ecol 41:128–136
Restovich SB, Andriulo AE, Portela SI (2012) Introduction of cover crops in a maize-soybean rotation of the humid Pampas: effect on nitrogen and water dynamics. Field Crop Res 128:62–70
Rodriguez H, Fraga R, Gonzalez T et al (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21
Roslycky EB (1982) Glyphosate and the response of the soil microbiota. Soil Biol Biochem 14:87–92
Ruffo M, Parsons A (2003) Cultivos de cobertura en sistemas agrícolas. INPOFOS. Inf Agronómicos 21:13–20
Ruffo M, Parsons A (2004) Cultivos de cobertura en sistemas agrícolas. Informaciones Agronómicas Cono Sur 21:13–16
Salsal MC (2012) Factores condicionantes de La evolución estructural de suelos limosos bajo siembra directa. Efecto sobre el balance de agua. Tesis doctoral, Universidad de Buenos Aires
Salsal MC (2013) Nota: La sustentabilidad de sistemas bajo SD depende de la secuencia de cultivos implementada. Rev Investig Agropecuarias RIA 39(2):120–121
Sasal MC, Andriulo AE, Taboada MA (2006) Soil porosity characteristics and water movement under zero tillage in silty soils in Argentinean Pampas. Soil Tillage Res 87:9–18
Satorre E (2003) Las posibilidades ambientales y tecnológicas de la pradera pampeana para la producción de granos. Las Ciento y Una Hacia los 100 millones de toneladas de granos y la exportación de 1 millón de toneladas de carne. Bolsa de Cereales de Buenos Aires, p 37–38
Schlesinger WH (1997) Biogeochemistry: an analysis of global change. Academic, Nueva York, EEUU. Geol Mag 135(6):819–842
Scianca C, Barraco M, Álvarez C (2009) Estrategias de manejo de centeno utilizado como cultivo de cobertura en un argiudol típico del noroeste bonaerense. Memoria técnica 2008–2009. Ediciones INTA EEA General Villegas. p 22. ISSN 1850-6038
Scianca C, Perez M, Barraco M, et al (2011) Cultivos de cobertura en sistemas de producción Orgánica. Producción de materia seca e impacto sobre algunas propiedades edaficas y poblaciones de malezas. En memoria técnica. Ediciones INTA EEA General Villegas. p 38–45
Shushkova T, Ermakova I, Leontievsky A (2009) Glyphosate bioavailability in soil. Biodegradation 21:403–410
Siddiqui ZA (2006) PGPR: biocontrol and biofertilization, Springer, Dordrecht 318. 1402040024
Steinrücken HC, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 94:1207–1212
Studdert GA, Echeverría HE (2000) Crop rotations and nitrogen fertilization to manage soil organic carbon dynamics. Soil Sci Soc Am J 64:1496–1503
Teasdale JR (1996) Contribution of cover crops to weed management in sustainable agricultural systems. J Prod Agric 9:475–479
Teasdale JR, Brandsaeter LO, Calegari A, et al (2007) Cover crop and weed managment. In: Upadhyaya MK, Blackshaw RE. p 49–64
Tejada M (2009) Evolution of soil biological properties after addition of glyphosate, difluenican and glyphosate more difluenican herbicides. Chemosphere 76:365–373
Urquiaga S, Jantalia CP, Alves BJR et al (2004) Importancia de la FBN en el secuestro de carbono en el suelo y en la sustentabilidad agrícola. En: Monzón de Asconegui MA, García de

Salamone IE, Miyazaki SS (eds) Biología del Suelo. Transformación de la materia orgánica. Usos y biodiversidad de los organismos edáficos. Editoral FAUBA, Universidad de Buenos Aires, p 1–6

Villalba A (2009) Resistencia a herbicidas. Glifosato. En: Ciencia, docencia y tecnología 39: 169–186

Wan MT, Rahe JE, Watts RGA (1998) New technique for determining the sublethal toxicity of pesticides to the vesicular-arbuscular mycorrhizal fungus glomus intraradices. Environ Toxicol Chem. Pensacola 17(7):1421–1428

Wardle D (2002) Communities and ecosystems: linking the aboveground and belowground components. Princeton Univ. Press, Princeton

Zabaloy MC, Gómez MA (2005) Diversity of rhizobia isolated from an agricultural soil in Argentina based on carbon utilization and effects of herbicides on growth. Biol Fertil Soils 42:83–88

Zabaloy MC, Garland JL, Gomez MA (2008) An integrated approach to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region. Argent Appl Soil Ecol 40:1–12

Zablotowicz RM, Reddy KN (2004) Impact of glyphosate on the Bradyrhizobium japonicum simbiosis with glyphosate-resistant transgenic soybean: a mini review. J Environ Qual 33:825–831

Zotarelli L, Torres E, Boddey RM, et al (2002) Role of legumes in the N economy of cereal production in crop rotation under conventional and no-tillage. In: World congress of soil science. Proceeding of the 17PthP World Congress of Soil Science. Bangkok

Zucchi M, Angiolini L, Borin L et al (2003) Response of bacterial community during bioremediation of an oil-polluted soil. J Appl Microbiol 94:248–257

Soil–Plant–Microbe Interactions: Use of Nitrogen-Fixing Bacteria for Plant Growth and Development in Sugarcane

3

Rajesh Kumar Singh, Pratiksha Singh, Hai-Bi Li, Li-Tao Yang, and Yang-Rui Li

Abstract

Sugarcane is an important industrial agricultural crop cultivated worldwide for the production of sugar, ethanol, and other related by-products. More than 50 diseases were observed in sugarcane caused by different plant pathogenic microbes, i.e., fungi, bacteria, viruses, phytoplasmas, and nematodes. Sugarcane is a lengthy crop, so it requires more amounts of plant nutrients, i.e., N, P, and K, as well as other micro- and macronutrients. Thus, the chances of diseases are more to adapt the favorable conditions for pathogens survival. Nitrogen is one of the greatest limiting nutritional aspects for the growth of plants. An abundant supply of nitrogen is required for the plant's early growth. Higher doses of fertilizers, chemicals, and pesticides are applied by farmers to sugarcane to promote early growth and development of crops, to control the diseases, and to increase the yield in many countries. But the continuous use of these chemicals leads to resistance development against the pathogens and may cause negative effects on the environment and contamination of soil and water in addition to a serious hazard to human and animal health. Because of these facts, we focus to find an alternative method for chemical usage. It has been acknowledged that a large

R.K. Singh (✉) • P. Singh • H.-B. Li • L.-T. Yang
Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning 530005, China
e-mail: rajeshsingh999@gmail.com

Y.-R. Li
Agricultural College, State Key Laboratory of Subtropical Bioresources Conservation and Utilization, Guangxi University, Nanning 530005, China

Guangxi Key Laboratory of Sugarcane Biotechnology and Genetic Improvement, Ministry of Agriculture, Sugarcane Research Center, Chinese Academy of Agricultural Sciences; Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_3

number of naturally occurring plant growth-promoting nitrogen-fixing microbes are present in soil/rhizosphere. A wide variety of mechanisms are used by these bacteria to colonize in the rhizosphere such as biological control against plant pathogens, biological nitrogen fixation, and phytohormone production, as well as their ability to enhance nutrient availability. A biological nitrogen-fixing microbe has massive potential to replace the chemical fertilizers and can be used as biofertilizer in plants. In this chapter, the role of bacteria associated with nitrogen fixation and colonizing the internal parts of the sugarcane plant without exerting any core destruction to their host plant is described.

Keywords
Biofertilizer • GFP • Nitrogen-fixing microbes • PGPR • Sugarcane

3.1 Introduction

Nowadays, the increasing global population is increasing the demand for food production worldwide. Particularly in developing countries, there is regular demand for food to survive with appropriate amount for all (Singh et al. 2014). This need can be fulfilled by increasing the cultivated area, using barrel lands for agriculture, managing the environmental stresses, increasing soil fertility, controlling diseases caused by plant pathogens, designing and developing better seed and crop varieties, etc. Therefore, it is our responsibility to research and focus on managing these problems and increasing the agricultural products to serve the increasing needs of the population in our country. Biotic and abiotic stresses are negatively influencing the crop growth and yield conditions. Abiotic stress has constantly played a major role in reducing agricultural products. The abiotic stresses such as soil pH, drought, salinity, environmental temperature, metal toxicity, overflowing, smog, and contamination are a key cause to reduced crop growth and production (Ladeiro 2012; Rengasamy 2006; Lawlor and Cornic 2002). On the other hand, the living organisms, such as bacteria, viruses, fungi, nematodes, phytoplasma, and parasitic diseases in fields, which reduce the agricultural products, and causes biotic stresses. Among them, fungus is one of the important plant pathogens, causing two-thirds of the total disease. An annual loss of agricultural products caused by these diseases is at least 30% worldwide (Fisher et al. 2012). However, the farmers use higher doses of fertilizers, chemicals, as well as pesticides for growth and development and disease control of crops, to increase production. A higher dose of fertilization causes serious environmental pollution and also raises the production cost (Herridge et al. 2008; Li and Yang 2015). It may have adverse and random effects on the environment, in addition to the pollution of soil, water, and natural areas. Such effects carry severe hazard to human and animal health, and in addition, developing countries have to face the demand of high costs for such technologies and chemical utilization (Pedraza 2008). Therefore, we need to find a viable alternative method in place of chemicals to protect the soil and environment.

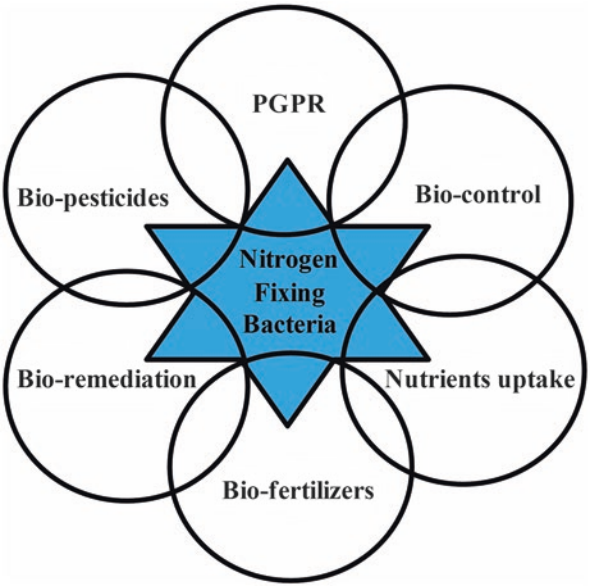


Fig. 3.1 Model for nitrogen-fixing bacteria showing the relationship among PGPR, biocontrol, nutrients uptake, biofertilizers, bioremediation, and biopesticides

It is essential to develop and design improved crop varieties that allow the unsafe possession of frequently dangling environmental issues (Begcy et al. 2012) and disease resistance. It is also essential to isolate and identify a functional applicant microbes that work in different environmental conditions and arid land to protect the crops from diseases and increase quality and productivity. There is enormous microbial flora inhabiting the earth, and they are present in all types of soils such as cultivated land, sand, desert, rock, thermal soil, snow soil, marsh and moorlands, sediments, semiaquatic ecosystems, etc. (Manoharachary and Mukerji 2006). We focused on nitrogen-fixing bacterial genera that are often found in large populations in rhizosphere soils/endophyte with general disease suppression and plant growth-promoting (PGP) activities. To solve the problems of chemicalization, biological nitrogen fixation (BNF) is the best substitute (Xing et al. 2015) (Fig. 3.1). Plant rhizosphere is a multipurpose and active biological environment of controlling plant–microbe interactions for binding important micro- and macronutrients from a limited nutrient pool (Solanki et al. 2012). Exploration of rhizosphere root colonization in grasses and verification of the fact that soil bacteria could change atmospheric nitrogen into functional forms in plants are reported (Hellriegel and Wilfarth 1888). Some Brazilian sugarcane cultivars are also capable of obtaining substantial nitrogen (N_2) from soil through BNF (Lima et al. 1987; Urquiaga et al. 1992, 2012). Nitrogen fertilizer usage is quite low in Brazil (~50 kg N ha^{-1}) in comparison to other manufacturer countries where N use is normally ~120 to 300 kg N ha^{-1} up to dangerous amounts of 700 kg N ha^{-1} (Robinson et al. 2011).

There are some bacteria related to roots and present in soil that are able to encourage progressive effects on the plant growth and development and are universally named plant growth-promoting rhizobacteria (PGPR). They can aggressively colonize the host and motivate the plant growth, i.e., directly/indirectly. Direct mechanisms (e.g., biological nitrogen fixation; solubilization of phosphate, other nutrients; production of phytohormones, ammonia, and siderophore) and indirect mechanisms (e.g., suppression of phytopathogens by production of antibiotic, HCN, chitinases, and antimicrobial properties) increase and support plant growth and yield as well as maintain soil fertility and health (Vessey 2003). Many of these bacteria also fix nitrogen from different mechanisms, which is necessary for growth and development of the plant. Nitrogen-fixing bacteria (NFB) have many characteristics as an excellent biofertilizer agent used in agriculture; they include N fixation, phytohormone production (auxins, cytokinins, and gibberellins), improved nutrient uptake, stress resistance, P-solubilization, siderophores, IAA production, ACC (aminocyclopropane carboxylic acid) deaminase activity, bioremediation, and biocontrol. For developing countries lack of fund for procuring chemical fertilizer and pesticides also forces to enhance the use of PGPB/PGPR where they could play a significant role in improving agricultural products in many countries (Glick 2012).

Thus, the major objectives of this chapter are (1) to focus and summarize our current knowledge about nitrogen-fixing bacteria that are often found in large volume in soils and (2) to evaluate their beneficial activities in plant–microbe interactions in order to use them further as an excellent biofertilizer with general disease suppression and PGPR in agriculture.

3.2 Sugarcane

Sugarcane (*Saccharum* spp.) is one of the world's largest commercial crops (Dotaniya and Datta 2014; Choudhary et al. 2016), and it is one of the most significant agronomic crops globally and is also a source of sugar, renewable energy, and biomaterials. Sugarcane is harvested in more than 109 countries and cultivated on about 26.9 million hectares of 1.91 billion tons worldwide (Factfish 2016), and 50% of global production generated in Brazil and India (Fischer et al. 2012). It is economically important in several countries, and over 60% of the world's sugar is extracted from sugarcane (Grivet and Arruda 2001). Sugarcane is a tropical and subtropical crop plant belonging to the grass family (3–4 m), Gramineae, which originates from Indian subcontinent (Table 3.1), where it has been cultivated for over 4000 years (Orlando et al. 2005). It is a lengthy cash crop so high rates of nitrogen fertilizer are often applied to maximize yields. Large amounts of plant macro- and micronutrients are required for sugarcane. In 1 ha land measured, 140 (N), 34 (P), and 332 (K) ha^{-1} kg is removed from 100 tons of sugarcane (Bokhtiar et al. 2001). Currently there is an increasing attentiveness on this crop because it is a rich source of carbohydrates, used as a food and fodder in various forms and as a

Table 3.1 Botanical classification of sugarcane

Classification		Description
Kingdom	Plantae	Plants
Subkingdom	Tracheobionta	Vascular plants
Division (phylum)	Magnoliophyta	Flower bearing plants
Super-division	Spermatophyta	Seed plants
Class	Liliopsida	Monocotyledons
Subclass	Commelinidae	–
Order	Poales	–
Family	Poaceae/Gramineae	Grass family
Subfamily	Panicoideae	–
Tribe	Andropogoneae	–
Subtribe	Saccharinae	–
Genus	*Saccharum* L.	Sugarcane
Species	*Saccharum officinarum* L.	Sugarcane

Table 3.2 Top ten largest sugarcane-producing countries in the world (2015)

Rank No.	Country	Production of sugarcane (tons)
1	Brazil	728.13
2	India	349.56
3	China	123.46
4	Thailand	96.50
5	Pakistan	58.49
6	Mexico	51.73
7	Colombia	38.75
8	Philippines	32.90
9	United States	28.00
10	Indonesia	27.40

fertilizer in various crop production across the globe. According to Dotaniya et al. (2016), all these products, food, fiber, fodder, fuel, and chemicals, are important, but economic value in crop production was mainly focused on sugarcane press mud, bagasse, and molasses.

The main sugarcane-producing country in the world market is Brazil, and the next major producers in reducing amounts are India, China, and Thailand (FAO 2016) (Table 3.2) for agribusiness and use in the production of sugar and alcohol. In India more than 45 million sugarcane cultivators and about 65% of the rural population depend on this industry. After textile industry the sugar industry is the second largest agricultural industry in the country (Dotaniya et al. 2016). Its importance in day-to-day life adds to its value. In this respect, it has a lot of importance in Indian agriculture. Sugar industry is one of the most remarkable and large-scale sugar industrial sectors in Maharashtra, India. A total of 80% sugar is produced by sugarcane and the rest from sugar beets in all over the world (Dotaniya et al. 2016).

3.3 Diseases in Sugarcane

In sugarcane more than 55 diseases are caused by different pathogens (fungi, bacteria, viruses, phytoplasmas, and nematodes) and have been reported from India (Rao et al. 2002; Croft and Magarey 2000). About 10–15% of the nation's sugarcane production is lost due to diseases. Among all the diseases, smut, red rot, wilt, and pineapple disease (sett rot) are very important fungal and bacterial diseases. Diseases like leaf scald disease (LSD) and ratoon stunting disease (RSD) are found to cause great yield loss in India (Viswanathan and Rao 2011). The main diseases are listed in Table 3.3 (Nasare et al. 2007; Rao and Ford 2000). Presently, rapid change in climate and population growth occurs, and therefore, to maintain and increase the crop yield sustainably, a substituted solution is necessary without simultaneous increase in cost and input utilization (Tikhonovich and Provorov 2011). The new approaches will require the application of biological solutions, including the manipulation and exploitation of beneficial soil microbe and plant interactions.

3.4 Chemicals Used

Nitrogen is an essential nutrient element that is required to all life on Earth. The rate and efficiency of soil nitrogen mineralization is highly dependent on soil physiochemical characteristics, including NH_4^+, nitrifying microorganisms, pH, aeration, and temperature present in the soil system. Soil nitrogen transformation processes are principally facilitated by interactions between functional communities of soil microorganisms and environment (Balser and Firestone 2005; Hogberg et al. 2013). Plants are able to use different N_2 sources, in both inorganic (nitrate and ammonium) and organic (urea, amino acids, and peptides) form using different mechanisms (Nacry et al. 2013; Zanin et al. 2014). Farmers use higher doses of fertilizers to increase the yield of crops and usually apply heavy N, P, K, and chemicals in sugarcane production. Aimed at minimizing these problems, attention in using PGPB to contribute in plant growth promotion was initiated in the 1980s and 1990s (Glick 2015). Other substitutes like compost, vermicompost, manure, farmyard manure, cover crops, and green manures can also help in enhancing crop yield and soil fertility and health under sustainable agriculture (Lim et al. 2016; Cabanillas et al. 2013). PGPB can replace the application of chemical fertilizers (20–25%) for crop production to many folds (Verma et al. 2014).

In agriculture, fertilization of plant with N_2 product is generally more and more practiced to raise the manufacture yield of food (Hurek and Reinhold-Hurek 2003). It is also another important reason for low productivity because of the decline in soil fertility. It is general practice to apply an average, sugarcane crop yielding 200–250 kg of N, 120–150 kg of P, and 175–225 kg of K from the soil in most of the cultivating countries. Soil fertility has declined in many sugarcane-growing areas due to inappropriate and inaccurate fertilizer schedules approved over the years under demanding cultivation of the crop. Hence, the use of high-chemical fertilizers caused serious problems for sustainable sugarcane cultivation. The agriculture

Table 3.3 List of major sugarcane diseases

Microorganisms	Disease name	Causing agents
Fungal diseases		
	Banded sclerotial disease	*Thanatephorus cucumeris*
	Black rot	*Ceratocystis adipose*
	Brown spot	*Cercospora longipes*
	Brown stripe	*Bipolaris stenospila*
	Downy mildew	*Sclerospora sacchari*
	Eyespot	*Helminthosporium sacchari*
	Fusarium sett and stem rot	*Gibberella fujikuroi*
		Fusarium moniliforme
		Gibberella subglutinans
	Leaf blast	*Didymosphaeria taiwanensis*
	Phytophthora rot of cuttings	*Phytophthora* spp.
		Phytophthora megasperma
	Pineapple disease	*Ceratocystis paradoxa*
		Chalara paradoxa
	Pokkah boeng	*Gibberella fujikuroi*
		Fusarium moniliforme
	Red leaf spot	*Dimeriella sacchari*
	Red rot	*Glomerella tucumanensis*
		Colletotrichum falcatum
	Red rot of leaf sheath and sprout rot	*Athelia rolfsii*
		Sclerotium rolfsii
	Rhizoctonia sheath and shoot rot	*Rhizoctonia solani*
	Root rots	*Marasmius sacchari*
		Pythium arrhenomanes
		Pythium graminicola Rhizoctonia sp.
	Rust, common	*Puccinia melanocephala*
	Rust, orange	*Puccinia kuehnii*
	Seedling blight	*Alternaria alternata*
		Bipolaris sacchari
		Cochliobolus hawaiiensis
		Bipolaris hawaiiensis
		Cochliobolus lunatus
		Curvularia lunata
		Curvularia senegalensis
		Setosphaeria rostrata
		Exserohilum rostratum
	Sheath rot	*Cytospora sacchari*
	Smut, culmicolous	*Ustilago scitaminea*
	Target blotch	*Helminthosporium* sp.
	Wilt	*Fusarium sacchari*

(continued)

Table 3.3 (continued)

Microorganisms	Disease name	Causing agents
Bacterial diseases		
	Gumming disease	*Xanthomonas axonopodis* pv. *vasculorum*
	Leaf scald	*Xanthomonas albilineans*
	Mottled stripe	*Herbaspirillum rubrisubalbicans*
	Ratoon stunting disease	*Leifsonia xyli* subsp. *xyli*
	Red stripe (top rot)	*Acidovorax avenae* subsp. *avenae*
Nematodes and Parasitic diseases		
	Lesion	*Pratylenchus* spp.
	Root-knot	*Meloidogyne* spp.
	Spiral	*Helicotylenchus* spp.
		Rotylenchus spp.
		Scutellonema spp.
Viral diseases		
	Chlorotic streak	Virus (assumed)
	Dwarf	Sugarcane dwarf virus
	Mosaic	Sugarcane mosaic virus
	Sereh	Virus (assumed)
	Streak disease	Maize streak virus, sugarcane strain
	Yellow leaf	Sugarcane yellow leaf virus
Phytoplasma diseases		
	Grassy shoot (SCGS)	Sugarcane grassy shoot phytoplasma
	Leaf chlorosis	
	Early bud sprouting	

yields are reduced due to nutrient deficiency for crop cultivation throughout the world. For plant growth, nitrogen plays a crucial role as a primary constituent for making nucleotides, proteins, and chlorophyll (Robertson and Vitousek 2009). Molecular nitrogen or dinitrogen (N_2) is present everywhere in the atmosphere but is metabolically unavailable directly to plants or animals (Fig. 3.2).

3.5 Soil–Plant–Microbe Interactions

On earth soil represents one of the richest microbial biomes (Gans et al. 2005). Soil provides the medium for plant growth and root development, and plants depend only on soil for all other nutrients and water for plant growth (Bhatia 2008). An environment rich with soil reduces the development of plant diseases, even when the pathogen is favored by the occurrence of a susceptible host. Previously, the classical definition of soil according to Cook and Baker (1983), "pathogen does not persist in soil established but causes less damage, or can cause disease in soils but later the disease is less important, though the pathogen can continue in the soil". In natural ecosystems primary macronutrients (total N, P, and K), secondary macronutrients (Ca, Mg), and micronutrients (Fe, Mn, Zn, B, SO_4^{2-}, and Cl^-) are present and

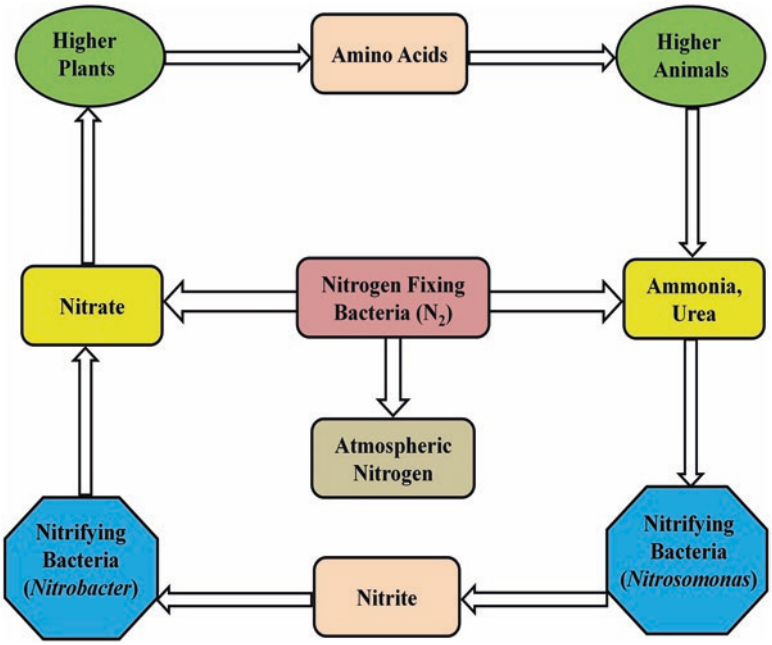


Fig. 3.2 Nitrogen cycle

maintain the fertility of soils which indicates soil health and soil biological activities. However, at high taxonomic rank, a few bacterial phyla including *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, and *Proteobacteria* summarize most of the diversity of distinct soil biomes (Fierer et al. 2009). Nevertheless, microbial biodiversity is an excellent indicator of soil health (Nielsen and Winding 2002). In soil, groups of microorganisms and other microbial agents are available, and bacteria, fungi, actinomycetes, protozoa, nematodes, algae, mycorrhiza, and arthropods play a significant role in nutrient cycling.

Plant-associated bacteria living in root exudates are classified as rhizospheric, while those colonizing the inner tissues of the plant are endophytic (Hardoim et al. 2008). To improve plant growth for the production of fiber, wood, biofuels, and significant molecules, plant–microbe interactions have been employed (Wu et al. 2009). For sustainable agricultural production, plant–bacteria interactions play a pivotal role in transformation, mobilization, solubilization, etc. in the rhizosphere from the limited nutrient pool of the soil and the plants to realize their full genetic potential following uptake of necessary plant nutrients (Compant et al. 2010). Research into plant–microbe interactions has focused on three categories of interactions, i.e., the ancient symbiosis between land plants and arbuscular mycorrhizae (Smith and Smith 2011), nitrogen fixation by rhizobia of legume roots within the nodules (Oldroyd et al. 2011), and pathogenesis (Dodds and Rathjen 2010; Kachroo and Robin 2013; Wirthmueller et al. 2013). The spectrum of plant–microbe interactions is extremely multifarious, including various microbial species, theoretically

acting as consortia (Hirsch 2004). Beneficial plant–microbe interactions are phytohormone production (PGP), BNF (biofertilizers), plant protection (biocontrol), abiotic stress tolerance (boost plant biomass on marginal land), phytoremediation (remediation of contaminated land), and endophytic specialization (novel pathways and reduced genomes for synthetic applications) (Farrar et al. 2014).

3.6 Mode of Action of PGPR as Biofertilizers

A common group of PGPR genera belonging to *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Rhizobium*, *Bradyrhizobium*, *Burkholderia*, *Frankia*, *Serratia*, *Thiobacillus*, *Pseudomonads*, and *Bacillus* (Glick 1995; Vessey 2003) is reported. Strains of bacterial genera, e.g., *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Serratia*, and *Azospirillum*, are already being used as biofertilizer for enhancing growth and yield of crops as well as maintaining soil fertility and health under cleaner production systems of agriculture (Souza et al. 2015). PGPB and PGPR are not a novel thing, and since 1930–1950, a number of different bacteria (*Azotobacter*, *Bacillus*, and *Pseudomonas* species) were applied in the agricultural field for enhancing crop growth and yield quality. PGPR has been increasing at a growing rate, but the term in research was first used by Kloepper and colleagues in the late 1970s (Kloepper and Schroth 1978; Suslow et al. 1979). Differently, bacteria, when correlated with roots, are able to encourage positive effects on the plant growth and ability; they are universally termed as PGPR. According to Vassey (2003), PGPRs enhance the nutrient status of host plants and can be classified into five areas: (1) BNF, (2) increasing the availability of nutrients from rhizosphere, (3) increasing the root surface area, (4) enhancing beneficial symbioses of the host, and (5) combining modes of action.

It is also stimulating that so many diazotrophs are PGPR; however, the fundamental mechanism of their growth-promoting effects is not only to fix N_2 supply to the host plant. This suggests that possibly:

(1) There is something other than the capability to fix N_2 that makes the organisms well adapted to living in the rhizosphere (e.g., lower mineral N_2 levels due to plant absorption)
(2) What may be considered insignificant levels of N_2 fixation (i.e., in agricultural terms) may actually be beneficial to host plants in nature
(3) Researchers generally select nitrogenase activity or use culture media well suited to diazotrophs (e.g., minimal N_2 media) while trying to isolate PGPR (Vessey 2003).

Some bacterial genera were identified to fix N_2 by reduction of atmospheric N_2 to ammonia (Table 3.4). All these species are able to colonize in the rhizosphere and endophytic environment of sugarcane. Recently, it was shown that the N_2-fixing bacterial species related to plants have great potential for agro-biotechnological applications, as they display the activities involved in PGP, biocontrol, or bioremediation (Caballero-Mellado et al. 2007) in addition to their role as nitrogen fixers.

Table 3.4 Plant growth-promoting rhizobacteria (PGPR) isolated from different parts of sugarcane, showing the evidence of their stimulation of plant growth and their ability to fix nitrogen in sugarcane plant

PGPR strains	Host	Crop	References
Bacillaceae, *Enterobacteriaceae*, *Paenibacillaceae*, and *Pseudomonadaceae* sp.	Endophytic	Sugarcane	Worarat Kruasuwan and Arinthip Thamchaipenet (2016)
B. tequilensis	Rhizosphere	Sugarcane	Li et al. (2015)
Burkholderia australis	Endophytic		Paungfoo-Lonhienne et al. (2014)
Achromobacter, *Agrobacterium*, *Burkholderia*, *Gluconacetobacter*, and *Stenotrophomonas* sp.	Endophytic/rhizosphere	Sugarcane	Anelise Beneduzi et al. (2013)
Pseudomonas, *Stenotrophomonas*, *Xanthomonas*, *Acinetobacter*, *Rahnella*, *Enterobacter*, *Pantoea*, *Shinella*, *Agrobacterium*, *Achromobacter*, *Microbacterium*, *H. seropedicae*, *H. rubrisubalbicans*, *Burkholderia*, and *G. diazotrophicus* sp.	Endophytic/roots/stems	Sugarcane	Taule et al. (2012); Lin et al. (2012), Urquiaga et al. (2012), and da Silva et al. (2012)
Stenotrophomonas sp. and *Herbaspirillum rubrisubalbicans*	Endophytic	Sugarcane	Ramos et al. (2011), Pedrosa et al. (2011)
Beijerinckia, *Bacillus*, *Klebsiella*, *Enterobacter*, *Erwinia*, *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter*, and *Pseudomonas* sp.	Endophytic/rhizosphere	Sugarcane	Abeysingha and Weerarathne (2010) and Mehnaz et al. (2010)
G. diazotrophicus	Roots/stems	Sugarcane	Taghavi et al. (2009), Oliveira et al. (2009),
Azospirillum, *Herbaspirillum*, *Burkholderia*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *K. pneumoniae*, and *Pantoea* sp.	Roots/stems/leaves	Sugarcane	Govindarajan et al. (2008)
Azospirillum, *Herbaspirillum*, *Burkholderia*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *K. pneumoniae*, and *Pantoea* sp.	Roots/stems/leaves	Sugarcane	Govindarajan et al. (2007) and Mendes et al. (2007)

(continued)

Table 3.4 (continued)

PGPR strains	Host	Crop	References
G. diazotrophicus and *Burkholderia* sp.	Roots/stems/rhizosphere	Sugarcane	Oliveira et al. (2006) and Govindarajan et al. (2006)
Gluconacetobacter diazotrophicus (*Acetobacter diazotrophicus*)	Endophytic	Sugarcane	Muthukumarasamy et al. (2005)
G. diazotrophicus	Nodal roots	Sugarcane	Loiret et al. (2004)
Gluconacetobacter diazotrophicus	Endophytic	Sugarcane	Boddey et al. (2003),
G. diazotrophicus, *H. seropedicae*, *H. rubrisubalbicans*, *A. amazonense*, and *Burkholderia* sp.	Endophytic	Sugarcane	Oliveira et al. (2002)
Gluconacetobacter diazotrophicus	Endophytic/roots/stems		Boddey et al. (2001) and Sevilla et al. (2001)
Enterobacter cloacae and *Klebsiella oxytoca*			
Gluconacetobacter diazotrophicus and	Endophytic/roots/ rhizosphere		Yamada et al. (1997) and Baldani et al. (1997)
Azospirillum sp.			
H. rubrisubalbicans	Endophytic	Sugarcane	Baldani et al. (1996)
Gluconacetobacter diazotrophicus	Endophytic	Sugarcane	Caballero-Mellado et al. (1995)
Gluconacetobacter diazotrophicus	Endophytic	Sugarcane	Fuentes Ramirez et al. (1993), Dobereiner et al. (1993), and Fuentes Ramirez et al. (1993)
Gluconacetobacter diazotrophicus and *Herbaspirillum* sp.	Endophytic	Sugarcane	Li and Mac-Rae. (1991) and Pimentel et al. (1991)
Gluconacetobacter sp.	Endophytic	Sugarcane	Gillis (1989)
Gluconacetobacter diazotrophicus	Endophytic	Sugarcane	Cavalcante and Dobereiner (1988)
Herbaspirillum seropedicae	Endophytic	Sugarcane	Baldani et al.(1986)

For evaluation with chemicals, pesticides, and fertilizers, microbial inoculants have various benefits; they (1) are more safe, (2) reduce environmental damages, (3) have potentially smaller risk to human and animal health, (4) have more targeted activity, (5) are effective in small quantities, (6) are controlled by the plant as well as pathogenic microbial populations, (7) decompose more quickly than chemical pesticides, (8) have resistance development, (9) are used as an integrated pest management (IPM) system, etc. (Berg 2009).

3.7 Microbial Effects on Plant Nutrient Acquisition

The soil background attached to the root is a hot spot of microbial richness and action owing to the occurrence of root exudates and rhizodeposits (Hiltner 1904). The rhizosphere is well known to host a variety of plant PGPB and PGPF (plant growth-promoting fungus) which are present. PGPB are distinct free-living soil, rhizospheric, rhizoplanic, endophytic, and phyllospheric bacteria that are under certain situations helpful for plants (Bashan and de-Bashan 2005). Single or more diverse direct and/or indirect mechanisms motivate the development of plants (Laslo et al. 2012). They can aggressively colonize the host and stimulate plant growth, either indirect (i.e., acting as biocontrol agents/antagonism against plant pathogens) or direct (i.e., enhancing nutrient acquisition/phytohormones production) mode of action (Weller et al. 2002; Vessey 2003; Glick 2012). They are capable of promoting plant growth through diverse mechanisms, and these bacteria may provide natural and harmless resources to increase the growth and yield of crops, thus reducing the use of agrochemicals (Pedraza 2008). PGPRs fix atmospheric nitrogen, phytohormones, siderophore production, phosphate, potassium and zinc solubilization, ACC deaminase enzyme production during stress, and disease control by inhibiting the growth of phytopathogens (Kumar et al. 2012) (Fig. 3.3). However, it is neither a single genus or species of microbes nor a single attribute relatively; it is a group of bacteria that have various PGP properties that supplement plant growth (Wu et al. 2009). Generally, 2–5% of rhizosphere bacteria are PGPR (Kumar et al. 2012).

3.8 Nitrogen-Fixing Microorganisms

BNF microorganisms are categorized into three groups: symbiotic nitrogen fixers such as *Rhizobium*; nonsymbiotic and free-living nitrogen-fixing bacteria such as *Agrobacterium*, *Klebsiella*, and *Azotobacter*; and nitrogen-fixing autotrophic cyanobacteria such as *Anabaena* (Iwata et al. 2010). The primary cause for reduction of crop growth and production is biotic and abiotic stresses such as drought, salinity, temperature, heavy metals, flooding, air pollution, chemicals, and radiation (Lawlor and Cornic 2002). Soil pH is a major limiting issue in yield production because they disturb virtually all plant functions (Veronica et al. 2009). Soil bacteria have positive influence on plant health, and PGPRs are free-living soil bacteria that are categorized into three major groups on the basis of their functions: PGPB, biocontrol PGPB (Bashan and Holguin 1998), and plant stress homeo-regulating bacteria (PSHB) (Cassan et al. 2009). Previously, not so much work has been reported for nitrogen-fixing microbial species which are not yet identified to provide nitrogen to grasses and other crops (de Oliveira et al. 2006). Although a lot of chemical fertilizers have been applied in sugarcane to promote plant growth in many countries. The N_2-fixing bacteria isolated from soils or roots can change nitrogen gas from the atmosphere into solid nitrogen complexes that can be used by the plants. Therefore, the use of beneficial microorganisms, mainly BNF bacteria to increase the sugarcane productivity by using this available expertise, is being promoted

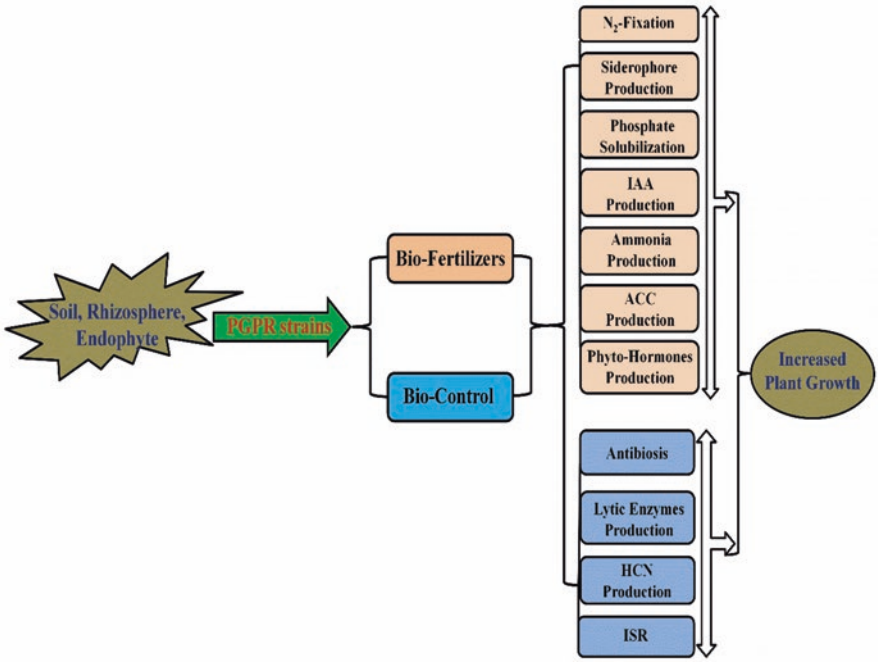


Fig. 3.3 Schematic presentations of selected microbes isolated from soil/rhizosphere/endophyte and their important mechanisms identified for plant growth promotion with PGPR traits. Different mechanisms can be generally studied in two categories; (**a**) biofertilization and (**b**) biocontrol are listed below: (**a**) biofertilization: (1) N_2 fixation, (2) siderophore production, (3) phosphate solubilization, (4) indole acetic acid production, (5) ammonia production, (6) aminocyclopropane carboxylic acid production, and (7) phytohormone production. (**b**) Biocontrol: (1) antibiosis, (2) lytic/hydrolytic enzymes (chitinase, protease, and endoglucanase), (3) hydrogen cyanide production, and (3) induced systemic resistance (ISR) of host plant

(Beneduzi et al. 2013), and the use of N_2-fixing microbes in agricultural field is a need. Researchers and scientists have explored and focused on identifying the N_2-fixing bacteria related to plants to decrease the harmful chemicals and protect the soil health and environment. Nitrogen fixation diazotrophic bacteria, either free-living or endophytes, may benefit the sugarcane through different mechanisms and promote plant growth.

The specific microorganisms responsible for the BNFs are unknown (James 2000). Various groups of bacteria, including species of *Azoarcus*, *Azospirillum*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum seropedicae*, *Klebsiella*, *Kosakonia*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Stenotrophomonas*, *Serratia*, and *Xanthomonas*, are among the main PGPRs used to promote the growth of several crops and sugarcane (Somers et al. 2004; Bhattacharyya and Jha 2012; Solanki et al. 2016). The most effective interactions between plants and microbes can affect plant development and environmental adaptation in the rhizosphere soil.

Several diazotrophic bacteria were isolated from sugarcane rhizosphere and inner tissues of roots and/or stems (Asis et al. 2000; Mirza et al. 2001; Reis et al. 2007; Taule et al. 2012). Earlier, some of the strains were confirmed for their use in inoculation trials, i.e., singly and in groups to support sugarcane growth (Oliveira et al. 2006, 2009; Taghavi et al. 2009; da Silva et al. 2012).

3.9 Interaction Studies Between Plant–Microbe Rhizosphere

Various mechanisms are intricate in plant–microbe interactions (Whipps 2001; Compant et al. 2005). For individual beneficial interactions, there are several mechanisms involved (Berg et al. 2002; Haas and Defago 2005; Muller et al. 2009), and a particular mechanism can vary within different pathosystems (Chet and Chernin 2002). However, for all successful plant–microbe interactions, the capability to colonize plant surroundings is essential (Lugtenberg et al. 2002; Kamilova et al. 2005). Root colonization by microbes increases the release of exudates usually in response to plants (Phillips et al. 2004) to produce some compounds that signal to quorum sensing for acting as stimulus to the bacterial population (Bauer and Mathesius 2004). Plant roots initiate crosstalk with microbes by producing signals that are accepted by the microbes, to produce signals that initiate root colonization (Bais et al. 2006). Visualization of root colonization was assessed by a variety of techniques, i.e., immunofluorescence microscopy, immunofluorescence colony-staining technique, scanning electron microscopy, and confocal laser scanning electron microscopy. All these techniques enable an easier study of interaction in the natural environment. One of the particularly popular marker genes that encode for the green fluorescent protein provides the cells with a GFP phenotype supporting them to be easily identified even in non-sterile sections (Unge et al. 1999) by using confocal microscopy.

Shimomura et al. (1962) first discovered green fluorescent protein (GFP) (Pacific Northwest protein) from *Aequorea victoria* jellyfish. In the last few decades, the GFP from jellyfish has been an evidence to fulfill its potential as an important molecular marker expressed in various natural environmental organisms (Errampali et al. 1999) and for the applications in cell biology, biochemistry, biotechnology, and microbiology as well as plant physiology. GFP technique can be used to quantify gene expression of distinct cells (Brand 1995), in plant hosts (Valdivia et al. 1996), in biofilms (Sternberg et al. 1999), on fermenters (Poppenborg et al. 1997), on leaf surfaces (Spear et al. 1999; Vanden Wymelenberg et al. 1997), and in soils (Bae and Knudson 2000). Many plant-associated rhizobacteria are well acknowledged for their ability to present plant growth promotion and to increase resistance toward diverse diseases, production of phytohormones, as well as work in different stress conditions. However, they habitually not succeed to present these advantageous effects practically in the fields (Stephane et al. 2010). Bacterial colonization of the internal tissues of plants has been described in almost all plant species in

every part. However, the use of microbial inoculations actively requires to observe the efficiency and colonization rate to track and identify the inoculated strains within the host plant (Fig. 3.4).

3.10 Phytohormone Signaling

Phytohormones are organic compounds which play a significant role as growth regulator for the development of plants. The roles of phytohormones are essential, diverse, and multifarious merging both default growing pathways and active responses to the environment (Durbak et al. 2012). It may be predictable that phytohormones are key components of plant–microbe interactions. There are mainly five groups: abscisic acid, auxins, cytokinins, ethylene, and gibberellins. Certain bacteria have the capacity to produce phytohormones including indole-3-acetic acid (IAA), auxin, gibberellin (GA), and cytokinin (CK) (Bottini et al. 2004; Tsavkelova et al. 2006) and play a significant role. These hormones can be produced by the plants themselves and similarly by the microorganisms. It has been imagined that phytohormones might be used as signaling molecules between bacteria and host. It exists as a crosstalk among IAA and ethylene biosynthesis oppressed as a resource of message (Spaepen et al. 2007; Yuan et al. 2008). On the other side, low quantities of IAA prompted confrontation in the plant (Hartmann et al. 2004). IAA is involved in many plant–bacteria signaling (Spaepen et al. 2007). Ethylene is an essential hormonal, and at low levels, it can encourage plant progress in numerous species, and it is generally measured as an inhibitor of plant growth and identified as a senescence hormone (Pierik et al. 2006). Furthermore, bacteria can also stimulate and control phytohormone production in the plant.

3.11 Agricultural Research Industry in India

Scientific developments in biotechnology, microbiology, and globalization of food and agricultural markets changed the agriculture for private industries to contribute food and agricultural research worldwide (Fuglie 2012). The Indian government has increased the funding for research which doubled between 1996 and 2009 (Pal et al. 2012) and is trying to boost more private sectors' research and development through different rules such as stronger intellectual property rights. These policies look to have some achievement with private sector, increasing their percentage in total research in agriculture from 17% (1995) to 31% (2009) in India (Pray and Nagarajan 2012). Most private company's gaining interests in food and agricultural areas and spending high investment to earn more profit. Developing countries need some important changes, payable to reserves by international companies and local companies with significant technology (Pray and Fuglie 2015) in agriculture sector such as seeds, chemicals, pesticides, bio-formulations, etc. An evidence from India shows that the total private agricultural research increased from $24 million in the mid-1980s to $250 million in 2008–2009 (Pray and Nagarajan 2014). Among these

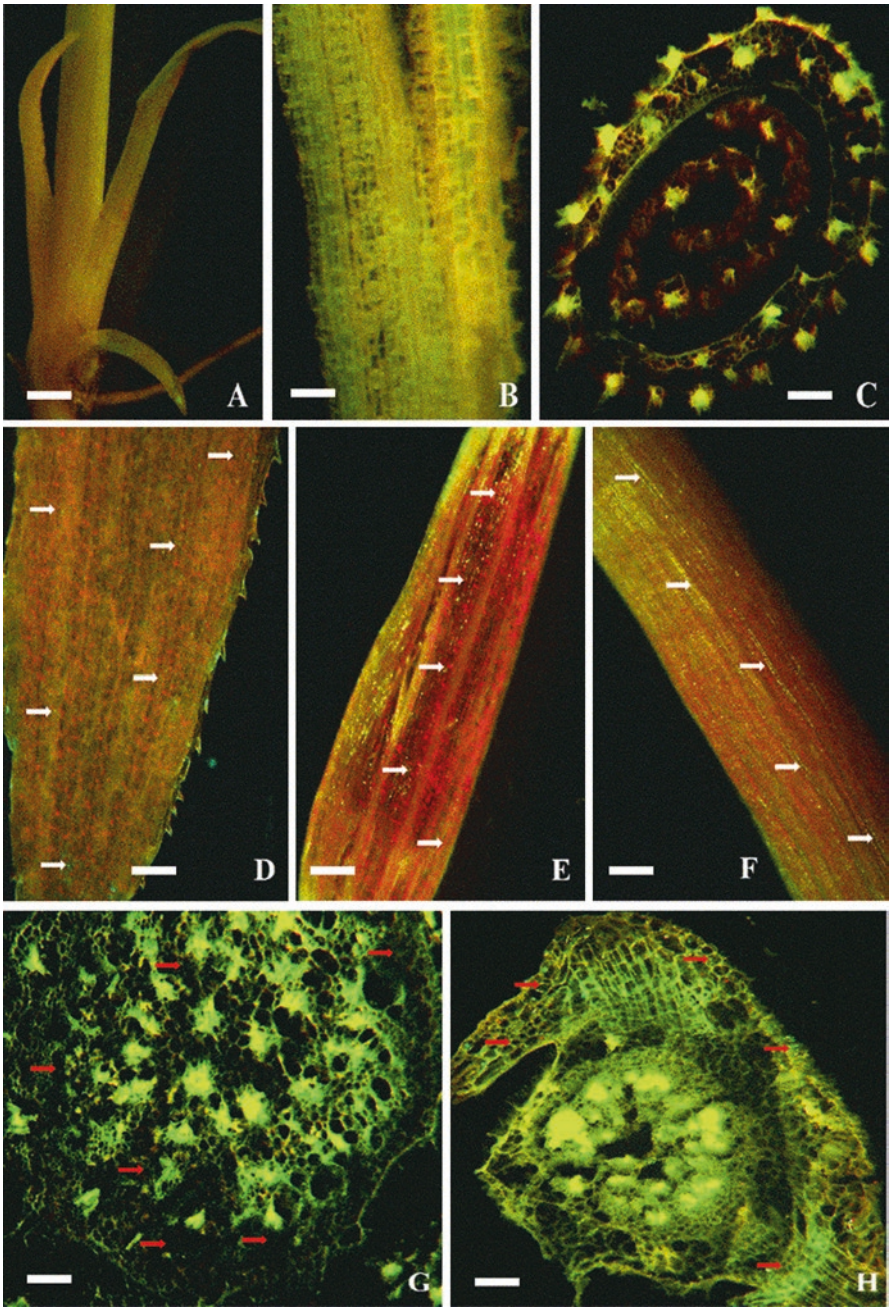


Fig. 3.4 Laser scanning confocal microscopic images present GFP-tagged bacteria, fluorescence green in color. (**a–c**) is a control sample;(**d–h**) is GFP-tagged selected nitrogen-fixing microbes and colonization of strain in sugarcane plant. An *arrowhead* points to bacteria which infect the leaf, stem, and root. Scale bars: 50 μm in all pictures

Table 3.5 Evolution of agricultural input use in India (Pray and Nagarajan 2014)

	Year				
Agricultural input	1971	1981	1991	2001	2011
Quality seed distribution (in thousands of tons)	52	450	575	918	2773
Fertilizer consumption (in thousands of tons of NPK nutrients)	2000	5300	12,000	18,000	28,300
Pesticide consumption (in thousands of tons of active ingredients)	26	47	72	44	56

industries, the seed and biotechnology industry spent the most on R&D in 2008–2009 and grew the fastest. This category includes research on sugar mills, tea and coffee firms, and biofuel manufacturers and includes improving crop productivity and quality. The demand for latest agricultural inputs in developing countries has increased rapidly because of increase in research factors. For example, in India, farm purchases of quality seeds and fertilizers have raised steadily over the past 40 years (Table 3.5). In the 1980s, India controlled the making and circulation of seed, pesticide, and agricultural implements. In the late 1980s, the Indian government started permitting huge national and overseas privately maintained companies to join in the market. In the 1990s, overseas companies were certified to have mainstream ownership in agribusinesses. The agricultural investments in India, Brazil, and China were breakthroughs in biotechnology to motivate private sector research. Therefore, many scientists, researchers, industries, and other public and private agencies have focused and made an effort to alter the microbial flora of agricultural soils in order to favor the plant growth development and better yield quality production.

Farmers increased their use of industrial inputs dramatically after 1971, which is when the green revolution really took off. From 1971 to 1991, the distribution of certified and truthfully labeled seeds increased tenfold, fertilizer consumption increased from 2 to 12 million tons, and consumption of pesticides almost tripled (Pray and Nagarajan 2014). Demand for inputs in private research is often driven by the expectation that there will be future markets for the inventions developed by research. Sales in the agricultural input industries have grown extremely rapidly. Research intensity in the fertilizer industry started low and went lower during this period (Pray and Nagarajan 2014).

3.12 Future Work

It is clear that researchers have done so much of work and confirm that soil, rhizosphere, and endophytic microbes are responsible for PGPR as well as biological nitrogen fixation in sugarcane and other crops. Choice of the selected strains is a very critical task, and selection takes many years because an efficient strain of the nitrogen-fixing microorganisms is important in biofertilizer production. To find out the alternative use, the strains from chemical fertilizers as a reliable source along

with all PGP activities in addition to long survivability in any environmental conditions in the soil then go for large-scale production of biofertilizer with the use of nitrogen-fixing microorganisms.

3.13 Conclusion

Numerous scientific literature is available today on soil–plant–microbe interactions which are natural facts and occur everywhere in the soil. The rhizospheric community is highly complex and comprises numerous organisms available for interactions. Several species of nitrogen-fixing organisms have been identified and developed as biofertilizer for plant growth and development. However, there is considerable interest in the exploitation of successful nitrogen fixers for a greater understanding of their ecosystem. Nitrogen-fixing organisms improve the tolerance of plants to abiotic stresses such as increased tolerance or resistance to cold, heat, drought, salinity, heavy metal, etc. There could be another moving and demanding job for microbes that may offer superior opportunities to execute the concept of biofertilizer in the field under the natural environments. The use of N fixers can play an important role in crop protection and integrated pest management programs. With the rising acceptance and awareness of the benefits conferred, the efforts should be focused forward on the developing technology and improving environment-friendly biofertilizer for agronomically important crops, i.e., sugarcane, rice, and wheat. Despite the recent advancement in technology, microbial inoculants' commercialization needs more optimization and a comprehensive study from the angle of discovery, fermentation, formulation, and function. This technology is promising if we take into consideration the rising cost and declining environmental pollution as well as maintenance of the soil structure and fertility problems.

References

Abeysingha NS, Weerarathne CS (2010) A preliminary study on quantification of biological nitrogen fixation in sugarcane grown in Sevanagala in Sri Lanka. J Natl Sci Found Sri Lanka 38:207–210

Asis CAJ, Kubota M, Ohta H et al (2000) Isolation and partial characterization of endophytic diazotrophs associated with Japanese sugarcane cultivar. Soil Sci Plant Nutr 46:759–765

Bae YS, Knudson GR (2000) Co-transformation of *Trichoderma harzianum* with beta-glucuronidase and green fluorescent protein genes provides a useful tool for monitoring fungal growth and activity in natural soils. Appl Environ Microbiol 66:810

Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:234–266

Balser TC, Firestone MK (2005) Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73:395–341

Bashan Y, de-Bashan LE (2005) Plant growth-promoting. In: Hillel D (ed) Encyclopedia of soils in the environment, vol 1. Elsevier, Oxford, pp 103–115

Bashan Y, Holguin G (1998) Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil Biol Biochem 30:1225–1228

Bauer WD, Mathesius U (2004) Plant responses to bacterial quorum sensing signals. Curr Opin Plant Biol 7:429–433

Begcy K, Mariano ED, Gentile A et al (2012) A novel stress-induced sugarcane gene confers tolerance to drought, salt and oxidative stress in transgenic tobacco plants. PLoS One 7(9):e44697. doi:10.1371/journal.pone.0044697

Beneduzia A, Moreirab F, Costab PB et al (2013) Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Appl soil Ecol 63:94–104

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berg G, Roskot N, Steidle A et al (2002) Plant dependent genotypic and phenotypic diversity of antagonistic *Rhizobacteria* isolated from different *Verticillium* host plants. Appl Environ Microbiol 68:3328–3338

Bhatia CR (2008) Molecular mechanisms of plant and microbe coexistence. In: Nautiyal CS, Dion P. (eds) ISBN: 978–3–540-75574-6Soil biology, 15th edn. ©Springer-Verlag, Berlin, p 53

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bokhtiar SM, Paul GC, Rashid MA et al (2001) Effect of press mud and organic nitrogen on soil fertility and yield of sugarcane grown in high Ganges river flood plain soils of Bangladesh. Indian Sugar 51(4):235–240

Bottini R, Cassan F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65(5):497–503

Brand A (1995) GFP in drosophila. Trends Genet 11:324–325

Caballero-Mellado J, Fuentes-Ramirez LE, Reis VM et al (1995) Genetic structure of Acetobacter diazotrophicus populations and identification of a new genetically distant group. Appl Environ Microbiol 61:3008–3013

Caballero-Mellado J, Onofre-Lemus J, Estrada-de los Santos P et al (2007) The tomato rhizosphere, an environment rich in nitrogen-fixing Burkholderia species with capabilities of interest for agriculture and bioremediation. Appl Environ Microbiol 73:5308–5319

Cabanillas C, Stobbia D, Ledesma A (2013) Production and income of basil in and out of season with vermin composts from rabbit manure and bovine ruminal contents alternatives to urea. J Clean Prod 47:77–84

Cassan F, Maiale S, Masciarelli O et al (2009) Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. Eur J Soil Biol 45:12–19

Chet I, Chernin L (2002) Biocontrol, microbial agents in soil. In: Bitton G (ed) Encyclopedia of environmental microbiology. Willey, New York, pp 450–465

Choudhary RL, Wakchaure GC, Minhas PS et al (2016) Response of ratoon sugarcane to stubble shaving, off-barring, root pruning and band placement of basal fertilizers with a multipurpose drill machine. Sugar Tech. doi:10.1007/s12355-016-0438-x

Compant S, Duffy B, Nowak J et al (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Compant S, Clement C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Cook RJ, Baker KF (1983) The nature and practice of biological control of plant pathogens. APS Press, St Paul

Croft BJ, Magarey RC (2000) *Pachymetra* root rot. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (eds) A guide to sugarcane diseases. CIRAD/ISSCT, CIRAD Publication Services, Montpellier, pp 126–130

Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548

Dotaniya ML, Datta SC (2014) Impact of bagasse and press mud on availability and fixation capacity of phosphorus in an inceptisol of north India. Sugar Tech 16(1):109–112
Dotaniya ML, Datta SC, Biswas DR et al (2016) Use of sugarcane industrial by-products for improving sugarcane productivity and soil health. Int J Recycl Org Waste Agri 5(3):185–194
Durbak A, Yao H, Mc-Steen P (2012) Hormone signaling in plant development. Curr Opin Plant Biol 15:92–96
Errampali D, Leung K, Cassidy MB et al (1999) Application of the green fluorescent protein as a molecular marker in environmental microorganisms. J Microbiol Methods 35:187–1999
Farrar K, Bryant D, Selby NC (2014) Understanding and engineering beneficial plant–microbe interactions: plant growth promotion in energy crops. Plant Biotechnol J 12:1193–1206
Fierer N, Strickland MS, Liptzin D et al (2009) Global patterns in below ground communities. Ecol Lett 12:1238–1249
Fischer D, Pfitzner B, Schmid M et al (2012) Molecular characterisation of the diazotrophic bacterial community in uninoculated and inoculated field-grown sugarcane (*Saccharum* sp.) Plant Soil 356:83–99
Fuglie K (2012) Productivity growth and technology capital in the global agricultural economy. In: Fuglie K, Wang SL, Ball VE (eds) Productivity growth in agriculture: an international perspective. CABI, Washington, DC, pp 335–368
Gans J, Wolinsky M, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309:1387–1390
Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117
Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica (Cairo): 963401 doi:10.6064/2012/963401
Glick BR (2015) Issues regarding the use of PGPB. In: Glick BR (ed) Beneficial plant bacterial interaction. Springer Publishing, Switzerland, pp 223–243
Govindarajan M, Balandreau J, Muthukumarasamy R et al (2006). Improved yield of micropropagated sugarcane following inoculation by endophytic *Burkholderia vietnamiensis*. Plant Soil 280:239–252
Govindarajan M, Kwon SW, Weon HY (2007) Isolation, molecular characterization and growth-promoting activities of endophytic sugarcane diazotroph Klebsiella sp. GR9. World J Microbiol Biotechnol 23:997–1006
Govindarajan M, Jebanesan A, Reetha D et al (2008) Antibacterial activity of Acalypha indica L. Eur Rev Med Pharm Sci 12:299–302
Grivet L, Arruda P (2001) Sugarcane genomics: depicting the complex genome of an important tropical crop. Curr Opin Plant Biol 5:122–127
Haas D, Defago G (2005) Biological control of soil-borne pathogens by *fluorescent pseudomonads*. Nat Rev Microbiol 3:307–319
Hardoim PR, Van Overbeek LS, Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16(10):463–471
Hartmann A, Gantner S, Schuhegger R et al (2004) N-acyl homoserine lactones of rhizosphere bacteria trigger systemic resistance in tomato plants. In: Lugtenberg B, Tikhonovich I, Provorov N (eds) Biology of molecular plant–microbe interactions, vol 4. APS, St. Paul, pp 554–556
Hellriegel H, Wilfarth H (1888) Untersuchungen uber die Stickstoffnahrung der Gramineen und Leguminosen. Beilageheft zu der Zeitschrift des Vereins fur Rubenzucker-Industrie Deutschen Reichs, p 234
Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 3(11):1–18
Hiltner L (1904) Uberneue Erfahrungen und Probleme auf dem Gebiet der Bodenba kteriologie und unterbesonderer Berucksichtigung der Grundungungund Brache. Arb Dtsch Landwirtschafts-Ges 98:59–78
Hirsch AM (2004) Plant–microbe symbioses: a continuum from commensalism to parasitism. Symbiosis 37:345–363

Hogberg MN, Hogbom L, Kleja DB (2013) Soil microbial community indices as predictors of soil solution chemistry and N leaching in *Picea abies* (L.) Karst forests in S. Sweden. Plant Soil 372(1):507–522

Hurek T, Reinhold-Hurek B (2003) *Azoarcus* spp. strain BH72 as a model for nitrogen fixing grass endophytes. J Biotechnol 106:169–178

Iwata K, Azlan A, Yamakawa H et al (2010) Ammonia accumulation in culture broth by the novel nitrogen-fixing bacterium, *Lysobacter* sp. E4. J Biosci Bioeng 110(4):415–418

James EK (2000) Nitrogen fixation in endophytic and associative symbiosis. F Crop Res 65:197–209

Kachroo A, Robin GP (2013) Systemic signaling during plant defense. Curr Opin Plant Biol 16:527–533

Kamilova F, Validov S, Azarova T et al (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7:1809–1817

Kruasuwan W, Thamchaipenet A (2016) Diversity of culturable plant growth-promoting bacterial endophytes associated with sugarcane roots and their effect of growth by co-inoculation of diazotrophs and actinomycetes. J Plant Growth Regul 35:1074–1087

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Station de Pathologie Vegetaleet Phyto-Bacteriologie (ed). Proceedings of the 4th International Conference on Plant Pathogenic Bacteria, vol. II. Gilbert-Clarey, Tours, France, p 879–882

Kumar P, Dubey RC, Maheshwari DK (2012) *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 167:493–499

Ladeiro B (2012) Saline agriculture in the 21st century: Using salt contaminated resources to cope food requirements. J Bot. doi:10.1155/2012/310705

Laslo E, Gyorg Y, Mara E et al (2012) Screening of plant growth promoting rhizobacteria as potential microbial inoculants. Crop Prot 40:43–48

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25:275–294

Li YR, Yang LT (2015) Sugarcane agriculture and sugar industry in China. Sugar Tech 17(1):1–8

Li YR, Zhou XZ, Yang LT (2015) Biological nitrogen fixation in sugarcane and nitrogen transfer from sugarcane to cassava in an intercropping system. Inter J Sci Nat 6:214–218

Lim SL, Lee LH, Wu TY (2016) Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis. J Clean Prod 111:262–278

Lima E, Boddey RM, Dobereiner J (1987) Quantification of biological nitrogen fixation associated with sugar cane using a ^{15}N-aided nitrogen balance. Soil Biol Biochem 19:165–170

Lugtenberg BJJ, Chin-A-Woeng TFC, Bloemberg GV (2002) Microbe–plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek 81:373–383

Manoharachary C, Mukerji KG (2006) Microbial activity in the rhizosphere. In: Mukerji KG, Manoharachary C, Singh J (eds) Soil biology, vol 7. ©Springer-Verlag, Berlin

Mehnaz S, Baig DN, Lazarovits G (2010) Genetic and phenotypic diversity of plant growth promoting rhizobacteria isolated from sugarcane plants growing in Pakistan. J Microbiol Biotechnol 20:1614–1623

Mendes R, Pizzirani-Kleiner AA, Araujo WL et al (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of Burkholderia cepacia complex isolates. Appl Environ Microbiol 73:7259–7267

Mirza MS, Ahmad W, Latif F et al (2001) Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micropropagated sugarcane in vitro. Plant Soil 237:47–54

Muller H, Westendorf C, Leitner E et al (2009) Quorum-sensing effects in the antagonistic rhizosphere bacterium *Serratia plymuthica* HRO-C48. FEMS Microbiol Ecol 67:468–467

Nacry P, Bouguyon E, Gojon A (2013) Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil. doi:10.1007/s11104-013-1645–9

Nasare K, Yadav A, Singh AK et al (2007) Molecular and symptom analysis reveal the presence of new phytoplasmas associated with sugarcane grassy shoot disease in India. Plant Dis 91:1413–1418

Nielsen MN, Winding A (2002) Microorganisms as indicators of soil health. NERI Technical Report No. 388. National Environmental Research Institute, Ministry of the Environment, Denmark

Oldroyd EDG, Murray JD, Poole PS et al (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144

de Oliveira ALM, de Canuto EL, Urquiaga S et al (2006) Yield of micropropagated sugarcane varieties in different soil types following with diazotrophic bacteria. Plant Soil 284(1):23–32

Oliveira ALM, Canuto EL, Urquiaga S et al (2006) Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. Plant Soil 284:23–32

Oliveira ALM, Stoffels M, Schmid M et al (2009) Colonization of sugarcane plantlets by mixed inoculations with diazotrophic bacteria. Eur J Soil Biol 45:106–113

Orlando BH, ThommaBart PHJ, Carmona E et al (2005) Identification of sugarcane genes induced in disease resistant somaclones upon inoculation with Ustilago scitaminea or Bipolaris sacchari. Plant Physiol Biochem 43:1115–1121

Pal S, Rahija M, Nienke-Beintema N (2012) India: recent developments in agricultural research, country note. International Food Policy Research Institute (IFPRI), Washington, DC

Paungfoo-Lonhienne C, Lonhienne TGA, Yeoh YK et al (2014) A new species of Burkholderia isolated from sugarcane roots promotes plant growth. Microb Biotechnol 7:142–154

Pedraza RO (2008) Recent advances in nitrogen-fixing acetic acid bacteria. Int J Food Microbiol 125:25–35

Pedrosa FO, Monteiro RA, Wassem R et al (2011) Genome of Herbaspirillum seropedicae Strain SmR1, a Specialized Diazotrophic Endophyte of Tropical Grasses. PLoS Genet 7, e1002064. doi:10.1371/journal.pgen.1002064

Phillips DA, Fox TC, King MD et al (2004) Microbial products trigger amino acid exudation from plant roots. Plant Physiol 136:2887–2894

Pierik R, Tholen D, Poorter H et al (2006) The Janus face of ethylene: growth inhibition and stimulation. Trends Plant Sci 4:176–183

Poppenborg L, Friehs K, Flaschel E (1997) The green fluorescent protein is a versatile reporter for bioprocess monitoring. J Biotechnol 58:79–88

Pray CE, Fuglie KO (2015) Agricultural research by the private sector. Annu Rev Resour Econ 7:399–424

Pray CE, Nagarajan L (2012) Innovation and research by private agribusiness in India. IFPRI Discussion Paper 01181, IFPRI, Washington, DC

Pray CE, Nagarajan L (2014) The transformation of the Indian agricultural input industry: has it increased agricultural R and D. Agric Econ 45:145–156

Ramos PL, Van Stefanie T, Fabiano TL et al (2011) Screening for endophytic nitrogen-fixing bacteria in Brazilian sugar cane varieties used in organic farming and description of Stenotrophomonas Pavanii Sp. Nov. Int J Syst Evol Microbiol 61:926–931

Rao GP, Ford RE (2000) Vectors of virus and phytoplasma diseases of sugarcane: an overview. In: Rao GP, Ford RE, Tosic M, Teakle DS (eds) Sugarcane pathology, Vol. III. Virus and phytoplasma diseases. Science Publishers, Hamshere, pp 265–314

Rao GP, Viswanathan R, Singh SB (2002) Current situation of sugarcane diseases in India. In: Singh SB, Rao GP, Easwaramoorthy S (eds) Sugarcane crop management. SCI Tech Publishing LLC, Houstan, p 734

Reis VM, Lee S, Kennedy C (2007) Biological nitrogen fixation in sugarcane. In: Elmerich C, Newton WE (ed) Associative and endophytic nitrogen-fixing bacteria. Springer. p 213–232

Rengasamy P (2006) World salinization with emphasis on Australia. J Exp Bot 57:1017–1023

Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: balancing the cost of an essential resource. Annu Rev Environ Resour 34:97–125

Robinson N, Brackin R, Vinall K et al (2011) Nitrate paradigm does not hold up for sugarcane. PLoS One 6(4):e19045. doi:10.1371/journal.pone.0019045

Shimomura O, Johnson FH, Saiga Y (1962) Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequorea*. J Cell CompPhysiol 59:223–239

da Silva M, Antonio C, de Oliveira P et al (2012) Survival of endophytic bacteria in polymer-based inoculants and efficiency of their application to sugarcane. Plant Soil 356:231–243

Singh RK, Kumar DP, Singh P et al (2014) Multifarious plant growth promoting characteristics of chickpea rhizosphere associated Bacilli help to suppress soil-borne pathogens. Plant Growth Regul 73(1):91–101

Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu Rev Plant Biol 62:227–250

Solanki MK, Kumar S, Pandey AK et al (2012) Diversity and antagonistic potential of *Bacillus* spp. associated to the rhizosphere of tomato for the management of *Rhizoctonia solani*. Biocontrol Sci Tech 22:203–217

Solanki MK, Wang Z, Wang FY (2016) Intercropping in sugarcane cultivation influenced the soil properties and enhanced the diversity of vital diazotrophic bacteria. Sugar Tech. doi:10.1007/s12355-016-0445-y

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Souza R, de Ambrosini A, Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38:401–419

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Spear RN, Cullen D, Andrews JH (1999) Fluorescent labels, confocal microscopy, and quantitative image analysis in study of fungal biology. Methods Enzymol 307:607–623

Stephane C, Christophe C, Angela S (2010) Plant growth-promoting bacteria in the rhizo and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Sternberg C, Christensen BB, Johansen T et al (1999) Distribution of bacterial growth activity in flow-chamber biofilms. Appl Environ Microbiol 65:4108–4117

Suslow TV, Kloepper JW, Schroth MN et al (1979) Beneficial bacteria enhance plant growth Rhizobacteria. Calif Agric Exp Stn 33:15–17

Taghavi S, Garafola C, Monchy S et al (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75(3):748–757

Taule C, Mareque C, Barlocco C et al (2012) The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community. Plant Soil 356:35–49

Tikhonovich IA, Provorov NA (2011) Microbiology is the basis of sustainable agriculture: an opinion. Ann Appl Biol 159:155–116

Tsavkelova EA, Klimova SY, Cherdyntseva TA et al (2006) Hormones and hormone-like substances of microorganisms. A review. Appl Biochem Microbiol 42:229–235

Unge AR, Molback TL, Jansson J (1999) Simultaneously monitoring of cell number and metabolic activity of specific bacterial populations with a dual *gfp-lux AB* marker system. Appl Enviorn Microbiol 65:813–821

Urquiaga S, Cruz KHS, Boddey RM (1992) Contribution of nitrogen fixation to sugarcane: nitrogen-15 and nitrogen-balance estimates. Soil Sci Soc Am J 56(105–11):4

Urquiaga S, Xavier RP, de Morais RF et al (2012) Evidence from field nitrogen balance and ^{15}N natural abundance data for the contribution of biological N_2 fixation to Brazilian sugarcane varieties. Plant Soil 356:5–21

Valdivia R, Hromockyj A, Monack D et al (1996) Applications for green fluorescent protein (GFP) in the study of host-pathogen interactions. Gene 173:47–52

Vanden Wymelenberg AJ, Cullen D, Spear RN et al (1997) Expression of green fluorescent protein in Aureobasidium pullulans and quantification of the fungus on leaf surfaces. Bio Techniques 23:686–690

Verma JP, Jaiswal DK, Sagar R (2014) Pesticide relevance and their microbial degradation: a state of art. Rev Environ Sci Biotechnol 13:429–466

Veronica S, Fabricio C, Oscar M et al (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. Appl Microbiol Biotechnol 85:371–381

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2):571–586

Viswanathan R, Rao GP (2011) Disease scenario and management of major sugarcane diseases in India. Sugar Tech 13(4):336–353

Weller DM, Raaijmakers JM, Gardener BBM et al (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Whipps J (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Wirthmueller L, Maqbool A, Banfield MJ (2013) On the front line: structural insights into plant–pathogen interactions. Nat Rev Microbiol 11:761–776

Wu CH, Bernard SM, Andersen GL et al (2009) Developing microbe-plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. Microb Biotechnol 2:428–440

Xing Y, Yang L, Huang S et al (2015) A new nitrogen fixing endo-bacterium strain isolated from sugarcane stem. In: Guilin PR (ed) Proceedings of international symposium on technologies to improve sugar productivity in developing countries. China Agricultural Science, Beijing, pp 487–490

Yuan ZC, Haudecoeur E, Faure D et al (2008) Comparative transcriptome analysis of *Agrobacterium tumefaciens* in response to plant signal salicylic acid, indole-3-acetic acid and gamma-amino butyric acid reveals signalling cross-talk and *Agrobacterium*–plant co-evolution. Cell Microbiol 10:2339–2354

Zanin L, Tomasi N, Wirdnam C et al (2014) Isolation and functional characterization of a high affinity urea transporter from roots of *Zea mays*. BMC Plant Biol 14:222. doi:10.1186/s12870-014-0222–6

Webliography

Classification of Sugarcane (n.d.) http://theagricos.com/agriculture/crops/sugarcane/scientific-classification-sugarcane/

Factfish (2016) Sugarcane: (May, 2016) for all countries http://www.factfish.com/statistic/sugarcane

FAO (2016) Top Sugarcane Production: FAO of the United Nations: (2016) http://faostat.fao.org/site/339/default.aspx

Sugarcane diseases cite ref (n.d.) SCGS, India, VSI, 1–0 https://en.wikipedia.org/wiki/List

Microbial Interactions and Plant Health

4

Amrita Sengupta, Sunil Kumar Gunri, and Tapas Biswas

Abstract

With augmented population, hasty industrialization, and urbanization worldwide, land for agricultural production is declining faster, and there is a huge demand for ecologically viable and environmentally affable techniques in agriculture, competent of providing adequate sustenance for the increasing human inhabitants and of improving the quality as well as quantity of certain agricultural harvests. A great deal of endeavor focusing on soil biology and the agroecosystem as a whole is required, enabling better perception of the complex processes and communications governing the stability of agricultural lands and plant kingdom. The scientific advances in modern times, researching biodiversity, have revealed that microbial miscellany is of massive potential that can be explored through careful assortment of the same and their booming use may solve critical agricultural and environmental issues. Here, we promote the thought that considering the mechanism by which plants select and interact with their microbiomes may have a direct or indirect effect on plant health that further may lead to establishment of novel microbiome-driven strategies that can embark upon the development of a more sustainable agriculture.

Keywords

Association • Biofertilizers • Crop production • Inoculation • Microorganisms • PGPR • Phyllosphere • Rhizosphere • Symbiosis

A. Sengupta (✉) • S.K. Gunri
Department of Agronomy, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252, India
e-mail: amritasenbckv@gmail.com

T. Biswas
Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_4

4.1 Introduction

The ultimate threats of the twenty-first century have become quite comprehensible in the last few decades. Climate change due to the enormous increase in the production of greenhouse gases is real (Crowley 2000). A typical characteristic of modern intensive agriculture worldwide, i.e., application of synthetic chemicals like fertilizers, fungicides, herbicides, and pesticides, has been reported as non-sustainable and having multiple harmful impacts on both human or animal and plant health as well as environmental well-being (Franks et al. 2006; Glick 2014). There is a legitimate need for renewable energy supplies (Cook et al. 1991; Jackson 1999). Under such circumstances, prospective alternatives to the use of chemical or synthetic inputs are microbial inoculants, environment-friendly microbial formulations that act as biofertilizers, phyto-stimulants, and/or microbial biocontrol agents (Olubukola et al. 2012).

Surplus microbes are there in each gram of soil, and microbial cells are found extensively in plant and animal tissues (Andreote et al. 2014). Microorganisms execute various metabolic activities indispensable for their own survival (Sengupta and Gunri 2015), and useful properties of such microbial inoculants could be manifested either by direct endorsement of plant growth through nutrient recycling or indirectly by defending plants from phytopathogens, or by invigorating tolerance to some of the abiotic strain in plants, which grow under nonoptimal ecological factors including soil, higher or lower temperature, acidity, salinity, drought, and heavy metals as well (Penrose and Glick 2003; Kang et al. 2014) .The varied community of microbes develop a metagenome of information that also extends to both outside and inside of the human body (Ahmed et al. 2011). Microbes are also capable of playing major roles in the development of soil aggregates that help in stabilization of the topsoil (van Veen et al. 1997) and improvement of soil health and can help in ecological detoxification, wastewater treatment, etc. (Ahmad et al. 2011). The mechanisms governed by microbes in the regulation of physiological processes of their hosts have been comprehensively studied in the light of latest findings on microbiomes. Even though there is no lucid depiction of the overall function of the plant microbiome, there is considerable confirmation that these communities are involved in infection control, enhanced nutrient attainment, and influence stress tolerance. Thus, currently, noteworthy venture is being exerted on research to build up such microbial inoculants which have positive plant growth properties in environmentally responsive sustainable cultivation (Barriuso et al. 2008a, b).

A large portion of favorable soil microorganisms are still undiscovered, and their environmental functions are pretty indefinite till date. Thus, enormous assays of microbial activities are the fundamental steps toward progress in innovative technologies for proficient exploitation of microorganisms for realization of sustainability in agriculture. Microbial involvement in combination with advancements in digital imaging, nanotechnology, and electronics may play a key role in solving universal challenges of the twenty-first century together with climate change (Ahmad et al. 2011).

This book chapter sums up features of microbial community that make up the plant microbiome and further presents a chain of studies recounting the underneath factors that contour the phylogenetic and useful plant-associated communities.

4.2 Microbial Interactions

Microbial populations interrelate and establish relations with each other and with higher organisms. Usually the relationship is nutritional, though other benefits may accrue, and the association can turn essential to the survival of one or both partners. There are several sorts of associations, viz., amensalism and competition, mutualism, parasitism protocooperation, synergism and commensalism, etc., between the organisms.

Odum (1971) has proposed the following relations:

(a) *Neutralism*, where the two microorganisms perform entirely autonomously
(b) *Symbiosis*, the two symbionts relying upon one another and mutually benefiting the affiliation
(c) *Protocooperation*, a relationship of reciprocal advantage to the two species but devoid of the cooperation being mandatory for their survival or for performance of some response
(d) *Commensalism*, in which only one species derives profit while the other is unaltered
(e) *Competition*, a situation in which there is a repression of one organism as the two species fight for restraining quantities of O_2, space, nutrients, or other common necessities
(f) *Amensalism*, in which one species is covered up while the following is not affected, often the result of toxin production
(g) *Parasitism* and *Predation*, the direct assault of one individual upon another
(h) *Synergism*, in field conditions the probable synergistic effect in the plant between inducing virus and other non-linked viruses which could be brought to those plants from outside sources

4.3 Microbe–Plant Interactions

Plants are exposed to huge numbers of microorganisms that are present in the topsoil and are found on leaves and stems. Plants are the major resource of nutrients for microorganisms being the prime source of organic carbon. Plants provide nutrients through shedding of leaves, pollen, etc., or through exudates or dead tissues in an indirect manner (Sivakumar and Thamizhiniyan 2012). In few instances, nutrients are provided straightway to microbes that form close relations with plants. Associations with plants can vary from those that are tremendously damaging to the plant, such as those with dangerous pathogens, through exchanges, which do not come out to influence plant growth, to advantageous ones such as those formed with

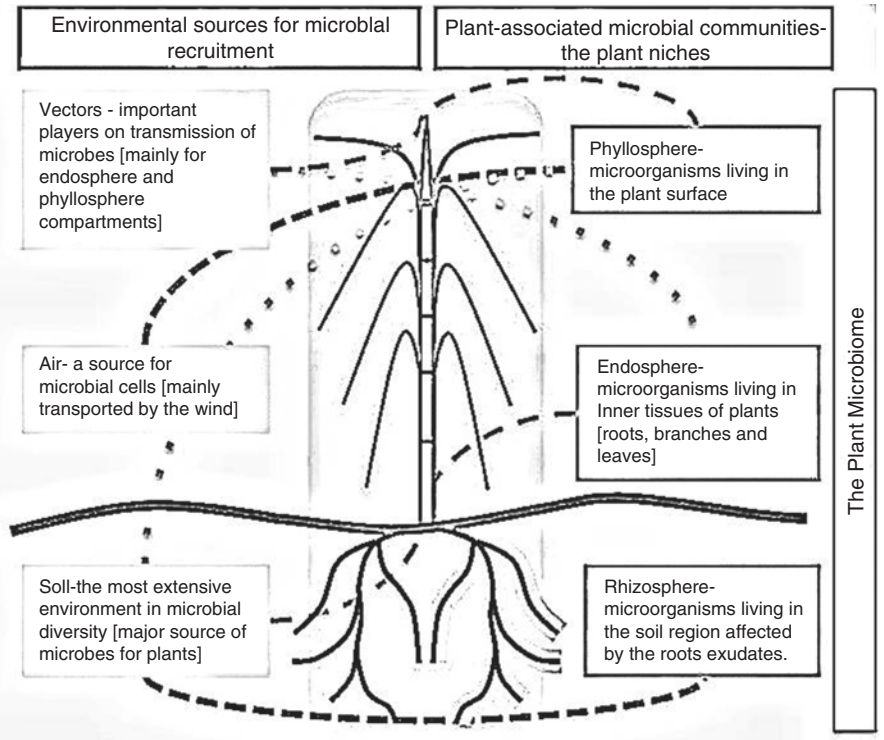


Fig. 4.1 Schematic depiction of the key sources for microbes that compose the plant-associated communities: the rhizosphere, endosphere, and phyllosphere. Width and fill of connections point out the role of ecological sources for the composition of microbial communities in plant-harboring niches

mycorrhizal fungi or nitrogen-fixing bacteria. For most microorganisms, exchanges with growing plants expand no further than the colonization of the surfaces of stems, leaves, and roots because these are regions where exudates are accessible.

Such microbiomes in plants may form divergent communities, like the ones from the rhizosphere, endosphere, or the phyllosphere (Hirsch and Mauchline 2012; Hardoim et al. 2008) (Fig. 4.1). In each of these niches, the "microbial tissue" is established by, and responds to, specific selective pressures (Andreote et al. 2014).

4.3.1 Rhizosphere and Root Exudates

The rhizosphere is the frontier between plant roots and soil where communications among numerous invertebrates as well as microbes influence plant growth, biogeochemical cycles, and indulgence to biotic and abiotic strain (Philippot et al. 2013).

In rhizosphere microbial action is generally high. Hiltner (1904) observed the zone of extreme microbial commotion around the roots and named it as rhizosphere

(as cited by Hartmann et al. 2008). Roots emit considerable amounts of sugars, amino acids, hormones, and vitamins, which promote such a widespread growth of fungi and bacteria that these organisms often form microcolonies on the surface of the roots. Primarily roots contain little or no microbial colonization but with advancement in plant growth, in the soil, root exudates, comprising a combination of 10 sugars, 10 organic acids, about 18 amino acids, mucilage, etc., along with other cell exerts or root caps that influence on microbial colonization (Griffin et al. 1976). The chemicals in the forms of root exudates, released in the proximity of plant rhizosphere, are known to belong from the vital group of carbohydrates, phenols, organic acids, protein, and lipid along with other cellular components (Nguyen 2003; Dini-Andreote and Elsas 2013). Root exudates have been grouped and are primarily classified into two major classes, viz., compounds of high molecular weight like polysaccharides and proteins and that of low molecular weight like amino acids, organic acids, sugars, phenolic compounds, and other secondary metabolites (Bais et al. 2006; Badri and Vivanco 2009; Narasimhan et al. 2003). From these molecules, few are linked with establishment of key portions of the microbial community (generally metabolized by a good number of soil organisms, e.g., glucose), but other compounds released are capable of activating precise groups of organisms (those related to signaling and chemo taxis, e.g., flavonoids) (Nguyen 2003; Jones et al. 2004).

Quantitative and qualitative compositions of exudes from plant roots are generally determined by the plant species, plant developmental stage, cultivar, and various environmental factors, including soil pH, temperature, type of soil, as well as presence of microorganisms in soil (Badri and Vivanco 2009; Uren 2000). These differences fabricate microbial communities in the rhizosphere that have a definite degree of specificity for each plant species.

4.3.1.1 Mechanism of Root Exudation

Plants communities employ a variety of transportation mechanisms to export and exude compounds in the soil rhizosphere (Badri and Vivanco 2009; Weston et al. 2012). Usually, roots can release root exudates through active or passive mechanisms by means of secretion or diffusions, respectively. Majority of low-molecular-weight organic compounds are released from plants through a passive process. Small polar and uncharged molecules are elated by direct passive diffusion, a procedure that depends on membrane permeability, the polarization of the exuded compounds, and cytosolic pH (Badri and Vivanco 2009). Plant root cells release additional substances, like resultant polysaccharides, proteins, and other metabolic derivatives, with the help of various membrane-bound proteins (Weston et al. 2012). These carrier proteins comprise the ATP-binding cassette (ABC) transporters (Badri et al. 2008, 2009a; Loyola-Vargas et al. 2007; Sugiyama et al. 2008), multidrug and toxic compound extrusion (MATE) family (Yazaki 2005), the key facilitator super family (Reddy et al. 2012), and the aluminum-activated malate transporter family (Weston et al. 2012). Though the detailed functions of these membrane-bound transport proteins are not well stated, they have been connected with the transfer of a wide range of compounds into the rhizosphere. Badri et al. (2008, 2009a) found

that 25 ABC transporter genes were notably overexpressed in the *Arabidopsis thaliana* (L.) Heynh. roots and played significant roles in these discharge processes. Adding up to ABC transporters, MATEs are also dynamic transporters that export a large variety of substrates across membranes by using the electrochemical gradient of other ions (Weston et al. 2012). Many MATE genes play important role in exporting different compounds, such as plant-derived alkaloids, toxic compounds, antibiotics, citrate anions, and phenolic compounds, out of the cells of plant roots, which have been identified as well as characterized in *Arabidopsis* (Diener et al. 2001; Li et al. 2002; Liu et al. 2009), sorghum (Magalhaes et al. 2007), barley (Furukawa et al. 2007), and rice (Ishimaru et al. 2011).

4.3.1.2 Rhizosphere Microbes Influence Plant Root Exudation

Plant root exudation is also influenced by the microbes (fungi and bacteria), colonized in the rhizosphere (Jones et al. 2004; Leyval and Berthelin 1993; Matilla et al. 2010a, b). Several studies have shown that the arbuscular mycorrhizal fungi colonization can alter plant root exudation qualitatively, e.g., augmenting secretions of N, phenolics, and gibberellins and minimizing secretions of total sugars, potassium ions, and phosphorus (Jones et al. 2004).

Preceding studies have revealed that various ectomycorrhizal fungal taxa have discrete influence on profusion and specifications root exudes of plants (Fransson and Johansson 2010; Rosling et al. 2004). The inoculation with ectomycorrhizal fungus and/or rhizobacteria can modify root exudation in both quantitative and qualitative aspects (Leyval and Berthelin 1993). Another latest research has revealed that both the profusion and individuality of root-associated fungi influence plant root exudation rates (Meier et al. 2013). Furthermore, in reaction to pathogen attack, plants discharge compounds as root exudates, such as oxalic acids, phytoalexins, proteins, and other unknown substances (Nelson 1990; Steinkellner et al. 2007). In addition to fungi, bacteria influence plant root exudation too. For instance, *A. thaliana* was found to produce distinct root exudation profiles when cultured with *Pseudomonas putida* KT2440 compared with the plant without *P. putida*, suggesting that bacteria are also modulating plant root exudation (Matilla et al. 2010a, b). In addition to plant root exudation, the soil microbiome may also influence the plant metabolome (Badri et al. 2013b). Distinct soil microbiomes were applied to *A. thaliana*, and this not only affected plant growth but also influenced the leaf metabolome, which in turn influenced the feeding behavior of the larvae of the herbivore *Trichoplusia ni* (Badri et al. 2013b). Similarly, inoculation of *Arabidopsis* plants under drought stress with distinct microbial communities originating from pine, corn, and *Arabidopsis* soils demonstrated that a sympatric microbiome, with a history of *Arabidopsis* growth, was able to alter the plant's ability to detect drought stress and increased its biomass production compared with the pine and corn microbial communities (Zolla et al. 2013). This may be due to the ability of soil microbes to modulate ethylene levels by degrading the ethylene precursor 1-aminocycloprop ane-1-carboxylic acid (ACC) using the enzyme ACC deaminase (Glick 2005). The plant hormone ethylene is involved in a large number of plant responses particularly related to plant stress, and its production is synchronized by nutrition, light,

temperature, and even the status and levels of other plant hormones (Glick 2005). High levels of ethylene aggravate stress responses and even weaken plant root growth (Argueso et al. 2007). A large number of soil microbes are able to ease plant stress responses to ethylene production by catalyzing the cleavage of ACC, the direct precursor to ethylene, into α-ketobutyrate and ammonia (Glick 2005; Stearns et al. 2012). Thus, lowering plant ethylene levels improves the plants' capacity to defend against a variety of abiotic and biotic stresses. ACC deaminase activity helps in ameliorating drought stress (Arshad et al. 2008), water stress, salinity stress (Mayak et al. 2004), and overall abiotic stress and also helps in growth promotion function of plants (Glick et al. 2007; Yang et al. 2009). For example, the soil bacterium *Achromobacter piechaudii* ARV8 that has ACC deaminase activity was able to increase tomato and pepper seedling biomass (Mayak et al. 2004). Recently, Stearns et al. (2012) studied the response of *Brassica napus* to ACC deaminase bacteria and showed that genes involved in auxin production were upregulated in the plant, while genes involved in ethylene stress response were downregulated. This provides a clear signal to the benefits ACC deaminase-containing bacteria have on the plant. Determining how the overall bacterial community is involved in mediating and reducing ethylene-mediated stress could create technologies to help the plant deal with abiotic stress.

4.3.1.3 Rhizospheric Interactions

4.3.1.3.1 Root Exudates and Plant–Microbe Interactions

In the last decade, the means by which root exudates mediate rhizospheric interactions have been extensively studied (Fig. 4.2) (Badri et al. 2013a; Broeckling et al. 2008; Chaparro et al. 2013; Doornbos et al. 2012; Micallef et al. 2009a, b).

Plant root-exuded phytochemicals can intervene a number of connections, such as plant–plant, plant–microbe, and plant–faunal. These interactions differ from neutral to advantageous or harmful (Mercado-Blanco and Bakker 2007; Raaijmakers et al. 2009). In few cases, microbes can change from pathogenic to symbiotic depending upon the environmental conditions (Newton et al. 2010). For example, rhizobia, symbiotic nitrogen (N)-fixing bacteria, range from a symbiotic to a neutral interaction with plants based on nitrogen levels in soils (Davidson and Robson 1986; Zahran 1999). Furthermore, under N-limiting conditions, legumes exude more flavones and flavonols to attract and initiate legume–rhizobia symbiosis (Coronado et al. 1995; Zhang et al. 2009).

In a similar way, mycorrhizal symbiotic relationships are governed by an equal exchange of nutrients and benefits for each member (Kiers et al. 2011). As, for example, in experiments on *Medicago truncatula* Gaertn., it was found that as more carbon was given to the mycorrhizal partner, the mycorrhiza in turn provided the plant with more phosphorous (Kiers et al. 2011).

4.3.1.4 Functions of Rhizosphere Microbiome

Microorganisms from the rhizosphere play significant roles in ecological vigor of the plant hosts. Significant microbial processes that are likely to take place in the

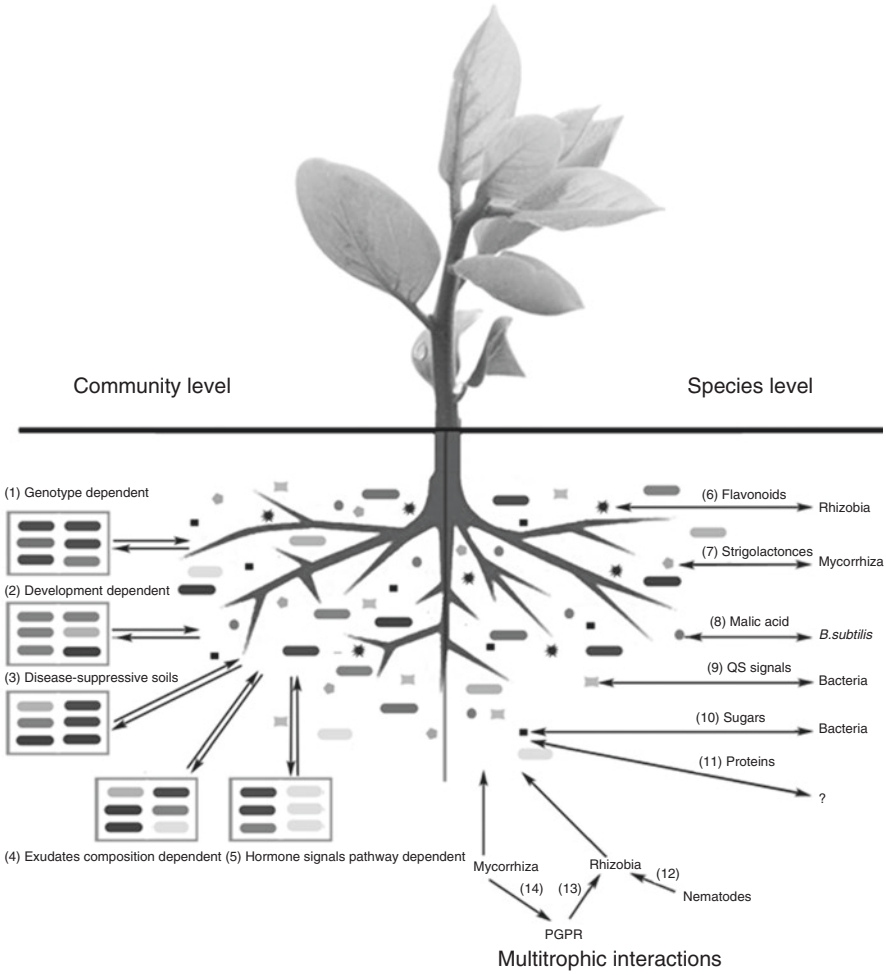


Fig. 4.2 Root exudates intervene a large number of rhizospheric interactions: at the species level (*right* side), multitrophic interactions (*bottom*), and at the community level (*left* side)

rhizosphere consist of pathogenesis and its counterpart, plant protection/growth promotion, along with synthesis of antibiotics, colonization of plants, and recycling of natural resources (Kent and Triplett 2002). Plant–microbe interactions may thus be considered as advantageous, neutral, or detrimental to the plant, depending on the specific microorganisms and plants concerned and on the existing environmental situation (Bais et al. 2006). Exploring the microbes, through sorting out their probable interactions with plant communities, has opened up a new interesting area for experimentations in rhizosphere research.

4.3.1.4.1 Beneficial Functions

Plant beneficial microbial interactions can be more or less divided into three categories. Firstly, microorganisms those, in association with plants, are accountable for its nutrition (i.e., microorganisms that augment the supply of mineral nutrients to the plant). In this case, though majority of microorganisms may not intermingle directly with the plant, their impact on soil abiotic and biotic factor undoubtedly has impacts on plant growth. Again, there are group of microbes, documented as biocontrol agents, which can stimulate plant growth and development in an indirect manner, by prevention of activities of pathogens. The third group comprising microbes, known to produce phytohormones, is responsible for direct plant growth promotion. On the other hand, it seems that neutral connections are found broadly in the rhizosphere of all crop plants. Saprophytes are accountable for different crucial soil processes, like mineralization of associated soil nutrient or turnover processes and decomposition of organic residues in soil. While such organisms neither benefit nor harm the plants straightway, absence of such microbes would undoubtedly influence plant health and productivity, and their presence is evidently essential for soil dynamics (Brimecombe et al. 2007).

Bacteria, living in the rhizosphere, exert their favor on growth of the plants globally and are referred as PGPR, i.e., plant growth-promoting rhizobacteria (Kloepper and Schroth 1978). The number of bacteria, recognized as PGPR, has been found to increase on a recent time, due to various advanced studies in bacterial taxonomy and better understanding regarding mechanisms of actions of various PGPR, covering a broader collection of plant species as well. Presently, PGPR comprise members from varied bacterial taxonomic classes (Lucy et al. 2004), and we are going to discuss a few instances in order to illustrate the mode of functioning and biodiversity of such bacterial community. A wide range of beneficial PGPR have been utilized profitably for inoculation of crop plants that include members from the genera *Azospirillum* (Cassán and García Salamone 2008), *Pseudomonas* (Loper and Gross 2007), *Bacillus* (Jacobsen et al. 2004), *Stenotrophomonas* (Ryan et al. 2009), *Serratia* (De Vleeschauwer and Höfte 2007), *Streptomyces* (Schrey and Tarkka 2008), and *Rhizobium* (Long 2001). *Rhizobium* (Long 2001) and some fungi from the genera *Trichoderma*, *Coniothyrium*, and *Ampelomyces* have also been described to be beneficial for the host plant (Harman et al. 2004). The mode of functioning of these PGPR has complex mechanisms to promote plant growth, development, and protection. Important among them are biofertilization (improving nutrient availability to plants), phytostimulation (plant growth promotion through production of phytohormones), and biocontrol (control of diseases, primarily through production of various antibiotic as well as antifungal metabolites and lytic enzymes and induction of plant defense responses). The genera *Bacillus* and *Pseudomonas* are known to be the predominant ones among PGPR groups from the rhizosphere (Morgan 2005). It is mentioned that in several instances regarding individual benevolent connections between plants and microbes, a wide range of mechanisms are actually implicated therein (Muller et al. 2009). The direct plant growth promotion functions are complicated enough to discriminate from disease control, and the comparative significance on a specific method can differ within dissimilar pathogen systems (Chet and Chernin 2002).

4.3.1.4.2 Pathogen Inhibition

Bacteria and fungi live in the region of roots and get nourished on root exudates and dead root cells. Competition amid microbial species in this region is rigid. In the fight for perseverance and establishment in the niche, bacteria use a number of strategies.

4.3.1.4.3 Antagonism

Root colonization not only results in high PGPR inhabitant densities in the root system, it also delivers antagonistic metabolites that are concerned in straight inhibition of plant pathogens (Shoda 2000; Raaijmakers et al. 2002). This includes inhibitions of growth of microbes, i.e., antibiosis, by use of diffusible antibiotics or organic volatile compounds, biosurfactants, and toxins and the mechanism of parasitism, which perhaps could involve synthesis of extracellular enzymes that can degrade cell walls, such as chitinase and β1,3-glucanase (Compant et al. 2005; Haas and Défago 2005). Degradation of the pathogenicity factors for the pathogens, like toxic substances released by favorable organisms, has also been recorded as mechanism for protection (Haas and Défago 2005). To exhibit the function of antibiotics in the process of biocontrol, the mutants impaired in the process of biosynthesis or mutants with overproducing habit have been utilized together with, in few cases, reporter genes, or probes have been used to explain efficient production of the compound in rhizosphere. For example, *Bacillus subtilis* strains were found to develop a number of strong antifungal metabolites, viz., kanosamine, zwittermicin A, and lipopeptides from fengycin, iturin, and surfactin families (Emmert and Handelsman 1999; Ongena and Thonart 2006). Excess synthesis of the extracellular protease in *Stenotrophomonas maltophilia* W81, a mutant strain, has been reported to exert improved biocontrol of *Pythium ultimum* (Dunne et al. 2000). Release of glucanase and chitinase by *Streptomyces* and *Trichoderma* species has been reported to play a pivotal role in myco-parasitism of phytopathogenic fungi (Whipps 2001).

4.3.1.4.4 Colonization

For all thriving plant–microbe connections, the capability to colonize plant habitats is vital (Lugtenberg et al. 2002; Kamilova et al. 2005). Distinct bacterial cells can affix to surfaces and, after cell division and propagation, form dense aggregates normally referred to as macro-colonies or biofilms. Steps of colonization comprise of attraction, detection, adherence, incursion (pathogenic microbes and endophytes only), followed by colonization and growth, along with some other strategies for establishment of connections. Roots start cross talk with soil microbes by generation of signals that are accepted by the microbes, which in turn produce signals that set off colonization (Berg 2009). PGPR get to surfaces of the root through active motility using flagella and are guided by chemotactic responses (Pinton et al. 2007). This proves that ability of PGPR highly depends upon their capacity to take benefit of a precise situation or on their abilities to become accustomed to varying conditions or plant species. In the majority cases, after 2–3 weeks, the population of PGPR declines progressively with time after inoculation from 107,109 cells per gram dry soil to 105,106 cells per gram dry soil (DeFlaun and Gerba 1993). However

such population threshold remains adequate to provide positive effects (Raaijmakers et al. 2002). As a result, the rhizosphere proficiency of the biocontrol agents involves successful root colonization along with the aptitude to live and proliferate by the side of growing plant roots over a long period, in presence of native microflora (Weller 1988; Lugtenberg and Dekkers 1999).

4.3.1.4.5 Competition

Competition for resources such as oxygen and nutrients occurs generally between soil-inhabiting organisms. For biocontrol, competition occurs, while antagonists compete straightway with the pathogenic microbes for various resources. Root-inhabiting microorganisms compete for appropriate sites at the surfaces of roots. Competition for nutrient elements, such as carbon, is considered to be accountable for the incidence of fungistasis, leading toward suppression of germination of fungal spore (Alabouvette et al. 2006). Given, the comparative profusion of substrates from the rhizosphere, the efficacies of uptake of nutrients, and catabolism by the bacterial community are a major factor for competitiveness (Chin-A-Woeng et al. 2003). The capacity for rapid growth when substrates are encountered is not the only factor affecting rhizosphere competence, as rhizobacteria deploy many other metabolic strategies. As, for example, the capacity for extracellular conversion of glucose to gluconic acid and 2-ketogluconic acid enables some bacteria, together with quite a few species from the genera *Pseudomonas*, in order to impound glucose successfully and gives some aggressive advantage over microbes that lack the capability to utilize these compounds (Gottschalk 1986).

Competition for tracer elements, like as iron, zinc, manganese, copper, etc., too occurs in soils. As, for instance, iron is an indispensable element for growth of all existing organisms and the lack of its bioavailable form in soil habitats results in an enraged competition (Loper and Henkels 1997). Siderophores, the compounds with lower molecular weight and higher affinity for iron, are synthesized by some of the microbes or mostly biocontrol agents in order to solubilize and obtain the ferric ions competitively under iron-restraining conditions that further render the very element unavailable to other microbes from soil that are unable to thrive without iron (Loper and Henkels 1997; Haas and Défago 2005). The microbes, having properties of siderophore production, on the contrary, can take up iron–siderophore complex by means of using a particular receptor located in the outer cell membrane. Suppression of the soilborne pathogens of various plants, by *Pseudomonas*, through siderophore production has also been reported by many authors (Loper 1988; Weger et al. 1988; Buysens et al. 1996).

4.3.1.4.6 Induced Resistance

Bacteria, associated with plants, reduce the actions of pathogens by means of microbial antagonism along with by activating the plant to better defense mechanism, a phenomenon termed “induced systemic resistance (ISR)” (Shoda 2000; Van Loon 2007).

Sometimes, the methods of induced systemic resistance, elicited by plant growth-promoting rhizobacteria, overlap to some extent to that of systemic acquired

resistance, i.e., SAR of pathogens. Both of the mechanisms stand for a condition of improved basal confrontation of the plant, which depends upon signaling compounds such as jasmonic acid, ethylene, and salicylic acid (Van Loon 2007). Natural defense response against stresses from biotic or abiotic origin such as physical stresses (heat or frost), inoculation by pathogenic or nonpathogenic organisms, and chemical molecules from natural or synthetic origins is exhibited by all plants (Alabouvette et al. 2006).

4.3.1.4.7 Plant Growth Promotion

Biofertilization

The system of escalating the performance of crop plants by PGPR is not finely comprehended yet. A number of PGPR inoculants are commercialized at present that appear to support augmentation in plant growth, through one of the following mechanisms:

1. Production of bio-stimulants or phytohormones
2. Inhibition of plant infection as bioprotectant
3. Enhancement of nutrient acquirement as biofertilizers

PGPR as biofertilizer perform both directly and indirectly by serving to make nutrient available to the host plant and influencing growth of plant root and morphology positively or by additional favorable symbiotic interactions (Vessey 2003). The major instance of such kind of relationship is fixation of nitrogen by bacteria. The symbiosis between legume host and rhizobia is one of the significant examples of plant growth-promoting rhizobacteria (PGPR). Bacteria from this cluster can metabolize root exudates that are mainly carbohydrates and supply nitrogen to the host plant in return for production of amino acids. The free-living bacteria like *Azospirillum*, *Burkholderia*, and *Stenotrophomonas* have nitrogen-fixing ability as well (Dobbelare et al. 2003). One more nutrient element that can be provided to the crop plants through oxidation by bacteria is sulfate (Banerjee and Yesmin 2002). Bacteria can also supply plant nutrition by releasing phosphorous from organic sources like phytates and hence help in plant growth promotion indirectly (Unno et al. 2005). Use of *Azospirillum* resulted in augmentation of root growth and activities that increase uptake of phosphorous along with other macro- and microelements (Dobbelaere and Okon 2007). *Pseudomonas fluorescens* CHA0 has capability of acidification of its surroundings and solubilization of mineral phosphate, which strongly depends on its aptitude of gluconic acid production (De Werra et al. 2009).

4.3.1.4.8 Phytostimulation

Phytostimulation enhances plant growth in a direct way. Phytohormones [e.g., production of indole-3-acetic acid (IAA), auxins, cytokinins, and gibberellins] play an important role in processes of plant growth. Such phytohormones can be produced by the plants themselves as well as by their allied microbes, as, for example, *Azospirillum* spp., in addition to its capacity of fixing the atmospheric nitrogen

(Steenhoudt and Vanderleyden 2000). Species from the genera *Bacillus* and *Pseudomonas* can synthesize the plant growth regulators or phytohormones that help crops in having greater amount of fine roots which have the effect of increasing the absorptive surface of plant roots for uptake of water and nutrients. They can produce phytohormones like gibberellins, cytokinins, indoleacetic acid, and ethylene production inhibitors. Indole-3-acetic acid is a phytohormone that is involved in cell division, root initiation, as well as enlargement of plant cells (Salisbury 1994). Auxins are most plentiful phytohormones quantitatively, which are exuded by *Azospirillum* spp., and their synthesis, more willingly than fixation of nitrogen, is the prime factor that is accountable for the encouragement of profuse rooting of plants and, thereby, enhanced plant growth (Bloemberg and Lugtenberg 2001). Furthermore, plant-associated bacteria can influence the hormonal balance of the plant. Ethylene is the significant instance to illustrate the fact that the stability is most imperative for the result of hormones: at lower level, it can endorse growth of plant in quite a few species together with *Arabidopsis thaliana*, whereas it is generally considered as an inhibitor toward plant development and known as a senescence hormone (Pierik et al. 2006). The general effect on the plant can be direct, that is, through plant growth promotion, or indirect, that is, through improving plant nutrition via the better development of the roots, and it is hard to differentiate between them. The increase in root IAA level for plantlets of lodgepole pine, infected with *Paenibacillus polymyxa*, as well as root concentration of dihydroxyzeatin riboside in case of plants inoculated using *Pseudomonas fluorescens* (Fuentes-Ramirez and Caballero-Mellado 2005), may be accredited to the orientation of plant hormone synthesis by the bacterial species. However, the uptake of bacterial synthesized phytohormones cannot be excluded, since both *P. polymyxa* and *Pseudomonas* produce IAA and cytokinins in vitro (Fuentes-Ramirez and Caballero-Mellado 2005).

4.3.1.4.9 Pathogenic Functions

Root exudates can attract both favorable and pathogenic populations (Schroth and Hildebrand 1964) that may be virulent for a few hosts. Many pathogens, fungi and bacteria, have evolved and exhibited a higher level of host specificity (Raaijmakers et al. 2009). Plants are also not out of defense. In fact, it is found that approximately 2% of the identified fungal species are capable of colonization in plants and thereby can cause infection in plant body (Buchanan et al. 2000). Though plants remain in constant contact with virulent fungal, bacterial, or viral pathogens, successful contamination is hardly recognized. This is because a common confrontation in opposition to most of such pathogens, named as "nonhost resistance" or "horizontal resistance," is found in plant bodies (Heath 1981). This reinforces the concept that the plants are not always fit targets for infection by a definite group of pathogens owing to reflexive opposition mechanisms ensuing "basic incompatibility." Such resistance mechanisms consist of configurational barriers and poisonous chemicals that are there in the strong plants, bound triumphant infection to specific pathogens, which have the abilities to conquer these factors and thus reveal "basic compatibility." However, even if contact is recognized with the plant, pathogenic microbes are frequently confronted with toxic compounds named phytoanticipins (van Etten

et al. 1994). This phrase consists of a range of components fashioned by various biosynthetic pathways that obtain antimicrobial characteristics. Such resultant metabolites of low molecular mass are primarily stored in inert forms in the organelles or vacuoles and are exuded upon demolition of the cells. While destruction of the integrity of the host plant tissue is a component of the colonization mechanism by fungal bodies, phytoanticipins symbolize a significant confrontation strategy in opposition to such pathogens. Though, in some cases, pathogenic bodies conquer the preformed hindrances from host plants and may expand virulent contamination leading toward ailment in plant bodies. Plant diseases participate directly in the eradication of ordinary possessions from agriculture. Particularly, soilborne pathogens impart more losses, as fungi remain most hostile from soil. Their detrimental effects range from placid symptoms to catastrophes where entire fields with agricultural produce can be ruined. Consequently, they become persistent and foremost threats toward stability of ecosystem and food production function worldwide. Most common bacterial agents comprise of Gram-positive bacterium *Streptomyces scabies* and the Gram-negative bacteria *Ralstonia* spp., *Erwinia carotovora*, and *Pseudomonas*. The oomycetes and fungal phytopathogens include members from the genus *Rhizoctonia*, *Rhizopus*, *Fusarium*, *Pythium*, *Phytophthora*, and *Verticillium* (Tournas and Katsoudas 2005). Among the woodland pathogens, the significant ones are the filamentous fungi like *Phytophthora* spp. (Rizzo et al. 2005) and *Armillariella* and *Heterobasidion* (Asiegbu and Nahalkova 2005).

4.3.2 Phyllosphere: Plant Community with Microbiome

A second component of plant–microbiome interaction consists of microbes colonizing the aboveground area or exterior of plant tissues, i.e., the phyllosphere. The phyllosphere is a massive ecology that is likely to attain an area of 6.4 × 108 km^2 and is heavily colonized by microbes (Morris and Kinkel 2002). The terminology is generally used to describe the surface of the leaf (Vorholt 2012) though it is applicable to any aerial plant tissue.

The microbial communities from the phyllosphere have indispensable roles in plant growth and development. Protecting plant community from invading pathogens, fixation of atmospheric nitrogen, biosynthesis of phytohormones (Jones 1970; Freiberg 1998; Brandl et al. 2001; Kishore et al. 2005), carbon sequestration (Bulgarelli et al. 2013), etc., are some of such functions that are essential for sustainable agricultural practices.

Lindow and Brandl (2003) reported that community of phyllosphere is mainly comprised of bacteria, algae, fungi, and nematodes or protozoa in a few instances. Bacteria are the most plentiful community among these microbes that are found between 105 and 107 cells per cm^2 (Beattie and Lindow 1995; Andrews and Harris 2000) in phyllosphere. These communities are sometimes to be found far away from the rhizosphere, prime resource of plant-associated microorganisms, and are found to exhibit higher rates of colonization, mostly promoted by the movement of airstream as well as vectors (Bulgarelli et al. 2013). Organisms from the phyllosphere

can flourish and survive even under oligotrophic ecological surroundings with ultraviolet radiation, restricted nutrient accessibility, and varied pH, temperature, and moisture conditions (Andrews and Harris 2000). Air along with aerosols, earth, and moisture are the prime sources that frame the communities from the phyllosphere (Bulgarelli et al. 2013). The interaction among different ecological factors can amend the microbial communities from phyllosphere. Genomic structure of plants is one of the key drivers, determining the composition of bacterial communities in the phyllosphere in temperate (Redford et al. 2010) and tropical forests (Lambais et al. 2006). Diverse plant communities anchor different microbes, owing to the creation of precise niche and confined circumstances that are governed by the inherited and the efficient metabolic activities of the plants (Redford et al. 2010). The uniqueness of the phyllosphere was reported in plants of beans, lettuce, cucumber, maize, and grasses with alteration in the profusion and constitution of bacterial community (O'Brien and Lindow 1989; Kinkel et al. 2000; Rastogi et al. 2012). Geological remoteness is also another significant player in configuring microbial communities in the phyllosphere (Bokulich et al. 2014). The diverse bacterial communities anchored by grapevines manipulate superiority of the produced wine. In a more comprehensive outlook, the intraspecific alteration in the composition of the microbial community in the phyllosphere can be noticed, primarily governed by the heterogeneous nutritional condition, found in leaf surfaces, where the heterogeneous carbon sources like glucose, fructose, and sucrose lead to precise microbial colonization on the leaf veins, the regions close to the exterior appendages and stomata (Lindow and Brandl 2003; Vorholt 2012). According to Davey and O'Toole (2000) and Lindow and Brandl (2003), such heterogeneity in few instances is endorsed by the microbial association in biofilms that are general characteristic of organisms from the phyllosphere, functioning as the defender and aggregator of bacterial cells under the regular uncongenial circumstances. Regardless of such instances, it is likely to detect a "core" for the microbial population from the phyllosphere that colonize host plants, from the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Redford et al. 2010; Vorholt 2012). These phyla consist of the most plentiful and well-examined microbes that signify the fact that additional researches regarding this issue should be planned to assess taxonomic ranks beyond phylum. Consequently, this core is assumed to be made of microbes depicting a coevolving history with the plant communities, along with the host structure that is complementary to the specifications found inside the bacterial cells. Such microbial reserve can be utilized for the benevolence of farming practices that endorse the movement and odd or synergistic associations, which may kindle plant growth and/or defense in opposition to attack of pathogenic communities.

4.3.3 Endosphere: A Forte Meant for Close Friends

The existence of microbial cells in plant inner tissues was explained long ago as the same as plant infection. At that time, the microbes within plant tissues were individuals that are able to contaminate the host plants, leading toward difficulties in

growth and development of plants as well as losses in yield. Perhaps, such association was caused due to the accessibility of methods for detection of microbial connections that time that were merely proficient in making out microbes that are easy to cultivate or found in large quantities. The occurrence of nonpathogenic microbes within plant tissues was explained by De Bary (1866) for the first time, who revealed that microbes are present in microscopically examined plant tissues. Such examination remained unknown until the endophytes were defined. The endophytes are generally defined based on the capability to perceive the microbial cells from plant tissues that have been surface sterilized formerly (Hallmann et al. 1997). Petrini (1991) has given a functional description for endophytes as "organisms that at some part of their life cycle colonize internal plant tissues without causing apparent harm to the host." On a more exhaustive examination, endophytes are divided in the subgroups "obligate" and "facultative" by the researchers. Endophytes, which depend on metabolism of host plants for their endurance, and are transmitted among plants through the activity of vectors or by vertical transmission, are classified as obligate ones (Hardoim et al. 2008). Endophytes, those living on the outer surface of the host plants at some point of their life cycle, are known as facultative ones. They are recruited by the host plants from neighboring communities from the soil mass, mainly from the rhizosphere. Endophytes are there in every plant inner tissues (Rosenblueth and Martinez-Romero 2006). The existence of endophytes in plants cultured in vitro has been explained, where such organisms seem to be closely connected with host plants not in by means of colonizing the culture media but preferably living inside the tissues of the plant (Almeida et al. 2009; Abreu-Tarazzi et al. 2010). Mendes et al. (2007) showed that the endophytic *Burkholderia* spp. have the capacity to regulate the growth of the pathogenic *Fusarium moniliforme*. Ferrara et al. (2012) showed that the endophytic diazotrophs from roots of sugarcane are capable of producing substances related to plant growth-promoting functions and can exude greater amount of amino acids that could aid in plant nutrition. Araújo et al. (2002) showed that the whole endophytic community is influenced by the occurrence of the pathogen and incidence of disease like variegated chlorosis in citrus is a consequence of the dealings between the endophytic community and pathogenic *X. fastidiosa* and not with the host only. The capability of genetically customized endophytes that generate the heterologous protein cry1Ac7 can control *Diatraea saccharalis*, a sugarcane pest (Quecine et al. 2014). Though numerous individual abilities have been explained for endophytes, such organisms, as a community, are competent enough for several other functions that cannot be detected from separate case studies on the microbes.

Numerous studies were made to find the origin of endophytic organisms (Hallmann et al. 1997; Saikkonen et al. 1998; Mitter et al. 2013). The origin of microbes, residing in rhizosphere or the seed-borne ones, is firmly associated to the strategy of preservation of the same within the host plants that confirms the diffusion of endophytic microbes between plants. The evidence of the mechanism of transmission as well as survival of specific endophytes and their interaction with plant bodies are indicated through their genomic organization. Dini-Andreote et al. (2012) studied over sizes and origins of numerous endophytic genomes. The

scientists related the lifestyle of microbes to the genome size for detection of the deviation in ecological conditions as one of the key drivers of expansion or shrinking of genome. Endosymbionts usually possess more compacted genomes, while bacteria from niches of variable ecological conditions such as rhizosphere need to harbor the complete cache of genes to survive under diverse environmental situations, leading toward dominance of larger genomes. Apparently, endophytes appear to fit in the former portion of the theory since they exist within plant bodies, where the surroundings are more secure in comparison with the rhizosphere. Nevertheless, taking into account the origin and transmissions of endophytes, it is said that probably few endophytes must deal with distinct environments during their course of life cycle when they remain outside host plants. Mitter et al. (2013) showed a greater deviation in the genome size of bacterial endophytes, which suggests that the community of endophytes consist of microbes from various origin. Those with bigger genomes are likely to live in varied environments like soil or rhizosphere, and the ones with smaller genomes are to be transmitted vertically within stable surroundings.

4.4 Conclusion

If microbiome–plant interactions are understood and described in a more improved and detailed manner, such data could be accessible for the invention of newer technologies, concentrating on a superior investigation of the characteristic in agricultural strata, influenced by microbes. Alteration in the configuration of microbial population, for example, by injection of distinct exogenous microbes or by means of influencing ecological circumstances toward benevolence of specific sets of microbiomes, heading toward improved plant opposition or effectiveness in the nutrient uptake could be a reality. In this manner, the progress of "microbiome-driven cropping systems" may effect in the subsequent uprising in agricultural field, offering a further sustained structure for plant production.

References

Abreu-Tarazi MF, Navarrete AA, Andreote FD, Almeida CV, Tsai SM, Almeida M (2010) Endophytic bacteria in long-term *in vitro* cultivated axenic pineapple microplants revealed by PCR DGGE. World J Microbiol Biotechnol 26:555–560

Ahmad I, Ahmad F, Pichtel H (eds) (2011) Microbes and microbial technology: agricultural and environmental applications doi 10.1007/978-1-4419-7931-5_1

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the European situation. Eur J Plant Pathol 114:329–341

Almeida CV, Andreote FD, Yara R, Ossamu FA, Azevedo JL, Almeida M (2009) Bacteriossomes in axenic plants: endohytes as stable symbionts. World J Microbiol Biotechnol 25:1757–1764

Andreote FD, Gumiere T, Durrer A (2014) Exploring interactions of plant microbiomes. Sci Agric 71(6):528–539

Andrews JH, Harris RF (2000) The ecology and biogeography of microorganisms on plant surfaces. Annu Rev Phytopathol 38:145–180

Araújo WL, Marcon J, Maccheroni W, van Elsas JD, van Vuurde JWL, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914

Argueso C, Hansen M, Kieber J (2007) Regulation of ethylene biosynthesis. J Plant Growth Regul 26(2):92–105. doi:10.1007/s00344-007-0013-5

Arshad M, Shaharoona B, Mahmood T (2008) Inoculation with *Pseudomonas* spp containing ACC-deaminase partially eliminates the effects of drought stress on growth yield and ripening of pea (*Pisum sativum* L). Pedosphere 18(5):611–620. doi:10.1016/S1002-0160(08)60055-7

Asiegbu FO, Nahalkova JLG (2005) Pathogen-inducible cDNAs from the interaction of the root rot fungus *Heterobasidion annosum* with scots pine (*Pinus sylvestris* L). Plant Sci 168:365372

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32(6):666–681. doi:10.1111/j1365-3040200901926x. PMID: 19143988

Badri DV, Loyola-Vargas VM, Broeckling CD, De-la-Pena C, Jasinski M, Santelia D, Martinoia E, Sumner LW, Banta LM, Stermitz F, Vivanco JM (2008) Altered profile of secondary metabolites in the root exudates of Arabidopsis ATP-binding cassette transporter mutants. Plant Physiol 146(2):762–771. doi:10.1104/pp107109587 PMID:18065561

Badri DV, Quintana N, El Kassis EG, Kim HK, Choi YH, Sugiyama A, Verpoorte R, Martinoia E, Manter DK, Vivanco JM (2009) An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiol 151(4):2006–2017. doi:10.1104/pp109147462. PMID:19854857

Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013a) Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J Biol Chem 288(7):4502–4512. doi:10.1074/jbcM112433300. PMID:23293028

Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM (2013b) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytol 198(1):264–273. doi:10.1111/nph12124. PMID: 23347044

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57(1):233–266. doi:10.1146/annurevarplant57032905105159. PMID:16669762

Banerjee M, Yesmin L (2002) Sulfur-oxidizing plant growth promoting *Rhizobacteria* for enhanced canola performance US Patent 20080070784

Barriuso J, Ramos-Solabo B, Guierrez M (2008a) Protection against pathogen and salt stress by four plant growth promoting rhizobacteria isolated from *Pinus* sp on *Arabidopsis thaliana*. Biol Control 98(6):666–672

Barriuso J, Solano BR, Lucas JA, Lobo AP, Villaraco AG, Mañero FJG (2008b) In: Ahmad I, Pichtel J, Hayat S (eds) Ecology genetic diversity and screening strategies of Plant Growth Promoting Rhizobacteria (PGPR). WILEY-VCH Verlag GmbH, Co KGaA, Weinheim, pp 1–17

Beattie GA, Lindow SE (1995) The secret life of foliar bacterial pathogens on leaves. Annu Rev Phytopathol 33:145–172

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Bokulich NA, Thorngate JH, Richardson PM, Mills DA (2014) Microbial biogeography of wine grapes is conditioned by cultivar vintage and climate. Proc Natl Acad Sci USA 111:E139–E148

Brandl MT, Quinones B, Lindow SE (2001) Heterogeneous transcription of an indoleacetic acid biosynthetic gene in *Erwinia herbicola* on plant surfaces. Proc Natl Acad Sci USA 98:3454–3459

Brimecombe MJ, De Leij FAAM, Lynch JM (2007) Rhizodeposition and microbial populations. In: Pinton R, Veranini Z, Nannipieri P (eds) The rhizosphere biochemistry and organic substances at the soil-plant *interface*. Taylor, Francis Group, New York

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74(3):738–744. doi:10.1128/AEM02188-07

Buchanan R, Gruissem W, Jones RL (2000) Biochemistry and molecular biology of plants. American Society of Plant Biologists, Rockville

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64(1):807–838

Buysens S, Heungens K, Poppe J, Höfte M (1996) Involvement of pyochelin and pyoverdin in suppression of *Pythium* induced damping off of tomato by Pseudomonas aeruginosa 7NSK2. Appl Environ Microbiol 62:865–871

Cassán F, García Salamone I (2008) Azospirillum sp: cell physiology plant response agronomic and environmental research in Argentina. Asociacion Argentina de Microbiologia, Buenos Aires

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One 8(2):e55731. doi:10.1371/journalpone 0055731. PMID:23383346

Chet I, Chernin L (2002) Biocontrol microbial agents in soil. In: Bitton G (ed) Encyclopedia of environmental microbiology. Willey, New York, pp 450–465

Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJ (2003) Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol 157:503–523

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Cook JH, Beyea J, Keeler KH (1991) Potential impacts of biomass production in the United States on biological diversity. Annu Rev Energ Environ 16:401–431

Coronado C, Zuanazzi J, Sallaud C, Quirion JC, Esnault R, Husson HP, Kondorosi A, Ratet P (1995) Alfalfa root flavonoid production is nitrogen regulated. Plant Physiol 108(2):533–542. doi:10.1104/pp1082533 PMID:12228491

Crowley TJ (2000) Causes of climate change over the past 1000 years. Science 289:270–277

Davey ME, O'Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867

Davidson IA, Robson MJ (1986) Effect of contrasting patterns of nitrate application on the nitrate uptake N2-fixation nodulation and growth of white clover. Ann Bot 57(3):331–338

De Bary A (1866) Morphologie und Physiologie Pilze Flechten und Myxomyceten Hofmeister's handbook of physiological botany Engelmann Leipzig Germany

De Vleeschauwer D, Höfte M (2007) Using *Serratia plymuthica* to control fungal pathogens of plants. CAB Rev 2:4–6

De Werra P, Péchy T, Keel C, Maurhofer M (2009) Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas fluorescens* CHA0. Appl Environ Microbiol 75:4162–4174

DeFlaun MF, Gerba CP (1993) Monitoring recombinant DNA microorganisms and viruses in soil. In: Metting FBJ (ed) *Soil microbial* ecology: application in agricultural and environmental management. Marcel Dekker, Washington, DC, pp 131–150

Diener AC, Gaxiola RA, Fink GR (2001) Arabidopsis *ALF5* a multidrug efflux transporter gene family member confers resistance to toxins. Plant Cell. Online 13(7):1625–1638. doi:10.1105/tpc1371625

Dini-Andreote F, van Elsas JD (2013) Back to the basics: the need for ecophysiological insights to enhance our understanding of microbial behaviour in the rhizosphere. Plant Soil 373:1–15

Dini-Andreote F, Andreote FD, Araújo WL, Trevors JT, van Elsas JD (2012) Bacterial genomes: habitat specificity and uncharted organisms. Microbial Ecol 64:1–7

Dobbelaere S, Okon Y (2007) The plant growth promoting effects and plant responses In: Elmerich C, Newton WE (eds) Associative and endophytic nitrogen fixing bacteria and cyanobacterial associations (Nitrogen fixation: origins applications and research progress) Heidelberg. Springer, Verlag

Dobbelare S, Vanderleyden J, Okon Y (2003) Plant growth promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149
Doornbos R, Loon L, Bakker PHM (2012) Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere A review. Agron Sust Dev 32(1):227–243. doi:10.1007/s13593–011-0028-y
Dunne C, Moenne Loccoz Y, de Bruijn FJ, O'Gara F (2000) Overproduction of an inducible extracellular serine protease improves biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* strain W81. Microbiology 146:2069–2078
Emmert EAB, Handelsman J (1999) Biocontrol of plant disease: a (gram) positive perspective. FEMS Microbiol Lett 171:1–9
Ferrara FIS, Oliveira ZM, Gonzales HHS, Floh EIS, Barbosa HR (2012) Endophytic and rhizospheric enterobacteria isolated from sugar cane have different potentials for producing plant growth-promoting substances. Plant Soil 353:409–417
Franks A, Ryan RP, Abbas A, Mark GL, O'Gara F (2006) Molecular tools for studying plant growth promoting rhizobacteria. In: Cooper JE, Rao JR (eds) Molecular approaches to soil rhizosphere and plant microorganisms analysis. Biddes Ltd Kings, Lynn, pp 116–131
Fransson PMA, Johansson EM (2010) Elevated CO2 and nitrogen influence exudation of soluble organic compounds by ectomycorrhizal root systems. FEMS Microbiol Ecol 71(2):186–196. doi:10.1111/j1574-6941200900795x. PMID:19889031
Freiberg E (1998) Microclimatic parameters influencing nitrogen fixation in the phyllosphere in a Costa Rican premontane rain forest. Oecologia 117:9–18
Fuentes-Ramirez LE, Caballero-Mellado J (2005) Bacterial biofertilizers. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 143–172
Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y, Sato K, Katsuhara M, Takeda K, Ma JF (2007) An aluminum-activated citrate transporter in barley. Plant Cell Physiol 48(8):1081–1091. doi:10.1093/pcp/pcm091. PMID: 17634181
Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251(1):1–7. doi:10.1016/jfemsle2005 07030. PMID:16099604
Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39
Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. In: Bakker PAHM, Raaijmakers JM, Bloemberg G, Hofte M, Lemanceau P, Cooke BM (eds) New perspectives and approaches in plant growth-promoting rhizobacteria research. Springer, Dordrecht, pp 329–339
Gottschalk G (1986) Bacterial metabolism. Springer, Berlin/Heidelberg/New York
Griffin GJ, Hale MG, Shay FJ (1976) Nature and quantity of sloughed organic matter produced by roots of axenic peanut plants. Soil Biol Biochem 8:29–32
Haas D, Défago G (2005) Biological control of soilborne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319
Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914
Hardoim P, van Overbeek L, van Elsas J (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471
Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) Trichoderma species – opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56
Hartmann A, Rothballer M, Schmid M (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil 312(1-2):7–14
Heath MC (1981) A generalized concept of host-parasite specificity. Phytopathology 7:1121–1123
Hiltner L (1904) Uber neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brachte. Arbeiten der Deutschen Landwirtschaftlichen Gesellschaft 98:59–78
Hirsch PR, Mauchline TH (2012) Who's who in the plant root microbiome? Nat Biotechnol 30:961–962
Ishimaru Y, Kakei Y, Shimo H, Bashir K, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK (2011) A rice phenolic efflux transporter is essential for solubilizing precipitated apoplas-

mic iron in the plant stele. J Biol Chem 286(28):24649–24655. doi:10.1074/jbcM111221168. PMID: 21602276

Jackson T (1999) Renewable energy Summary paper for the renewables series. Energy Policy 20:861–883

Jacobsen BJ, Zidack NK, Larson BJ (2004) The role of *Bacillus*-based biological control agents in integrated pest management systems: plant diseases. Phytopathology 94:1272–1275

Jones K (1970) Nitrogen fixation in phyllosphere of Douglas Fir *Pseudotsuga-Douglasii*. Ann Bot 34:239–244

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163(3):459–480. doi:10.1111/j1469–81372004 01130x

Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7:1809–1817

Kang SM, Khan AL, Waqs M, You YH, Kim JH, Kim GK, Hamayun M, Lee IJ (2014) Plant growth promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. J Plant Interact 9:673–682

Kent AD, Triplett EW (2002) Microbial communities and their interactions in soil and rhizosphere ecosystems. Ann Rev Microbiol 56:211–236

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bucking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333(6044):880–882. doi:10.1126/science1208473. PMID:21836016

Kinkel LL, Wilson M, Lindow SE (2000) Plant species and plant incubation conditions influence variability in epiphytic bacterial population size. Microb Ecol 39:1–11

Kishore GK, Pande S, Podile AR (2005) Biological control of late leaf spot of peanut (*Arachis hypogaea*) with chitinolytic bacteria. Phytopathology 95:1157–1165

Kloepper JW, Schroth MN (1978) Plant growth promoting rhizobacteria on radishes In: Proceedings of the IVth international conference on plant pathogenic bacteria Argers France:Station de Pathologie Vegetale et Phytobacteriologyie INRA, pp 879–882

Lambais MR, Crowley DE, Cury JC, Bull RC, Rodrigues RR (2006) Bacterial diversity in tree canopies of the Atlantic forest. Science 312:1917–1917

Leyval C, Berthelin J (1993) Rhizodeposition and net release of soluble organic compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. Biol Fertil Soils 15(4):259–267. doi:10.1007/ bf00337210

Li L, He Z, Pandey GK, Tsuchiya T, Luan S (2002) Functional cloning and characterization of a plant efflux carrier for multidrug and heavy metal detoxification. J Biol Chem 277(7):5360–5368. doi:10.1074/jbcM108777200. PMID:11739388

Lindow SE, Brandl MT (2003) Microbiology of the phyllophere. Appl Environ Microbiol 69:1875–1883

Liu J, Magalhaes JV, Shaff J, Kochian LV (2009) Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. Plant J 57(3):389–399. doi:10.1111/j1365-313X200803696x. PMID:18826429

Long SR (2001) Genes and signals in the *Rhizobium*-legume symbiosis. Plant Physiol 125:69–72

Loper JE (1988) Role of fluorescent siderophore production in biological control of *Pythium ultirnum* by a *Pseudomonas fluorescens* strain. Phytopathology 78:166–172

Loper JE, Gross H (2007) Genomic analysis of antifungal metabolite production by *Pseudomonas fluorescens* Pf5. Eur J Plant Pathol 119:265–278

Loper JE, Henkels MD (1997) Availability of iron to *Pseudomonas fluorescens* in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. Appl Environ Microbiol 63:99–105

Loyola-Vargas V, Broeckling C, Badri D, Vivanco J (2007) Effect of transporters on the secretion of phytochemicals by the roots of *Arabidopsis thaliana*. Planta 225(2):301–310. doi:10.1007/s00425–006–0349-2. PMID:16868775

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86(1):1–25

Lugtenberg BJJ, Dekkers LC (1999) What makes *Pseudomonas* bacteria rhizosphere competent. Environ Microbiol 1:9–13

Lugtenberg BJJ, Chin A, TFC W, Bloemberg GV (2002) Microbe plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek 81:373–383

Magalhaes JV, Liu J, Guimaraes CT, Lana UGP, Alves VMC, Wang Y-H, Schaffert RE, Hoekenga OA, Pineros MA, Shaff JE, Klein PE, Carneiro NP, Coelho CM, Trick HN, Kochian LV (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat Genet 39(9):1156–1161. doi:10.1038/ng2074. PMID: 17721535

Matilla MA, Ramos JL, Bakker PAHM, Doornbos R, Badri DV, Vivanco JM, Ramos-Gonzalez MI (2010a) *Pseudomonas putida* KT2440 causes induced systemic resistance and changes in Arabidopsis root exudation. Environ Microbiol 2(3):381–382

Matilla MA, Ramos JL, Bakker PAHM, Doornbos R, Badri DV, Vivanco JM, Ramos-Gonzalez MI (2010b) *Pseudomonas putida* KT2440 causes induced systemic resistance and changes in Arabidopsis root exudation. Environ Microbiol Rep 2(3):381–388. doi:10.1111/j1758–22292009 00091x. PMID:23766110

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42(6):565–572. doi:10.1016/jplaphy200405009. PMID:15246071

Meier IC, Avis PG, Phillips RP (2013) Fungal communities influence root exudation rates in pine seedlings. FEMS Microbiol Ecol 83(3):585–595. doi:10.1111/1574–694112016. PMID:23013386

Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JM (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of Burkholderia cepacia complex isolates. Appl Environ Microbiol 73(22):7259–7267

Mercado-Blanco J, Bakker P (2007) Interactions between plants and beneficial *Pseudomonas* spp: exploiting bacterial traits for crop protection. Antonie van Leeuwenhoek 92(4):367–389. doi:10.1007/s10482–007–9167-1

Micallef SA, Channer S, Shiaris MP, Colon-Carmona A (2009a) Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. Plant Signal Behav 4(8):777–780. doi:10.4161/psb489229. PMID:19820328

Micallef SA, Shiaris MP, Colon-Carmona A (2009b) Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. J Exp Bot 60(6):1729–1742. doi:10.1093/jxb/erp053. PMID:19342429

Mitter B, Petric A, Shin MW, Chain PSG, Hauberg-Lotte L, Reinhold-Hurek B, Nowak J, Sessitsch A (2013) Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. Front Plant Sci 4:120

Morgan JAW (2005) Biological costs and benefits to plant-microbe interactions in the rhizosphere. J Exp Bot 56(417):1729–1739

Morris CE, Kinkel LL (2002) Fifty years of phyllosphere microbiology: significant contributions to research in related fields. In: Lindow SE, Hecht-Poinar EI, Elliott V (eds) Phyllosphere microbiology. APS Press, St Paul, pp 365–375

Müller H, Westendorf C, Leitner E, Chernin L, Riedel K, Schmidt S, Eberl L, Berg G (2009) Quorumsensing effects in the antagonistic rhizosphere bacterium *Serratia plymuthica* HROC48 FEMS. Microbiol Ecol 67:468–478. doi:10.1111/j.1574-6941.2008.00635.x. PMID: 19220861

Narasimhan K, Basheer C, Bajic VB, Swarup S (2003) Enhancement of plant–microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls. Plant Physiol 132(1):146–153. doi:10.1104/pp102016295. PMID:12746520

Nelson E (1990) Exudate molecules initiating fungal responses to seeds and roots. Plant Soil 129:61–73

Newton AC, BDL F, Atkins SD, Walters DR, Daniell TJ (2010) Pathogenesis parasitism and mutualism in the trophic space of microbe–plant interactions. Trends Microbiol 18(8):365–373. doi:10.1016/jtim201006002. PMID:20598545

Nguyen C (2003) Rhizodeposition of organic C by plants:mechanisms and controls. Agronomie 23:375–396
O'Brien RD, Lindow SE (1989) Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. Phytopathology 79:619–627
Odum EP (1971) Fundamentals of ecology. Saunders, Philadelphia
Olubukola O, Babalola O, Glick BR (2012) The use of microbial inoculants in African agriculture. Food Agric Environ 10:540–549
Ongena M, Thonart P (2006) Resistance induced in plants by nonpathogenic microorganisms: elicitation and defense responses. In: Teixeira da Silva JA (ed) Floriculture ornamental and plant biotechnology: advances and topical issues. Global Science Books, London, pp 447–463
Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase containing plant growth-promoting rhizobacteria. Physiol Plant 118:10–15.
Petrini O (1991) Fungal endophytes of tree leaves. In: Fokkema NJ, van den Heuvel (eds) Microbial ecology of the leaves. Cambridge University Press, Cambridge, pp 185–187
Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799
Pierik R, Tholen D, Poorter H, Visser EJW, Laurentius ACJ, Voesenek (2006) The Janus face of ethylene: growth inhibition and stimulation. Trends Plant Sci 11:176–183
Pinton R, Veranini Z, Nannipieri P (2007) The rhizosphere biochemistry and organic substances at the soil plant *interface*. Taylor, Francis Group LLC, New York
Quecine MC, Araujo WL, Tsui S, Parra JRP, Azevedo JL, Pizzirani-Kleiner AA (2014) Control of *Diatraea saccharalis* by the endophytic *Pantoea agglomerans* 331 expressing cry1Ac7. Arch Microbiol 196:227–234
Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81:537–547
Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, van Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341–361
Rastogi G, Sbodio A, Tech JJ, Suslow TV, Coaker GL, Leveau JHJ (2012) Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. ISME J 6:1812–1822
Reddy VS, Shlykov MA, Castillo R, Sun EI, Saier MH (2012) The major facilitator superfamily (MFS) revisited. FEBS J 279(11):2022–2035. doi:10.1111/ j1742-4658201208588x. PMID:22458847
Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N (2010) The ecology of the phyllosphere: geographic and phyllogenetic variability in the distribution of bacteria on tree leaves. Environ Microbiol 12:2885–2893
Rizzo DM, Garbelotto M, Hansen EA (2005) *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. Annu Rev Phytopathol 43:309–335
Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts molecular plant-microbe interactions 19:827–837
Rosling A, Lindahl BD, AFS T, Finlay RD (2004) Mycelial growth and substrate acidification of ectomycorrhizal fungi in response to different minerals. FEMS Microbiol Ecol 47(1):31–37. doi:10.1016/S0168–6496(03)00222–8. PMID:19712344
Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, van der Lelie D, Dow JM (2009) Versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat Microbiol Rev 7:514–525
Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Ann Rev Ecol Syst 29:319–343
Salisbury FB (1994) The role of plant hormones. In: Wilkinson RE (ed) Plant-environment interaction. Dekker, New York, pp 39–81
Schrey SD, Tarkka MT (2008) Friends and foes:streptomycetes as modulators of plant disease and symbiosis. Antonie Van Leeuwenhoek 94:11–19

Schroth MN, Hildebrand DC (1964) Influence of plant exudates on root infecting fungi. Annu Rev Phytopathol 2(10):11–32
Sengupta A, Gunri S (2015) Microbial intervention in agriculture: an overview. Afr J Microbiol Res 9(18):1215–1226
Shoda M (2000) Bacterial control of plant diseases. J Biosci Bioeng 89:515–521
Sivakumar PV, Thamizhiniyan P (2012) Enhancement in growth and yield of tomato by using AM fungi and Azospirillum. Int J Environ Biol 2(3):137–141
Stearns JC, Woody OZ, McConkey BJ, Glick BR (2012) Effects of bacterial ACC deaminase on *Brassica napus* gene expression. Mol Plant Microb Interact 25(5):668–676. doi:10.1094/MPMI-08-11-0213. PMID:22352713
Steenhoudt O, Vanderleyden J (2000) *Azospirillum* a free-living nitrogen-fixing bacterium closely associated with grasses: genetic biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506
Steinkellner S, Lendzemo V, Langer I, Schweiger P, Khaosaad T, Toussaint JP, Vierheilig H (2007) Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant–fungus interactions. Molecules 12(7):1290–1306. doi:10.3390/12071290. PMID:17909485
Sugiyama A, Shitan N, Yazaki K (2008) Signaling from soybean roots to *Rhizobium*:an ATP-binding cassette-type transporter mediates genistein secretion. Plant Signal Behav 3(1):38–40. doi:10.4161/psb314819. PMID:19704765
Tournas VH, Katsoudas E (2005) Mould and yeast flora in fresh berries grapes and citrus fruits. Int J Food Microbiol 105:1117
Unno Y, Okubo K, Wasaki J, Shinano T, Osaki M (2005) Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of lupin analysed by phytate utilization ability. Environ Microbiol 7:396–404
Uren NC (2000) Types amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil–plant interface. Marcel Dekker, New York, pp 19–40
van Etten HD, Mansfield JW, Bailey JA, Farmer EE (1994) Two classes of plant antibiotics: phytoalexins *versus* phytoanticipins. Plant Cell 6:1191–1192
Van Loon LC (2007) Plant responses to plant growth promoting bacteria. Eur J Plant Pathol 119:243–254
van Veen JA, van Overbeek LS, van Elisas JD (1997) Fate and activity of microorganisms introduced into soil. Microbiol Mol Biol Rev 61:121–135
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev 10:828–840
Weger LA, van Arendonk JJCM, Recourt K, van der Hofstad GAJM, Weisbeek PJ, Lugtenberg B (1988) Siderophore mediated uptake of Fe3+ by the plant growth stimulating *Pseudomonas putida* strain WCS358 and by other rhizosphere microorganisms. J Bacteriol 170:4693–4698
Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:379–407
Weston LA, Ryan PR, Watt M (2012) Mechanisms for cellular transport and release of allelochemicals from plant roots into the rhizosphere. J Exp Bot 63(9):3445–3454
Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511
Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4. doi:10.1016/jtplants2008 10004. PMID:19056309
Yazaki K (2005) Transporters of secondary metabolites. Curr Opin Plant Biol 8(3):301–307. doi:10.1016/jpbi200503011. PMID: 15860427
Zahran HH (1999) Rhizobium–legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63(4):968–989. PMID: 10585971
Zhang J, Subramanian S, Stacey G, Yu O (2009) Flavones and flavonols play distinct critical roles during nodulation of *Medicago truncatula* by *Sinorhizobium meliloti*. Plant J 57(1):171–183. doi:10.1111/j1365-313X2008 03676x. PMID:18786000
Zolla G, Badri DV, Bakker MG, Manter DK, Vivanco JM (2013) Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. Appl Soil Ecol 68:1–9. doi:10.1016/japsoil201303007

"I've Got the Magic in Me": The Microbiome of Conventional vs Organic Production Systems

5

Andrea Sanchez-Barrios, Mohammad Radhi Sahib, and Seth DeBolt

Abstract

The term microbiome refers to the existence of multiple microbial genomes present in an environment in an association with a host. With the development of more precise sequencing approaches, identification of genus and families that were uncultivable microbes has been made possible. The current chapter explores the importance of understanding microbial communities and their association with agricultural production systems with particular attention to endophytic microorganisms. Agri-management practices and their relationship to the selection of microbial variation of taxa by plants and soil have been discussed in detail. The article also discusses how farming practices such as cover cropping and mulching mediate microbial community dynamics. Future perspectives on advancing sustainability by microbiome optimization are discussed.

Keywords

Soil • Microbiome • Plant growth • Expansion • Endophyte • Organic

A. Sanchez-Barrios • S. DeBolt (✉)
Department of Horticulture, University of Kentucky,
309 Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546-0312, USA
e-mail: sdebo2@uky.edu

M.R. Sahib
Department of Horticulture, University of Kentucky,
309 Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546-0312, USA

Al Qasim Green University, Babylon, Iraq

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_5

5.1 Introduction: Evolving Concepts of the Plant Microbiome

5.1.1 General

The soil is a complex environment where there is a vast mix of organic matter, minerals, nutrients, and gases, among others, enclosing a myriad of organisms – micro and macro – that are capable of supporting and retarding plant life and growth. The heterogeneity that exists in these environments is controlled by a series of biological and ecological interactions combined by soil properties, which allow for the proliferation and establishment of certain groups of microbial organism, changing the dynamics of the ecosystem (Gale et al. 2000).

The importance of understanding microbial communities and their association with agricultural production systems lies on the premises of a future with more sustainable approaches to challenges in agriculture. Although many efforts have been directed toward a better understanding on how these microbial communities work, there are still a great number of questions related to the most influential factors dictating the identity or core participants, the diversity and niche specificity, the establishment and maintenance of association with plants, and retrograde signaling networks that could functionalize associations.

5.1.2 Looking Deeper into the Plant Microbiome Using Developing Technologies

The term microbiome refers more to the existence of multiple microbial genomes present in an environment in an association with a host. For the purpose of this chapter, we are focused on the plant bacterial microbiome in an agricultural context. The soil microbial community has received an abundance of attention over past decades, but the broader plant microbiome includes organisms that dwell in the phyllosphere, inside the plant as endophytic organisms, as well as in the rhizosphere and soil. Bacterial organisms are classified as endophytic if they inhabit plant tissue during its life cycle. In contrast, some rhizospheric bacteria colonize plants as opportunistic organisms that interact at some point with the plant but don't inhabit it in an obligate manner. An interest in endophytes, particularly obligate endophytes and the benefits they are able to confer to plants, and how some of these changes may be transferred genetically has emerged recently.

Recent advances in next-generation sequencing (NGS) technology have advanced our understanding of this community (Lundberg et al. 2012; Bulgarelli et al. 2012; Wagner et al. 2016). In terms of the plant microbiome and its relationship to agricultural production, studies have proved that the presence of certain groups of organisms is capable of processing and absorbing nutrients (Manzoni et al. 2008) rendering them available for plant growth (Schardl et al. 2004; Barrow et al. 2008; Xia et al. 2013), repression of disease, and the capacity to mediate the impact of

extreme environmental stress factors (Plett and Martin 2011). What remains complicated is how to foment the presence of those beneficial groups and how they could be used for improvement of many important agronomical crops. Indeed, it will important to establish how soil conditions and agronomical practices affect the selection of these microbial organisms by the plant. Technologies such as NGS accompanied by *fluorescence* in situ *hybridization* (CARD-FISH) for specific microbiome components have broadened what we can identify and how we associate them with the host plant. Agri-management practices and their relationship to the selection for variation of taxa by plants and soil are the main reasons for the development of this chapter. We will be looking at how managing practices could be important when trying to understand the strengths or weaknesses of these relationships, since they are able to influence the development and dominance of a bacterial community.

5.2 The Microbiome and Agriculture

The interaction between plants and individual microorganisms has been studied for the last several decades. Isolation and testing of strains present in soil and plants have largely aimed to understand the capacity that these microorganisms have for plant improvement or pathogenicity. Until the last 5 years, most of the isolation and identification was done via culture-dependent techniques. However, with the development of more precise sequencing approaches, identification of genus and families that were unculturable has been made possible, even to the point of looking at functional genes (Tsurumaru et al. 2015). These advances have provided more insight into the selection and structure of bacterial communities by plants under different environments (Lundberg et al. 2012, 2013; Lebeis et al. 2015; Birtel et al. 2015; Ding and Melcher 2016). Identifying the variability as well as functionality of communities that colonize plants could be used to select for bacteria (or groups of bacterial community members) that can positively modify the plant morphology or interaction with its environment. Despite the attractiveness of being able to inject a single or collection of microorganisms into an agricultural production system to enhance crop performance, there are many reasons that this will be challenging in practice. The complexity of the microbial community and competitiveness of a single microbial factor are unlikely to be dominant enough to sustain any influence on a cropping system. Furthermore, the ability to genetically optimize or engineer microbes to enhance agricultural systems will be a regulatory and environmental containment challenge. As related to agricultural production systems, the notion that understanding the plant microbiome and how it functions and then adapting our management practices to maximize the most interesting members of the microbiome is perhaps the most rational area for future work. Furthermore, plant breeding has not taken into account any influence of a microbiome, and it remains possible that the intersection between plant breeding and microbiome functionality will be a fruitful area for research (Gopal and Gupta 2016). Finally, knowledge of the

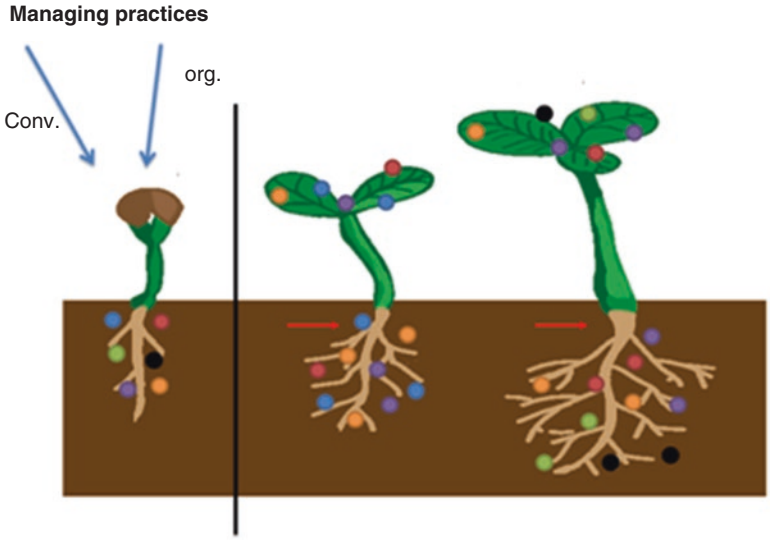


Fig. 5.1 Schematic presentation of the microbiome. Image of a broadleaf seedling planted (*left*) and conceptualizing the overlay of management practices. As the seedling grows, bacterial community members from the soil, which are represented as orange, *blue*, *purple*, and *red dots*, occupy various components of the root (*red arrow*) and phyllosphere (*green* components of plant aerial tissue)

mechanisms by which a microbiome element influences the plant anatomy is still developing and should shed light on hormonal networks and functional gene networks influenced by the microbiome.

How a bacterial microbiome colonizes and establishes itself in living plant tissue will involve not just the physical entry into the plant but also how to avoid the plant immune system (friend versus foe association) (Downie and Walker 1999; Iniguez et al. 2005). As the field of microbial inoculums matures, it will be important to understand the complexity of this association window and whether it is under passive or active control by the host plant. It is expected that numerous non-obligate bacterial genera enter the plant during germination and seedling establishment. As the main contact point for the plant with the microbe-rich soil, microbes are thought to enter into their host (plant) through the root system due to their vast adhering area with soil particles (Hansen et al. 1997; Tokala et al. 2002; Iniguez et al. 2005; Rosenblueth and Martinez-Romero 2006; Seipke et al. 2012) (Fig. 5.1). The rhizosphere is the area that is described as the zone of the soil that is subjected to the influence of the roots. At the same time, another term that will be highly important to mention while talking about entrance of microbial organism to the plant is the spermosphere. This is related to the seed exterior layers that are in contact with the soil and over which microbes will be interacting before germination.

5.3 Inspection Between Agricultural Management Practices and Microbiome

It seems that through the use of culturing and next-generation sequencing, there have been signs that point toward a more consistent and numerous amounts of organisms being identified as endophytic microbiome elements in organic production systems when compared to conventional farming practices (Xia et al. 2015; Schlaeppi and Bulgarelli 2015; Hartmann et al. 2015). The reasons behind those differences among bacterial communities still remain slightly unclear, but data supporting increased soil microbial diversity in organically managed soils have been well documented (Wang et al. 2016). More work has been put toward the elucidation of the effect that the systems may have on the selection of the taxa present in the soil. These results supported findings by Soltani et al. (2010) and Bacon and White (2016) that many endophytic bacterial genotypes increased plant growth and induced a defense system with low cost.

As mentioned before, the differences found among isolates identified as endophytic microbial species comparing conventional and organic crops are of interest as they may be linked to if they can link crop productivity, since one of the main goals is to be able to replicate these environments for crop enhancements or at least to influence selection by plants toward some of these communities. Hall and Davis (1990) suggest that certain bacilli move through the plant using the vascular system rather than symplastic movement. Base on physiological aspects, the older the plant may be, the harder will be for certain endophytic bacteria to translocate from tissue type to tissue, and therefore it is anticipated that as we develop a more sophisticated understanding of tissue-type endophyte colonization, we may see different levels of abundance or community members. Some research supports that the age of the plant may not be one of the limitations for the colonization of obligated bacteria when tissue type was held consistent (roots) (Lundberg et al. 2012). This could be due to the fact that some of these endophytes may be present at early stages and stay there and that the variation of the presence or absence of other species may be related to those that are not strictly necessary to inhabit the plant. Interestingly, it was found by Lundberg et al. (2012) that genotype was a critical determinant in root microbiome community analysis suggesting that the intersection between breeding and agricultural farming practices may be critical for future work.

An interesting concept to examine is how farming practices and the types of crops that are being produced display variance in microbial community metrics. For instance, cover cropping, mulching and soil composition (Kumar et al. 2014), the use of alternative tillage systems (Carbonetto et al. 2014), and overall soil nutrient composition (Stagnari et al. 2014) have an impact in the structure and composition of the soil microbial communities. Carbonetto and co-workers (2014) suggested that soils exposed to high use of fertilizers displayed a shift in the metabolic strategies used by the microbial communities which exasperated community shifts. Metabolism seems to also become more "flexible" for those organisms that were present under tillage practices vs those in non-till areas, but the metabolic flexibility does not mean that they were better adapted; on the contrary, they showed that if

conditions were considered unfavorable (e.g., lower nutrient content in soil), some of those microbial organisms are unlike to adapt, which differed from the non-tillage system. Similar results were found in cotton crops that were maintained under conventional tillage and no tillage (Feng et al. 2003). It seems like the use of non-tillage, for example, and not so many applications of fertilizers, among other things, can have a positive effect in microbial communities in the soil. Kennedy and Smith (1995) support that heavy tillage as a farming practice can be negative for microbial diversity and abundance by the alteration of the properties of the soil. Overall, high population and biodiversity of microorganisms in the soil is an indicator of soil health. Healthy soil has a normal amount of aggregation and percent of air, water, and nutrients; thus, the soil does not need many fertilizers or pesticides to increase plant productivity or to control stresses as the plant will be tolerant (Paul 2007). This parlays with good farming practices, not necessarily organic versus conventional practices.

Both practices, organic and conventional, have systems that follow the application of chemicals to treat and maintain their crops during their production process. Some of the chemicals used tend to be more long lasting within the farming system than others and could have small but progressive impacts on an indigenous microbial community present in the soil. Thus, when comparing results in this area, one must consider numerous environment and cultural factors that vary greatly and are different to compare. A question remains whether the use of pesticides affects microbial communities in the soil in a nontarget manner and in turn influences the selection of the plant microbiome. Even though pesticides are made to target insects and other types of organism that have no relationship with the fungi or bacteria present in soil, it is feasible that in a more individual scale, some species in particular may be affected (Foley et al. 2005). To date, further research is needed on a case-by-case basis to interrogate this postulate.

Herbicides or the surfactants used in their application to a target crop may also have an impact in the microbial communities since some of these, for instance, octylamines, can be slightly bacteriotoxic (https://www.echa.europa.eu/sv/web/guest/registration-dossier/-/registered-dossier/1996/7/7/2) but are nontarget and have been unstudied as environmental risk factors in agricultural microbiome systems. Other herbicidal or pesticidal molecules will remain in the soil (predominantly in conventional systems) for years, for example, the preemergent herbicide used on railroad lines indaziflam (Brabham and Debolt 2013) has an extremely long residual time. While off-target influences of commercially available pesticides and herbicides are typically nonlethal and modest, if a product can be mildly class specific bacteriotoxic, it can easily be envisioned how this could shift the balance in an agricultural crop microbiome (Wilkinson and Lucas 1969). To date, we have an unsatisfactory understanding of this process and whether subtle influences could even alter a microbiome in an agricultural setting.

It is important to take in consideration that longtime exposure to a specific managing practice could alter the soil environment by a simple selection mechanism. It seems that although change is part of both systems, organic farming may be a better option to also increase richness, among others, by shifting the structure of the

microbiota compared to conventional practices (Hartmann et al. 2015). Still, more parameters and variables need to be tested to fully confirm these hypotheses and address better the full impact that these practices have on the microbial communities' structure (Hartmann and Widmer 2006).

5.4 Employing Microbial Elements in Agricultural Systems

It is known that obligated microbes have to follow usually a more elaborated process for their colonization. They can be considered pathways, which usually ramify into production of exudates, rates of production of them, quorum sensing, and hormone metabolisms among others. Exudates are considered to be molecules produced and released either by the plant or bacteria to the rhizosphere (Li et al. 2016 PNAS). Some of the molecules present in these exudates are combination of sugars, amino acids, alkaloids, flavonoids, and others (Biedrzycki et al. 2010; Kumar and Bais 2012). Rates of the exudate production can also have an impact on how the plant selects the microbes from the rhizosphere. Now, the fact that some microbes are capable to produce their own chemicals and modulate the communication with the plant through molecule signaling, it is probably one of the future uses of studying the microbiomes of different systems. Indeed, some endophytic microbiome elements have been used to identify target herbicides in plants (Xia et al. 2014). The idea will be to find ways into isolating, producing, or stimulating the production of these chemicals for the manipulation of the selection power of the plant and at least inhabit it for a small time frame (or long, depending on the effect that it has in the host development and health). It may be suitable to bypass the microbial soil feature and grow it in vitro to harvest the target chemical for organic farming purposes, which is already the case for *Bacillus thuringiensis*.

Promoting plant growth by manipulating microbiomes may have a modest capacity to support the positive traits in a cropping species, thus decreasing the use of synthetic chemicals or nutrients (Singh et al. 2010). Using microbes in agriculture as bio-fertilizers and biopesticides has been well established, but lately it has received more attention, and scientists are currently focusing on the plant microbiome itself instead of just using microbes (Deakeret al. 2004). Using microbes is less practical than using synthetic chemicals because variation in soil and environmental conditions will almost certainly be a selection force and will therefore require regional solutions in agriculture. Modern agriculture has not accepted regionality of trait solutions from major crop biotechnology companies, and therefore it is unclear whether microbial systems will be poorly accepted. Organic farmers may be more willing to work with such regional/environment-specific products simply due to scale (Bacon and White 2016).

There are select studies that show that application of bacterial isolates could support plant growth and productivity under specific conditions, possibly modulating plant microbiomes (Xia et al. 2015). However, these rarely translate from greenhouse or in vitro conditions to the field and even more rarely into a wide variety of agricultural eco-zones. The plant growth-promoting fungal inoculum *Trichoderma*

sp. is still the best example of a successful strategy for this (Altmore et al. 1999). It is hoped that the use of beneficial microbes in organic production system could buffer plant productivity by providing nutrients and other growth-promoting compounds to the crop not only for a short time but also for many seasons because this organic system maintains soil fertility and health.

Treatments and inoculation with bacterial organisms showed in Xia et al. (2014) that plant cell walls are susceptible to the colonization and production of certain chemicals (exudates) by the bacteria. This is a good growth indicator for studies of interactions between plant and microbes because of the importance of the plant cell wall, since it plays an essential role in being a barrier against stresses, connecting extracellular and intracellular environments, and regulating plant growth. Their work also showed that the combination of techniques for identification and isolation of organisms was crucial for a proper selection of candidate strains and their capacity of inhabiting the plant during long periods of its life. Even though manipulating the microbiome is important to increase plant productivity, it is currently a challenge to adopt bacterial strains grown in a lab environment and implement their use in the farmers' fields. These artificially cultured "strains may lack key characteristics for widespread distribution in sustainable and productive agricultural systems" (Parnell et al. 2016). Most of the studies related to bacterial strains as an alternative to synthetic chemicals represent either lab or greenhouse experiments (Adesemoye et al. 2009), making the results obtained from these approaches not an accurate representation of the real environment that plants may be exposed to in a farm setting (Parnell et al. 2016). Although microbial organisms have potential for changing agriculture, there are still a lot of questions that will need to be answered before their acceptance.

5.5 Conclusion

The overall outcome of studies into the functionality of the plant microbiome has been satisfactory to maintain research and agricultural interest. The compelling idea of establishing a more sustainable production system through increasing the abundance or functionality of members of a natural community is highly attractive and potentially cost-effective. Several conclusions and future directions exist. A combined focus on plant breeding in association with detailed microbiome assessment is needed based on the genotype specificity identified in recent studies (Lundberg et al. 2012). Organic farming systems are modestly less likely to drive force and selection on the microbiome community due their inherent focus on soil quality rather than external inputs. Because genotype and environmental conditions both influence the microbiome in plants, long-term studies are needed across numerous species and eco-zones to adequately assess results.

Acknowledgment Work was supported by the National Science Foundation under Cooperative Agreement No. 1355438 and IOS-1256029. US Department of Agriculture Hatch funding and Altria Graduate Research Fellowship to ASB also supported this work.

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58(4):921–929

Altmore C, Norvell WA, Bjorkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai. Appl Environ Microbiol 65:2926–2933

Bacon CW, White JF (2016) Functions, mechanisms and regulation of endophytic and epiphytic microbial communities of plants. Symbiosis 68(1):87–98

Barrow JR, Lucero ME, Reyes-Vera I, Havstad KM (2008) Do symbiotic microbes have a role in plant evolution, performance and response to stress? Commun Integr Biol 1:69–73

Biedrzycki ML, Jilany TA, Dudley SA, Bais HP (2010) Root exudates mediate kin recognition in plants. Commun Integr Biol 3:28–35

Birtel J, Walser J-C, Pichon S, Bürgmann H, Matthews B (2015) Estimating bacterial diversity for ecological studies: methods, metrics, and assumptions. PLoS One 10(4):e0125356. doi:10.1371/journal.pone.0125356

Brabham C, Debolt S (2013) Chemical genetics to probe the cell wall. Front Plant Biotechnol 3:309

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren, van Themaat E et al (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488:91–95

Carbonetto B, Rascovan N, Álvarez R, Mentaberry A, Vázquez MP (2014) Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in Argentine Pampas. PLoS One 9:1–11

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology – a review. Soil Biol Biochem 36(8):1275–1288

Ding T, Melcher U (2016) Influences of plant species, season and location on leaf endophytic bacterial communities of non-cultivated plants. PLoS One 11(3):e0150895. doi:10.1371/journal.pone.0150895

Downie JA, Walker SA (1999) Plant responses to nodulation factors. Curr Opin Plant Biol 2:483–489

Feng Y, Motta AC, Reeves DW, Burmester CH, Van Santen E, Osborne JA (2003) Soil microbial communities under conventional till and no-till continuous cotton systems. Soil Biol Biochem 35:1693–1703

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR et al (2005) Global consequences of land use. Science 309:570–574

Gale WJ, Cambardella CA, Bailey TB (2000) Surface residue and root-derived carbon in stable and unstable aggregates. Soil Sci Soc Am J 64:196–201

Gopal M, Gupta A (2016) Microbiome selection could spur next-generation plant breeding strategies. Front Microbiol 7:1971–1977

Hall TJ, Davis WEE (1990) Survival of *Bacillus subtilis* in silver sugar maple seedlings over a two-year period. Plant Dis 74:608–609

Hansen ML, Kregelund L, Nybroe O, Sorensen J (1997) Early colonization of barley roots by Pseudomonas fluorescens studied by immunofluorescence technique and confocal laser scanning microscopy. FEMS Microbiol Ecol 23:353e360

Hartmann M, Widmer F (2006) Community structure analyses are more sensitive to differences in soil bacterial communities than anonymous diversity indices. Appl Environ Microbiol 72:7804–7812

Hartmann M, Frey B, Mayer J, Mader P, Widmer F (2015) Distinct soil microbial diversity under long-term organic and conventional farming. ISME J 9:1177–1194

Iniguez LA, Dong Y, Carter HD, Ahmer BMM, Stone JM, Triplett E (2005) Regulation of enteric endophytic bacterial colonization by plant defenses. Mol Plant-Microbe Interact 18:169–178

Kennedy A, Smith K (1995) Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170:75–86

Kumar AS, Bais HP (2012) Wired to the roots: impact of root beneficial microbe interactions on the above ground plant physiology and protection. Plant Sign Behav 72:694–706
Kumar A, Maurya BR, Raghuwanshi R (2014) Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.) Biocatal Agric Biotechnol 3:121–128
Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Glavina del Rio T, Jones CD, Tringe SG, Dangl JL (2015) Plant microbiome. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science 349:860–864
Li B, Lia Y-Y, Wua H-M, Zhanga F-F, Lia C-J, Lia X-X, Lambersb H, Long L (2016) Root exudates drive interspecific facilitation by enhancing nodulation and N2 fixation. PNAS 113:236496–236501
Lundberg D et al (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86–90
Lundberg DS, Yourstone S, Mieczkowski P, Jones CD, Dangl DL (2013) Practical innovations for high-throughput amplicon sequencing. Nat Methods 10:999–1002
Manzoni S, Jackson RB, Trofymow JA, Porporato A (2008) The global stoichiometry of litter nitrogen mineralization. Science 321:684–686
Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV (2016) From the lab to the farm: an industrial perspective of plant beneficial microorganisms. Frontiers Plant Sci 7:1110. doi:10.3389/fpls.2016.01110
Paul EA (2007) Soil microbiology, ecology, and biochemistry in perspective. Soil microbiology, ecology and biochemistry, 3rd edn. Academic, San Diego, pp 3–24
Plett JM, Martin F (2011) Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. Trends Genet 27:14–22
Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19:827–837
Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seed borne fungal endophytes. Annu Rev Plant Biol 55:315–340
Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. Mol Plant-Microbe Interact 28:212–217
Seipke RF, Kaltenpoth M, Hutchings MI (2012) Streptomyces as symbionts: an emerging and widespread theme? FEMS Microbiol Rev 36:862–876
Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nat Rev Micro 8:779–790
Soltani AA, Khavazi K, Asadi-Rahmani H, Omidvari M, Abaszadeh DP, Mirhoseyni H (2010) Plant growth promoting characteristics in some *Flavobacterium* spp. isolated from soils of Iran. J Agr Sci 4:106–115
Stagnari F, Perpetuini G, Tofalo R, Campanelli G, Leteo F, Della Vella U, Schirone M, Suzzi G, Pisante M (2014) Long-term impact of farm management and crops on soil microorganisms assessed by combined DGGE and PLFA analyses. Front Microbiol 5:644
Tokala R, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey FJ, Morra MJ (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol 68:2161–2171
Tsurumaru H et al (2015) Metagenomic analysis of the bacterial community associated with the taproot of sugar beet. Microbes Environ 30:63–69
Wagner MR, Lundberg DS, del Rio T, Tringe SG, Dangl JF, Mitchell-Olds T (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Comm 7:12151
Wang W, Wang H, Feng Y, Wang L, Xiao X, Xi Y, Luo X, Sun R, Ye X, Huang Y, Zhang Z, Cui Z (2016) Consistent responses of the microbial community structure to organic farming along the middle and lower reaches of the Yangtze River. Scientific Reports 6: Article number 35046
Wilkinson V, Lucas RL (1969) Effects of herbicides on the growth of soil fungi. New Phytol 68:709–719

Xia Y, Greissworth E, Mucci C, Williams MA, DeBolt S (2013) Characterization of culturable bacterial endophytes of switchgrass (*Panicum virgatum* L.) and their capacity to influence plant growth. GCB Bioenergy 5:674–682

Xia Y, Petti C, Williams MA, DeBolt S (2014) Experimental approaches to study plant cell walls during plant-microbe interactions. Front Plant Sci 5:540

Xia Y, Debolt S, Dreyer D, Scott D, Williams M (2015) Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Front Plant Sci 6. doi:10.3389/fpls.2015.00490

Plant-Microbe Interactions: Current Perspectives of Mechanisms Behind Symbiotic and Pathogenic Associations

6

Muhammad Sohail Akram, Muhammad Shahid, Muhammad Tahir, Faisal Mehmood, and Muhammad Ijaz

Abstract

The phyllosphere and rhizosphere of plants have been a reservoir of microorganisms of both symbiotic and pathogenic nature. The interplay between plants and associated microbes involves complex and dynamic mechanisms, many of which are unexplored. The unraveling of these mechanisms is a big challenge for plant biologists. The consequence of such interactions may be beneficial, detrimental, or neutral for the hosts. There are many known mechanisms through which microorganisms especially bacteria support plant growth, i.e., fixation of atmospheric nitrogen, solubilization of inorganic phosphate, modulated phytohormones synthesis, production of stress-responsive enzymes like 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and biocontrol of many plant diseases. Both above- and underground plant organs are frequently exposed to a plethora of microorganisms, including viruses, bacteria, oomycetes, fungi, and eukaryotic protozoans. Phytopathogens defend their habitat and infect

M.S. Akram
Department of Botany, Government College University, Faisalabad 38000, Pakistan

M. Shahid (✉)
Department of Bioinformatics and Biotechnology, Government College University, Faisalabad 38000, Pakistan
e-mail: shahidmpg@yahoo.com

M. Tahir
Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari Campus, Islamabad, Pakistan

F. Mehmood
Department of Environmental Sciences and Engineering, Government College University, Faisalabad 38000, Pakistan

M. Ijaz
College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Layyah, Pakistan

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_6

plants by a variety of compounds (toxins) that are broad spectrum in their activity. In response, plants initiate defensive mechanisms that resist pathogen penetration and subsequent infection. Thus, various events of molecular crosstalk take place between plants, and both friendly and hostile microbes trigger a series of highly dynamic plant cellular responses. Such mechanisms are very crucial for pathogen recognition and induction of adequate defense signal transduction cascades in the plant. More research insights are required to unravel the molecular basis behind these mechanisms. Also, to support the plant life, many complex mechanisms initiated after the association of symbiotic or pathogenic microorganisms need to be explored.

Keywords
Mechanisms • Plant-associated microbes • Symbiosis • Pathogenesis • Defense responses

6.1 Introduction

Plant-microbe interactions play a vital role to ensure sustainability in agriculture and ecosystem restoration (Badri et al. 2009). The interactions may be categorized as positive, negative, or neutral which largely depends on the nature of microorganisms associating the host. Positive interactions stimulate plant growth by conferring abiotic and/or biotic stress tolerance and help the plants for the revitalization of nutrient-deficient and contaminated soils. Negative interactions involve host-pathogen interactions resulting in many plant diseases and adverse effects and host life (Abhilash et al. 2012). Moreover, some microbes reside in the soil surrounding the plant roots just to obtain their nutrition from root exudates. They do not influence the plant growth or physiology in a positive or negative way, thus forming neutral interactions. Apart from that, the resource allocation between different plant parts is greatly affected by beneficial microorganisms. Also, many above- and belowground interactions with herbivores and other natural enemies of the plants are modulated by the microorganisms. In addition, the physicochemical and biological soil properties are modified in response to physiological, biochemical, and molecular dialogues between plants and associated microbes (Dubey et al. 2015). Plant root exudates are rich in low molecular weight compounds like amino acids, organic acids, polymerized sugars, root border cells, and dead root cap cells. Moreover, plant roots secrete many phyto-siderophores which sequester the metallic micronutrients from the soil, resulting in enhanced plant nutrition. Some secondary metabolites present in plant root exudates also play key role in plant-microbe communications (Bais et al. 2006). Different interfaces of rhizosphere, phyllosphere, and endosphere possibly exist in such complex and largely unexplored interactions. Thus, the complex and interconnected process that takes place at the abovementioned interfaces must be explored to understand the contribution of each and every player to the well-being of the ecosystem. Therefore, the complete

knowledge of mechanisms underlying plant-microbe communications is necessary to decipher the interfaces among host plant microorganisms for sustainable agriculture, increased biomass and bioenergy production, and restoration of soil properties (Saleem and Moe 2014). For microorganisms, colonization is a vital step for effective establishment leading to friendly or pathogenic relationship with plants (Kamilova et al. 2006; Lugtenberg et al. 2002). Successful colonization involves host cell surface recognition, adherence, invasion, growth, and multiplication along with several unexplored mechanisms. The crosstalk between plants and microbes is initiated by the production of plant signals that are perceived by the microbes and stimulate synthesis of chemicals for colonization (Ali et al. 2016; Bais et al. 2006). Motile microorganisms are best suited to participate actively in this crosstalk (Lugtenberg et al. 2002). Moreover, a confirmation of microbial structure greatly influences the intensity, duration, and outcome of plant-microbe interactions (Danhorn et al. 2004; Shahid et al. 2015). In this chapter, we attempted to highlight all the known mechanisms that drive strong association of microorganisms and hosts. The outcomes of these mechanisms on growth and physiology of plant have also been discussed.

6.2 Mechanisms Behind Plant-Symbiont Interactions

6.2.1 Biological Nitrogen Fixation

Plants uptake nitrogen either as inorganic form (NH_4^+ and NO_3^-) or as low molecular weight dissolved organic nitrogen (DON), particularly amino acids (Murphy et al. 2003; Streeter et al. 2000). Atmospheric dinitrogen (N_2) cannot be incorporated into plant metabolism until reduced to a more useable form like ammonia (NH_3) by some diazotrophic microorganisms (Rovira 1991). Nitrogen cycle also contains biological nitrogen fixation (BNF) as a vital process (Stevenson and Cole 1999). One type of nitrogen fixation that does not include any biological activity involves industrial fixation and fixation through natural lightening process, which converts atmospheric N_2 to NO_3^-. The other type, biological nitrogen fixation (BNF), is a process by which atmospheric N_2 is converted to NH_3, and this reaction is catalyzed by nitrogenase enzyme present in diazotrophic microorganisms. The later type contributes more than 2×10^{13} g nitrogen annually, worldwide. From this amount, 80% is contributed by symbiotic fixation, and the remaining 20% is made available by free-living or associative nitrogen-fixing systems (Falkowski 1997).

The ability to convert atmospheric N_2 into plant usable form exists only in bacteria and Archaea (Young 1992). For BNF, bacterial species of *Acinetobacter*, *Alcaligenes*, *Arthrobacter, Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Burkholderia*, *Pseudomonas*, *Serratia*, and (*Brady*)*Rhizobium* are mainly involved in establishing symbiotic and associative-symbiotic interactions with plant roots where ultimately both partners are benefited (Egamberdiyeva 2005; Tilak et al. 2005). There are two main types of BNF:

1. Symbiotic nitrogen fixation, e.g., (*Brady*)*Rhizobium* and *Frankia* in leguminous and nonleguminous actinorhizal plants, respectively
2. Non-symbiotic nitrogen fixation, e.g., *Cyanobacteria*, *Azotobacter*, *Azospirillum*, etc.

6.2.1.1 Symbiotic Nitrogen Fixation

Rhizobia are classically defined as symbiotic bacteria that invade the roots and stems of leguminous plants to fix nitrogen (van Rhijn and Vanderleyden 1995). It is a synthesis of NH_4^+ (a plant usable form of N) using atmospheric N_2 (plant non-usable form of N) by *rhizobia* in nodules of leguminous plants. The important nitrogen-fixing *rhizobial* genera in legumes are *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, *Azorhizobium*, and *Sinorhizobium*, each of which belongs to a distinct and well-established phylogenetic group (Hungria and Vargas 2000; Sy et al. 2001). Besides rhizobia, many *Frankia* species has also been reported to form nodules in nonleguminous actinorhizal plants for N_2 fixation (Zhang et al. 2012). Moreover, non-rhizobial strains may also occupy nodule cells and benefit the plants. Hameed et al. (2004) reported the occupation of a phosphate solubilizing *Agrobacterium* strain Ca-18 with the nodule cells. Many other studies have also described the existence of non-rhizobial bacteria in root nodules of leguminous crops (Rajendran et al. 2012; Tariq et al. 2012).

6.2.1.2 Non-symbiotic Nitrogen Fixation

The atmospheric dinitrogen fixation without the formation of nodules is termed as non-symbiotic nitrogen fixation. The non-symbiotic diazotrophic genera include *Azotobacter*, *Azospirillum*, *Acetobacter*, *Azoarcus*, *Bacillus*, and *Pseudomonas* (Saharan and Nehra 2011). Many cyanobacterial genera have been identified as free-living diazotrophic bacteria (Fiore et al. 2005). In a recent study, conducted in China, the non-symbiotic nitrogen-fixing bacterial diversity was categorized as *Proteobacteria* (63.9%), *Actinobacteria* (32.2%), *Firmicutes* (1.9%), and *Bacteriodetes* (1.9%), and many bacterial genera were found as free-living nitrogen fixers such as *Arthrobacter*, *Mitsuaria*, *Burkholderia*, *Sinorhizobium*, *Pseudomonas*, and *Rhizobium* at lower taxonomic level (Xu et al. 2012).

6.2.1.3 Biochemistry and Genetics of Biological Nitrogen Fixation

The process of BNF is complex and involves many functional and regulatory genes (Dixon and Kahn 2004). The reduction of atmospheric nitrogen is performed by a nitrogenase enzyme, a dimer of two metalloproteins: nitrogenase iron protein (Fe protein) and nitrogenase molybdenum-iron protein (Mo-Fe protein) (Einsle et al. 2002; Strop et al. 2001). Nitrogen, as a substrate, is bound to molybdenum-iron-sulfur homocitrate clusters of Mo-Fe protein, and the same phenomenon is utilized by other substrates such as acetylene, protons, etc. (Postgate 1982). The second protein (Fe-protein) shuttles electrons to Mo-Fe-protein, and this process consumes two Mg-ATP for each electron (Halbleib and Ludden 2000). The complete process of BNF can be expressed as follows:

$N_2 + 10H^+ + 8e^- + nMgATP \rightarrow 2\,NH_4^+ + H_2 + nMgADP + nPi\,, n \geq 16$ (Dean and Jacobson 1992)

Thus, the process is energy demanding and consumes 8 mol of ATP to produce 1 mol of NH_4^+. This ratio may be much higher under natural conditions (Hill 1992). The diazotrophic ability of soil bacteria can be measured in vitro through acetylene reduction assay (Dilworth 1966).

6.2.1.4 Genes Involved in Biological Nitrogen Fixation

Different species have different number and arrangement of genes engaged in the process of BNF. The Mo-Fe is a tetrameric ($\alpha_2\beta_2$) protein, encoded by *nifDK*, and Fe-protein is a homodimer (α_2), encoded by *nifH* (Halbleib and Ludden 2000). So these structural genes along with many regulatory and accessory genes are responsible synthesis of *nif* regulon (Dean and Jacobson 1992). The *nifD* and *nifK* genes are part of same operon, while *nifH* is also considered, in some studies, a part of the same operon. Nitrogenase metal clusters, synthesized by *nifE* and *nifN*, are found together on the operon *nifEN*, which may have arisen from the duplication of two operons *nifDK* and *nifEN*, considered as the core operons (Fani et al. 2000). A number of other genes are also present to supplement these operons which are responsible for coding proteins responsible for electron transport (*nifF*, *nifJ* in *Klebsiella pneumoniae*), regulation (*nifA*) or Fe-Mo cofactor (*nif*B, *nif*V in *Klebsiella pneumoniae*) synthesis (Triplett et al. 1989). In rhizobial species, *fix* and *nod* genes are present which control nitrogen fixation and nodule formation, respectively. While in free-living diazotrophs like *Klebsiella pneumonia*, many of these genes are absent (Dean and Jacobson 1992). Apart from the standard nitrogenase nitrogen-fixing system (*nifDK* and *nifH*), two alternative nitrogen-fixing systems have also been well characterized (Bishop and Premakumar 1992). These systems do not carry molybdenum; instead one carry vanadium (*vnfDK* and *vnfH*) while the other contain only iron and no unusual metal (*anfDK* and *anfH*). All these systems share significant sequence homology but still enough difference for identification. The two alternative systems are regulated under Mo-deficient conditions (Bishop and Premakumar 1992).

6.2.2 Phosphate Solubilization and Mobilization

Plant phosphate availability is improved by arbuscular mycorrhizae through the increase in root surface area, thus forming channels of phosphate nutrition (Osorio and Habte 2015). Plant growth-promoting rhizobacteria (PGPR) facilitate plants to obtain nutrition from inorganic and organic pools of soil through mineralization and solubilization processes (Hilda and Fraga 1999). If all the microbial population of soil is considered, phosphate solubilizing bacteria (PSB) constitute 1–50% (Chen et al. 2006).

Thus, they stimulate phosphorus uptake and significantly modulate plant growth, physiology, and yield (Arcand and Schneider 2006; Chen et al. 2006; Perez et al. 2007). Various strains of *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Rhizobium*

along with *Aspergillus* and *Penicillium* fungi were found to be the most influential P solubilizers (Whitelaw 1999). The mechanism of P-solubilization is associated with organic acids released by P-solubilizing bacteria, lowering the pH of rhizosphere. Thus, the organic acid production causes the chelation of H^+ ions in the root microenvironment, and insoluble phosphates are transformed into soluble form (Mullen 2005; Trivedi and Sa 2008). Two major mechanisms of bacterial phosphate solubilization are:

1. Mineral phosphate solubilization
2. Organic phosphate solubilization

6.2.2.1 Mineral Phosphate Solubilization

Mostly, microbial mineral phosphate solubilizing ability corresponds to production of organic acids (Rodriguez et al. 1999). Phosphate solubilizing bacteria are known to produce many organic acids like malic acid, oxalic acid, gluconic acid, 2-keto gluconic acid, citric acid, lactic acid, propionic acid, succinic acid, and formic acid, and most of these organic acids especially 2-ketogluconic acid, malic acid, oxalic acid, and citric acid are found in rhizosphere of various crops and vegetables (Jaeger III et al. 1999; Chen et al. 2006; Gulati et al. 2010; Shahid et al. 2015). In Gram-negative bacteria, glucose is oxidized to gluconic acid (GA), and biosynthesis of GA is catalyzed by glucose dehydrogenase (GDH) enzyme. Pyrroloquinoline quinone (PQQ) acts as cofactor of GDH (Goldstein 1994). A gene cluster consisted of six open reading frames (pqqA, B, C, D, E, and F) leads to the formation of PQQ (Kim et al. 1998a; Meulenberg et al. 1992). The *pqqE* coding sequence is the most conserved and is considered to be responsible in mineral phosphate solubilization (Perez et al. 2007; Shahid et al. 2012).

Goldstein and Liu (1987) cloned mineral phosphate solubilizing (MPS) gene from Gram-negative bacteria *Erwinia herbicola* for the first time. Expression of this gene in *E. coli* HB101 resulted in the production of GA and solubilization of hydroxyapatite. *E. coli* has the capability of synthesizing GDH but is unable to synthesize PQQ and GA. The protein encoded by cloned 1.8 kb fragment is similar to the gene III product encoded by PQQ synthesis gene complex from *Acinetobacter calcoaceticus* and to *pqqE* of *Klebsiella pneumonie* (Liu et al. 1992). Moreover, a 7 kb fragment from genomic DNA of *Rahnella aquatilis*, responsible of inducing hydroxyapatite solubilization in *E. coli*, was analyzed, and two complete and one partial ORFs were found. One of the complete ORFs was cloned and was found analogous to *pqqE* of *E. herbicola, K. pneumoniae*, and *A. calcoaceticus* (Kim et al. 1998a), and the partial ORF was found similar to *pqqC* of *K. pneumoniae.* Another gene (*gabY*) of *Pseudomonas cepacia*, carrying GA production capacity and MPS ability, has been characterized (Babu-Khan et al. 1995), and the recombinant protein sequence showed no similarity with the previously cloned gene carrying GA production ability but was identical to histidine permease protein. Containing this gene, *E. coli* was capable of producing the GA only when functional glucose dehydrogenase gene (*gcd*) was expressed. Thus, it was speculated that the synthesis of

PQQ was accomplished through an alternative pathway, or the production of *gcd* cofactor was different from PQQ (Babu-Khan et al. 1995).

Other genes related to MPS do not relate to *pqq* DNA or *gcd* biosynthesis mechanism. According to another report, a DNA segment isolated from *Enterobacter agglomerans* demonstrated MPS in *E. coli* JM109 without changing the pH of medium (Kim et al. 1997). Thus, acid production is not the only choice for MPS by bacteria (Illmer and Schinner 1995). Mineral phosphate solubilization capability of PGPB was attempted to be improved by the cloning technique using PQQ synthetase gene of *Erwinia herbicola* (Rodríguez et al. 2000). The gene was isolated by Goldstein and Liu (1987) and was subcloned in a broad host range vector (*p*KT230). Thus, the expression of recombinant molecule was obtained in *E. coli* and then transformed to PGPB strains (*Burkholderia cepacia* and *Pseudomonas aeruginosa*) by tri-parental conjugation. Many exconjugants selected on the specific medium produced larger halo zones on medium with tricalcium phosphate as a sole P source. So, heterologous expression of PQQ synthetase gene resulted in increased MPS ability in PGPB.

6.2.2.2 Organic Phosphate Solubilization

Organic form of soil phosphorus can be released by three groups of enzymes:

1. Nonspecific acid phosphatases: by dephosphorylation of phospho-ester and/or phosphor-anhydride bonds in organic matter
2. Phytases: involved in the release of P from phytic acid
3. Phosphonatases and C-P lyases: by cleavage of C-P bond in organo-phosphonates

Currently, the main focus of research is on acid phosphatases and phytases as their substrates are present in huge amounts in soil.

6.2.2.2.1 Nonspecific Acid Phosphatases (NSAPs)

Bacterial nonspecific acid phosphatases consist of three molecular families designated as A, B, and C (Thaller et al. 1995). During the last decade, these enzymes were studied for their biotechnological applications, and class A NSAPs were successfully used for environmental bioremediation of uranium-contaminated wastewater (Macaskie et al. 1997). NSAPs may also be used for gene expression in PGPB by recombinant DNA technology for improved phosphate solubilization. Several phosphate solubilizing genes from Gram-negative bacteria have been isolated and characterized (Rossolini et al. 1998). These genes, when expressed in PGPB, can enhance phosphate solubilizing ability of bacteria. Some of these genes code for the acid phosphatases which perform the same function in soil. For instance, the *acpA* gene of *Francisella tularensis* encodes for a substrate-specific acid phosphatase with maximum efficiency at pH 6 (Reilly et al. 1996). In addition, *PhoC* gene, coding for NSAP class A and *napA* gene for class B, was very promising and isolated from *Morganella morganii* (Maria et al. 1995; Thaller et al. 1994). In rhizobacteria,

a gene was isolated from *Burkholderia cepacia* which codes for two acid phosphatases *napD* and *napE* along with an outer membrane protein responsible for P transport to the cell (Deng et al. 2001, 1998).

6.2.2.2.2 Phytases

Phytases are not yet potentially exploited for organic phosphate solubilization of soil. A significant portion of soil organic phosphorus is comprised of phytate (Richardson 1994). Plants are not directly dependent on phytate for their P requirements. A significant improvement in growth and P uptake in *Arabidopsis* plants was observed when phytase gene (*phyA*) from *Aspergillus niger* was genetically engineered (Richardson et al. 2001a). It has also been reported that microbial inoculation increases the inositol phosphate utilization by plants (Richardson et al. 2001b). Therefore, development of high phytase producing inoculants would be of great importance for enhancing plant growth and phosphorus contents (Hanif et al. 2015). Phytases are also produced by roots of several plant species (Li et al. 1997; Lung et al. 2008). *E. coli* phytase genes (*appA* and $appA_2$) have been isolated and characterized (Golovan et al. 1999; Rodriguez and Fraga 1999). Similarly, phytase genes have been cloned from *B. subtilis* and *B. licheniformis* (Tye et al. 2002). A *phyA* gene of *B. amyloliquefaciens* FZB45 stimulated the maize growth in the presence of phytate and under limited phosphate conditions (Idriss et al. 2002). In addition, thermally tolerant phytase gene (*phy*) has been reported and characterized from *Bacillus* sp. DS11 (Kim et al. 1998b) and *B. subtilis* VTT E-68013 (Kerovuo et al. 1998).

6.2.3 Plant Growth Hormone Production

Plant growth hormones are organic compounds that act as messengers and help plants to respond to their environment. They are very effective even if synthesized in a very small quantity and may inhibit plant growth if present in large amounts (Arshad and Frankenberger 1991). They are synthesized in one plant part and then transported to the other, where they may cause the physiological response and effect on growth and fruit ripening. In this way, they are also referred to as plant growth regulators (Davies 2010). There are five main groups of plant growth regulators:

1. Auxins
2. Gibberellins
3. Cytokinins
4. Ethylene
5. Abscisic acid

Among these, indole-3-acitic acid (IAA) is considered to be the most important phytohormone which plays a major role in cell growth and division and known to increase the lateral root development in plants (Seo and Park 2009). Many bacterial genera, including *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, and

Pseudomonas, have been reported to produce a considerable amount of IAA in vitro and in vivo (Chen et al. 2006; Nahas 1996; Venieraki et al. 2011). The bacterial genus *Azospirillum* is known to produce a good concentration of IAA for plant growth (Saharan and Nehra 2011). IAA acts as signal molecule for plant development including organogenesis and has a potential role in cell division, expansion, elongation, and gene regulation (Ryu and Patten 2008). Phytohormones has been reported to be synthesized by phosphate solubilizing bacteria (Chen et al. 2006; Vassilev et al. 2006). Diverse bacterial species are known to produce IAA in pure culture and soil, and their interactions with plant roots have been widely studied (Akram et al. 2016; Leveau and Lindow 2005; Rodriguez et al. 2004; Venieraki et al. 2011). IAA is responsible for the phyto-stimulation, and many microorganisms use it as a tool for interacting with plants. Thus, IAA is also involved in bacterial colonization with plant roots. It also acts as a signaling molecule in bacteria and directly affect bacterial physiology (Barazani and Friedman 1999; Spaepen et al. 2007). On the basis of potential for auxin production by rhizosphere microorganisms, effective plant growth-promoting bacteria from plant rhizosphere can be screened and reinoculated on plants for growth and yield improvement (Khalid et al. 2004). Some microorganisms like *Azospirillum* produce IAA in the presence of L-tryptophan. Tryptophan acts as a precursor for the production of IAA (Tien et al. 1979). Inoculation of crop plants with IAA-producing bacterial isolates augments plant growth. A significant increase in root proliferation and root dry matter was observed in eucalyptus cuttings when grown on a substrate inoculated with IAA-producing rhizobacteria (Teixeira et al. 2007). Other phytohormones like gibberellins, cytokinins, ethylene, and abscisic acid are also reported to be produced by plant-associated bacteria and stimulate plant growth and development. The most important microbially produced gibberellin is gibberellic acid. Similarly, cytokinins are adenine derivatives, and microbial synthesis of cytokinins in the rhizosphere and its effect on plant physiological pathways are being investigated (Baca and Elmerich 2007).

6.2.4 Biocontrol

Microorganism, being indigenous to soil and rhizosphere, play a vital role in the biocontrol of phytopathogens. They can suppress a broad range of bacterial, fungal, and nematode diseases and are also effective against viral diseases. PGPR are being used as biocontrol agents all over the world. They have produced significant results against plant pathogens in vitro and in greenhouse, but their performance in the field are still inconsistent. They have also been successfully used in integrated pest management programs (Siddiqui 2006). They also have natural ability to restrain soilborne pathogens (Weller et al. 2002). Shoda (2000) reviewed that bacterial genera *Pseudomonas*, *Bacillus*, *Alcaligenes*, and *Agrobacterium* have been successfully identified as biocontrol agents. In addition, many other bacteria such as *Micromonospora*, *Streptomyces*, *Streptosporangium*, and *Thermobifida* are reported to act as biocontrol agents (Franco-Correa et al. 2010). Genus *Pseudomonas* is the

largest group considered to have biocontrol activity, and *P. fluorescens* strain WCS374 increased the reddish yield up to 40% by suppressing the *Fusarium* wilt disease (Bakker et al. 2007; Kremer and Kennedy 1996). *Pseudomonas* has many traits which are involved in plant growth promotion and biocontrol (Weller 1988). Bhattacharyya and Jha (2012) reviewed the following characteristics of *Pseudomonas* making them as potential biocontrol agents:

1. Rapid in vitro production to construct a mass growth
2. Ability to utilize metabolites and exudates of seed and roots
3. Ability to colonize the rhizosphere and spermatosphere
4. Capability of producing a wide range of bioactive metabolites (antibiotics, siderophores, volatiles, and other growth promoting substances)
5. A strong competitive ability with other microorganisms in environment
6. Ability to adapt environmental stresses
7. Development of induced systemic resistance in plants
8. Production of hydrogen cyanide (HCN)

6.2.5 Production of ACC Deaminase

An appropriate amount of ethylene is essential for plant growth and development, but its high concentration may affect plant cellular processes and retard plant growth. PGPR were able to regulate the ethylene level in root zone of *Arabidopsis thaliana* using their 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase, which actually prevents ACC to take part in ethylene biosynthesis pathway (Desbrosses et al. 2009). Using this mechanism, plants were able to tolerate environmental stresses by keeping a normal amount of ethylene in their root zone. A number of PGPR strains like *Achromobacter*, *Azospirillum*, *Pseudomonas*, *Enterobacter*, *Bacillus*, and *Rhizobium* have been found to show this mechanism (Duan et al. 2009; Ghosh et al. 2003; Govindasamy et al. 2008).

Ghosh et al. (2003) reported the enhanced root length in *Brassica campestris* by three *Bacillus* spp. (*Bacillus circulans* DUC1, *Bacillus firmus* DUC2, and *Bacillus globisporus* DUC3), carrying ACC deaminase activity. Similarly, root and shoot dry matter was increased in *Brassica napus* after the inoculation with *Pseudomonas asplenii* which contain ACC deaminase gene (Reed and Glick 2005). Thus, PGPR possessing ACC deaminase activity increase plant biomass in a stressed environment like salinity, temperature, drought, waterlogging, pathogenicity, and contaminants (Saleem et al. 2007). PGPR can also be genetically modified to perform this function. The efforts to express ACC deaminase gene to plant genome have been made to modify the plant species, but these efforts have yet not come up with complete success due to certain constraints like international trade agreements and proprietary rights on genetically modified crops and also due to some limitations in recombinant DNA technology.

6.3 Mechanisms Behind Plant-Pathogen Interactions

Plants constantly remain under threat of array of pathogens that have the capability of provoking disease. Pathogens include diverse organisms (bacteria, fungi, oomycetes, and viruses) which usually share common infection strategies but may also have pathogenicity determinants unique to each. Plants, in turn, defend themselves from possible damages of infection. However, plants do not possess a mobile defense system, and they largely depend on the inherited immunity patterns and systemic signals originating in response of pathogens (Ausubel 2005; Jones and Dangl 2006). The pathogen-plant interaction is a two-way process. Pathogen attempts to manipulate the biology and physiology of the host cell for generating an environment favoring pathogen growth. The plant cell responds by recognizing and targeting potential pathogen landing on its surface. Both plant and pathogen genes evolve together over a course of time, with emergence of new elicitors/effectors and corresponding plant resistance analogs, enabling this two-way communication to continue.

The following sections will highlight our current understanding about the plant-pathogen interaction, both at physiological and molecular levels.

6.3.1 General Classification of Plant Pathogens

Plant pathogens can be divided into three groups, i.e., biotrophs, necrotrophs, and hemi-biotrophs (Li et al. 2013):

1. Biotrophs tend to keep plant tissues alive as they majorly feed on living cells. Their penetration and infection strategy are such that they induce minimum damage to cell. PAMP-triggered immunity (PTI) is mainly involved in responses to biotrophs. (Lazniewska et al., 2012).
2. Necrotrophs release cell wall-degrading enzymes (CWDE) making host cells vulnerable which mostly lead to cell death. They feed on the materials released from the infected tissue. DAMP-triggered immunity (DTI) is primarily involved in providing resistance against necrotrophs (Wen, 2013).
3. Hemi-biotrophs are given the name because of the presence of an initial biotrophic phase pursued by a necrotrophic stage where they can live as saprophytes. Both PTI and DTI may get activated in response to the attack of a hemi-biotroph (Fawke et al. 2015).

6.3.2 Pathogen Infection to Host Cell

A successful infection requires entry of a pathogen into host cell. Stomata, hydathodes, and wounded tissues are the main cell entry points for pathogenic bacteria, and majority of the invaded bacteria proliferate in apoplast regions, only. Oomycetes and pathogenic fungi develop specialized feeding structures called haustoria which

invaginate into the host plasma membrane. The plasma membrane of host cell as well as of haustoria and contiguous extracellular matrix together constitute an intimate interface that determines the outcome of host-pathogen communication. In addition, pathogens also release compounds like cell wall-degrading enzymes (CWDE) and extracellular polysaccharides (EPS) which make tissue soft, enhance maceration, prevent desiccation, provide defense against host resistance factors, and, hence, facilitate pathogen invasion.

6.3.3 Plant Defense Responses

Plants respond to a potential pathogen at two levels (Jones and Dangl 2006). The first level include inherited basal responses (known as PAMP-triggered immunity, PTI) immediately after a pathogen invades host surface and attempts to penetrate inside. The second defense level (called effector-triggered immunity, ETI) is represented by host resistance against the pathogen-released effectors. Both these levels are crucial to minimize the pathogenicity but at different phases of infection (Li et al. 2013).

6.3.3.1 Basal Resistance or PAMP-Triggered Immunity (PTI)

It is the first line of active plant defense and is activated by the recognition of a virulent pathogen itself or released elicitors called pathogen-associated molecular patterns or microbe-associated molecular patterns (PAMPs or MAMPs), hence named as PAMP-triggered immunity (PTI). PAMPs are generated by microbial molecules, i.e., activators of XA21-mediated immunity, methylated DNA, double-stranded DNA, elongation factor peptides, flagellar proteins, lipopolysaccharides, and peptidoglycans (Li et al. 2013; Zeng et al. 2010).

PAMP triggers are perceived by plant pattern-recognition receptors (PRRs) localized at host cell surface (Dodds and Rathjen 2010). PRRs are receptor-like transmembrane proteins with most having a ligand-binding ectodomain (for PAMPs recognition) and a cytoplasmic kinase signaling domain (catalytic domain). Certain plant-generated signal molecules such as ethylene, jasmonic acid, and salicylic acid regulate the role of PRRs against a particular infection. Failure of proper perception of PAMPs results in high disease incidence, signifying the importance of PRR-based perception and PTI patterns. Plant and animals PRRs possess analogous structural domains, indicating their convergent evolution in two different domains of life.

Being a first line of defense, PTI is often phenotypically reflected by callose deposition, cell wall thickening, and stomata closure, as well as physiologically by production of antimicrobial compounds and reactive oxygen species (ROS). Moreover, PTI activates the mitogen-activated protein kinases and calcium signaling and induces changes in expression of pathogen-responsive genes (Nürnberger et al. 2004). This basal resistance strategy minimizes spread of further infection to nearby tissues (Chisholm et al. 2006). However, pathogens are equipped with mechanisms to counter plant-produced antimicrobial compounds and ROS. *Xanthomonas*

campestris pv. campestris showed enhanced synthesis of catalase and peroxidases while, *X. campestris* pv. phaseoli synthesizes alkyl hydroperoxidase reductases for neutralization of plant produced anti-pathogen compounds/ROS.

Besides this, PTI may get activated by damage-associated molecular patterns (DAMPS). DAMPs serve as signals to trigger the PTI response in infected host plants, in a similar way as for PAMPs. DAMPs are triggered by synthesis of endogenous small peptides and/or cell wall fragments that are released from damaged or stressed cells (Li et al. 2013).

6.3.3.2 Pathogen-Induced Resistance or Effector-Triggered Immunity (ETI)

After successful penetration, a pathogen tries to suppress the components of PTI by release of certain effectors. A large set of effectors have been characterized (Mesarich et al. 2016; Stergiopoulos and de Wit 2009). Effectors manipulate host metabolism and defense mechanisms to facilitate further spread and virulence by various ways.

6.3.3.2.1 Effectors

To overcome plant defense mechanisms, pathogens produce a wide range of virulence factors like cell wall-degrading enzymes, effector proteins, plant hormones, and certain toxins. Among all of these, effector proteins [avirulence (Avr) proteins] play pivotal role. These are expressed by avirulence (Avr) genes which are associated with genomic islands and/or transposable elements. In addition, lateral gene transfer through bacteriophages, integrative or conjugative elements, and bacterial plasmids helped in acquisition of Avr genes.

The bacterial effectors are released into plant cells via type 3 secretion system (T3SS) to suppress plant defense mechanisms. In order to weaken the host defense programs, effectors must target host components involved in immune responses. These may alter the physiology of host plant for enhanced pathogen infestation and/or disturb the host plant defense mechanisms. Virulence mechanism of a variety of effectors has been explored at the molecular level. The AvrPto effector of Pseudomonas syringae targets plant FLS2, and AvrPto/FLS2 interaction modulates flagellin induced PTI responses and, in turn, enhances pathogen virulence in tomato and *Arabidopsis* (Xiang et al. 2008). The C-terminal E3 ligase domain of *P. syringae* AvrPtoB effector ubiquitinates plant produced FLS2 and suppresses PTI by degrading FLS2 (Göhre et al. 2008). In addition, AvrPtoB also targets CERK1 for degradation by ubiquitination of CERK1 kinase domain (Gimenez-Ibanez et al. 2009).

Similarly, *Arabidopsis* MAP kinases (MPK3 and MPK6) are inactivated by HopAI1 through removal of phosphate group from phosphor-threonine leading to suppression of PTI responses. In *Arabidopsis*, the AvrB effector of *P. syringae* mimics coronatine leading to activation of jasmonate signaling cascade, and resultantly flg22-induced deposition of callose reduced and cells become more susceptible (Gimenez-Ibanez et al. 2009; He et al. 2004; Shang et al. 2006). The effector AvrAC of *Xanthomonas campestris* pv. campestris (Xcc) is delivered into host cell as an

uridylyl transferase that catalyzes addition of uridine monophosphate onto BIK1 and RIPK (receptor like cytoplasmic kinases involved in PTI). The conserved phosphorylation sites present in the activation loop of BIK1 and RIPK are modulated and, therefore, reduce their kinase activity and capability of downstream signaling (Deslandes and Rivas 2012).

Effectors from filamentous fungal pathogens and their host targets are comparatively less characterized. The results of the study reveal that fungal pathogens use almost the same strategies as used by bacterial pathogens. A strong virulence effect by leaf mold fungus *Cladosporium fulvum* and rice blast fungus *Magnaporthe oryzae* is found to be dependent on synthesis of Ecp6 and Slp1 effectors, respectively. Both of the effectors (Ecp6 and Slp1) compete with receptors CEBiP and CERK1, for chitin binding, to block host PTI responses (de Jonge et al. 2010). The effector AvrPiz-t of *M. oryzae* enhances virulence by suppressing PTI through targeting host RING E3 ubiquitin ligase APIP6. *Ustilago maydis* (corn smut fungal pathogen) synthesizes and releases an apoplastic effector "Pep1" to suppress ROS burst, a typical PTI response, by directly targeting the apoplastic peroxidase "POX12."

6.3.3.2.2 Resistance Proteins

Plants possess corresponding R proteins, the product of resistance (R) genes, to recognize the pathogen-produced Avr protein. There are eight major variants of R genes (and hence R proteins) as reviewed by (Gururani et al. 2012). The R protein variants have major differences in their organization of amino acid motif as well as their membrane spanning domains (Fig. 6.1). It has been observed that leucine-rich repeats (LRRs) are common in majority of the R proteins indicating their importance in recognition of a specific pathogen.

The first class of resistance proteins is a group of cytoplasmic proteins which possess LRR and nucleotide-binding site (NBS) motifs along with an N-terminal domain with homology to the toll-interleukin-1-receptor (TIR) domain present in mammalian proteins. The flax L6, tobacco N, and RPP5 R proteins are grouped in the first class of R proteins. The second major class of R genes includes the genes encoding for cytoplasmic proteins with a C-terminal LRR, a supposed N-terminus coiled coil domain (CC) and a NBS. These have been identified in *Arabidopsis* (RPS2 and RPM1 resistance proteins against *P. syringae*) and tomato (resistance protein I2 against *Fusarium oxysporum*). The third class of R gene family consists of extra cytoplasmic leucine-rich repeats (eLRR), associated to a transmembrane domain (TrD). The proteins are devoid of NBS motif. eLRRs are not directly engaged in pathogen recognition and/or activation of host defense genes. However, eLRRs play a significant role in certain defense proteins like polygalacturonase inhibiting proteins (PGIPs). The representative genes of this class include *C. fulvum* R genes (Cf-2, Cf-4, and Cf-9) that possess an eLRR, a membrane-spanning domain and a short cytoplasmic C-terminus region. The fourth class of resistance genes is characterized by Xa21, a rice R gene against Xanthomonas. The Xa21 consists of eLRR, TrD, and an intracellular serine-threonine kinase (KIN) domain as shown in Fig. 6.1. The group of proteins having a TrD fused to a putative CC domain (e.g., The *Arabidopsis* RPW8) constitutes the fifth class of R proteins. The sixth class of

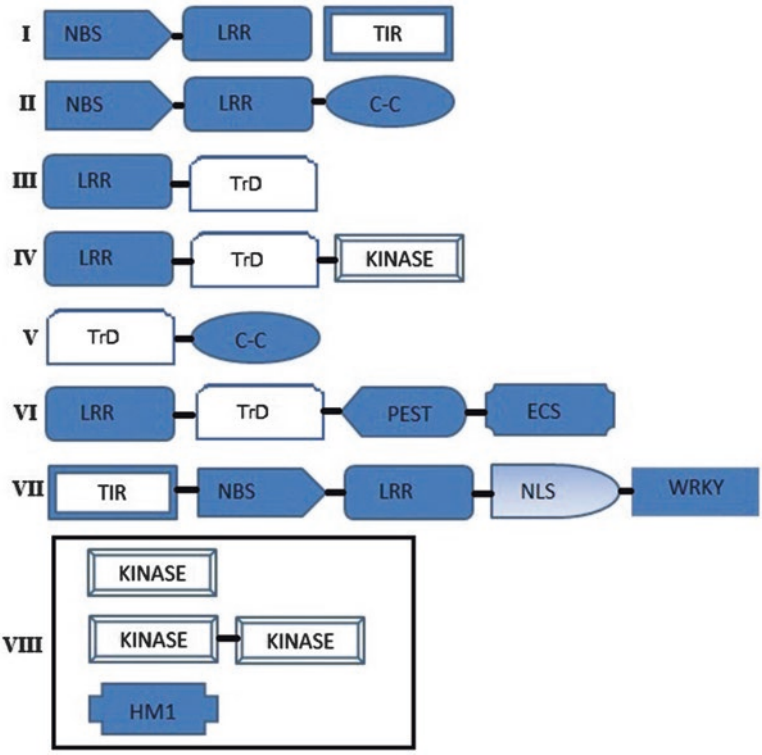


Fig. 6.1 Plant resistance proteins (R proteins): types and position of important domains. Nucleotide-binding site (*NBS*), leucine-rich repeats (*LRR*), coiled coil (*C-C*), transmembrane domain (*TrD*), interleukin-1-receptors (*TIRToll*), protein degradation domain (proline-glycine-serine-threonine, *PEST*), endocytosis cell signaling domain (*ECS*), nuclear localization signal (*NLS*), amino acid domain (*WRKY*), helminthosporium carbonum toxin reductase enzyme (*HM1*)

R proteins contains putative eLRRs associated to a PEST (Pro-Glu-Ser-Thr) domain for protein degradation and short protein motifs (ECS) that can target the protein for receptor mediated endocytosis. The examples of this group include tomato Ve1 and Ve2 R proteins. However, in few studies, Ve1 and Ve2 proteins have been classified as PAMP receptors. The *Arabidopsis* RRS1-R that confers resistance against phytopathogen *Ralstonia solanacearum* is a good representative of the seventh class of R proteins. These proteins have a C-terminal extension together with a WRKY domain as well as a presumed nuclear localization signal (NLS) sequence. The WRKY domain is the given name because of the presence of a conserved N-terminal amino acid (WRKYGQK) sequence along with a zinc finger-like motif.

Few enzymatic proteins have been categorized in the eighth class of plant R proteins. These are devoid of LRR or NBS domains. The enzyme HC toxin reductase, encoded by the maize Hm1 gene, detoxifies a specific cyclic tetrapeptide toxin, essentially required for pathogenicity, of the fungus (HC toxin). Hence, HC toxin reductase provides protection against southern corn leaf blight caused by

Cochliobolus carbonum (a fungal pathogen). Similarly, the Rpg1 gene from barley encodes a receptor kinase-like protein having two tandem protein kinase (kinase-kinase) domains. The protein does not have any membrane-spanning domain and other known sequences present in classical R proteins. However, the protein provides barley resistance against stem rust, hence considered a potential R protein (Jones et al. 1994).

6.3.3.2.3 Avr/R Protein Interaction

The Avr/R protein interaction determines the host specificity, pathogenicity level, degree of damage, and subsequent pathogen spread to nearby tissues. In addition, differentiation of pathogen as biotroph or necrotroph is also achieved by Avr/R protein interaction by initiating a crosstalk between response pathways and regulating balance of salicylic acid (a signal for resistance against biotrophs) and level of jasmonic acid along with ethylene (both promote defense against necrotrophs). However, it has been found that NB-LRR protein-mediated disease resistance is much more effective against biotrophs or hemi-biotrophs but not against necrotrophs (Glazebrook 2005).

In response to effectors, plants exhibit a second line of defense initiated by the recognition of a specific effector followed by triggering a stronger resistance response named as effector-triggered immunity (ETI). ETI is a quicker and robust version of PTI (Tao et al. 2003; Thilmony et al. 2006; Truman et al. 2006) that usually culminates in hypersensitive response (HR) characterized as death of infected cells. It generally does not extend beyond the infected area and help in restriction of pathogen growth. However, it is not always observed and not a requirement for triggering ETI. This Avr/R gene recognition pattern has been historically termed as "gene-for-gene resistance" (Gururani et al. 2012). Under pathogen favorable circumstances, the effector modulates the effector-mediated signal cascade and suppresses ETI (instead of activating it) which leads to effector-triggered susceptibility (ETS).

6.3.3.3 Mechanism of R Protein-Effector Interaction

6.3.3.3.1 Direct Interaction

The R protein may directly recognize pathogen-released effector, in a similar way like ligand binds its receptor (Fig. 6.2). This was elucidated by studying the interaction between rice Pita CC-NB-LRR immune receptor and *Magnaporthe grisea* (a fungus) AVR-Pita effector (Jia et al. 2000). A single amino acid substitution in the LRR abolished this interaction and resulted in loss of resistance. Similarly, RRS1-R immune receptor of *Arabidopsis* recognizes directly PopP2 (a bacterial effector) (Deslandes et al. 2003). Yeast two-hybrid analysis revealed a significant direct interaction between flax rust fungus AvrL effectors with corresponding plant immune receptors (encoded from L locus) leading to activation of resistance (Dodds et al. 2006).

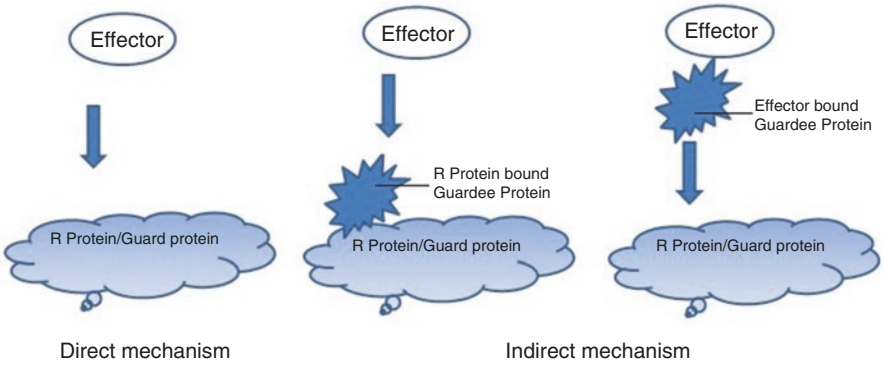


Fig. 6.2 Effector(s) and plant R (resistance) proteins: elucidation of direct and indirect mechanisms

6.3.3.3.2 Indirect Interaction: Guard Hypothesis

The R proteins may also interact with effectors indirectly in a more complex way. The guard hypothesis suggests that effectors induce modulations in certain host proteins (called guardee proteins) which are assessed by R proteins (guard protein). A guard-guardee protein interaction then activates a signal cascade for initiation of protective measures (Jones and Dangl 2006). Two variations exist in this hypothesis:

1. The guardee protein remains bound, constitutively, to guard protein even before an effector come and modulate the guardee.
2. The guard protein receptor interacts with its guardee, only after the later come in contact with an effector.

6.3.3.4 Evolution of Effector and Resistance Specificities

Interaction and two-way communications between pathogen and host plant cells led to the evolution and emergence of new groups of effectors and corresponding R proteins generating an arms race termed as gene-for-gene concept. The plant-microbe coevolution and plant immune responses can be described in a "zigzag" model consisted of four phases, initially proposed by (Jones and Dangl 2006):

Phase I: Plant recognition receptors (PRRs), located at cell surface, recognize PAMPs (or MAMPs) released from invading microbe(s) to trigger first line of defense, i.e., PTI that attempts to limit the invasion of a potential pathogen.

Phase II: Within a host cell, a successfully invaded pathogen synthesizes and releases effectors to initiate virulence by suppressing PTI. The interaction determines the plant-microbe relationship leading to effector-triggered susceptibility (ETS).

Phase III: Plants deploy intracellular immune proteins (i.e. R proteins/ NB-LRR proteins) to detect pathogen-initiated effectors. These proteins detect effectors either directly or indirectly. This triggers ETI, a stronger immune response that

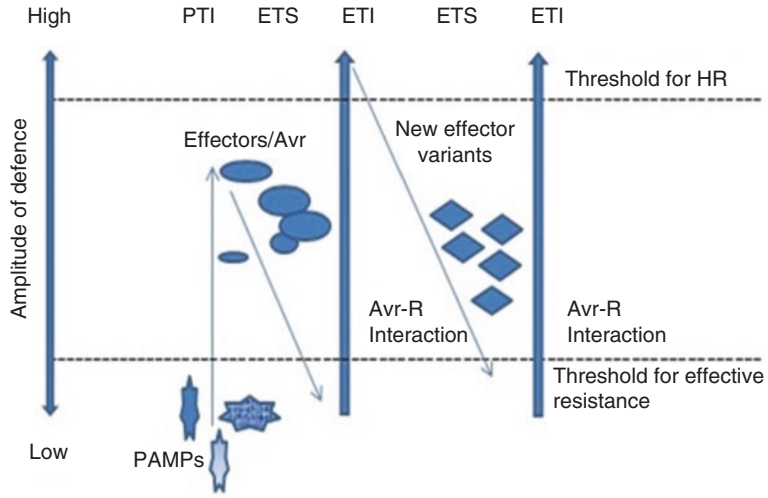


Fig. 6.3 Zigzag model: a depiction of coevolution of pathogen effectors and plant R (resistance) proteins as proposed by Jones and Dangl (2006)

provides resistance against a pathogen and often climaxes in tissue hypersensitive response (HR), i.e., a programmed cell death at the infection site.

Phase IV: In response to plant-induced pressure, the effectors undergo modifications to escape ETI. New variants of effectors evolve suppressing host ETI and triggering ETS again. The process of natural selection plays a vital role in the development of new effector and corresponding R specificities.

Hence, at population level, the coevolutionary arms race between a plant species and a pathogen determines the consequences of a pathogen attack with ETI and ETS occurring alternately as represented in Fig. 6.3.

6.3.3.5 Non-host Resistance

Majority of the pathogens fail to infect plants as these can resist an invaded pathogen and, hence, are supposed to be non-hosts. This non-host resistance is distinctly different from pathogen-mediated resistance, complex, durable, and a multigenic trait. There are two possible ways that lead to two types of non-host resistance mechanisms, i.e., type I and type II (Mysore and Ryu 2004).

6.3.3.5.1 Type I

A pathogen fails to suppress PTI and grow on a new evolutionarily divergent host due to its ineffective effectors. The host displays a strong PTI response but no ETI or HR. The attack of nonadapted barley pathogen, *B. graminis* f. sp. *hordei* (Bgh) on *Arabidopsis* (a non-host plant), results in enhanced synthesis of cell wall appositions (act as physical barriers) and antimicrobial metabolites to limit pathogen entry. But *Arabidopsis* did not display HR response in this non-host resistance mechanism (Thordal-Christensen 2003).

6.3.3.5.2 Type II

This non-host resistance involves recognition of pathogen effectors; its mechanism resembles ETI and often culminates at HR. It was observed that soybean, a non-host for *P. syringae* pv. tomato, recognized AvrA and AvrD effectors through Rpg2 and Rpg4 proteins when infected with *P. syringae* pv. tomato. Similarly, *AvrRxo1* produced by *Xanthomonas oryzae* pv. oryzae was recognized by maize (a non-host for the said pathogen) *Rxo1*. In addition, *Arabidopsis* displayed resistance to a fungal pathogen of Brassica (*Leptosphaeria maculans*) which was achieved by unlinked R proteins. Hence, poorly explored R protein-mediated responses also play significant role in broadening the resistance mechanisms and may minimize pathogen host specificity (Senthil-Kumar and Mysore 2013).

6.4 Conclusion

It can be concluded that a variety of mechanisms are utilized by microbes to interact with plants. These mechanisms are broadly classified into two categories, viz., symbiotic interactions and pathogenic interactions. Symbiotic interactions between plants and microbes involve a variety of activities of mutual benefits like nitrogen fixation, P solubilization, growth hormone production, and biocontrol with a variety of genetic and metabolic pathways involved. Plant defense responses against a pathogen can be categorized into two levels, i.e., basal resistance and pathogen-induced resistance. The former are early level responses initiated upon a pathogen recognition by host cell surface localized receptors, while the latter are induced by

Table 6.1 Some mechanisms of plant growth promotion by microorganisms

Type	Definition	Mechanism	References
Biofertilizer	A biological substance which improves the plant growth through increased nutrient acquisition	Biological nitrogen fixation, nutrient solubilization, and mobilization	Hanif et al. (2015), Kapoor et al. (2008), Rinaldi et al. (2008), Shahid et al. (2012), Somers et al. (2004), and Vessey (2003)
Phytostimulator	The substances with the ability to produce plant growth hormones like IAA, gibberillic acid, Cytokinins and ethylene	Production of phytohormones	Akram et al. (2016), Hanif et al. (2015), Lugtenberg et al. (2002),and Somers et al. (2004)
Biopesticide	The biological substances that indirectly promote plant growth by suppressing the plant diseases	Production of lytic enzymes, siderophores, antibiotics, HCN, and induced systemic resistance	Chandler et al. (2008), Somers et al. (2004), Vessey (2003), Ali et al. (2016), Somers et al. (2004), and Yasmeen et al. (2012)

Table 6.2 List of Avr genes (with plant pathogens) and corresponding R genes (with host plants)

Avr gene(s) (plant pathogen species)	R gene(s) (host plant species	References
Bacteria		
Avr-Bs2 (Xanthomonas campestris)	*Bs2 (Capsicum annuum)*	Minsavage et al. (1990)
Avr-Xa1 (X. oryzae)	*Xa1 (Oryza sativa)*	Yoshimura et al. (1998)
Avr-Pto, Avr-PtoB (Pseudomonas syringae pv. *tomato)*	*Pto (Lycopersicum esculentum)*	Abramovitch et al. (2003)
AvrRpm1 (P. syringae)	*RPM1 (Arabidopsis thaliana)*	Hubert et al. (2003)
AvrRpt2 (P. syringae)	*RPS2 (A. thaliana)*	Bent et al. (1994) and Whalen et al. (1991)
AvrRps4 (P. syringae)	*RPS4 (A. thaliana)*	Gassmann et al. (1999) and Hinsch and Staskawicz (1996)
AvrPphB (P. syringae)	*RPS5 (A. thaliana)*	Jenner et al. (2003) and Swiderski and Innes (2001)
Fungi		
AvrMla (Blumeria graminis)	*Mla (Hordeum vulgare)*	Zhou et al. (2001)
Avr2 (Cladosporium fulvum)	Cf-*2 (Lycopersicum esculentum)*	Rooney et al. (2005) and van Esse et al. (2008)
Avr4 (C. fulvum)	Cf-*4 (L. esculentum)*	Thomas et al. (1997)
Avr5 (C. fulvum)	Cf-*5 (L. esculentum)*	(Dixon et al. 1998)
Avr9 (C. fulvum)	Cf-*9d (L. esculentum)*	Jones et al. (1994)
Avr1 (Fusarium oxysporum)	*I2 (L. esculentum)*	Ori et al. (1997) and Simons et al. (1998)
AyrL AvrN, AvrL567 genes (Melampsora lini)	*L N, L5, L6, and L7 (Linum usitatissimum)*	Dodds et al. (2006) and Lawrence et al. (1995)
Avr-Pita (Magnaporthe grisea)	*Pi-ta (Oryza sativa)*	Jia et al. (2000) and Kang et al. (2005)
AvrRP-I-D (Puccinia sorghi)	*Rp1 (Zea mays)*	Collins et al. (1999)
Avr-Rpg1 (Puccinia graminis f sp. *tritici)*	*Rpg1 (Hordeum vulgare)*	Brueggeman et al. (2002) and Horvath et al. (2003)
Oomycetes		
Avr3 (Bremia lactucae)	*Dm3 (Lactuca sativa)*	Meyers et al. (1998) and Michelmore and Wong (2008)
ATR1 (Hyaloperonospora arabidopsis)	*RPP1-Nd/WsB (Arabidopsis thaliana)*	Rehmany et al. (2005)
ATR13 (H. arabidopsis)	*RPP13-Nd (A. thaliana)*	Alfano and Collmer (1996) and Bittner-Eddy et al. (2000)
AvrB, AvrRPP1A, AvrRPP1B, AvrRPP1C, AvrRPP2	*RPP1, RPP2, RPP4, RPP5, RPP8 (A. thaliana)*	Botella et al. (1998), McDowell et al. (1998), Parker et al. (1997), Van Der Biezen et al. (2002)
AvrRPP4, AvrRPP5, AvrRPP8 (Prenospora parasitica)		
Avr1 (Phytophthora infestans)	*R1 (Solanum tuberosum)*	Ballvora et al. (2002)

(continued)

Table 6.2 (continued)

Avr gene(s) (plant pathogen species)	R gene(s) (host plant species	References
Avr-blb1 (P. infestans)	*Rpi-blb1 (S. tuberosum)*	Vleeshouwers et al. (2008)
PiAvr2 (P. infestans)	*Rpi (S. tuberosum)*	Lokossou et al. (2009) and Vossen et al. (2005)
Avr3a (P. infestans)	*R3a (S. demissum)*	(Armstrong et al. 2005)
Ipio, Ipib, Ipi-o4 (P. infestans)	*RB (S. bulbocastanum)*	Champouret et al. (2009) and van West et al. (1998)
Avr3b-Avr10-Avr11 locus (P. infestans)	*R3b, R10, R11 (S. tuberosum)*	Jiang et al. (2006)
Avr1a, Avr3a and Avr3c (P. sojae)	*Rps1a, Rps3a, Rps3c (Glycine max)*	Dong et al. (2009), Mao and Tyler (1996), and Qutob et al. (2009)
Viruses		
Bean dwarf mosaic virus (Bdm)	*BV1 protein (Phaseolus vulgaris)*	Garrido-Ramirez et al. (2000)
Coat protein (cucumber mosaic virus)	*RCY1 (Arabidopsis thaliana)*	Takahashi et al. (2001)
Vpg (cucumber mosaic virus)	*At-eIF4E1 (cum1)/ At-eIF4G (cum2) (A. thaliana)*	Gallois et al. (2010) and Yoshii et al. (2004)
3'half of genome (lettuce mosaic virus)	*mo1, mol2 (Lactuca sativa)*	Gao et al. (2004) and Nicaise et al. (2003)
Vpg (pea seed borne mosaic virus)	*sbm1 (Pisum sativum)*	Keller et al. (1998)
Nla protease (potato virus X)	*Ry (S. tuberosum)*	Mestre et al. (2000)
Vpg (tobacco etch virus)	*Pot-1 (*Lycopersicon spp.	Moury et al. (2004)
Vpg (rice yellow mottle virus)	*eIF(iso)4G1 (Oryza sativa)*	Hébrard et al. (2010)
Hc-pro and P3 cistron (soybean mosaic virus)	*Rsv1 (Glycine max)*	Eggenberger and Hill (1997)
VPg (turnip mosaic virus)	*At-eIF(iso)4E (A. thaliana)*	Wittmann et al. (1997)
TuRBO1, TuRBO3, TuRBO4, TuRBO5, TuMV P3 (*Turnip mosaic virus)*	P3, CI *(Brassica napus)*	Jenner et al. (2000) and Jenner et al. (2003)

pathogen produced effectors and are largely controlled by plant resistance (R) proteins. There are both direct and indirect mechanisms by which pathogen effects interact with host resistance proteins. Moreover, the pathogen (or its effectors) and host resistance specificities continuously evolve together making an arms race named as "gene-for-gene concept" (Tables 6.1 and 6.2).

Acknowledgments We acknowledge Khadija Rafiq for critical reading and an anonymous reviewer for his/her suggestions to improve this chapter.

References

Abhilash P, Powell JR, Singh HB, Singh BK (2012) Plant–microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends Biotechnol 30:416–420

Abramovitch RB, Kim YJ, Chen S, Dickman MB, Martin GB (2003) *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J 22:60–69

Akram MS, Tariq M, Shahid M, Azeem M, Javed MT, Saleem S, Riaz S (2016) Deciphering Staphylococcus sciuri SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (Zea mays L.) Front Microbiol 7:867

Alfano JR, Collmer A (1996) Bacterial pathogens in plants: life up against the wall. Plant Cell 8:1683

Ali A, Hameed S, Imran A, Iqbal M, Iqbal J, Oresnik IJ (2016) Functional characterization of a soybean growth stimulator *Bradyrhizobium* sp. strain SR-6 showing acylhomoserine lactone production. FEMS Microbiol Ecol 92(9)

Arcand MM, Schneider KD (2006) Plant-and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. An Acad Bras Cienc 78:791–807

Armstrong MR et al (2005) An ancestral oomycete locus contains late blight avirulence gene Avr3a, encoding a protein that is recognized in the host cytoplasm. Proc Natl Acad Sci U S A 102:7766–7771

Arshad M, Frankenberger WT (1991) Microbial production of plant hormones. Plant Soil 133:1–8

Ausubel FM (2005) Are innate immune signaling pathways in plants and animals conserved? Nat Immunol 6:973–979

Babu-Khan S, Yeo TC, Martin WL, Duron MR, Rogers RD, Goldstein AH (1995) Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia*. Appl Environ Microbiol 61:972–978

Baca BE, Elmerich C (2007) Microbial production of plant hormones, in: associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Springer, Berlin

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant–microbe interactions. Curr Opin Biotechnol 20:642–650

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Bakker PAHM, Pieterse CMJ, Van Loon LC (2007) Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology 97:239–243

Ballvora A et al (2002) The R1 gene for potato resistance to late blight (Phytophthora infestans) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant J 30:361–371

Barazani OZ, Friedman J (1999) Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? J Chem Ecol 25:2397–2406

Bent AF et al (1994) RPS2 of Arabidopsis thaliana: a leucine-rich repeat class of plant disease resistance genes. Sci-New York Then Washington 2:1856–1856

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bishop PE, Premakumar R (1992) Alternative nitrogen fixation systems. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman & Hall, New York, pp 736–762

Bittner-Eddy PD, Crute IR, Holub EB, Beynon JL (2000) RPP13 is a simple locus in Arabidopsis thaliana for alleles that specify downy mildew resistance to different avirulence determinants in Peronospora parasitica. Plant J 21:177–188

Botella MA et al (1998) Three genes of the Arabidopsis RPP1 complex resistance locus recognize distinct Peronospora parasitica avirulence determinants. Plant Cell 10:1847–1860

Brueggeman R et al (2002) The barley stem rust-resistance gene Rpg1 is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci 99:9328–9333

Champouret N et al (2009) Phytophthora infestans isolates lacking class I ipiO variants are virulent on Rpi-blb1 potato. Mol Plant-Microbe Interact 22:1535–1545

Chandler D, Davidson G, Grant WP, Greaves J, Tatchell GM (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Technol 19:275–283

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Collins N, Drake J, Ayliffe M, Sun Q, Ellis J, Hulbert S, Pryor T (1999) Molecular characterization of the maize Rp1-D rust resistance haplotype and its mutants. Plant Cell 11:1365–1376

Danhorn T, Hentzer M, Givskov M, Parsek MR, Fuqua C (2004) Phosphorus limitation enhances biofilm formation of the plant pathogen Agrobacterium tumefaciens through the PhoR-PhoB regulatory system. J Bacteriol 186:4492–4501

Davies PJ (2010) The plant hormones: their nature, occurrence, and functions. In: Davies PJ (ed) Plant hormones physiology, biochemistry and molecular biology. Kluwer Academic Publishers, Dordrecht, pp 1–15

de Jonge R et al (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science 329:953–955

Dean DR, Jacobson MR (1992) Biochemical genetics of nitrogenase. In: Stacey G, Burris RH, Evans DJ (eds) Biological nitrogen fixation. Chapman & Hall, New York, pp 763–834

Deng S, Summers ML, Khan ML, McDermott TR (1998) Cloning and characterization of a *Rhizobium meliloti* nonspecific acid phosphatase. Arch Microbiol 170:18–26

Deng S, Elkins JG, Da LH, Botero LM, McDermott TR (2001) Cloning and characterization of a second acid phosphatase from *Sinorhizobium meliloti* strain 104A14. Arch Microbiol 176:255–263

Desbrosses G, Contesto C, Varoquaux F, Galland M, Touraine B (2009) PGPR-Arabidopsis interactions is a useful system to study signaling pathways involved in plant developmental control. Plant Signal Behav 4:321–323

Deslandes L, Rivas S (2012) Catch me if you can: bacterial effectors and plant targets. Trends Plant Sci 17:644–655

Deslandes L et al (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc Natl Acad Sci 100:8024–8029

Dilworth MJ (1966) Acetylene reduction by nitrogen-fixing preparations from *Clostridium pasteurianum*. Biochim Biophys Acta 127:285–294

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD (1998) The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 10:1915–1925

Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548

Dodds PN et al (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proc Natl Acad Sci 103:8888–8893

Dong S, Qutob D, Tedman-Jones J, Kuflu K, Wang Y, Tyler BM, Gijzen M (2009) The Phytophthora sojae avirulence locus Avr3c encodes a multi-copy RXLR effector with sequence polymorphisms among pathogen strains. PLoS One 4:e5556

Duan J, Müller KM, Charles TC, Vesely S, Glick BR (2009) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase genes in rhizobia from southern Saskatchewan. Microb Ecol 57:423–436

Dubey RK, Tripathi V, Abhilash P (2015) Book review: principles of plant-microbe interactions: microbes for sustainable agriculture. Front Plant Sci 6

Egamberdiyeva D (2005) Plant-growth-promoting rhizobacteria isolated from a Calcisol in a semi-arid region of Uzbekistan: biochemical characterization and effectiveness. J Plant Nutr Soil Sci 168:94–99

Eggenberger A, Hill J (1997) Analysis of resistance-breaking determinants of soybean mosaic virus. Phytopathology 87:S27

Einsle O, Tezcan FA, Andrade SLA, Schmid B, Yoshida M, Howard JB, Rees DC (2002) Nitrogenase MoFe-protein at 1.16 a resolution: a central ligand in the FeMo-cofactor. Science 297:1696–1700

Falkowski PG (1997) Evolution of the nitrogen cycle and its influence on the biological sequestration of CO2 in the ocean. Nature 387:272–275

Fani R, Gallo R, Liò P (2000) Molecular evolution of nitrogen fixation: the evolutionary history of the nifD, nifK, nifE, and nifN genes. J Mol Evol 51:1–11

Fawke S, Doumane M, Schornack S (2015) Oomycete interactions with plants: infection strategies and resistance principles. Microbiol Mol Biol Rev 79:63–65

Fiore MF, Neilan BA, Copp JN, Rodrigues JLM, Tsai SM, Lee H, Trevors JT (2005) Characterization of nitrogen-fixing cyanobacteria in the Brazilian Amazon floodplain. Water Res 39:5017–5026

Franco-Correa M, Quintana A, Duque C, Suarez C, Rodríguez MX, Barea JM (2010) Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl Soil Ecol 45:209–217

Gallois J-L et al (2010) Single amino acid changes in the turnip mosaic virus viral genome-linked protein (VPg) confer virulence towards Arabidopsis thaliana mutants knocked out for eukaryotic initiation factors eIF (iso) 4E and eIF (iso) 4G. J Gen Virol 91:288–293

Gao Z, Johansen E, Eyers S, Thomas CL, Noel Ellis T, Maule AJ (2004) The potyvirus recessive resistance gene, sbm1, identifies a novel role for translation initiation factor eIF4E in cell to cell trafficking. Plant J 40:376–385

Garrido-Ramirez E, Sudarshana M, Lucas W, Gilbertson R (2000) Bean dwarf mosaic virus BV1 protein is a determinant of the hypersensitive response and avirulence in *Phaseolus vulgaris*. Mol Plant-Microbe Interact 13:1184–1194

Gassmann W, Hinsch ME, Staskawicz BJ (1999) The Arabidopsis RPS4 bacterial resistance gene is a member of the TIR NBS LRR family of disease resistance genes. Plant J 20:265–277

Ghosh S, Penterman JN, Little RD, Chavez R, Glick BR (2003) Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica campestris*. Plant Physiol Biochem 41:277–281

Gimenez-Ibanez S, Hann DR, Ntoukakis V, Petutschnig E, Lipka V, Rathjen JP (2009) AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. Curr Biol 19:423–429

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227

Göhre V et al (2008) Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. Curr Biol 18:1824–1832

Goldstein AH (1994) Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous mineral phosphates by gram-negative bacteria. In: Torriani-Gorini A, Yagil E, Silver S (eds) Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington, DC, pp 197–203

Goldstein AH, Liu ST (1987) Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Nat Biotechnol 5:72–74

Golovan S, Wang G, Zhang J, Forsberg CW (1999) Characterization and overproduction of the *Escherichia coli* appA encoded bifunctional enzyme that exhibits both phytase and acid phosphatase activities. Can J Microbiol 46:59–71

Govindasamy V, Senthilkumar M, Gaikwad K, Annapurna K (2008) Isolation and characterization of ACC deaminase gene from two plant growth-promoting rhizobacteria. Curr Microbiol 57:312–317

Gulati A, Sharma N, Vyas P, Sood S, Rahi P, Pathania V, Prasad R (2010) Organic acid production and plant growth promotion as a function of phosphate solubilization by *Acinetobacter rhizosphaerae* strain BIHB 723 isolated from the cold deserts of the trans-Himalayas. Arch Microbiol 192:975–983

Gururani MA, Venkatesh J, Upadhyaya CP, Nookaraju A, Pandey SK, Park SW (2012) Plant disease resistance genes: current status and future directions. Physiol Mol Plant Pathol 78:51–65
Halbleib CM, Ludden PW (2000) Regulation of biological nitrogen fixation. J Nutr 130:1081–1084
Hameed S, Yasmin S, Malik KA, Zafar Y, Hafeez FY (2004) *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* strains isolated from cultivated legumes. Biol Fertil Soils 39:179–185
Hanif K, Hameed S, Imran A, Naqqash T, Shahid M, Van Elsas JD (2015) Isolation and characterization of a β-propeller gene containing phosphobacterium *Bacillus subtilis* strain KPS-11 for growth promotion of potato (*Solanum tuberosum* L.) Front Microbiol 6:1–12. doi:10.3389/fmicb.2015.00583
He P et al (2004) Activation of a COI1-dependent pathway in Arabidopsis by Pseudomonas syringae type III effectors and coronatine. Plant J 37:589–602
Hébrard E et al (2010) Direct interaction between the Rice yellow mottle virus (RYMV) VPg and the central domain of the rice eIF (iso) 4G1 factor correlates with rice susceptibility and RYMV virulence. Mol Plant-Microbe Interact 23:1506–1513
Hilda R, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–359
Hill S (1992) Physiology of nitrogen fixation in free-living heterotrophs, vol 87. Chapman & Hall, New York
Hinsch M, Staskawicz B (1996) Identification of a new Arabidopsis disease resistance locus, RPS4, and cloning of the corresponding avirulence gene, avrRps4, from *Pseudomonas syringae* pv. *pisi*. Mol Plant-Microbe Interact 9:55–61
Horvath H, Rostoks N, Brueggeman R, Steffenson B, von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the Rpg1 gene. Proc Natl Acad Sci 100:364–369
Hubert DA, Tornero P, Belkhadir Y, Krishna P, Takahashi A, Shirasu K, Dangl JL (2003) Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. EMBO J 22:5679–5689
Hungria M, Vargas MAT (2000) Environmental factors affecting N2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Res 65:151–164
Idriss EE et al (2002) Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology 148:2097–2109
Illmer P, Schinner F (1995) Solubilization of inorganic calcium phosphates – solubilization mechanisms. Soil Biol Biochem 27:257–263
Jaeger CH III, Lindow SE, Miller W, Clark E, Firestone MK (1999) Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Appl Environ Microbiol 65:2685–2690
Jenner CE, Sanchez F, Nettleship S, Foster G, Ponz F, Walsh J (2000) The cylindrical inclusion gene of turnip mosaic virus encodes a pathogenic determinant to the Brassica resistance gene TuRB01. Mol Plant-Microbe Interact 13:1102–1108
Jenner CE, Wang X, Tomimura K, Ohshima K, Ponz F, Walsh JA (2003) The dual role of the potyvirus P3 protein of turnip mosaic virus as a symptom and avirulence determinant in brassicas. Mol Plant-Microbe Interact 16:777–784
Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J 19:4004–4014
Jiang RH, Weide R, van de Vondervoort PJ, Govers F (2006) Amplification generates modular diversity at an avirulence locus in the pathogen Phytophthora. Genome Res 16:827–840
Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329
Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JD (1994) Isolation of the tomato Cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. Sci-New York Then Washington 3:789–789

Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B (2006) Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. Mol Plant-Microbe Interact 19:250–256

Kang BC, Yeam I, Frantz JD, Murphy JF, Jahn MM (2005) The pvr1 locus in *Capsicum* encodes a translation initiation factor eIF4E that interacts with tobacco etch virus VPg. Plant J 42:392–405

Kapoor R, Sharma D, Bhatnagar A (2008) Arbuscular mycorrhizae in micropropagation systems and their potential applications. Sci Hortic 116:227–239

Keller KE, Johansen E, Martin RR, Hampton R (1998) Potyvirus genome-linked protein (VPg) determines pea seed-borne mosaic virus pathotype-specific virulence in *Pisum sativum*. Mol Plant-Microbe Interact 11:124–130

Kerovuo J, Lauraeus M, Nurminen P, Kalkkinen N, Apajalahti J (1998) Isolation, characterization, molecular gene cloning, and sequencing of a novel phytase from *Bacillus subtilis*. Appl Environ Microbiol 64:2079–2085

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96:473–480

Kim KY, McDonald GA, Jordan D (1997) Solubilization of hydroxypatite by *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. Biol Fertil Soils 24:347–352

Kim KY, Jordan D, Krishnan HB (1998a) Expression of genes from *Rahnella aquatilis* that are necessary for mineral phosphate solubilization in *Escherichia coli*. FEMS Microbiol Lett 159:121–127

Kim YO, Lee JK, Kim HK, Yu JH, Oh TK (1998b) Cloning of the thermostable phytase gene (phy) from *Bacillus* sp. DS11 and its overexpression in *Escherichia coli*. FEMS Microbiol Lett 162:185–191

Kremer RJ, Kennedy AC (1996) Rhizobacteria as biocontrol agents of weeds. Weed Technol 10:601–609

Lawrence GJ, Finnegan EJ, Ayliffe MA, Ellis JG (1995) The L6 gene for flax rust resistance is related to the Arabidopsis bacterial resistance gene RPS2 and the tobacco viral resistance gene N. Plant Cell 7:1195–1206

Leveau JHJ, Lindow SE (2005) Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. Appl Environ Microbiol 71:2365–2371

Li M, Osaki M, Rao IM, Tadano T (1997) Secretion of phytase from roots of several plant species under phosphorus-deficient conditions. Plant Soil 195:161–169

Li Y, Huang F, Lu Y, Shi Y, Zhang M, Fan J, Wang W (2013) Mechanism of plant–microbe interaction and its utilization in disease-resistance breeding for modern agriculture. Physiol Mol Plant Pathol 83:51–58

Liu ST et al (1992) Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101: nucleotide sequence and probable involvement in biosynthesis of the coenzyme pyrroloquinoline quinone. J Bacteriol 174:5814–5819

Lokossou AA et al (2009) Exploiting knowledge of R/Avr genes to rapidly clone a new LZ-NBS-LRR family of late blight resistance genes from potato linkage group IV. Mol Plant-Microbe Interact 22:630–641

Lugtenberg BJJ, Chin-A-Woeng TFC, Bloemberg GV (2002) Microbe–plant interactions: principles and mechanisms. Antonie Leeuwenhoek 81:373–383

Lung S, Leung A, Kuang R, Wang Y, Leung P, Lim B (2008) Phytase activity in tobacco (*Nicoana tobacum*) root exudates is exhibited by purple acid phosphatase. Phytochemistry 69:365–373

Macaskie LE, Yong P, Doyle TC, Roig MG, Diaz M, Manzano T (1997) Bioremediation of uranium-bearing wastewater: biochemical and chemical factors affecting bioprocess application. Biotechnol Bioeng 53:100–109

Mao Y, Tyler BM (1996) The *Phytophthora sojae* genome contains tandem repeat sequences which vary from strain to strain. Fungal Genet Biol 20:43–51

Maria CT, Lombardi G, Berlutti F, Schippa S, Gian MR (1995) Cloning and characterization of the NapA acid phosphatase/phosphotransferase of *Morganella morganii*: identification of a new family of bacterial acid-phosphatase-encoding genes. Microbiology 141:147–154

McDowell JM, Dhandaydham M, Long TA, Aarts MG, Goff S, Holub EB, Dangl JL (1998) Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of Arabidopsis. Plant Cell 10:1861–1874
Mesarich CH et al (2016) A conserved proline residue in Dothideomycete Avr4 effector proteins is required to trigger a Cf4 dependent hypersensitive response. Mol Plant Pathol 17:84–95
Mestre P, Brigneti G, Baulcombe DC (2000) An Ry-mediated resistance response in potato requires the intact active site of the NIa proteinase from potato virus Y. Plant J 23:653–661
Meulenberg JJM, Sellink E, Riegman NH, Postma PW (1992) Nucleotide sequence and structure of the *Klebsiella pneumoniae pqq* operon. Mol Gen Genet 232:284–294
Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore RW (1998) Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 10:1833–1846
Michelmore R, Wong J (2008) Classical and molecular genetics of *Bremia lactucae*, cause of lettuce downy mildew. Eur J Plant Pathol 122:19–30
Minsavage G, Dahlbeck D, Whalen M, Kearney B, Bonas U, Staskawicz B, Stall R (1990) Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. vesicatoria—pepper interactions. Mol Plant-Microbe Interact 3:41–47
Moury B et al (2004) Mutations in potato virus Y genome-linked protein determine virulence toward recessive resistances in *Capsicum annuum* and *Lycopersicon hirsutum*. Mol Plant-Microbe Interact 17:322–329
Mullen MD (2005) Phosphorus in soils: biological interactions. In: Hillel D (ed) Encyclopedia of soils in the environment. Elsevier Ltd., Oxford, pp 210–215
Murphy DV, Recous S, Stockdale EA, Fillery IRP, Jensen LS, Hatch DJ, Goulding KWT (2003) Gross nitrogen fluxes in soil: theory, measurement and application of< sup> 15 N pool dilution techniques. Adv Agron 79:69–118
Mysore KS, Ryu CM (2004) Non-host resistance: how much do we know? Trends Plant Sci 9:97–104
Nahas E (1996) Factors determining rock phosphate solubilization by microorganisms isolated from soil. World J Microbiol Biotechnol 12:567–572
Nicaise V et al (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the potyvirus lettuce mosaic virus. Plant Physiol 132:1272–1282
Nürnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev 198:249–266
Ori N et al (1997) The I2C family from the wilt disease resistance locus I2 belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. Plant Cell 9:521–532
Osorio NW, Habte M (2015) Effect of a phosphate-solubilizing fungus and an arbuscular mycorrhizal fungus on leucaena seedlings in tropical soils with contrasting phosphate sorption capacity. Plant Soil 389:375–385
Parker JE et al (1997) The Arabidopsis downy mildew resistance gene RPP5 shares similarity to the toll and interleukin-1 receptors with N and L6. Plant Cell 9:879–894
Perez E, Sulbaran M, Ball MM, Yarzabal LA (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem 39:2905–2914
Postgate JR (1982) The fundamentals of nitrogen fixation. Cambridge University Press, Cambridge
Qutob D et al (2009) Copy number variation and transcriptional polymorphisms of Phytophthora sojae RXLR effector genes Avr1a and Avr3a. PLoS One 4:e5066
Rajendran G, Patel MH, Joshi SJ (2012) Isolation and characterization of nodule-associated *Exiguobacterium* sp. from the root nodules of fenugreek (*Trigonella foenum-graecum*) and their possible role in plant growth promotion. Int J Microbiol 2012:256–261
Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51:1061–1069
Rehmany AP et al (2005) Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two Arabidopsis lines. Plant Cell 17:1839–1850

Reilly TJ, Baron GS, Nano FE, Kuhlenschmidt MS (1996) Characterization and sequencing of a respiratory burst-inhibiting acid phosphatase from *Francisella tularensis*. J Biol Chem 271:10973–10983

Richardson AE (1994) Soil microorganisms and phosphorous availability. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Soil biota: Management in sustainable farming systems. CSIRO, East Melbourne, pp 50–62

Richardson AE, Hadobas PA, Hayes JE (2001a) Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. Plant J 25:641–649

Richardson AE, Hadobas PA, Hayes JE, O'Hara CP, Simpson RJ (2001b) Utilization of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil micro-organisms. Plant Soil 229:47–56

Rinaldi A, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: seperating the wheat from the chaff fungal divers. Fungal Divers 33:1–45

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rodriguez E, Han Y, Lei XG (1999) Cloning, sequencing, and expression of an *Escherichia coli* acid phosphatase/phytase gene (*appA2*) isolated from pig colon. Biochem Biophys Res Commun 257:117–123

Rodriguez H, Gonzalez T, Selman G (2000) Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. J Biotechnol 84:155–161

Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91:552–555

Rooney HC, van't Klooster JW, van der Hoorn RA, Joosten MH, Jones JD, de Wit PJ (2005) Cladosporium Avr2 inhibits tomato Rcr3 protease required for Cf-2-dependent disease resistance. Science 308:1783–1786

Rossolini GM, Schippa S, Riccio ML, Berlutti F, Macaskie LE, Thaller MC (1998) Bacterial nonspecific acid phosphohydrolases: physiology, evolution and use as tools in microbial biotechnology. Cell Mol Life Sci 54:833–850

Rovira AD (1991) Rhizosphere research-85 years of progress and frustration. In: Kleister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Amsterdam, pp 3–13

Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in *Enterobacter cloacae* UW5. J Bacteriol 190:7200–7208

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21:1–30

Saleem M, Moe LA (2014) Multitrophic microbial interactions for eco-and agro-biotechnological processes: theory and practice. Trends Biotechnol 32:529–537

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Senthil-Kumar M, Mysore KS (2013) Non-host resistance against bacterial pathogens: retrospectives and prospects. Annu Rev Phytopathol 51:407–427

Seo PJ, Park CM (2009) Auxin homeostasis during lateral root development under drought condition. Plant Signal Behav 4:1002–1004

Shahid M, Hameed S, Imran A, Ali S, van Elsas JD (2012) Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11. World J Microbiol Biotechnol 28:2749–2758. doi:10.1007/s11274-012-1086-2

Shahid M, Hameed S, Tariq M, Zafar M, Ali A, Ahmad N (2015) Characterization of mineral phosphate-solubilizing bacteria for enhanced sunflower growth and yield-attributing traits. Ann Microbiol 65:1525–1536. doi:10.1007/s13213-014-0991-z

Shang Y et al (2006) RAR1, a central player in plant immunity, is targeted by *Pseudomonas syringae* effector AvrB. Proc Natl Acad Sci 103:19200–19205

Shoda M (2000) Bacterial control of plant diseases. J Biosci Bioeng 89:515–521

Siddiqui Z (2006) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui Z (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 111–142
Simons G et al (1998) Dissection of the Fusarium I2 gene cluster in tomato reveals six homologs and one active gene copy. Plant Cell 10:1055–1068
Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240
Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448
Stergiopoulos I, de Wit PJ (2009) Fungal effector proteins. Annu Rev Phytopathol 47:233–263
Stevenson FJ, Cole MA (1999) Cycles of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients. Wiley, Chichester
Streeter TC, Bol R, Bardgett RD (2000) Amino acids as a nitrogen source in temperate upland grasslands: the use of dual labelled (13C, 15N) glycine to test for direct uptake by dominant grasses. Rapid Commun Mass Spectrom 14:1351–1355
Strop P, Takahara PM, Chiu HJ, Angove HC, Burgess BK, Rees DC (2001) Crystal structure of the all-ferrous [4Fe-4S] 0 form of the nitrogenase iron protein from *Azotobacter vinelandii*. Biochemistry 40:651–656
Swiderski MR, Innes RW (2001) The Arabidopsis PBS1 resistance gene encodes a member of a novel protein kinase subfamily. Plant J 26:101–112
Sy A et al (2001) Methylotrophic methylobacterium bacteria nodulate and fix nitrogen in symbiosis with legumes. J Bacteriol 183:214–220
Takahashi H et al (2001) Mapping the virus and host genes involved in the resistance response in cucumber mosaic virus-infected *Arabidopsis thaliana*. Plant Cell Physiol 42:340–347
Tao Y et al (2003) Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen Pseudomonas syringae. Plant Cell 15:317–330
Tariq M, Hameed S, Yasmeen T, Ali A (2012) Non-rhizobial bacteria for improved nodulation and grain yield of mung bean [*Vigna radiata* (L.) Wilczek]. Afr J Biotechnol 11:15012–15019
Teixeira DA, Alfenas AC, Mafia RG, Ferreira EM, Siqueira L, Maffia LA, Mounteer AH (2007) Rhizobacterial promotion of eucalypt rooting and growth. Braz J Microbiol 38:118–123
Thaller MC, Berlutti F, Schippa S, Lombardi G, Rossolini GM (1994) Characterization and sequence of PhoC, the principal phosphate-irrepressible acid phosphatase of *Morganella morganii*. Microbiology 140:1341–1350
Thaller MC, Berlutti F, Schippa S, Iori P, Passariello C, Rossolini GM (1995) Heterogeneous patterns of acid phosphatases containing low-molecular-mass polypeptides in members of the family *Enterobacteriaceae*. Int J Syst Bacteriol 45:255–261
Thilmony R, Underwood W, He SY (2006) Genome wide transcriptional analysis of the Arabidopsis thaliana interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli* O157: H7. Plant J 46:34–53
Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones J (1997) Characterization of the tomato Cf-4 gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. Plant Cell 9:2209–2224
Thordal-Christensen H (2003) Fresh insights into processes of non-host resistance. Curr Opin Plant Biol 6:351–357
Tien TM, Gaskins MH, Hubbell DH (1979) Plant growth substances produced by *Azospirillum brazilense* and their effect on the growth of pearl millet. Appl Environ Microbiol 37:1016–1024
Tilak K et al (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci 89:136–150
Triplett EW, Roberts GP, Ludden PW, Handelsman J (1989) What's new in nitrogen fixation. ASM News 55:15–21
Trivedi P, Sa T (2008) *Pseudomonas corrugata* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. Curr Microbiol 56:140–144
Truman W, Zabala MT, Grant M (2006) Type III effectors orchestrate a complex interplay between transcriptional networks to modify basal defence responses during pathogenesis and resistance. Plant J 46:14–33

Tye A, Siu F, Leung T, Lim B (2002) Molecular cloning and the biochemical characterization of two novel phytases from *B. subtilis* 168 and *B. licheniformis*. Appl Microbiol Biotechnol 59:190–197

Van Der Biezen EA, Freddie CT, Kahn K, Jones JD (2002) Arabidopsis RPP4 is a member of the RPP5 multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components. Plant J 29:439–451

van Esse HP et al (2008) The Cladosporium fulvum virulence protein Avr2 inhibits host proteases required for basal defense. Plant Cell 20:1948–1963

van Rhijn P, Vanderleyden J (1995) The Rhizobium-plant symbiosis. Microbiol Rev 59:124–142

van West P, de Jong AJ, Judelson HS, Emons AMC, Govers F (1998) TheipiO gene of *Phytophthora infestans* is highly expressed in invading hyphae during infection. Fungal Genet Biol 23:126–138

Vassilev N, Vassileva M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71:137–144

Venieraki A et al (2011) Characterization of nitrogen-fixing bacteria isolated from field-grown barley, oat, and wheat. J Microbiol 49:525–534

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vleeshouwers VG et al (2008) Effector genomics accelerates discovery and functional profiling of potato disease resistance and Phytophthora infestans avirulence genes. PLoS One 3:e2875

Vossen EA et al (2005) The Rpi-blb2 gene from *Solanum bulbocastanum* is an Mi-1 gene homolog conferring broad spectrum late blight resistance in potato. Plant J 44:208–222

Weller DM (1988) Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:379–407

Weller DM, Raaijmakers JM, Gardener BBMS, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Whalen MC, Innes RW, Bent AF, Staskawicz BJ (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell 3:49–59

Whitelaw MA (1999) Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv Agron 69:99–151

Wittmann S, Chatel H, Fortin MG, Laliberté J-F (1997) Interaction of the viral protein genome linked of turnip mosaic potyvirus with the translational eukaryotic initiation factor (iso) 4E ofArabidopsis thalianaUsing the yeast two-hybrid system. Virology 234:84–92

Xiang T et al (2008) Pseudomonas syringae effector AvrPto blocks innate immunity by targeting receptor kinases. Curr Biol 18:74–80

Xu CW et al (2012) Changes in non-symbiotic nitrogen-fixing bacteria inhabiting rhizosphere soils of an invasive plant *Ageratina adenophora*. Appl Soil Ecol 54:32–38

Yasmeen T, Hameed S, Tariq M, Iqbal J (2012) *Vigna radiata* root associated mycorrhizae and their helping bacteria for improving crop productivity. Pak J Bot 44:87–94

Yoshii M, Nishikiori M, Tomita K, Yoshioka N, Kozuka R, Naito S, Ishikawa M (2004) The Arabidopsis cucumovirus multiplication 1 and 2 loci encode translation initiation factors 4E and 4G. J Virol 78:6102–6111

Yoshimura S et al (1998) Expression of Xa1, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci 95:1663–1668

Young JPW (1992) Phylogenetic classification of nitrogen-fixing organisms. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman & Hall, New York, pp 43–86

Zeng W, Melotto M, He SY (2010) Plant stomata: a checkpoint of host immunity and pathogen virulence. Curr Opin Biotechnol 21:599–603

Zhang X, Shen A, Wang Q, Chen Y (2012) Identification and nitrogen fixation effects of symbiotic *Frankia* isolated from *Casuarina* spp. in Zhejiang, China. Afr J Biotechnol 11:4022–4029

Zhou F et al (2001) Cell-autonomous expression of barley Mla1 confers race-specific resistance to the powdery mildew fungus via a Rar1-independent signaling pathway. Plant Cell 13:337–350

Nucleic Acid Extraction for Studying Plant-Microbe Interactions in Rhizosphere

7

Gautam Anand, Abhineet Sain, Virendra S. Bisaria, and Shilpi Sharma

Abstract

Studies on diversity of microbial community in the field of rhizosphere ecology vastly rely on nucleic acid markers for analysis. The extraction can be a tedious and complicated task owing to the vast heterogeneity present in soil in terms of organic and inorganic constituents, texture and moisture content, and also the huge repertoire of life forms that it nurtures. There is no universal method of extraction for all soil types. The various challenges presented by the soil constituents make it an ever-evolving process. Cell lysis is an inherent part of any extraction process, with the extraction methodology exerting a huge impact on purity and yield of nucleic acid. Different extraction methods employed so far can be classified under two main categories based on the step of lysis: indirect lysis and direct lysis methods. Humic acid is a persistent contaminant that has the maximum impact on nucleic acid quality, along with its interference with several downstream analyses. Several methods have been optimized for removal of soil organic content. With the realization of the importance of RNA component in providing a deeper insight into the functionality of the system, the co-extraction of DNA and RNA is a trending technique, with vast emphasis on the removal of humic acid, and purity of the extracted DNA/RNA.

Keywords

Cell lysis • Humic acid • Co-extraction • DNA • RNA

G. Anand • A. Sain • V.S. Bisaria • S. Sharma (✉)
Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India
e-mail: shilpi@dbeb.iitd.ac.in

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_7

7.1 Introduction

Soil is a multiphasic complex of minerals, organic components, porous components, and a huge repertoire of life forms. Microbes are the most abundant living organisms in soil. These microbes play a crucial role in various biogeochemical cycles. Numerous studies have targeted the diversity of soil microflora in order to develop a deeper understanding of the biogeochemical cycles that are vital for the maintenance of ecosystem. Changes in soil microbial community structure have been linked to functional capabilities of soil (Nannipieri et al. 2003). Cultivation-dependent techniques have been employed over the years to tap the soil microbial diversity. Despite the advances in media formulation and other technical steps, only 1–2% of the total soil microbial community has been elucidated (Amann et al. 1995). Cultivation-independent approaches have offered a deeper insight in capturing the total soil microbial community. Analyses using molecular markers such as nucleic acid that can be directly extracted from the environment are extensively in use for elucidation of microbial community. The extraction procedure mainly includes cell lysis, purification of nucleic acid from other cell constituents, and finally extraction of the purified nucleic acid (Fig. 7.1). Extraction of nucleic acid from soil presents various challenges as soil is a complex amalgam of diverse substances, along with the vast abundance of microbes with different spatial adherences and anatomy that places a limitation in terms of targeting the soil community employing a common procedure. Soil organic contents are one of the major contaminants that affect the extraction process, and the downstream applications such as polymerase chain reaction (PCR), quantitative PCR (qPCR), metagenomic library preparation, etc., require nucleic acid with specific purity requirements (Tebbe and Vahajen 1993). Since different soil types have diverse heterogeneity, there is no universal methodology for all soil types. Consequently, constant standardization and optimization are needed for different soil types and also for the microbial community being targeted. This chapter focuses on different extraction procedures developed up till now for the extraction of nucleic acid from rhizospheric soil for understanding plant-microbe interactions in the system.

7.2 Cell Lysis

There are numerous methods for isolation of nucleic acid from soil that can largely be subdivided into two categories based on lysing strategies, i.e., indirect lysis method (cell extraction approach) and direct lysis method (based upon cell lysis in matrix). Both the methods have been well documented and cater to different downstream applications. The cell extraction method involves a prior extraction of cells from the matrix of the soil before being lysed. This method has been shown to be biased for certain cell types (mainly bacteria) (Steffan et al. 1988; Courtious et al. 2001). Cells strongly adhered to soil particles may be under-represented. Also, spores have less likelihood to be extracted via this approach (Roose-Amsaleg et al. 2001). Nevertheless, the cell extraction method enables the extraction of larger

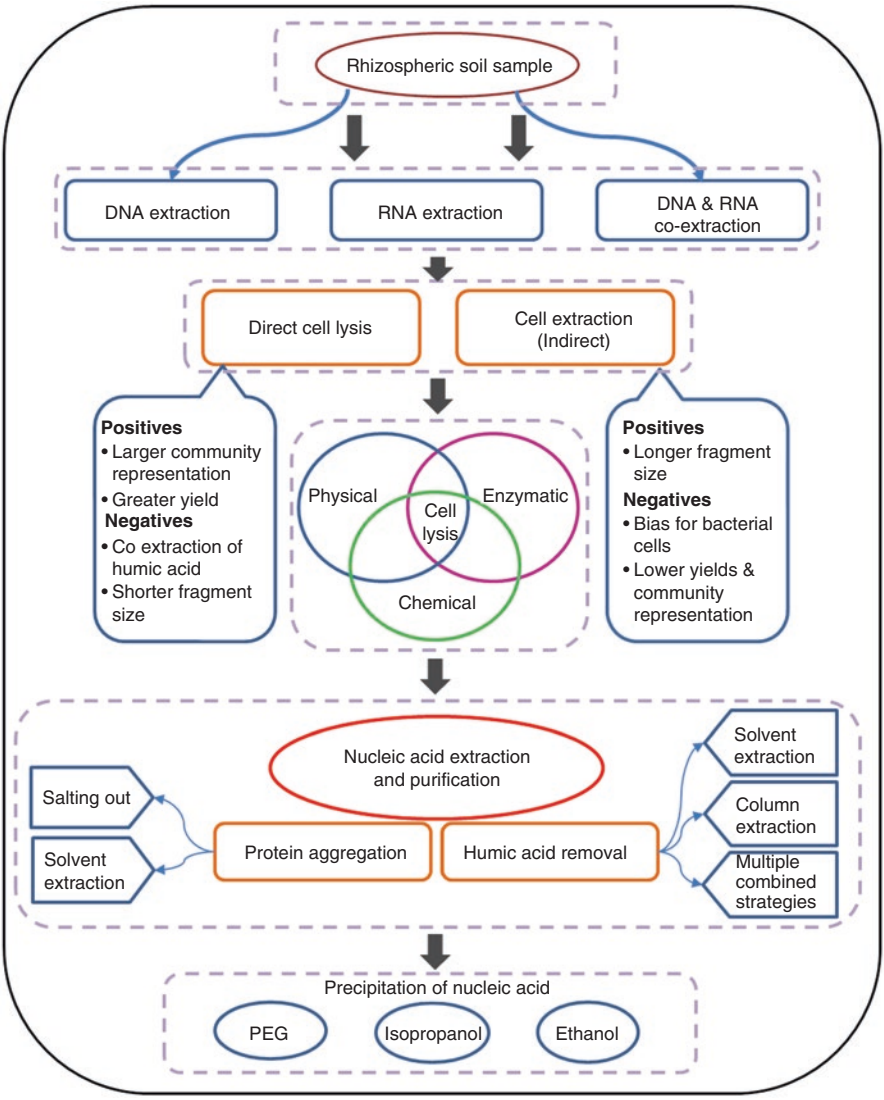


Fig. 7.1 Various steps in extraction of nucleic acid from rhizospheric soil

fragments of nucleic acid, which is preferred for downstream procedures such as construction of metagenomic library (Zhou et al. 1996; Robe et al. 2003; Hirsch et al. 2010). Additionally, the cell extraction method offers an advantage in terms of limited co-extraction of organic content (most common being humic acid).

Direct extraction method involves the lysing of cells in the matrix itself, followed by purification of nucleic acid from the lysed contents. The highlight of direct extraction method is a better representation, as well as quantification, of the

microbial community. However, it leads to smaller nucleic acid fragments (Smalla et al. 1993; Hirsch et al. 2010). The major drawback of this method is the greater possibility of co-extraction of soil organic components such as humic acid, fulvic acid, etc., which interfere with further analyses.

The choice of an extraction method can be based upon the target microbial community, the downstream application for which the nucleic acid is required, and the properties of soil from which nucleic acid is to be extracted. The method of cell lysis should be chosen so as to give a true representation of that community, taking care of the yield and purity. Studies have also shown that extraction efficiency differed with variation in types of dominant microbes, particularly gram-positive bacteria (Zhou et al. 1996; Kuske et al. 1998), and with cells at different growth stages (Frostegård et al. 1999).

7.2.1 Cell Extraction Method

As shortly discussed earlier, in this prior to cell lysis, the cells are separated from the matrix. Earliest reports employing cell extraction method are by Faegri et al. (1977) and Torsvik and Goksoyr (1978). The major steps involved in cell extraction method are, firstly, dislodging of cells from soil particles, and secondly, extraction of cells from the soil matrix before lysis.

7.2.1.1 Dispersion of Soil Particles

For dispersion of soil from cells, physical and chemical methods can be employed. Physical methods mainly involve the use of waring blenders (Faegri et al. 1977; Bakken and Lindahl 1995), sonication (Ramsay 1984), rotating pestle (Lindahl and Bakken 1995), and shaking (Turpin et al. 1993). The usage of waring blenders and rotating pestle was found to be the most effective in dispersion of soil particles (Lindahl and Bakken 1995; Robe et al. 2003). Chemical treatments include detergents such as sodium cholate and sodium deoxycholate for breakdown of bacterial lipopolysaccharides (Macdonald 1986), sodium dodecyl sulfate (SDS) with polyethylene glycol (PEG) for dissolution of hydrophobic material, and polyvinylpyrrolidone (PVP), which removes humic acid (Steffan et al. 1988). Cation exchange resins (Chelex 100) have also been employed for dispersion of soil particles (Jacobsen and Rasmussen 1992). Chemical dispersion methods have not been much advertised as they tend to have negative impact on the cell integrity which is a major drawback of using chemicals for dispersal methods. Therefore, chemical treatments are applied in conjunction with physical methods (Lindahl and Bakken 1995).

7.2.1.2 Separation of Cells from Soil Matrix

After the dispersal of soil particles, the cells are extracted from the dispersed medium. This is usually done by differential centrifugation methods as initially described by Faegri et al. (1977). This approach involves two successive rounds of centrifugation, first a low-speed centrifugation to eliminate soil debris, followed by high-speed centrifugation to collect the bacterial fraction. The low-speed

centrifugation should be dealt with precautions as cell recovery, and contamination can occur during this step (Steffan et al. 1988). Removal of contaminants like humic acid at this step by flocculation using $CaCl_2$ has been shown to result in lower cell recovery (Jacobsen and Rasmussen 1992). Holben et al. (1988) described that a cycle of dispersion and separation resulted in 10% cell recovery of the total bacterial fraction. Thus appropriate number of cycles of these steps can be used for higher recoveries (Robe et al. 2003). Successive centrifugation methods can be cumbersome and require constant optimization, especially with different soil types. Density gradient centrifugation method using a multi-gradient media, Nycodenz, with high-speed centrifugation provided extraction efficiency ranging between 20–50% (Bakken et al. 1995) and 6–25% (Mayr et al. 1999). Sucrose density gradient centrifugation methods can also be employed, which present a clean bacterial fraction, in comparison to the successive centrifugation methods (Pillai et al. 1991).

Cell extraction methods are usually preferred where the targeted microbial community is smaller and there is requirement of high molecular weight nucleic acid. It has been shown that with larger microbial community, the cell extraction methods introduce certain biases; hence, the true representative picture of the whole community is not captured.

7.2.2 Direct Lysis Method

Direct lysis method involves in situ lysis of cells within the soil matrix. The methodology was introduced by Ogram et al. (1987). All methods that can be classified under direct extraction method are derivations of a report by Ogram et al. (1987). Lysis in this method can be done by physical, chemical, or enzymatic means (Table 7.1). Physical methods employed for cell lysis mainly involve bead-beating method (Miller 2001; Niemi et al. 2001) and freeze thawing or freeze boiling (Moré et al. 1994; Degrange and Bardin 1995) for disruption of cell membrane. With bead-beating method, enhanced yields have been observed (Smalla et al. 1993; Moré et al. 1994; Cullen and Hirch 1998; Bürgmann et al. 2001). Other physical methods include thermal shock using microwaves (Orsini and Romano-Spica 2001), mortar mill grinding (Tebbe and Vajhen 1993), ultrasonication (Picard et al. 1992; Porteous et al. 1997), grinding with liquid nitrogen (Volossiouk et al. 1995; Zhou et al. 1996; Frostegård et al. 1999), simple grinding, and microwave thermal heating (Frostegård et al. 1999). In chemical methods, detergents (mostly SDS) have been used for lysis, supplemented with heating and chelating agents such as EDTA with Tris and sodium phosphate buffers (Robe et al. 2003). SDS can also be substituted with sarkosyl (Holben et al. 1988). These detergents have also been employed at varying temperatures: warm (60–70 °C) (Ogram et al. 1987; Kuske et al. 1998) and lower temperature (0–4 °C) to reduce the co-extraction of humic acid (Tebbe and Vahjen 1993). Cetyltrimethylammonium bromide (CTAB) and PVP are also commonly used as they help in removal of humic acid, though Zhou et al. (1996) demonstrated a reduction in yield with the use of PVP. In co-extraction methods, PVP has been shown to have higher ability to absorb humic acid without the loss of RNA (Mettel et al.

Table 7.1 Different treatments for cell lysis

Treatment	Features	References
Physical		
Freeze thaw/freeze boiling	Harsh yet inadequate	Tsai et al. (1991)
Microwave heating	Harsh	Orsini and Romano-Spica (2001)
Sonication	Mild	Ramsay et al. (1984)
Mortar mill grinding	Harsh	Tebbe and Vahjen (1993)
Bead mill homogenization	Adequate but lower yields than bead beating	Steffan et al. (1988), Miller et al. (1999, 2001)
Bead beating	Adequate and better yields	Smalla et al. (1993), Kuske et al. (1998), Miller et al. (1999), and Burgmann et al. (2001)
Chemical		
SDS	Denaturant	Ogram et al. (1987), Holben et al. (1988), and Kuske et al. (1998)
Sarkosyl	Denaturant	Holbe et al. (1988)
Guanidium isothiocyanate	Extraction from gram positives, and RNA	Porteous et al. (1997)
PVP	Removal of humic acid	Steffan et al. (1988) and Porteous and Armstrong (1991)
CTAB	Complexes humic acid	Zhou et al. (1996)
Sodium ascorbate	Removes phenolic compounds	Holben et al. (1988)
Enzymatic		
Lysozyme	Muropeptide hydrolysis	Tsai and Olson (1991), Tebbe and Vahjen (1993), and Martin-Laurent et al. (2001)
Proteinase K	Protease	Zhou et al. (1996)
Achromopeptidase	Protease	Liu et al. (1997)
Pronase	Protease	Jacobsen and Rammussen (1992)

2010; Sharma et al. 2012). Sodium ascorbate has also been successfully employed for the removal of phenolic compounds (Holben et al. 1988). Some studies have included guanidine isothiocyanate in extraction buffer for RNA isolation (Tsai et al. 1991; Orsini and Romano-Spica 2001), which aids in the stabilization of RNA. CTAB-DTT (dithiothreitol) has been reported to be better compared to treatment with SDS, despite the latter being more popular (Thakuria et al. 2008). Chemical treatments alone cannot always serve the purpose efficiently. Therefore, they are generally not employed in conjunction with physical or enzymatic methods (Robe et al. 2003). Lysozyme is the most commonly used enzyme that aids in dissolution of cell wall (Bruce et al. 1992; Tebbe and Vahjen 1993; Martin-Laurent et al. 2001). Other enzymes have also been used in the various protocols for lysis such as Simonet et al. (1984) used achromopeptidase, Jacobsen and Rasmussen (1992) utilized pronase, and Zhou et al. (1996) employed proteinase K.

7.3 Purification of Nucleic Acid

Soil organic content and proteins are the chief contaminants during extraction of nucleic acid. Deproteinization is mainly done by classical organic solvents such as phenol, chloroform, and isoamyl alcohol (Selenska and Klingmuller 1991; Smalla et al. 1993; Tebbe and Vahjen 1993). Salting out methods have also been in use, employing saturated solutions of sodium chloride (Holben et al. 1988; Harry et al. 1999), ammonium acetate (Steffan and Atlas 1988), and potassium chloride (Torsvik et al. 1990). Deproteinization happens under low-speed centrifugation at which the proteins are precipitated. Sodium chloride was found to be more effective as it precipitates soil particles along with proteins and cell constituents (Harry et al. 1999). Organic content, namely, humic acid, fulvic acid, etc., requires special considerations for its removal owing to reduction of yield and purity. Humic acid is polyphenolic in nature and inhibits the enzymes used in important downstream techniques such as PCR, qPCR, and RFLP, as the phenols bind to protein via hydrogen bonds resulting in altered conformation of the enzymes (Kreader 1996; Saleh-Lakha et al. 2011). Humic acid, which has similar physicochemical properties as that of nucleic acids, competes with nucleic acid when passed through minicolumns for adsorption (Harry et al. 1999). The minimum inhibitory concentration (MIC) of humic acid varies with its composition, source, and the enzyme used in downstream applications (Tebbe and Vahjen 1993). The presence of humic acid can be perceived by the naked eye as brownish coloration in the extracted nucleic acid (Robe et al. 2003). Hence the removal of humic acid is essential even if a compromise in yield is to be made upon employing subsequent purification procedures. Sometimes, even multiple elimination strategies are employed.

7.3.1 Purification Strategies

There are numerous strategies that have been employed for separation of nucleic acid from the cell constituents and the soil organics, especially humic acids (Table 7.2). The strategies have mostly used solvent-based purification or column purification. Cesium chloride (CsCl) gradient centrifugation was among the first purification strategies (Ogram et al. 1987) that enabled the purification of different components according to their densities into stable zones produced by centrifugation of CsCl. The use of CsCl gradient centrifugation is cumbersome and tedious and does not provide nucleic acid with considerable purity and yields (Ogram et al. 1987; Steffan et al. 1988). The other method that has also been utilized in the purification procedure is chromatography using gel filtration columns (Size exclusion chromatography). Size exclusion chromatography allows separation based on molecular weights. Different columns that have been used are Microspin Sephadex G50 (Van Elsas et al. 1991; Dijkmans et al. 1993), Sephadex G75 (Purdy et al. 1996), Sephadex G200 (Erb and Wagnerdobler 1993; Kuske et al. 1998), and Sepharose 4B (Jackson et al. 1997). Erb and Wagnerdobler (1993) reported a loss of 5–15% by employing column for purification and were able to successfully perform

Table 7.2 Purification methods for extraction of nucleic acid

Method	Remarks	References
Salting out (deproteinization) {NaCl, KCl, CH_3COONa, CH_3COOK, NH_3CH_3COOH}	Inadequate in itself, usually used in conjunction with solvent extraction method	Holben et al. (1988), Steffan and Atlas (1988), Torsvik et al. (1990), and Hilger and Myrold (1991)
Solvent extraction (phenol/chloroform/isoamyl alcohol{PCI}) (deproteinization)	Most commonly used but additional salting out methods can be employed for confirmation	Smalla et al. (1993) and Tebbe and Vahjen (1993)
CsCl density gradient centrifugation	Tedious and wearisome with lower yields	Ogram et al. (1987), Steffan et al. (1988), and Tebbe and Vahhen (1993)
Chromatography	Lower yields	Sephadex G50; Dijkmans et al. (1993)
		Sephadex G75; Cullen and Hirsch (1998)
		Sephadex 200; Kuske et al. (1998)
		Sepharose 4B; Jackson et al. (1997)
		Ion-exchange; Tebbe and Vahjen (1993)
		Hydroxyapatite; Torsvik et al. (1990)
Electrophoresis	Lower yields with persistence of small fraction of humic acid in organic rich samples	Young et al. (1993) and Harry et al. (1999)
Dialysis	Fast but inadequate by itself	Classical- Romanowski et al. (1992)
		Microconcentrators; Zhou et al. (1996), Clegg et al. (1997), and Porteous et al. (1997)

PCR. Kuske et al. (1998) employed PVP containing Sephadex G200 minicolumns that were able to purify large amounts of organic content from the nucleic acid fraction. However, Sepharose 4B columns were shown to be more effective than G50 and G200 with better PCR results (Jackson et al. 1997). Chromatography, using ion exchange columns to selectively elute nucleic acid, has also been employed (Torsvik 1980; Ogram et al. 1987; Tebbe and Vahjen 1993). The losses with ion exchange columns were 20–30% (Tebbe and Vahjen 1993), and the nucleic acid fraction required further purification (Torsvik et al. 1990). Steffan et al. (1988) used hydroxyapatite, which binds to nucleic acid, but the treatment exhibited significant losses. With the chromatography procedure, multiple rounds are needed to provide a clean

Table 7.3 Combined strategies for purification of nucleic acid

Combined strategy	References
CsCl treatment + hydroxyapatite	Orgam et al. (1987)
CsCl ultracentrifugation + microconcentrator + CsCl ultracentrifugation	Clegg et al. (1997)
CsCl treatment + potassium acetate precipitation + purification with glass milk	Smalla et al. (1993)
Gel electrophoresis + binding on silica matrix	Thornhill et al. (1995)
Gel electrophoresis (with PVP) + Elutip-d column	Boivin-Jahns et al. (1996)
CsCl treatment + potassium acetate precipitation + twofold purification through bind resin	Van Elsas et al. (1997)

fraction of nucleic acid. The major contaminant that competes with nucleic acid for sites on the minicolumns is humic acid (Harry et al. 1999). A conceptual model explaining the competition between humic acid, DNA-humic acid complex, and DNA for adsorption on minicolumns was developed by Roose-Amasleg et al. (2001). Electrophoresers have been used notably for nucleic acid purification and the removal of humic acid (Pitcher et al. 1989; Van Elsas et al. 1991; Zhou et al. 1996). With the use of lower melting point agarose (Young et al. 1993; Harry et al. 1999) and PVP (Young et al. 1993; Liu et al. 1997) for removal of humic acid, the yields were reduced significantly. Dialysis was first used to clean DNA (Romanowski et al. 1992), but the classical dialysis has given way to microconcentrators and minicolumns (Zhou et al. 1996; Clegg et al. 1997; Porteous et al. 1997). Porteous et al. (1997) successively used CTAB, PEG, and a microconcentrator to purify nucleic acid.

7.3.2 Combined Strategies

The numerous strategies enumerated above have been used but with little success when employed individually. Hence, it becomes imperative that multiple strategies be combined for the removal of various contaminants, mainly the organic content. The methods so far developed have been thus optimized for their rapidity, higher yields, and better purity. Numerous studies have compared and used multiple strategies with varying soil types (Table 7.3). Clegg et al. (1997) utilized a double CsCl density gradient ultracentrifugation with a microconcentrator in between the two centrifugation cycles. Nucleic acid recovered was of significant purity to be used for restriction and PCR-based studies. Smalla et al. (1993) employed a triple purification procedure, CsCl gradient centrifugation, followed by potassium acetate precipitation, subsequently followed by a purification step towards the end with glass milk (Geneclean). Nucleic acid so obtained was suitable for PCR-based studies. Thornhill et al. (1995) utilized gel electrophoresis and finally elution of DNA using a silica matrix. This method specifically removed the smaller fragments, thereby preventing the formation of chimeras during PCR amplification. Along with

electrophoresis, PVP was added for the removal of humic acid. However, the yields were significantly lower (Young et al. 1993; Liu et al. 1997). Boivin-Jahns et al. (1996) combined it with purification on Elutip-d columns to remove PVP, which was also seen to inhibit Taq polymerase. Upon individually employing Elutip-d column, recovery of only 40% was observed (Tsai et al. 1991). Four combined strategies were compared by Zhou et al. (1996) using only ion exchange resin column, agarose gel electrophoresis, and subsequent column purification and two resin column and agarose gel electrophoresis with subsequent concentration by a microconcentrator. The yield was the lowest in case of electrophoresis coupled with column, but the purity was observed to be the best. As documented, a single combination of purification strategies would not work for different soil types. Van Elsas et al. (1997) demonstrated that for five different soil types different strategies were required with the soil containing the highest organic content (30%), requiring the highest number of purification steps. Frostegård et al. (1999) compared four different protocols for six types of soils with varying clay and organic content. The four methods used were aqueous two-phase system comprising of PEG8000 and $(NH_4)_2SO_4$ and two successive Elutip-d columns, Sephacryl S400 column purification followed by Elutip-d column and Sephacryl S200 column purification followed by Elutip-d column. Solvent extraction involving two-phase system gave 56–80% recoveries. A comparison of four purification methods was reported by Miller et al. (1999) using only a single Spinbind column, combining gel electrophoresis with Spinbind column, Sephadex G200 column, and finally ammonium acetate precipitation. Only with the gel electrophoresis with Spinbind and Sephadex G200 column purification PCR amplification could be performed. The remaining humic acid content was advised to be removed by conjunction with CTAB purification step. Harry et al. (1999) made a comparison between different types of chromatography (gel exclusion, resin, and silica gel membrane) with gel electrophoresis (polyacrylamide and agarose). Also the combined methodology of using two minicolumns (silica gel and resin) and gel electrophoresis with silica gel minicolumn was evaluated. Electrophoresis followed by silica gel electrophoresis yielded best recovery even of the RNA fraction.

For the precipitation of nucleic acid, ethanol, isopropanol, and PEG-8000 can be used (Ogram et al. 1987; Porteous et al. 1997). PEG or isopropanol tends to decrease the sample volume (0.54 volume of isopropanol/PEG to 2 volumes of ethanol). Porteous et al. (1997) demonstrated that precipitation using alcohol favored the co-extraction of humic acid, whereas there was reduction in the co-extraction of humic acid with PEG. However, PEG can interfere with PCR; therefore, it needs to be removed by phenol extraction, thereby lowering the yields (Roose-Amsaleg et al. 2001). The purification strategies have now evolved as more studies have come up including RNA as marker. Many of these studies have also been used for co-extraction (Harry et al. 1999). However, the instability of RNA fraction reduces its occurrence in the final nucleic acid fraction.

7.4 Extraction of RNA and Co-extraction

RNA can specifically target the active participants of the soil microbial community. mRNA can provide us valuable information regarding the functional aspects of the soil community, but it is usually short lived. rRNA is much more stable because it tends to form secondary structures together with being stabilized with ribosomal proteins; thus a picture of dominant active population can be acquired from rRNA. Both mRNA and rRNA can be converted to cDNA which is a much more stable form. Extraction of RNA fraction including both mRNA and rRNA is a tedious process. As evident from the above sections, isolation of DNA is complexed by the presence of soil organics reducing yields and purity. The ubiquitous presence of RNases in the soil is an additional consideration, which hampers the extraction process when trying to extract RNA (Ogram et al. 1995; Saleh-Lakha et al. 2005). Extraction of only the RNA fraction has been documented in several studies (Moran et al. 1993; Filske et al. 1996; Miskin et al. 1999). Studies for the co-extraction of RNA and DNA have also gained momentum (Griffiths et al. 2000; Hurt et al. 2001; Costa et al. 2004; Peršoh et al. 2008; Mcllroy et al. 2009; Towe et al. 2011; Sharma et al. 2012). Different extraction procedures develop a bias if the gene abundances are linked to transcript rates; the co-extraction of DNA and RNA removes this bias (Towe et al. 2011). Various studies enlisted use different methods. Felske et al. (1996) involved mechanical lysis with centrifugation to prevent extraction of humic acid and further used PVP with BSA to remove residual humic acid. Moran et al. (1993) employed chemical enzymatic lysis using lysozyme phenol hot direct lysis method coupled with Sephadex G75 column to negate the co-extraction of humic acid in the extraction of RNA from soil. Miskin et al. (1999) used an indirect lysis extraction method with sodium phosphate, lysozyme, β-mercaptoethanol, and SDS in the extraction buffer followed by precipitation of nucleic acid with PEG. Duarte et al. (1998) modified the strategy of Ogram et al. (1995) of indirect lysis coupling with a Sephadex G75 column for the removal of humic acid. Griffiths et al. (2000) used a direct lysis bead-beating method with CTAB, NaCl, and potassium phosphate buffer using PEG for precipitation of nucleic acid. Peršoh et al. (2008) and Fang et al. (2014) employed aluminum sulfate $\{Al_2(SO_4)_3\}$ for flocculation of humic acid prior to lysis, whereas Braid et al. (2003) used ammonium aluminum sulfate $AlNH_4(SO_4)_2$. Sagova-Mareckova et al. (2008) compared seven different methods with 14 types of soils with variation in pH, moisture, bedrock, vegetation, texture, organic matter, and salinity. The innovative method involved modifications to the approach of Miller et al. (1999) along with the pretreatment of $CaCO_3$ and purification by $CaCl_2$. Towe et al. (2011) used a similar protocol as Griffiths et al. (2000) but with addition of β-mercaptoethanol to deactivate native DNases and RNase present in the soil during lysis and used silica-based columns to separate DNA and RNA fractions. Sharma et al. (2012) improved the protocol by Griffiths et al. (2000) by addition of PVP along with CTAB-NaCl in lysis and doubling the bead-beating step for better extraction of nucleic acid, followed by precipitation of nucleic acid using PEG on ice. With increasing number of researchers interested not only in gene abundance but also in transcript analysis using qRT-PCR, we would see the advent

Table 7.4 Strategies for extraction of nucleic acid

Strategy	References
Direct lysis bead-beating method (CTAB-NaCl + PCI + K2HPO4) + precipitation in PEG-6000	Griffiths et al. (2000)
Direct lysis bead-beating method (CTAB-NaCl +1% PVP + Tris)	Stach et al. (2001)
Pretreatment of $CaCO_3$ + Direct lysis bead-beating method (SDS-NaCl + Tris-HCl + Na_2HPO_4 + P-C-I) + purification by $CaCl_2$	Sagova-Mareckova et al. (2008)
Modified Griffiths protocol: addition of β-mercaptoethanol and purification and separation of DNA and RNA fractions using silica column	Towe et al. (2011)
Modified Griffiths protocol: Direct lysis bead-beating method (CTAB-NaCl +3.4% PVP + PCI + K_2HPO_4) + precipitation in PEG-6000, incubation on ice	Sharma et al. (2012)

of newer, sturdy protocols for RNA extraction. Nevertheless, the present protocols have substantial success in isolation of RNA (mRNA and rRNA), some of which are enlisted in Table 7.4.

7.5 Conclusion

The extraction of nucleic acid from soil is a process of constant optimization and standardization for different soil types. The major focus should be on the removal of contaminants, mainly soil organic content which reduces yield and interferes with the downstream applications. From the choice of cell lysis to the purification steps, nucleic acid extraction from soil aims for better yield and purity with enhanced representation of the target community. Majority of the extraction procedures focus on the DNA extraction; however, studies pertaining to the extraction of RNA as well as co-extraction of DNA and RNA have gained momentum. Studies utilizing RNA fraction for transcriptomic studies have increased due to developments in the extraction procedures. As efficient extraction procedures evolve, we would have deeper insight into the field of microbial ecology and plant-microbe interactions in a plant's rhizosphere.

Acknowledgment Funding received from DBT (BT/PR5499/AGR/21/355/2012) and SERB (YSS/2015/001437) is acknowledged.

References

Amann RI, Ludwig W, Schleifer K (1995) Phylogenetic identification and in-situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Bakken LR, Lindahl V (1995) Recovery of bacterial cells from soil. In: Nucleic acids in the environment. Springer, Heidelberg/Berlin, pp 9–27

Boivin-Jahns V, Ruimy R, Bianchi A et al (1996) Bacterial diversity in a deep-subsurface clay environment. Appl Environ Microbiol 62:3405–3412

Braid MD, Daniels LM, Kitts CL (2003) Removal of PCR inhibitors from soil DNA by chemical flocculation. J Microbiol Methods 52:389–393

Bruce KD, Hiorns WD, Hobman JL et al (1992) Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. Appl Environ Microbiol 58:3413–3416

Bürgmann H, Pesaro M, Widmer F, Zeyer J (2001) A strategy for optimizing quality and quantity of DNA extracted from soil. J Microbiol Methods 45:7–20

Clegg CD, Ritz K, Griffiths BS (1997) Direct extraction of microbial community DNA from humified upland soils. Lett Appl Microbiol 25:30–33

Costa R, Gomes NCM, Milling A, Smalla K (2004) An optimized protocol for simultaneous extraction of DNA and RNA from soils. Braz J Microbiol 35:230–234

Courtois S, Frostegård A, Göransson P et al (2001) Quantification of bacterial subgroups in soil: comparison of DNA extracted directly from soil or from cells previously released by density gradient centrifugation. Environ Microbiol 3:431–439

Cullen DW, Hirsch PR (1998) Simple and rapid method for direct extraction of microbial DNA from soil for PCR. Soil Biol Biochem 30:983–993

Degrange V, Bardin R (1995) Detection and counting of *Nitrobacter* populations in soil by PCR. Appl Environ Microbiol 61:2093–2098

Dijkmans R, Jagers A, Kreps S et al (1993) Rapid method for purification of soil DNA for hybridization and PCR analysis. Microb Releases 2:29–34

Duarte GF, Rosado AS, Seldin L et al (1998) Extraction of ribosomal RNA and genomic DNA from soil for studying the diversity of the indigenous bacterial community. J Microbiol Meth 32:21–29

Erb RW, Wagner-Dobler I (1993) Detection of polychlorinated biphenyl degradation genes in polluted sediments by direct DNA extraction and polymerase chain reaction. Appl Environ Microbiol 59:4065–4073

Fægri A, Torsvik VL, GoksÖyr J (1977) Bacterial and fungal activities in soil: separation of bacteria and fungi by a rapid fractionated centrifugation technique. Soil Biol Biochem 9:105–112

Fang C, Xu T, Ye C et al (2014) Method for RNA extraction and cDNA library construction from microbes in crop rhizosphere soil. World J Microbiol Biotechnol 30:783–789

Felske A, Engelen B, Nübel U, Backhaus H (1996) Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Appl Environ Microbiol 62:4162–4167

Frostegård ÅSA, Courtois S, Ramisse V et al (1999) Quantification of bias related to the extraction of DNA directly from soils. Appl Environ Microbiol 65:5409–5420

Griffiths RI, Whiteley AS, Anthony G et al (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66:5488–5491

Harry M, Gambier B, Bourezgui Y, Garnier-Sillam E (1999) Evaluation of purification procedures for DNA extracted from rich organic samples: interference with humic substances. Analusis 27:439–441

Hilger AB, Myrold DD (1991) Method for extraction of Frankia DNA from soil. Agric Ecosyst Environ 34(1-4):107–113

Hirsch PR, Mauchline TH, Clark IM (2010) Culture-independent molecular techniques for soil microbial ecology. Soil Biol Biochem 42:878–887

Holben WE, Jansson JK, Chelm BK, Tiedje JM (1988) DNA probe method for the detection of specific microorganisms in the soil bacterial community. Appl Environ Microbiol 54:703–711

Hurt RA, Qiu X, Wu L et al (2001) Simultaneous recovery of RNA and DNA from soils and sediments simultaneous recovery of RNA and DNA from soils and sediments. Appl Environ Microbiol 67:4495–4503

Jackson CR, Harper JP, Willoughby D et al (1997) A simple, efficient method for the separation of humic substances and DNA from environmental samples. Appl Environ Microbiol 63:4993–4995

Jacobsen CS, Rasmussen OF (1992) Development and application of a new method to extract bacterial DNA from soil based on separation of bacteria from soil with cation-exchange resin. Appl Environ Microbiol 58:2458–2462

Kreader CA (1996) Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. Appl Environ Microbiol 62:1102–1106
Kuske CR, Banton KL, Adorada DL et al (1998) Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. Appl Environ Microbiol 64:2463–2472
Lindahl V, Bakken LR (1995) Evaluation of methods for extraction of bacteria from soil. FEMS Microbiol Ecol 16:135–142
Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522
Macdonald RM (1986) Sampling soil microfloras: dispersion of soil by ion exchange and extraction of specific microorganisms from suspension by elutriation. Soil Biol Biochem 18:399–406
Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G, Catroux G (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. Appl Environ Microbiol 67(5):2354–2359
Maarit Niemi R, Heiskanen I, Wallenius K, Lindström K (2001) Extraction and purification of DNA in rhizosphere soil samples for PCRDGGE analysis of bacterial consortia. J Microbiol Methods 45(3):155–165
Mayr C, Winding A, Hendriksen NB (1999) Community level physiological profile of soil bacteria unaffected by extraction method. J Microbiol Meth 36:29–33
McIlroy SJ, Porter K, Seviour RJ, Tillett D (2009) Extracting nucleic acids from activated sludge which reflect community population diversity. Antonie van Leeuwenhoek Int J Gen Mol Microbiol 96:593–605
Mettel C, Kim Y, Shrestha PM, Liesack W (2010) Extraction of mRNA from soil. Appl Environ Microbiol 76:5995–6000
Miller DN (2001) Evaluation of gel filtration resins for the removal of PCR-inhibitory substances from soils and sediments. J Microbiol Methods 44:49–58
Miller DN, Bryant JE, Madsen EL, Ghiorse WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65:4715–4724
Miskin IP, Farrimond P, Head IM (1999) Identification of novel bacterial linages as active members of microbial populations in a freshwater sediment using a rapid RNA extraction procedure and RT-PCR. Microbiology 145:1977–1987
Moran MA, Torsvik VL, Torsvik T, Hodson RE (1993) Direct extraction and purification of rRNA for ecological studies. Appl Environ Microbiol 59:915–918
Moré MI, Herrick JB, Silva MC et al (1994) Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. Appl Environ Microbiol 60:1572–1580
Nannipieri P, Ascher J, Ceccherini MT et al (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655
Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. J Microbiol Meth 7:57–66
Ogram A, Sun W, Brockman FJ (1995) Isolation and characterization of RNA from low-biomass deep-subsurface sediments. Appl Environ Microbiol 61:763–768
Orsini M, Romano-Spica V (2001) A microwave-based method for nucleic acid isolation from environmental samples. Lett Appl Microbiol 33:17–20
Peršoh D, Theuerl S, Buscot F, Rambold G (2008) Towards a universally adaptable method for quantitative extraction of high-purity nucleic acids from soil. J Microbiol Meth 75:19–24
Picard C, Ponsonnet C, Paget E et al (1992) Detection and enumeration of bacteria in soil by direct DNA extraction and polymerase chain reaction. Appl Environ Microbiol 58:2717–2722
Pillai SD, Josephson KL, Bailey RL et al (1991) Rapid method for processing soil samples for polymerase chain reaction amplification of specific gene sequences. Appl Environ Microbiol 57:2283–2286
Pitcher DG, Saunders NA, Owen RJ (1989) Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 8:151–156

Porteous LA, Armstrong JL (1991) Recovery of bulk DNA from soil by a rapid, small-scale extraction method. Curr Microbiol 22:345–348

Porteous LA, Seidler RJ, Watrud LS (1997) An improved method for purifying DNA from soil for polymerase chain reaction amplification and molecular ecology applications. Mol Ecol 6:787–791

Purdy KJ, Embley TM, Takii S, Nedwell DB (1996) Rapid extraction of DNA and rRNA from sediments by a novel hydroxyapatite spin-column method. Appl Environ Microbiol 62:3905–3907

Ramsay AJ (1984) Extraction of bacteria from soil: efficiency of shaking or ultrasonication as indicated by direct counts and autoradiography. Soil Biol Biochem 16:475–481

Robe P, Nalin R, Capellano C et al (2003) Extraction of DNA from soil. Eur J Soil Biol 39:183–190

Romanowski G, Lorenz MG, Sayler G, Wackernagel W (1992) Persistence of free plasmid DNA in soil monitored by various methods, including a transformation assay. Appl Environ Microbiol 58:3012–3019

Roose-Amsaleg CL, Garnier-Sillam E, Harry M (2001) Extraction and purification of microbial DNA from soil and sediment samples. Appl Soil Ecol 18:47–60

Sagova-Mareckova M, Cermak L, Novotna J et al (2008) Innovative methods for soil DNA purification tested in soils with widely differing characteristics. Appl Environ Microbiol 74:2902–2907

Saleh-Lakha S, Shannon KE, Goyer C, Trevors JT (2011) Challenges in quantifying microbial gene expression in soil using quantitative reverse transcription real-time PCR. J Microbiol Meth 85:239–243

Saleh-Lakha S, Miller M, Campbell RG, Schneider K, Elahimanesh P, Hart MM, Trevors JT (2005) Microbial gene expression in soil: methods, applications and challenges. J Microbiol Methods 63(1):1–19

Selenska S, Klingmüller W (1991) Direct detection of *nif*-gene sequences of *Enterobacter agglomerans* in soil. FEMS Microbiol Lett 80:243–245

Sharma S, Mehta R, Gupta R, Schloter M (2012) Improved protocol for the extraction of bacterial mRNA from soils. J Microbiol Meth 91:62–64

Simonet P, Capellano A, Navarro E et al (1984) An improved method for lysis of *Frankia* with achromopeptidase allows detection of new plasmids. Can J Microbiol 30:1292–1295

Smalla K, Cresswell N, Mendonca Hagler LC et al (1993) Rapid DNA extraction protocol from soil for polymerase chain reaction mediated amplification. J Appl Bacteriol 74:78–85

Steffan RJ, Atlas RM (1988) DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Appl Environ Microbiol 54:2185–2191

Steffan RJ, Goksøyr J, Bej AK, Atlas RM (1988) Recovery of DNA from soils and sediments. Appl Environ Microbiol 54:2908–2915

Tebbe CC, Vahjen W (1993) Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. Appl Environ Microbiol 59:2657–2665

Thakuria D, Schmidt O, Mac Siúrtáin M et al (2008) Importance of DNA quality in comparative soil microbial community structure analyses. Soil Biol Biochem 40:1390–1403

Thornhill RH, Burgess JG, Matsunaga T (1995) PCR for direct detection of indigenous uncultured magnetic cocci in sediment and phylogenetic analysis of amplified 16S ribosomal DNA. Appl Environ Microbiol 61:495–500

Torsvik VL (1980) Isolation of bacterial DNA from soil. Soil Biol Biochem 12:15–21

Torsvik VL, Goksoyr J (1978) Determination of bacterial DNA in soil. Soil Biol Biochem 10:7–12

Torsvik V, Goksøyr J, Daae FL (1990) High diversity in DNA of soil bacteria. Appl Environ Microbiol 56:782–787

Towe S, Wallisch S, Bannert A et al (2011) Improved protocol for the simultaneous extraction and column-based separation of DNA and RNA from different soils. J Microbiol Meth 84:406–412

Tsai YL, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol 57:1070–1074

Tsai YL, Park MJ, Olson BH (1991) Rapid method for direct extraction of mRNA from seeded soils. Appl Environ Microbiol 57:765–768

Turpin PE, Maycroft KA, Rowlands CL, Wellington EM (1993) An ion-exchange based extraction method for the detection of salmonellas in soil. J Appl Bacteriol 74:181–190

Van Elsas JD, Van Overbeek LS, Fouchier R (1991) A specific marker, pat, for studying the fate of introduced bacteria and their DNA in soil using a combination of detection techniques. Plant Soil 138:49–60

Van Elsas JD, Mantynen V, Wolters AC (1997) Soil DNA extraction and assessment of the fate of *Mycobacterium chlorophenolicum* strain PCP-1 in different soils by 16S ribosomal RNA gene sequence based most-probable-number PCR and immunofluorescence. Biol Fertil Soils 24:188–195

Volossiouk T, Robb EJ, Nazar RN (1995) Direct DNA extraction for PCR-mediated assays of soil organisms. Appl Environ Microbiol 61:3972–3976

Young CC, Burghoff RL, Keim LG et al (1993) Polyvinylpyrrolidone-agarose gel electrophoresis purification of polymerase chain reaction-amplifiable DNA from soils. Appl Environ Microbiol 59:1972–1974

Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316–322

Plant–Fungi Association: Role of Fungal Endophytes in Improving Plant Tolerance to Water Stress

8

Khondoker M.G. Dastogeer and Stephen J. Wylie

Abstract

Plants are constantly being challenged with various biotic and abiotic stresses throughout their life cycle that exert profound deleterious effects on growth, development and health. Plants employ various physiological, biochemical and molecular mechanisms to combat these stress factors. Microorganism-mediated plant stress tolerance, particularly plant drought tolerance, is important in the study of plant–microbe interactions. Although relatively less well-known, fungal endophyte-mediated plant drought tolerance has been described for several cases. Unlike mycorrhizal fungi, non-mycorrhizal fungi may mediate the effects of water stress by adjusting, regulating or modifying plant physiological, biochemical and metabolic activities. We review the evidence for fungal endophyte-mediated plant drought tolerance and mechanisms.

Keywords

Abiotic stress • Water deficit • Endophyte • Growth • Photosynthesis • ROS • Osmotic adjustment

K.M.G. Dastogeer (✉)
Plant Biotechnology Research Group, Western Australian State Agricultural Biotechnology Centre (SABC), Murdoch University, Perth WA6150, Western Australia, Australia

Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
e-mail: k.dastogeer@murdoch.edu.au

S.J. Wylie
Plant Biotechnology Research Group, Western Australian State Agricultural Biotechnology Centre (SABC), Murdoch University, Perth WA6150, Western Australia, Australia

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_8

8.1 Introduction

Abiotic stress tolerance plays a vital role in determining crop productivity and distribution of plant species across the environment (Boyer 1982; Chaves et al. 2003). Environmental stresses such as drought, extreme temperatures, salinity or chemical toxicity bring serious consequences to crop production, causing collectively more than 50% yield losses worldwide (Bray et al. 2000; Wang and Frei 2011). Due to global climate change, abiotic stresses are expected to become more widespread in the coming decades and will pose serious threats to global food security (Ashmore et al. 2006; Battisti and Naylor 2009). Among the environmental stresses, water stress commonly, known as 'drought', is considered as one of the major challenges to crop production worldwide (Yue et al. 2006; IPCC 2007). If the crop is subjected to stress, particularly drought during its early stage of growth, germination and seedling establishment are severely arrested mainly owing to low water uptake, low energy supply and hindered enzyme functions (Okcu et al. 2005; Taiz and Zeiger 2010). The crop phenology is also affected by triggering a premature shifting of plant development from the vegetative stage to reproductive stage. This shortens the crop growth cycle (Desclaux and Roumet 1996). Moreover, all major attributes of plant–water relations, viz. leaf relative water contents (RWC), water potential, osmotic potential, pressure potential and transpiration rate, are significantly affected by drought, leading to impaired crop productivity (Kirkham 2005). Improving plant resistance to water stress and maintaining crop productivity are great challenges for achieving sustainable agriculture. Given the importance of drought stress to agriculture, plant reactions to stresses have been studied extensively. Such studies have added considerably to our understanding of plant response to stress at the whole-plant, morphological, physiological, cellular and molecular levels (Grover et al. 2001). Considerable research has been done to understand the mechanisms of abiotic stress responses in a wide variety of model and crop plant species. Now scientists are recognising that microbial partnerships are a ubiquitous part of plant biology. The presence and roles of microbes in plants are becoming clearer with high-throughput technologies such as genomics, functional genomics, proteomics and metabolomics. Plants form various associations with diverse kinds of microorganisms such as fungi, bacteria, viruses, archaea, protozoa, etc, and the form relationships ranging from mutualism to pathogenicity. One such interaction is the association of plants with fungal endophytes, which have been recorded from most plants studied in natural ecosystems. Fungal endophytes remain inside plant tissues without showing any disease symptoms (Rodriguez et al. 2009; Purahong and Hyde 2011). Besides mycorrhizal endophytes, non-mycorrhizal endophytes have been recovered from most plants. Non-mycorrhizal fungal endophytes (hereafter referred to as endophytes) form an intimate relationship with the host and provide various benefits including protection from drought stress (Lewis and Clements 1986; Rodriguez et al. 2004; Malinowski et al. 2004; Malinowski and Belesky 2006;

Zabalgogeazcoa 2008). Endophyte-mediated drought tolerance is associated with improving growth and productivity of the host. Endophytes also improve osmolyte production; influence plant–water relations and photosynthesis; adjust plant water potential, electrolyte balance, antioxidant synthesis and other structural and functional parameters; and thus enhance the plant's ability to tolerate stresses. This chapter presents an outline of the main studies in the area of water deficit stress responses in plants mediated by non-mycorrhizal fungal endophytes.

8.2 Plant Strategies to Withstand Water Stress

The underlying mechanisms of how plants respond to drought stress have been explored to a great extent from molecular to whole-plant levels. Researchers have identified hundreds of genes that are activated in plants in response to stress. A variety of tools including gene expression patterns and the use of transgenic plants has been developed to investigate the particular roles of these genes in plant responses to stress. Transgenic technologies and the advent of genomics and proteomics have offered a comprehensive profiling of the changes in gene and protein expression resulting from exposure to drought.

Plant reactions to water deficit stress are complicated since it is a function of time and space, and it involves multifaceted mechanisms from genomic, molecular and biochemical levels (Blum 1996; Chaves et al. 2003; Xu et al. 2009). Plants use different mechanisms to cope with the stress, and the way a plant behaves under drought can be explained by the following six broad stategies:

1. Escaping from drought by terminating plant life cycle prior to onset of severe stress, e.g. early flowering in annuals before the start of water deficit (Geber and Dawson 1990)
2. Drought avoidance through increasing water uptake and reducing water loss, e.g. developing root systems and reducing of stomata and canopy area (Schulze 1986; Jackson et al. 2000)
3. Drought tolerance chiefly through maintaining better osmotic balance and expanding elasticity of the cell wall to keep the tissue turgid (Morgan 1984)
4. Drought resistance via changing metabolic routes to thrive under stress condition (e.g. greater antioxidant metabolism) (Bartoli et al. 1999)
5. Drought abandonment by shedding one or more plant organ, e.g. detaching older foliage during drought (Chaves et al. 2003)
6. Drought-adapted physio-biochemical characters developed through plant evolution under long-term drought conditions via mutation and modifications at the genomic level (Hoffmann and Merilä 1999; Sherrard et al. 2009; Maherali et al. 2010)

8.3 Plant–Microbe Interactions and Drought Tolerance in Plant

The interaction of microbes with the plant can be traced back to the origin of plants. The early evolution of plants occurred in a diverse microbial world. Archaea, bacteria, fungi, and viruses had been evolving for billions of years (Reid and Greene 2012). The most well-known plant–microbe interaction is the mutualism between mycorrhizal fungi and plant where both partners generally benefit from each other. However, under natural settings, plants form relationships with endophytic fungi and viruses which can be beneficial or harmful for the partners depending on host types and natural and environmental situations (Bao and Roossinck 2013). Plants in natural systems and crop lands are simultaneously exposed to both biotic and abiotic stresses. Though stress research is mostly focusing on plant response to a particular environmental stress, research focusing to both biotic and abiotic stresses together has also been conducted (Xu et al. 2008; Garrett et al. 2006). Unravelling the complex mechanisms of plant–microbe relations and their effects in abiotic stress tolerance in plants could potentially advocate novel tactics to boost the productivity of crops (Schenk et al. 2012).

8.3.1 What Are Fungal Endophytes?

The term ‘endophyte’ refers to the fungi that live inside the plant intercellular and intracellular spaces for at least part of life cycle, causing no concurrent visible symptoms at any specific moment (Rodriguez et al. 2009; Purahong and Hyde 2011). This definition of endophyte is strictly operational and contextual since it takes into account the result of a specific fungus–host interaction only in a given time under the particular environmental settings, because symptomless endophytes can behave differently (e.g. as pathogens) under altered environmental conditions (Andrew et al. 2012; Sanchez-Marquez et al. 2012). The existence of fungal endophytes from fossil records suggests that endophyte–host associations may have evolved from the time of development of first higher plants on earth (Rodriguez and Redman 1997; Krings et al. 2012). Based on the survey conducted in the last 20 years on endophytes, it is thought that the majority, if not all plants, have one or more types of these endophytes and numerous endophytic species; in some cases, above a hundred can be found in a certain plant species (Arnold 2007). Fungal endophytes have been documented from healthy aerial tissues of conifers (Petrini and Fisher 1986) and grasses (Clay 1988). Further, fungal endophytes have also been reported from marine algae (Hawksworth 1988), lichens (Li et al. 2007), mosses and ferns (Fisher 1996), palms (Frohlich and Hyde 1999) and pteridophytes (Dhargalkar and Bhat 2009). Fungal endophytes can be grouped into three basic ecological groups: (1) mycorrhizal fungi, (2) balancious or ‘grass endophytes’ and (3) non-balancious endophytes (Schulz and Boyle 2005). However, Brundrett (2004) separated mycorrhizal from endophytic interactions in that mycorrhizas

have coordinated plant–fungus development and nutrient transfer at specialized interfaces. Later, Rodriguez et al. (2009) classified the endophytes under two major groups, viz. clavicipitaceous and non-clavicipitaceous on the basis of phylogeny and life history traits. Clavicipitaceous fungal endophytes are limited to certain grasses, while non-clavicipitaceous ones have a broad host range including both nonvascular and vascular plant species. In addition, recent reviews propose that members of the non-clavicipitaceous group can be segregated into three subgroups on the basis of host range, type of tissue infected, pattern spread, in planta infection and the establishment, diversity and benefits given to hosts (Rodriguez et al. 2009; Purahong and Hyde 2011). A diverse kind of relationships exists between the fungal endophytes and plant ranging from mutualistic (Redman et al. 2002), symbiotic and commensal (Deckert et al. 2001) to pathogens (Schulz et al. 1998). However, the state of the interaction between endophyte and host may be transitory, and many factors could make changes in their mode of interaction. In symbiotic associations, balansiaceous endophytes with their hosts are commonly considered as being mutualistic (Schardl and Clay 1997) even though some of them provide nothing to their hosts and can occasionally be antagonistic (Schardl et al. 2004a). Although most of the endophytes are regarded as being mutualistic with their hosts, some fungal endophytes may become pathogenic to plants, depending on the developmental stage of the partners, environmental conditions and plant defence reactions (Schulz and Boyle 2005). Endophytic fungi have been known to play a vital role in plant growth, especially grasses; however, few reports have elucidated their symbiosis with crops. Recently, the ecological roles of some endophytes have been explained (Redman et al. 2001; Waller et al. 2005; Arnold et al. 2007). In addition to providing nutritional benefits, fungal endophytes also confer significant physiological (Malinowski and Belesky 2000; Malinowski et al. 2004) and ecological (Malinowski and Belesky 2006) benefits, including protection from environmental stress (Rodriguez et al. 2004) as well as from an attack of pathogens (Zabalgogeazcoa 2008) and pests (Lewis and Clements 1986).

8.3.2 Mechanisms of Endophyte-Mediated Plant Drought Tolerance

Fungal endophytes have been shown to provide fitness benefit to plant when exposed to water-limiting conditions. Perhaps the most widely documented example of endophyte-mediated drought stress tolerance in plants is the enhanced drought tolerance of tall fescue and perennial ryegrass due to infection of the endophyte *Neotyphodium coenophialum*. Kane (2011) studied with the leaf-inhabiting endophyte *Neotyphodium lolii* to assess its potential benefits or harm in drought stress tolerance of native perennial ryegrass collections formerly obtained from the Mediterranean regions. Non-grass fungal endophytes have also been described to help plants alleviate drought stress (Redman et al. 2011; Khan et al. 2012; Waqas et al. 2012). The findings showed that endophyte colonization can help improve

abiotic stress tolerance such as drought in that host. It must be noted that endophytic symbiosis in plants does not always benefit the plant under drought or other abiotic stress conditions, and their interactions could cost for plants in terms of their ability to stand in stresses (Eerens et al. 1998; Cheplick et al. 2000; Cheplick 2004, 2006). Cheplick (2006) reviewed the role of fungal endophytes on potential drought tolerance and cited some studies where endophytes imparted no improvement in the host's ability to tolerate drought stress. For instance, Zaurov et al. (2001) inoculated fescue plants with *Neotyphodium* isolates collected from dissimilar hosts. They observed that some genotypic combinations affected negatively on plant mass, some had no effect and others increased plant biomass. Similarly, few combinations improved tolerance to soil aluminium; others have neutral or decreased tolerance compared to endophyte-free clones. This study revealed that genotype-specific interactions may increase or decrease or have no effect on plant adaptation and fitness. Thus, endophyte-mediated response to water stress is a complex phenomenon involving various metabolites and metabolic pathways. While the ability of fungal endophytes to provide drought tolerance in host plants has been described in many studies, the underlying mechanism(s) are incompletely characterized. In an effort to illuminate the underlying mechanism by which endophyte causes increased drought tolerance, researchers have reported few observations. Research so far studying the effect of endophyte on plant responses to drought stress have described certain physiological, biological and biochemical modifications such as (a) increased growth and development, (b) enhanced osmotic balance, (c) increased gaseous exchange and water-use efficiency and (d) improved defence against oxidative damage when water-limiting conditions may improve, alleviate and recompense the harmful effects of water stress in endophyte-colonized (EC) plants (Fig. 8.1). The present chapter aimed at outlining the recent advances in the study of improvement of drought tolerance by endophyte colonization in plant subjected to water stress.

8.3.2.1 Endophyte-Mediated Plant Growth Enhancement

Fungal endophytes have been shown to enhance growth and biomass of plants under water-limiting conditions. For example, inoculation of *Fusarium culmorum* and *Curvularia protuberata* resulted in higher biomass of drought-affected rice plants than non-inoculated plants (Redman et al. 2011). Endophytes *Chaetomium globosum* and *Penicillium resedanum* isolated from *Capsicum annuum* plants promoted shoot length and biomass of the host plants subjected to drought stress (Khan et al. 2012; Khan et al. 2014). Drought-challenged tomato plants showed higher root and shoot biomass when inoculated with class 2 fungal endophytes, including *Alternaria* sp. and *Trichoderma harzianum* (Azad and Kaminskyj 2016). Inoculating a *Trichoderma hamatum* isolate caused increased higher root fresh weight, dry weight and water content, regardless of water availability in *Theobroma cacao* (cacao) (Bae et al. 2009). The endophyte *Piriformospora indica* colonization in Chinese cabbage promoted root and shoot growth and lateral root development (Sun et al. 2010). Production of auxins by fungal endophytes is attributed to the increased growth of plants under stress (De Battista et al. 1990). Also, stress-induced endogenous abscisic acid and the genes involved, such as zeaxanthin epoxidase,

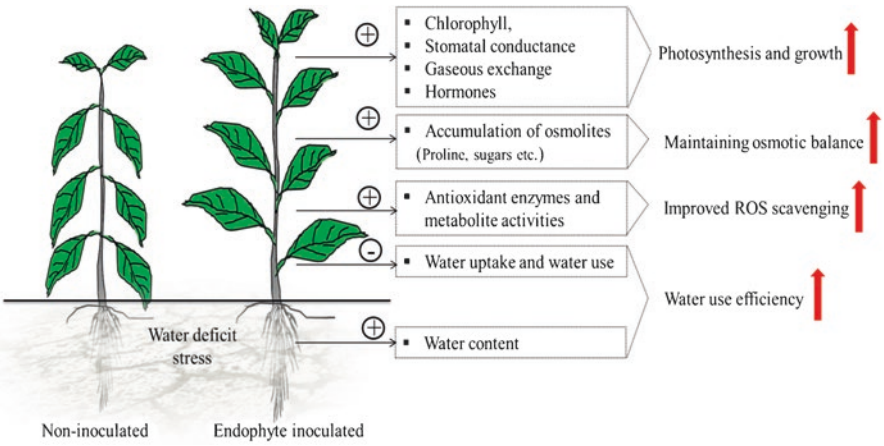


Fig. 8.1 Endophyte colonization can help plants better withstand in water deficit stress by exerting their effects, directly or indirectly, on plant functions at both above- and belowground. The plant on the left side which represents a drought-stressed non-colonized plant shows reduced growth and biomass due to lower photosynthetic rate, higher amount of oxidative damage, reduced uptake water and unbalanced osmoregulation. On the *right* side, a fungal endophyte-colonized plant under water deprivation stress is shown. Endophyte colonization shows increased growth and biomass due to enhanced photosynthetic rate, increased water-use efficiency and better osmotic balance. There is higher accumulation of osmolytes and lower degree of oxidative damage in the EC plants

9-cis-epoxycarotenoid dioxygenase 3 and ABA aldehyde oxidase 3, have been found to be significantly decreased in endophyte-colonized plants under stress, the effect of which could be comparable to that of the exogenous GA3 in terms of promoting plant growth and yield under stressed conditions by manipulating hosts' physiological processes (Khan et al. 2014). However, in some cases, it was recorded that endophytes do not show positive effects on host growth during drought stress, but they help with rapid recovery of host plant after water became available again (Ren and Clay 2008).

8.3.2.2 Endophyte-Mediated Improved Photosynthesis

Moisture stress causes decreased levels of photosynthesis in plants through decreased synthesis of ATP and other enzymes such as rubisco and sucrose–phosphate synthase as water availability decreases (Vassey and Sharkey 1989; Flexas and Medrano 2002; Parry et al. 2002; Ghannoum et al. 2003). Plant tolerance to water stress involves the management of extra radiation caused by reduced photosynthesis and CO_2 availability and a greater susceptibility to photo-damage (Powles 1984; Chaves et al. 2003). The endophyte-colonization results in higher chlorophyll content and leaf area in plants challenged by stress than non-colonized plant. Higher concentration of chlorophyll is associated with higher photosynthetic rate (Davies et al. 1993). The increased rate of photosynthesis was recorded from the drought-stressed *Capsicum annuum* plants colonized by endophytes *Chaetomium globosum* (Khan et al. 2012) and *Penicillium resedanum* (Khan et al. 2014). About twofold

increase in chlorophyll content and photosynthetic efficiency in *P. indica*-colonized *Arabidopsis* plants was measured when seedlings were challenged with water-limiting conditions (Sherameti et al. 2008). *P. indica* reduced the drought-induced decline in the photosynthetic rate and the denaturation of chlorophyll and thylakoid proteins (Sun et al. 2010). Although, the Fv/Fm values decreased in the non-EC plants under drought, no significant difference was observed for the *P. indica*-colonized plants indicating that EC plants suffer less from water stress than un-inoculated controls. In the same study, the total chlorophyll level was reported to be reduced by more than 50% in non-EC plant, but colonized plants showed only a slight decrease in total chlorophyll content (Sun et al. 2010). Additionally, a decrease in the protein levels of representative constituents of the thylakoid membrane and of enzymes situated in the plastid stroma in stressed plants was retarded when colonized with *P. indica* (Sun et al. 2010). Recently, Azad and Kaminskyj (2016) characterized a fungal endophyte that enhanced drought tolerance of the host and increased photosynthesis in the leaf. The mechanism of increased photosynthesis in EC plant under water stress is not fully understood. In one study, it was found that while the photosynthesis rate and stomatal conductance increased in drought-affected EC plants, initial rubisco activity and carboxylation efficiency did not differ from non-EC plants (Morse et al. 2002). It was suggested that endophyte colonization might result in reduced biochemical damage to the photosynthetic machinery plants subjected to water stress (Swarthout et al. 2009).

8.3.2.3 Plant–Water Relation and Osmotic Adjustment as Mediated by Endophyte

In the broad sense, decreasing water loss and maintaining water uptake are the key processes that plants employ to adapt to water-limiting environments. Maintaining water uptake is assisted within plant cells by osmotic adjustment (OA), a biochemical mechanism that helps plants to adapt to drought conditions. OA results in a net accrual of compatible solutes, also known as osmolytes in the cell so as to maintain the favourable gradient for water flow from soil into roots (Sanders and Arndt 2012). This accumulation of various ions, amino acids and sugars leads to a more negative osmotic potential, which is important for maintaining cell hydration and turgor, cellular development and growth, stomatal opening, photosynthesis and water uptake during drought (Chaves et al. 2003; Sanders and Arndt 2012). Endophyte-colonized plants consume significantly less water than non-colonized plants. For example, significantly less water use has been reported in endophyte-inoculated panic grass, rice, tomato and dune grass, indicative of their more efficient water usage. Reduced water consumption and improved water-use efficiency may offer a distinctive mechanism for endophyte-mediated drought resistance in plants (Rodriguez et al. 2008). Again, EC plants can maintain significantly greater water content than the non-inoculated under water stress, implying the ability of endophytes to delay desiccation and damage in stress. The endophyte association could help plant access larger volumes of water from sources not reachable to the non-infected plants which suffered from stress (Khan et al. 2013). Endophyte association resulted in a decreased level of electrolytic leakage inside the plant tissues upon exposure to water deficit

stress. Altered water potential and improved osmotic balance in drought-affected tall fescue infected with *N. coenophialum* endophyte have also been noted in some studies (Elmi and West 1995). Increased root water content was reported from *T. hamatum*-inoculated *T. cacao* plant subjected to water deficit stress compared to non-inoculated plants (Bae et al. 2009). A number of fungal endophytes have been reported to produce active biochemicals and metabolites that help the host plant withstand water deficit stress. Under drought conditions, significantly upregulation of free glucose, fructose, trehalose, sugar alcohols, proline and glutamic acid was detected in shoots and roots in tall fescue colonized by *Neotyphodium coenophialum* (Nagabhyru et al. 2013). Variable levels of proline accumulation were observed in EC plants subjected to water stress. While significantly more proline was accumulated in one genotype of tall fescue plant, no differences were observed in another genotype challenged with mimic drought in hydroponic culture (Bayat et al. 2009) when inoculated with *Neotyphodium* grass endophyte. Increased level of proline, soluble sugar and catalase (CAT) was observed in wheat colonized by endophyte *Chaetomium globosum* under water stress (Cong et al. 2015). Concentrations of aspartic acid and glutamic acid and of alanine and γ-aminobutyric acid were measured in drought-affected *Theobroma cacao* seedlings colonized by an isolate of *Trichoderma hamatum* (Bae et al. 2009). The changes in metabolites could be attributed to the strategies of EC plants towards drought tolerance or avoidance. Downregulation in osmolytes has previously been described as a strategy of drought avoidance, whereas the increase of osmoprotectants has been related to drought tolerance (Augé and Moore 2005; Ruiz-Sánchez et al. 2010).

8.3.2.4 Endophyte-Mediated ROS Scavenging

Reactive oxygen species (ROS) act as signalling molecules in plants. ROS is involved in many plant processes, including growth, stress response, cell cycle and programmed cell death by influencing the expression of related genes. Abiotic stresses cause excess synthesis of these highly reactive molecules, these ROS causing oxidative stress and damaging proteins, lipids and DNA (Gechev et al. 2006; Gill and Tuteja 2010). Manufacturing additional ROS, i.e. hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), singlet oxygen and superoxides ($^1O_2$), is one of the main mechanisms for plant cell damage or death in drought (Smirnoff 1993). Plants react against excess ROS through an intricate network of direct ROS-quenching activity or indirect hormone-mediated signalling activity. Various enzymatic and non-enzymatic antioxidant molecules are involved in scavenging ROS (revised in Miller et al. 2010; Scheibe and Beck 2011). Malfunctioning of these antioxidants' defence system results in oxidative damage in cells (Apel and Hirt 2004; Kwak et al. 2006). Endophyte colonization simulates a more powerful ROS-scavenging system in host plants under stress and reduces damage of biomolecules at the cellular level. For instance, a lower level ROS production has been documented in endophyte-colonized tomato plants than in control plants following water stress (Azad and Kaminskyj 2016). When plants were inoculated with *P. indica* and exposed to drought stress, up-regulation of peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) activities in the leaves was observed (Sun et al. 2010).

The level of another biomarker of oxidative stress, namely, malondialdehyde (MDA), was recorded to be lower in *P. indica*-colonized cabbage plants than in control plants. MDA is primarily produced through the ROS-induced degradation of polyunsaturated lipids (Pryor and Stanley 1975; Del Rio et al. 2005). It is suggested that *P. indica* could prevent or reduce the damage of these lipids by inhibiting excess ROS production under stress conditions. Endophytes that promote drought tolerance have also been found to have high levels of loline alkaloids (Schardl et al. 2004b). Further experiments could test if these molecules are associated with the prevention of damage of macromolecules or reduction of ROS effects. Endophyte-induced production of antioxidant enzyme in plants under stress is predominantly observed in leaves (Baltruschat et al. 2008; Vadassery et al. 2009). All these studies demonstrate that endophyte inoculation results in a strong defence response in plant in water stress, in which alleviation of oxidative stress might be a vital part. The study of nonvolatile compounds has been the major focus in most plant antioxidant research. However, plant leaves emitting volatile organic compounds could also play as a further defence system against stresses (Kesselmeier and Staudt 1999; Peñuelas and Munné-Bosch 2005). The effect of volatile compounds such as isoprenoids has been described, where these compounds act as protective agent against oxidative stress in plant through direct ROS scavenging and indirect alteration of ROS signalling in arbuscular mycorrhizal plants (Peñuelas and Munné-Bosch 2005; Rapparini et al. 2008; Lopez-Ráez et al. 2008; Vickers et al. 2009; Walter and Strack 2011; Asensio et al. 2012; Baslam and Goicoechea 2012). Endophyte-colonized plants could emit similar volatile organic compounds to cope with abiotic stress, but this aspect of the research has not been done till date. Further investigation is necessary to have the information on the fungal side as well as the knowledge of the fungal/plant interaction is paramount to elucidate underlying mechanisms regulating antioxidant defences that are crucial to improve the tolerance of plants to drought stress.

8.3.2.5 Molecular Mechanisms of Endophyte-Mediated Plant Drought Tolerance

Studies on the beneficial effects of endophyte symbiosis under drought have predominantly focused at the plant morpho-physiological level. Molecular tools have also been included in this type of studies. The responses of EC plants to stress can be regulated by the expression of drought-associated plant genes, e.g. those associated with signalling and regulatory pathways or those producing enzymes that synthesize various metabolic compounds. It was noted that, under drought conditions, EC and non-EC plants differently regulate the expression of several drought genes in the plant tissue, indicating the association of activation of Ca_2P signalling and related proteins (Singh et al., 2011) involved in the drought tolerance mechanisms. Among the genes regulated by the endophyte symbiosis during drought, delayed expression of drought-altered ESTs such as TcTPP, TcSOT, TcPR5 and TcNI in the leaves and TcPR5 and TcCESA3 in the roots has been described (Bae et al. 2009). Again, the expression a diverse array of stress-related genes, including 29A, ANAC072, DEHYDRATION-FINGER1, Ddelta, CBL1, HAT, etc. putatively

mediate drought tolerance of *Arabidopsis* plants inoculated with *P. indica* (Sherameti et al. 2008). Similarly, up-regulation of drought-associated genes DREB2A, CBL1, ANAC072 and RD29A was also reported in the drought-challenged leaves of *P. indica*-colonized Chinese cabbage plants. The contribution of endophyte to the enhanced drought tolerance of the host plant can be mediated by CAS protein and the thylakoid membrane CAS mRNA level associated with Ca2+ sensing regulator (Sun et al. 2010). Further research could encompass non-targeted screening of cDNA libraries from both endophyte and host plants. Such an approach could allow the detection of stress-induced genes that offer increased stress tolerance in endophyte-colonized hosts. Employing microarrays and next-generation sequencing technologies to elucidate stress tolerance mechanisms (physiological and molecular) involved in endophyte colonization will be used to compare EC and non-EC plants of the same host genotype.

8.4 Future Directions

Studies indicate that fungal endophytes occur in most plant species studied so far. Endophytes that exhibit non-mutualistic lifestyles in particular hosts may form mutualistic symbioses with genetically dissimilar plant species and confer stress tolerance. If this is true for all the endophytes, it may be promising to isolate endophytes from the plant living in harsh environments and exploit their role in genetically different stress-sensitive plant species. To achieve this, identification of novel endophytes from plant of diverse habitat and genotypes is paramount since it is assumed that many endophytes have not yet been identified, and the ecological functions have not been thoroughly studied. The effects of endophytes in improving of drought stress on plants have typically been investigated using pot cultures under greenhouse or growth chamber conditions where interactions between the partners were studied in a controlled manner. However, under natural conditions, endophyte colonization is affected by factors that are absent in controlled greenhouse or laboratory conditions. With a view to fully comprehend the endophyte effects on plant stress tolerance, future research must include field trials. These investigations could include varying levels of stress treatment and nutrition supplement as well as at various geographical locations so as to reveal the effects of endophyte, stress, soil nutrition and their interaction effects. Promising endophyte isolates could also be tested with various crop species under various cropping practices that resembles those used by growers. The proportion of fungal endophytes capable of forming an effective symbiosis with the host under drought stress and enhance tolerance is generally unknown. A thorough investigation of endophyte colonization of various plants and extensive screening of endophyte isolates to select the most promising ones is the first step towards utilizing their full potential. Morphologically, similar strains of the same fungal species can have differential roles on host growth and development as influenced by temperature, pH, water, nutrient availability and other factors (Picone 2003). Such conditional phenomena demand that beneficial endophyte isolates may need to be tested with various host genotypes and local

agroecosystem settings. Again, most studies have been taken place on the plant side, but efforts should be made to study the effect on the endophyte side and how they function at the different circumstances. Therefore, mutants of both partners will be valuable tools to elucidate the fundamental processes involved. Combined efforts where various disciplines as plant physiology, ecology, mycology, biochemistry, molecular biology and biotechnology could meet together are still needed. These investigations should also be united with a thorough analysis of the transmission of this knowledge to natural environments, considering the fact that knowledge of the roles endophytic fungi play in ecosystems is important as parts of the earth are warm and dry.

8.5 Concluding Notes

In nature, plants do not live as independent entities, but form a complex community with diverse organisms including microbes. These organisms, in particular, fungal endophytes, provide significant advantage to the plants that grow in inhospitable environments. From the studies reviewed in this chapter, it is evident that endophyte colonization can significantly improve plant drought stress tolerance. We focused our review on plant growth, photosynthesis, osmotic balance, water relation, metabolic changes and antioxidant production. All these parameters are interrelated and will influence each other, especially at the plant physiological level. How endophytic fungi affect these parameters under drought is still unclear. Molecular approaches will help elucidate the whole response of plant–endophyte interactions at different levels. Further, in-depth investigation involving a combination of approaches, including physiological, biochemical and molecular data and 'omics' techniques, will clarify the interrelated molecular mechanisms and novel metabolic pathways of endophyte-mediated plant drought tolerance.

References

Andrew M, Barua R, Short SM, Kohn LM et al (2012) Evidence for a common toolbox based on necrotrophy in a fungal lineage spanning necrotrophs, biotrophs, endophytes, host generalists and specialists. PLoS One 7:e29943

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fung Biol Rev 21:51–66

Asensio D, Rapparini F, Peñuelas J et al (2012) AM fungi root colonization increases the production of essential isoprenoids vs nonessential isoprenoids especially under drought stress conditions or after jasmonic acid application. Phytochemistry 77:149–161

Ashmore M, Toet S, Emberson L et al (2006) Ozone-a significant threat to future world food production? New Phytol 170:201–204

Augé RM, Moore JL (2005) Arbuscular mycorrhizal symbiosis and plant drought resistance. In: Mehrotra VS (ed) Mycorrhiza: role and applications. Allied Publishers Limited, New Delhi, pp 136–157

Azad K, Kaminskyj S (2016) A fungal endophyte strategy for mitigating the effect of salt and drought stress on plant growth. Symbiosis 68(1):73–78. doi:10.1007/s13199-015-0370-y

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL et al (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60:3279–3295. http://dx.doi.org/10.1093/jxb/erp165

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schäfer P, Schwarzinger I, Zuccaro A, Skoczowski et al (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol 180(2):501–510. doi:10.1111/j.1469-8137.2008.02583.x

Bao X, Roossinck MJ (2013) Multiplexed interactions: viruses of endophytic fungi. Adv Virus Res 86:37–58. doi:10.1016/B978-0-12-394315-6.00002-7

Bartoli CG, Simontacchi M, Tambussi E, Beltrano J, Montaldi E, Puntarulo S (1999) Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. J Exp Bot 50:375–385

Baslam M, Goicoechea N (2012) Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza 22:347–359

Battisti DS, Naylor RL (2009) Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323:240–244

Bayat F, Mirlohib A, Khodambashic M et al (2009) Effects of endophytic fungi on some drought tolerance mechanisms of tall fescue in a hydroponics. Russ J Plant Physiol 56(4):510–516

Blum A (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regul 20:135–148

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Bray EA, Bailey-Serres J, Weretilnyk E et al (2000) Responses to abiotic stresses. In: Gruissem W, Buchannan B, Jones R (eds) Biochemistry and molecular biology of plants. Am Soc Plant Physiol, Rockville, pp 1158–1249

Brundrett MC (2004) Diversity and classification of mycorrhizal associations. Biol Rev 79:473–495

Chaves MM, Maroco JP, Pereira JS et al (2003) Understanding plant responses to drought- from genes to the whole plant. Funct Plant Biol 30:239–264

Cheplick GP (2004) Recovery from drought stress in *Lolium perenne* (Poaceae): are fungal endophytes detrimental? Am J Bot 91:1960–1968

Cheplick GP (2006) Costs of fungal endophyte infection in *Lolium perenne* genotypes from Eurasia and North Africa under extreme resource limitation. Environ Exp Bot 60:202–210

Cheplick GP, Perera A, Koulouris K et al (2000) Effect of drought on the growth of *Lolium perenne* genotypes with and without fungal endophytes. Funct Ecol 14:657–667

Clay K (1988) Fungal endophytes of grasses-a defensive mutualism between plants and fungi. Ecology 69:10–16

Cong GQ, Yin CL, He BL, Li L, Gao KX et al (2015) Effect of the endophytic fungus *Chaetomium globosum* ND35 on the growth and resistance to drought of winter wheat at the seedling stage under water stress. Acta Ecol Sin 35:6120–6128

Davies FT, Potter JR, Linderman RG et al (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P concentration response in gas exchange and water relations. Physiol Plant 87:45–53

De Battista JP, Bacon CW, Severson RF, Plattner RD, Bouton JH et al (1990) Indole acetic acid production by the fungal endophyte of tall fescue. Agron J 82:878e880

Deckert RJ, Melville LH, Peterson L et al (2001) Structural features of a *Lophodermium* endophyte during the cryptic life-cycle phase in the foliage of *Pinuss trobus*. Mycol Res 105:991–997

Del Rio D, Steward AJ, Pellegrini N et al (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 15:316–328

Desclaux D, Roumet P (1996) Impact of drought stress on the phenology of two soybean (*Glycine max* L. Merril) cultivars. Field Crops Res 46:61–70

Dhargalkar S, Bhat DJ (2009) *Echinosphaeria pteridis* sp. and its *Vermiculariopsiella* anamorph. Mycotaxon 108:115–122

Eerens JPJ, Lucas RJ, Easton S, White JGH et al (1998) Influence of the endophyte (*Neotyphodium lolii*) on morphology, physiology, and alkaloid synthesis of perennial ryegrass during high temperature and water stress. N Z J Agric Res 41(2):219–226. doi:10.1080/00288233.1998.9513305

Elmi AA, West CP (1995) Endophyte effects on tall fescue stomatal response, osmotic adjustment, and tiller survival. New Phytol 131:61–67

Fisher PJ (1996) Survival and spread of the endophyte *Stagonospora pteridiicola* in *Pteridium aquilinum*, other ferns and some flowering plants. New Phytol 132:119–122

Flexas J, Medrano H (2002) Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Ann Bot 89:183–189

Frohlich J, Hyde KD (1999) Biodiversity of palm fungi in the tropics: are global fungal diversity estimates realistic? Biodivers Conserv 8:97Z–1004

Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE et al (2006) Climate change effects of plant disease: genomes to ecosystems. Annu Rev Phytopathol 44:489–509

Geber MA, Dawson TE (1990) Genetic variation in and covariation between leaf gas exchange, morphology and development in *Polygonum arenastrum*, an annual plant. Oecologia 85:153–158

Gechev TS, Van Breusegem F, Stone JM, Denev I, Laloi C et al (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays 28:1091–1101

Ghannoum O, Conroy JP, Driscoll SP, Paul MJ, Foyer CH, Lawlor DW et al (2003) Non-stomatal limitations are responsible for drought-induced photosynthetic inhibition in four C_4 grasses. New Phytol 159:835–844

Gill S, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochim 48:909–930

Grover A, Kapoor A, Laksmi OS, Agarwal S, Sahi C, Katiyar-Agarwal S, Agarwal M, Dubey H et al (2001) Understanding molecular alphabets of the plant abiotic stress responses. Curr Sci 80(2):206–216

Hawksworth DL (1988) The variety of fungal-algal symbioses, their evolutionary significance, and the nature of lichens. Bot J Linn Soc 96:3–20

Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol Evol 14:96–101

IPCC (2007) Climate change 2007: synthesis report. In: Pachauri RK, Reisinger A (eds) Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. IPCC, Geneva

Jackson RB, Sperry JS, Dawson TE et al (2000) Root water uptake and transport: using physiological processes in global predictions. Trends Plant Sci 5:482–488

Kane KH (2011) Effects of endophyte infection on drought stress tolerance of *Lolium perenne* accessions from the Mediterranean region. Environ Exp Bot 71(3):337–344

Kesselmeier J, Staudt M (1999) Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. J Atmos Chem 33:23–88

Khan AL, Shinwari ZK, Kim Y, Waqas M, Hamayun M, Kamran M, Lee IJ et al (2012) Role of endophyte *Chaetomium globosum* lk4 in growth of *Capsicum annuum* by production of gibberellins and indole acetic acid. Pak J Bot 44:1601–1607

Khan AL, Waqas M, Khan AR, Hussain J, Kang SM, Gilani SA, Hamayun M, Shin JH, Kamran M, Al-Harrasi A, Yun BW, Adnan M, Lee IJ et al (2013) Fungal endophyte *Penicillium janthinellum* LK5 improves growth of ABA-deficient tomato under salinity. World J Microbiol Biotechnol 29(11):2133–2144. doi:10.1007/s11274-013-1378-1

Khan AL, Waqas M, Lee IJ et al (2014) Resilience of *Penicillium resedanum* LK6 and exogenous gibberellin in improving *Capsicum annuum* growth under abiotic stresses. J Plant Res 128(2):259–268. doi:10.1007/s10265-014-0688-1

Kirkham MB (2005) Principles of soil and plant water relations. Elsevier Academic Press, Burlington
Krings M, Taylor TN, Dotzler N et al (2012) Fungal endophytes as a driving force in land plant evolution: evidence from the fossil record. In: Southworth D (ed) Biocomplexity of plant-fungal interactions. Wiley, New York, pp 5–28
Kwak JM, Nguyen V, Shroeder JI et al (2006) The role of active oxygen species in hormonal responses. Plant Physiol 141:323–329
Lewis GC, Clements RO (1986) A survey of ryegrass endophyte (*Acremonium loliae*) in the U.K. and its apparent ineffectuality on a seedling pest. J Agric Sci 107:633–638
Li WC, Zhou J, Guo SY, Guo LD et al (2007) Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. Fungal Divers 25:69–80
Lopez-Ráez JA, Charnikhova T, Gomez-Roldan V, Matusova R, Kohlen W, De Vos R et al (2008) Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytol 178:863–874
Maherali H, Caruso CM, Sherrard ME, Latta RG et al (2010) Adaptive value and costs of physiological plasticity to soil moisture limitation in recombinant inbred lines of A*vena barbata*. Am Nat 175:211–224
Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci 40:923–940
Malinowski DP, Belesky DP (2006) Ecological importance of *Neotyphodium* spp. grass endophytes in agroecosystems. Grassl Sci 52(1):1–14. doi:10.1111/j.1744-697X.2006. 00041.x
Malinowski DP, Zuo H, Belesky DP et al (2004) Evidence for copper binding by extracellular root exudates of tall fescue but not perennial ryegrass infected with *Neotyphodium* spp. endophytes. Plant Soil 267(1):1–12. doi:10.1007/s11104-005-2575-y
Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R et al (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33(4):453–467
Morgan JM (1984) Osmoregulation and water stress in higher plants. Ann Rev Plant Physiol 35:299–319
Morse LJ, Day TA, Faeth SH et al (2002) Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. Environ Exp Bot 48:257–268
Nagabhyru P, Dinkins RD, Wood CL, Bacon CW, Schardl CL et al (2013) Tall fescue endophyte effects on tolerance to water-deficit stress. BMC Plant Biol 13:127
Okcu G, Kaya DM, Atak M et al (2005) Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.) Turk J Agric For 29:237–242
Parry MA, Andralojc PJ, Khan S, Lea PJ, Keys AJ et al (2002) Rubisco activity: effects of drought stress. Ann Bot 89:833–839
Peñuelas J, Munné-Bosch S (2005) Isoprenoids: an evolutionary pool for photoprotection. Trends Plant Sci 10:166–169
Petrini O, Fisher PJ (1986) Fungal endophytes in *Salicornia perennis*. Trans Br Mycol Soc 87(4):647–651
Picone C (2003) Managing mycorrhizae for sustainable agriculture in the tropics. In: Vandermeer JH (ed) Tropical agroecosystems. CRC, Boca Raton, pp 95–132
Powles SB (1984) Photoinhibition of photosynthesis induced by visible light. Annu Rev Plant Physiol 35:15
Pryor WA, Stanley JP (1975) Letter: a suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Non-enzymatic production of prostaglandin endoperoxides during autoxidation. J Org Chem 40:3615–3617
Purahong W, Hyde KD (2011) Effects of fungal endophytes on grass and non-grass litter decomposition rates. Fungal Divers 47:1–7
Rapparini F, Llusià J, Peñuelas J et al (2008) Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of *Artemisia annua*. Plant Biol 10:108–122
Redman RS, Dunigan DD, Rodriguez RJ (2001) Fungal symbiosis: from mutualism to parasitism, who controls the outcome, host or invader? New Phytol 151:705–716

Redman RS, Kim YO, Woodward CJDA, Greer C, Espino L, Doty S, Rodriguez RJ et al (2011) Increased fitness and adaptation of rice plants to cold, drought and salt stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6:E14823

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM et al (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298:1581

Reid A, Greene SE (2012) How microbes can help feed the world. Report on an American Academy of Microbiology Colloquium. Washington, DC

Ren A, Clay K (2008) Impact of a horizontally transmitted endophyte, *Balansia henningsiana*, on growth and drought tolerance of *Panicum rigidulum*. Int J Plant Sci 170:599e608

Rodriguez RJ, Redman RS (1997) Fungal life-styles and ecosystem dynamics: biological aspects of plant pathogens, plant endophytes and saprophytes. In: Andrews JH, Tommerup L (eds) Advances in botanical research. Academic, London

Rodriguez RJ, Redman RS, Henson JM et al (2004) The role of fungal symbioses in the adaptation of plants to high stress environments. Mitig Adapt Strateg Glob Chang 9(3):261–272

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Rodriguez RJ, White JF, Arnold AE, Redman RS et al (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Ruiz-Sánchez M, Aroca R, Muñoz Y, Armada E, Polón R, Ruiz-Lozano JM et al (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J Plant Physiol 167:862–869

Sanchez-Marquez S, Bills GF, Herrero N, Zabalgogeazcoa I et al (2012) Nonsystemic fungal endophytes of grasses. Fungal Ecol 5:289–297

Sanders GJ, Arndt SK (2012) Osmotic adjustment under drought conditions. In: Aroca R (ed) Plant responses to drought stress. Springer, New York, pp 199–229

Schardl CL, Clay K (1997) Evolution of mutualistic endophytes from plant pathogens. In: Carroll, Tudzynski (eds) The mycota V: Part B. Springer, Berlin, pp 1–17

Schardl CL, Leuchtmann A, Spiering MJ et al (2004a) Annu Rev Plant Biol 55:315–340

Schardl CL, Leuchtmann A, Spiering MJ et al (2004b) Symbioses of grasses with seed borne fungal endophytes. Ann Rev Plant Biol 55:315–340

Scheibe R, Beck E (2011) Drought, desiccation, and oxidative stress. In: Lüttge U, Beck E, Bartels D (eds) Plant desiccation tolerance, Ecological studies, vol 215. Springer, Heidelberg, pp 209–232

Schenk PM, Carvalhais LC, Kazan K et al (2012) Unraveling plant–microbe interactions: can multi-species transcriptomics help? Trends Biotechnol 30(3):177–184

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686

Schulz B, Guske S, Dammann U, Boyle C et al (1998) Endophyte-host interactions. II Defining symbiosis of the endophyte-host interaction. Symbiosis 25:213–227

Schulze ED (1986) Carbon dioxide and water vapor exchange in response to drought in the atmosphere and the soil. Ann Rev Plant Physiol 37:247–274

Sherameti I, Tripathi S, Varma A, Oelmuller R et al (2008) The root-colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. Mol Plant-Microbe Interact 21:799–807. doi:10.1094/MPMI-21-6-0799

Sherrard ME, Maherali H, Latta RG et al (2009) Water stress alters the genetic architecture of functional traits associated with drought adaptation in *Avena barbata*. Evolution 63:702–715

Singh LP, Gill SS, Tuteja N (2011) Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal Behav 6:175–191

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Sun CA, Johnson J, Cai DG, Sherameti I, Oelmuller R, Lou BG et al (2010) *Piriformosapora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017. doi:10.1016/j.jplph.2010.02.013

Swarthout D, Harper E, Judd S, Gonthier D, Shyne R, Stowe T, Bultman T et al (2009) Measures of leaf-level water-use efficiency in drought stressed endophyte infected and non-infected tall fescue grasses. Environ Exp Bot 66(1):88–93

Taiz L, Zeiger E (2010) Plant physiology, 5th edn. Sinauer Associates, Sunderland

Vadassery J, Ranf S, Drzewiecki C, Mithöfer A, Mazars C, Scheel D, Lee J, Oelmüller R (2009) A cell wall extract from the endophytic fungus Piriformospora indica promotes growth of Arabidopsis seedlings and induces intracellular calcium elevation in roots. Plant J 59:193–206

Vassey TL, Sharkey TD (1989) Mild water stress leads to reduced extractable sucrose-phosphate synthase activity in leaves of *Phaseolus vulgaris* L. Plant Physiol 89:1066–1070

Vickers CE, Gershenzon J, Lerdau MT, Loreto F et al (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nat Chem Ecol 5:283–291

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, Wettstein D, Franken P, Kogel KH et al (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A 102:13386–13391. doi:10.1073/pnas.0504423102

Walter MH, Strack D (2011) Carotenoids and their cleavage products: biosynthesis and functions. Nat Prod Rep 28:663–692

Wang Y, Frei M (2011) Stressed food—the impact of abiotic environmental stresses on crop quality. Agric Ecosyst Environ 141:271–286

Waqas M, Khan AL, Kamran M, Hamayun M, Kang SM, Kim YH, Lee IJ et al (2012) Endophytic fungi produce gibberellins and indole acetic acid and promotes host-plant growth during stress. Molecules 17:10754–10773. doi:10.3390/molecules170910754

Xu P, Chen F, Mannas JP, Feldman T, Sumner LW et al (2008) Virus infection improves drought tolerance. New Phytol 180:911–921

Xu ZZ, Zhou GS, Shimizu H (2009) Effects of soil drought with nocturnal warming on leaf stomatal traits and mesophyll cell ultrastructure of a perennial grass. Crop Sci 49:1843–1851

Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, Jin D et al (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics 172(2):1213–1228

Zabalgogeazcoa I (2008) Fungal endophytes and their interaction with plant pathogens. Span J Agric Res 6:138–146

Zaurov DE, Bonos S, Murphy JA, Richardson M, Belanger FC et al (2001) Endophyte infection can contribute to aluminium tolerance in fine fescues. Crop Sci 41:1981–1984

Root-Associated Bacteria: Rhizoplane and Endosphere

9

Reeta Goel, Vinay Kumar, Deep Kumar Suyal, Biplab Dash, Prahalad Kumar, and Ravindra Soni

Abstract

Root-associated microbiota, primarily from the region of rhizoplane and endosphere, have an influential role for promoting plant growth and development. These microbial communities either directly or indirectly affect the root and subsequently the whole plant. However, several studies have been conducted to explore the hidden bacterial world found in rhizoplane and endosphere through several novel techniques for determining their role in enhancing plant growth. In the following sections of this chapter, we are going to discuss the present status of root-associated microbial research.

Keywords

Rhizosphere • Rhizoplane • Endosphere • Bacteria

9.1 Introduction

Microbiota in and around the plant roots are significantly influenced by biophysical and biogeochemical properties of rhizosphere (Hinsinger et al. 2009). It is expected that plant identity can make up these root-associated microbial communities, but

R. Goel • D.K. Suyal
Department of Microbiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

V. Kumar
ICAR-National Institute of Biotic Stress Management, Baronda farm, Raipur, Chhatisgarh, India

B. Dash • P. Kumar • R. Soni (✉)
Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi VishvaVidyalaya, Raipur, Chhatisgarh, India
e-mail: rs31693@gmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_9

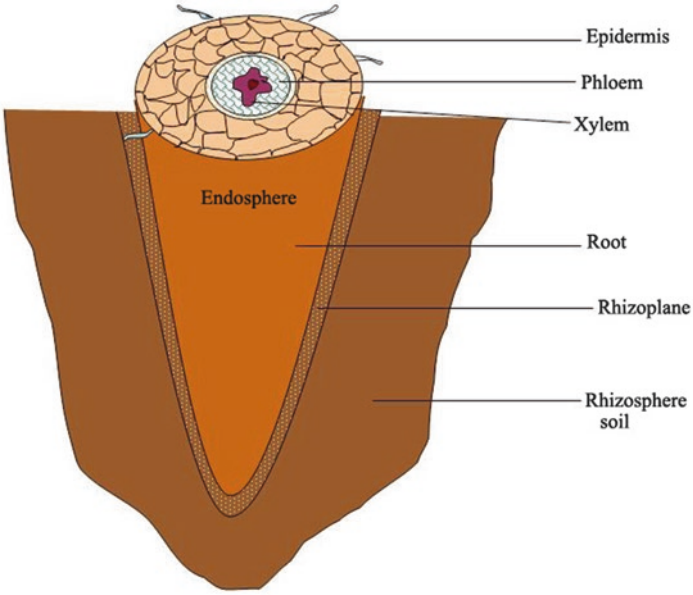


Fig. 9.1 Different root-soil compartments where microbes, generally, make their association with plant

studies have assessed how the diversity of root microbiota varies with plant diversity growing under native habitats (Aleklett et al. 2015). Based on the area of colonization, bacteria associated with plants are grouped into associative bacteria that include communities present in the rhizosphere (in the surrounding area of root) and in rhizoplane (on root surface) and endosphere (Fig. 9.1). Steep competition is observed between roots of adjacent plants for three basic things, i.e., space, water, and nutrients along with soil microorganisms including bacteria and fungi that thrive on organic material for their source of energy (Ryan and Delhaize 2001).

If we talk about the microbiota associated with roots only, then the root has two separate niches, i.e., rhizoplane and endosphere. Rhizoplane harbors diverse group of microbes that are attached on the surface of the root, while at the same time, microbes residing inside the root comes under endosphere (Hacquard et al. 2015). Further, the implications of root–microbe interactions in agriculture (Schlaeppi and Bulgarelli 2015) and for the ecology of plants (Lau and Lennon 2011, 2012; Wagner et al. 2014) have stimulated great interest in the area that shape root-associated microbiota. Our discussion in the sections to come would highlight the successive changes and views of scientific groups in these frontier areas of root-associated microbiota, i.e., rhizoplane and endosphere microbiology.

9.2 Rhizoplane

Rhizoplane is the part of conversion where soil particles along with bacterial and fungal hyphae adhere to the epidermal and cortical layers of the root (Sylvia et al. 2005; Singer and Donald 2006). A very thin boundary is present in-between the rhizoplane and rhizosphere which makes it an obvious choice (Johri et al. 2003). A sizeable number of studies on plant communities are based on soil microbes residing in rhizosphere which are affected by plant roots (Nunan et al. 2005); few have investigated rhizoplane communities, as a separate ecological niche (Kowalchuk et al. 2002; Nunan et al. 2005). Several mechanisms of bacterial colonization are being put forward depending upon the type of bacteria and their colonization of host rhizoplane (Hardoim et al. 2008; Compant et al. 2008, 2010). Probably, the adherence of bacteria to the rhizoplane, possibly, supported by biofilm formation (Villacieros et al. 2003; Reinhold-Hurek et al. 2015), discontinued biofilm or extensive microcolonies (Villacieros et al. 2003; Maurya et al. 2014). However, Barahona et al. (2010) reported that the root-colonizing ability of a bacterial strain cannot be generally contingent from its ability of forming biofilms over abiotic surfaces under standard laboratory conditions. Furthermore, the microbial communities of rhizoplane are being influenced by several aspects except plant species, for example, the constituents of vegetation in the vicinity of these communities, which would also provide bacterial communities with their required substrate and affect other soil constraints such as the nutrient status of nearby soil and factors such as pH (Nunan et al. 2005). In addition, the microbial heterotrophic activity is invigorated by root exudates (Nguyen 2003) and thus there is an increased microbial O_2 consumption in the rhizoplane.

9.2.1 Rhizoplane-Associated Bacterial Population

The epiphytic microbial communities are simultaneously most numerous as well as functionally most prevalent over the rhizoplane, which had various similarities to the surrounding bulk soils. According to Normander and Prosser (2000), rhizoplane bacteria have originated from soil, surrounding the rhizoplane. They have also found out a close resemblance in-between the degenerate gradient gel electrophoresis (DGCE) profiles of rhizoplane and soil as against the profiles found in the root tissue or on the seed, therefore indicating that the primary locus of origin of rhizoplane bacteria is none other than the surrounding soil. This report was also supported by other studies (Bulgarelli et al. 2012). Further, rhizoplane generally has a good amount of bacterial population. In the rhizoplane, the population densities range from 10^5 to 10^7 CFU g/1 of fresh weight (Bais et al. 2006). Dibbern et al. (2014) revealed that most members of the β-*Proteobacteria* (Methylophilaceae, Oxalobacteraceae, Comamonadaceae), the α-*Proteobacteria* (Sphingomonadaceae, Bradyrhizobiaceae), the *Gammaproteobacteria* (Legionellaceae), and the *Bacteroidetes* (Sphingobacteriaceae) were mobilized, all characteristic taxa for the rhizoplane (rhizoplane). Whereas the 16S RDNA clone library from a microbial

rhizoplane community of oilseed rape was dominated by α-*Proteobacteria* and bacteria of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum, less than 17% of the cultured bacteria belonged to these two groups. Allocation in excess of 64% of the cultivated isolates was made to the γ- and γ-subclasses group of Proteobacteria which was present at about 14% in the clone library. Some major rhizoplane bacteria comprising of *Methylococcales*, *Pseudoalteromonadacea*, *Clostridium*, *Vibrio*, and *Desulfovibrio* were found out to have the potential of affecting nutrient cycles (Hong et al. 2015). Furthermore, it was observed that *Bacillus*, *Arthrobacter*, *Listeria*, and *Sporolactobacillus* were the predominant genera in the maize plant, followed by *Azotobacter*, *Micrococcus*, and *Pseudomonas* genera (Cavaglieri et al. 2009). As per the investigation conducted by Bulgarelli et al. (2012), approximately 40% of the *Arabidopsis* root-inhabiting, bacterial population belongs to β-*Proteobacteria*. Recently, a novel bacterial strain designated as MRP-15T, belonging to the class *Sphingobacteria* (phylum *Bacteroidetes*), was isolated from the rhizoplane of *Dioscorea japonica* in South Korea, for which the name *Chitinophaga polysaccharide* sp. nov. was proposed by Han et al. (2014).

9.2.2 Role of Rhizoplane-Associated Bacteria

There is a general acceptance that rhizoplane microorganisms can influence plant growth (Giongo et al. 2010). With respect to plant growth, some rhizoplane microorganisms may be neutral or harmful, whereas other microbes support their host (Raaijmakers et al. 2002). It has been reported that in rhizoplane, a maximum number of salt, pH, and temperature-tolerant phosphate-solubilizing bacteria are found, followed by the rhizosphere and alkaline soils which are root-free (Johri et al. 1999). The genera *Arthrobacter*, *Bacillus*, *Enterobacter*, *Ochrobactrum*, *Pontola*, *Rhodococcus*, *Nocardia*, and *Pseudoxanthomonas* have been observed on the rhizoplanes of petroleum-contaminated soils (Al-Awadhi et al. 2009). Phytoremediation of bitumen-contaminated soil is carried out by plant microflora present in rhizoplane (Muratova et al. 2003). The rhizoplane also serves as a suitable source of antagonistic microorganisms for the biocontrol of soilborne phytopathogens. It is also evident that during in vitro interactions, antagonistic rhizoplane bacteria have the capability of inducing diverse morphological alterations in the phytopathogenic peronosporomycete hype (Deora et al. 2005; Islam et al. 2005).

9.3 Endosphere

Most of the microbes associated with plants are usually seen in rhizospheric soil where a fraction of them called “endophytes” are being able to infiltrate into plant tissues and resides within it (Brader et al. 2014; Mercado-Blanco 2015). All endophytic microbial species occupying the internal spaces of a plant are commonly referred to as “endosphere” which includes all plant parts along with its roots (van Overbeek and Saikkonen 2016). Based on their dependency on plants, endophytes

are classified into two groups, i.e., obligate endophytes and facultative endophytes (Rosenblueth and Martinez-Romero 2006). However, the interactions of microbes with rhizosphere are different and unique in the same plant (Turner et al. 2013). Rosenblueth and Martınez-Romero (2006) postulated that endophytes are generally better able to colonize plant tissues than rhizosphere isolate.

9.3.1 Endosphere-Associated Bacterial Population

High degree of variability is seen in population densities of endophytic bacteria in different plant tissues ranging from few hundreds to as high as 9×10^9 bacteria per gram of plant tissue (Chi et al. 2005). Mostly, throughout the α-, β-, and γ-*Proteobacteria* subgroups, endophytic species are being reported, and the latter is the most diverse and dominant group of them (Gottel et al. 2011; Miliute et al. 2015). Bacteria belonging to genera *Acidobacteria*, *Actinobacteria*, *Aquificae*, *Bacteroidetes*, *Cholorobi*, *Chloroflexi*, *Cyanobacteria*, *Deinococcus*, *Firmicutes*, *Fusobacteria*, *Thermus*, *Gemmatimonadetes*, *Nitrospira*, *Planctomycetes*, *Proteobacteria*, *Spirochaetes*, and *Verrucomicrobia*, representing a total of 16 phyla, are being reported as endophytes (Berg et al. 2006; Mengoni et al. 2009; Manter et al. 2010; Sessitsch et al. 2012; Kaewkla and Franco 2013). Some groups, such as *Mycobacterium* in roots, detected by molecular approach were not isolated by the cultivation method (Conn and Franco 2004). For exploring the bacterial genera, some of the important studies which were conducted after year 2000 are summarized in Table 9.1.

9.3.2 Role of Endosphere-Associated Bacteria

Endosphere microbes are known to have beneficial effects and at the same time are known to have deleterious effects on plant (Compant et al. 2010). Bacterial endophytes are suitable for biocontrol as they colonize and share similar ecological niche as that of phytopathogens (Berg et al. 2005). Additionally, endophytic microbes are known as possible useful sources of bioactive secondary metabolites (Strobel et al. 2004; Zhang et al. 2006) and as medicinally important agents (Silvia Fináková et al. 2007; Huang et al. 2008) in agriculture and industries as well (Joseph and Mini Priya 2011; Nair and Padmavathy 2014). A higher tolerance level for pathogens may occur by inducing plant defense reactions by endophytes (Zamioudis and Pieterse 2012). Useful applications such as phytoextraction and phytoremediation can be achieved by metal-resistant bacteria, isolated from the hyper-accumulator rhizosphere and endosphere (Andria et al. 2009; Visioli et al. 2015; Khan et al. 2015; Ma et al. 2016). Besides this, an enhancement in water retention and increase in biomass and leaf senescence are being carried out by endophytic microbes (Owen and Hundley 2004).

Table 9.1 Root-associated (rhizoplane and endosphere) bacterial genera from different crop/plants (studies conducted after year 2000)

Sr. No.	Name of the endophytic bacteria	Plant/crop	Root compartment	References
1	*Bradyrhizobium*, *Ensifer*, *Mesorhizobium*, *Burkholderia*, *Phyllobacterium*, *Devosia*, *and non-rhizobial bacteria*	Acacia (*Acacia salicina/ stenophylla*)	Endophytes	Hoque et al. (2011)
2	*Pantoea*, *Serratia*, *Acinetobacter*, *Bacillus*, *Agrobacterium*, *and Burkholderia*, *Bradyrhizobium elkanii*, *B. japonicum*, *Enterobacter agglomerans*, *E. sakazakii*, *Erwinia* sp., *Klebsiella oxytoca*, *K. pneumoniae*, *Pseudomonas citronellolis*, *Rhizobium* sp. *NGR 234*, *R. fredii*, *Ensifer* (*Sinorhizobium*) *fredii*, *Bacillus subtilis*, *Bacillus thuringiensis*	Soybean (*Glycine max* L.)	Endophytes	Bai et al. (2002, 2003), Li et al. (2008), Kuklinsky-Sobral et al. (2004, 2005), and Ikeda et al. (2009, 2010)
3	*Pantoea agglomerans*, *Pseudomonas fluorescens*, *R. leguminosarum* bv. *viciae*, *Streptomyces lydicus*	Pea (*Pisum sativum*)	Endophytes	Elvira-Recuenco and van Vuurde(2000) and Tokala et al. (2002)
4	*Bradyrhizobium* sp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* sp., *Burkholderia cepacia*, *Psoralea*	Pigeon pea (*Cajanus cajan*)	Endophytes	Kishore et al. (2005) and Ibanez et al. (2009)
5	*Azospirillum*, *Herbaspirillum*, *Pantoea*, *Methylobacterium*, *Burkholderia and Rhizobium*, *Bradyrhizobium elkanii*, *B. japonicum*, *Azorhizobium*, *Ensifer meliloti*, *Rhizobium oryzicola* sp. nov.	Rice (*Oryza sativa*)	Endophytes	Mano and Morisaki (2008) and Zhang et al. (2015)
6	*Psychrobacter* sp., *Pseudomonas putida*, *Pseudomonas fluorescens*, *Klebsiella terrigena*, *Pseudomonas chlororaphis*, *Bacillus megaterium*, *Rhizobium* sp., *Aminomonas* sp., *and Staphylococcus* sp.	Carrot (*Daucus carota*)	Endophytes	Surette et al. (2003)
7	*Klebsiella pneumoniae*, *Actinobacteria*, *Arthrobacter globiformis*, *Microbacterium testaceum*, *Bacillus megaterium*	Maize (*Zea mays*)	Endophytes	Chelius and Triplett (2000a, b) and Zinniel et al. (2002)

(continued)

Table 9.1 (continued)

Sr. No.	Name of the endophytic bacteria	Plant/crop	Root compartment	References
8	*α-Proteobacteria* (*Agrobacterium*, *Bradyrhizobium*, *Sphingomonas*), *β-Proteobacteria* (*Polaromonas*, *Variovorax*), *γ-Proteobacteria* (*Serratia*, *Stenotrophomonas*, *Pseudomonas*), *Actinobacteria*, *Bacteroidetes*, *Firmicutes*	Wheat (*Triticum aestivum*)	Endophytes	Robinson et al. (2016)
9	*Methylobacterium mesophilicuma*, *Enterobacter cloacae*, *Firmicutes*, *Bacillus* spp, *Curtobacterium flaccumfaciens*, *Nocardia* sp., *Pantoea agglomerans*	Citrus (*Citrus* species)	Endophytes	Araujo et al. (2001, 2002)
10	*Sphingomonas yanoikuyae*, *Pseudomonas pseudoalcaligenes*, *Serratia marcescens*, *Bacillus megaterium*, *Paenibacillus polymyxa*, *B. pumilus*, *B. cereus*, *S. yanoikuyae*, *Pseudomonas fluorescens*, *Arthrobacter globiformis*, and *Paenibacillus polymyxa*	Tomato (*Solanum lycopersicum*)	Endophytes	Hang et al. (2013)
11	*Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, and *Verrucomicrobia*	Duckweed (*Spirodela polyrrhiza*), aquatic plant	Rhizoplane	Matsuzawa et al. (2010)
12	*Bacillus*, *Burkholderia*, *Lysinibacillus*	Moso bamboo (*Phyllostachys edulis*)	Rhizoplane	Han et al. (2009)
13	*Agromyces allii*, *Microbacterium insulae*, *Mycobacterium neoaurum*, *Sphingopyxis witflariensis*, *Sphingobium estrogenivorans*, *Variovorax koreensis*, *Xanthomonas campestris* pv., *Agrobacterium tumefaciens*	Tree peony plants (*Paeonia ostii*)	Rhizoplane	Han et al. (2011)
14	*Chryseobacterium lactis*, *Chryseobacterium joostei*, *Chryseobacterium indologenes*, and *Chryseobacterium viscerum*	Maize (*Zea mays*)	Rhizoplane	Kämpfer et al. (2015)
15	*Streptomyces caeruleus* GIMN4T, *Streptomyces curacoi* NRRL B-2901T, *Streptomyces coeruleorubidus* NBRC 12761T, and *Streptomyces capoamus* JCM 4734T	Ginseng (*Panax ginseng*)	Rhizoplane	Lee et al. (2014)
16	*Rhodanobacter spathiphylli*, *R. panaciterrae*, *R. terrae*, *R. soli*, and *R. caeni*	Soybean (*Glycine max*)	Rhizoplane	Madhaiyan et al. (2014)

9.4 Techniques to Explore Rhizoplane and Endosphere

9.4.1 Exploration of Rhizoplane

Muraoka et al. (2000) observed microbial colonization by light microscopy and scanning electron microscopy on rice root surface. This could not present sufficient information on the variation in the community structure of rhizoplane microbiota among different nodal roots and along with growth stage. Nonetheless, to characterize multiple attributes in poorly characterized bacterial communities, samples are always needed. Transferring of bacteria with the help of a suitable medium from a sample surface for microbiological analysis can be achieved by washing or by blotting root surfaces with filter papers (Dennis et al. 2008; Ravikumar and Davi 2014). Dennis and colleagues reported a new sampling method for examining bacterial communities at the micro-spatial scale from rhizoplane or similar habitat. In this method, they used micro-sampling tungsten rods with laser-cut tips of 0.013 mm^2 surface areas, which were guided to sample sites using a micro-manipulator, while exposed plant root or soil surfaces were viewed with the help of a dissecting microscope (Dennis et al. 2008). Successful application of fluorescent in situ hybridization (FISH) technique has been made for studying the native microbial population of wetland rice in its rhizoplane (Eller and Frenzel 2001). FISH with double labeling of oligonucleotide probes (DOPE-FISH technique) was used by Compant and colleagues (2013) for visualizing the colonization behavior of grapevine root bacteria (rhizoplanic). Moreover, microbial cells were analyzed by using catalyzed reporter deposition-FISH (CARD-FISH) technique on the rhizoplane of wetland rice (Schmidt and Eickhorst 2014). Similarly, Bulgarelli et al. used CARD-FISH and scanning electron microscopy (SEM) for characterizing and visualizing bacteria attached to the rhizoplane (Bulgarelli et al. 2012).

Knief, in his study, used high-throughput 16S rRNA amplicon sequencing strategy for analyzing the rhizoplane-associated bacteria (Knief 2014). PCR-RFLP method was also applied for investigating the seasonal variation of the microbial community and the microbial succession of rice rhizoplane, and this method also proved useful for the purposes (Ikenaga et al. 2002). Ofek-Lalzar et al. (2014) analyzed microbial adaptation to the rhizoplane by a combinatorial approach of metagenomics and metatranscriptomics. Nonetheless, from a technical viewpoint, using advance approaches like metagenomics and high-throughput sequencing for analyzing the adhering root epiphytic compartment is indeed challenging.

9.4.2 Exploration of Endosphere

Visualization of endophytes in plant roots is most important for checking and locating their presence. Currently adopted techniques for visualization of endophytic bacteria in plants include transmission electron microscopy (TEM) (Vendramin et al. 2010), SEM (de Souza et al. 2004), FISH (Compant et al. 2011), and triphenyltetrazolium chloride vital staining (Thomas 2011), while tagging with labels such

as green fluorescent protein (GFP) facilitates the monitoring of externally applied organisms (Prieto et al. 2011). The second most important step in endophytic research is removal of rhizoplane-associated bacteria from the root surface. Around 45% of the rhizoplane population can be removed by vigorous washing as compared to untreated roots. Nonetheless, chemical or mechanical treatment can be used for removing the rhizoplane bacteria from the root surface, while endophytes are said to be present in the rest of the root materials (Gottel et al. 2011; Edwards et al. 2015). Approximately 30% of microbial population can be extracted from the rhizoplane by using chemicals but completely failed in removing microbial cells thereof. Further, studies focusing on endophytes should carefully apply toxic chemical agents such as sodium hypochlorite for removal of rhizoplane-associated cells from the surface of the root (Richter-Heitmann et al. 2016). The combination of 70% ethanol, 2% sodium hypochlorite, and 0.1% mercuric chloride was reported best for surface sterilization of roots (Anjum and Chandra 2015). Obligate endophytes are the most difficult to culture in the laboratory, as specific growth conditions are required for them. Moreover, reduced revival capacity of endophytes is seen even after several successful isolation. However, plant extracts were added to nutrient media for isolation and culture of bacterial endophytes, but despite of this effort, most of them remained uncultured (Alain and Querellou 2009; Eevers et al. 2015).

Therefore, molecular approaches seem to be the only way of analyzing endophytic diversity and their interaction with plants on the molecular level (Barea 2015; Raja et al. 2016). However, unculturable bacterial endophytic diversity revealed some technical limitations related to the separation of endophytic bacteria from plant nuclei, plastids, mitochondria, and plant-associated microbial DNA (Govindasamy et al. 2014). Moreover, culture-independent studies mainly depend upon good quality extracted metagenomic DNA. Here DNA extraction protocols may introduce significant biases in the endophytic microbial community diversities (Kgomotso et al. 2015). Therefore, for detection and identification of microbial communities in healthy plants, next-generation sequencing (NGS) might serve as a powerful tool (Trujillo et al. 2015). Quick analysis of composition and diversity of microbial communities in several habitats can be done by NGS. Similarly, suppression subtractive hybridization (SSH) recently showed its importance in differentiating closely related endophytic bacterial species. For analyzing the genetic diversity, SSH acts as an effective technique among microbes (Galbraith et al. 2004; Monteiro et al. 2012). Likewise, for differential expression analysis of endophytes, shotgun metagenomics (Sessitsch et al. 2012), microarray analysis and SOLiD-SAGE techniques have been used (Dinkins et al. 2010; Ambrose and Belanger 2012).

9.5 Conclusion

Plant roots are no longer considered an unexplored biological boundary, but still there is a lot of alive hope. Updated information on microbial interactions in plant roots can provide new dimensions for developing sustainable agricultural

production with minimal disturbance of the environment. Moreover, a better understanding of the root-associated microbial interaction is required for considering bacteria as a friend or foe to plant system since a number of factors are involved in it. It remains to make better use of traditional ecological values in attempting to better describe the microbial colonization of the root mainly rhizoplane and endosphere. Further, the ecosystem functioning where root-associated microbiota, especially bacterial community, play an important role for coexistence of plant species may prove helpful in exploring plant diversity. Finally, it is clear from the above discussion that the selection of the best strategy with respect to root microbiota exploration still remains a serious issue which has to be addressed with their respective research questions. Finally, for deriving a maximum benefit from the plant-associated bacterial genera, an extensive and intensive research on understanding rhizoplanic and endophytic ecology is required which will serve as the gateway for sustainable agriculture development in the times to come.

References

Alain K, Querellou J (2009) Cultivating the uncultured: limits, advances and future challenges. Extremophiles 13:583–594

Al-Awadhi H, El-Nemr I, Mahmoud H, Sorkhoh NA, Radwan SS (2009) Plant-associated bacteria as tools for the phytoremediation of oily nitrogen-poor soils. Int J Phytoremediation 11:11–27

Aleklett K, Leff J, Fierer N, Hart M (2015) Wild plant species growing closely connected in a subalpine meadow host distinct root-associated bacterial communities. Peer J 3:e804

Ambrose KV, Belanger FC (2012) Solid-Sage of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. PLoS ONE 7:e53214

Andria V, Reichenauer TG, Sessitsch A (2009) Expression of alkane monooxygenase (alkB) genes by plant-associated bacteria in the rhizosphere and endosphere of Italian ryegrass (*Lolium multiflorum* L.) grown in diesel contaminated soil. Environ Pollut 157:3347–3350

Anjum N, Chandra R (2015) Endophytic bacteria: optimization of isolation procedure from various medicinal plants and their preliminary characterization. Asian J Pharm Clin Res 8(4):233–238

Araujo WL, Maccheroni W Jr, Aguilar-Vildoso CI, Barroso PAV, Saridakis HO, Azevedo JL (2001) Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can J Microbiol 47:229–236

Araujo WL, Marcon J, Maccheroni W Jr, Elsas JDV, Vuurde JLV, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 48:230–238

Bai Y, Zhou X, Smith D (2003) Enhanced soybean plant growth due to coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci 43:1774–1781

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Barahona E, Navazo A, Yousef-Coronado F, de Cárcer DA, Martinez-Granero F, Espinosa-Urgel M, Martín M, Rivilla R (2010) Efficient rhizosphere colonization by Pseudomonas fluorescens f113 mutants unable to form biofilms on abiotic surfaces. Environ Microbiol 12(12):3185–3195

Barea JM (2015) Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J Soil Sci Plant Nutr 15:261–282

Berg G, Zachow C, Lottmann J, Gotz M, Costa R, Smalla K (2005) Impact of plant species and site on rhizosphere-associated fungi antagonistic to *Verticillium dahliae* Kleb. Appl Environ Microbiol 71(8):4203–4213

Berg G, Hallmann J. Schulz B, Boyle C, Sieber T (2006) Control of plant pathogenic fungi with bacterial endophytes. Springer, Berlin, pp 53–69

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488:91–95

Cavaglieri L, Orlando J, Etcheverry M (2009) Rhizosphere microbial community structure at different maize plant growth stages and root locations. Microbiol Res., 31 164(4):391–399

Chelius MK, Triplett EW (2000a) Immunolocalization of dinitrogenase reductase produced by *Klebsiella pneumoniae* in association with *Zea mays* L. Appl Environ Microbiol 66:783–787

Chelius MK, Triplett EW (2000b) Diazotrophic endophytes associated with maize. In: Triplett EW (ed) Prokaryotic nitrogen fixation: a model system for the analysis of a biological process. Horizon Scientific Press, Wymondham, pp 779–791

Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71(11):7271–7278

Compant S, Kaplan H, Sessitsch A, Nowak J, Ait Barka E, Clement C (2008) Endophytic colonization of *Burkholderia phytofirmans* strain PsJN in *Vitis vinifera* L.: from the rhizosphere to inflorescence tissues. FEMS Microbiol Ecol 63:84–93

Compant S, Clément C, Sessitsch A (2010) Plant growth promoting bacteria in the rhizo- and endosphere of plants. Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Compant S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A (2011) Endophytes of grapevine flowers, berries and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb Ecol 62:188–197

Compant S, Saima M, Lebrihi A, Florence M (2013) Visualization of grapevine root colonization by the Saharan soil isolate *Saccharothrix algeriensis* NRRL B-24137 using DOPE-FISH microscopy. Plant Soil 370(1–2):583–591

Conn VM, Franco CMM (2004) Analysis of endophytic actinobacterial population in the roots of wheat (*Triticum aestivum* L.) by terminal restriction fragment length polymorphism and sequencing of 16S rRNA clones. Appl Environ Microbiol 70:787–1794

De Souza AO, Pamphile A, De Mello-Sartori CL, Da Rocha C, Azevedo JL (2004) Plant-microbe interactions between maize (*Zea mays* L.) and endophytic microorganisms observed by scanning electron microscopy. Acta Scientiarum Biol Sci 26:357–359

Dennis Paul G, Miller AJ, Clark IM, Taylor RG, Valsami-Jones E, Hirsch PR (2008) A novel method for sampling bacteria on plant root and soil surfaces at the microhabitat scale. J Microbiol Methods 75:12–18

Deora A, Hashidoko Y, Islam MT, Tahara S (2005) Antagonistic rhizoplane bacteria induce diverse morphological alterations in *Peronosporomycete* hyphae during in vitro interaction. Eur J Plant Pathol 112:311–322

Dibbern D, Schmalwasser A, Lueders T, Totsche KU (2014) Selective transport of plant root-associated bacterial populations in agricultural soils upon snowmelt. Soil Biol Biochem 69:187–196

Dinkins RD, Barnes A, Waters W (2010) Microarray analysis of endophyte-infected and endophyte-free tall fescue. J Plant Physiol 167:1197–1203

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci 112(8):911–920

Eevers N, Gielen M, Sánchez-López A, Jaspers S, White JC, Vangronsveld J, Weyens N (2015) Optimization of isolation and cultivation of bacterial endophytes through addition of plant extract to nutrient media. Microb Biotechnol 8(4):707–715

Eller G, Frenzel P (2001) Changes in activity and community structure of methane-oxidizing bacteria over the growth period of rice. Appl Environ Microbiol 67:2395–2403

Elvira-Recuenco M, van Vuurde JWL (2000) Natural incidence of endophytic bacteria in pea cultivars under field conditions. Can J Microbiol 46:1036–1041

Firáková S, Šturdíková M, Múčková M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants, Section Botany, Institute of Botany, , Institute of Botany, Slovak Academy of Sciences. Biologia 62(3):251–257

Galbraith EA, Antonopoulos DA, White BA (2004) Suppressive subtractive hybridization as a tool for identifying genetic diversity in an environmental metagenome: the rumen as a model. Environ Microbiol 6:928–937

Giongo A, Anelise B, Adriana A, Kayser LV, Roberto SM, Luiz EF, Zanettini B, Helena M, Pereira PLM (2010) Isolation and characterization of two plant growth-promoting bacteria from the rhizoplane of a legume (*Lupinus albescens*) in sandy soil. Rev Bras Ciênc Solo 34(2):361–369

Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW (2011) Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. Appl Environ Microbiol 77:5934–5944

Govindasamy V, Franco CMM, Gupta VVSR (2014) Endophytic actinobacteria: diversity and ecology. In: Advances in endophytic research. Springer, New Delhi, India. pp 27–59

Hacquard S, Garrido-Oter R, Gonzalez A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R, Schulze-Lefert P (2015) Microbiota and host nutrition across plant and animal kingdoms. Cell Host Microbe 17:603–616

Han J, Xia D, Li L, Lang L, Zhang L (2009) Diversity of culturable bacteria isolated from root domains of Moso Bamboo (*Phyllostachys edulis*). Microb Ecol 58:363

Han J, Song Y, Liu Z, Hu Y (2011) Culturable bacterial community analysis in the root domains of two varieties of tree peony (*Paeonia ostii*). FEMS Microbiol Lett 322(1):15–24

Han SI, Lee HJ, Whang KS (2014) Chitinophaga polysaccharea sp. nov., an exopolysaccharide-producing bacterium isolated from the rhizoplane of *Dioscorea japonica*. Int J Syst Evol Microbiol 64(1):55–59

Hang F, Yanchang L, Qiongguang L (2013) Endophytic bacterial communities in tomato plants with differential resistance to *Ralstonia solanacearum*. Afr J Microbiol Res 7:1311–1318

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471

Hinsinger P, Bengough AG, Vetterlein D, Young I (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152

Hong Y, Liao D, Hu A, Wang H, Chen J, Khan S, Su J, Li (2015) Diversity of endophytic and rhizoplane bacterial communities associated with exotic *Spartina alterniflora* and native mangrove using Illumina amplicon sequencing. Can J Microbiol, 61 (10): 723–733

Hoque MS, Broadhurst LM, Thrall PH (2011) Genetic characterization of root nodule bacteria associated with *Acacia salicina* and *Acacia stenophylla* (Mimosaceae) across south eastern Australia. Int J Syst Evol Microbiol 61(2):299–309

Huang WY, Cai Y, Hyde KD, Corke H, Sun M (2008) Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Divers 33:61–75

Ibanez F, Angelini J, Taurian T, Tonelli ML, Fabra A (2009) Endophytic occupation of peanut nodules by opportunistic Gammaproteobacteria. Syst Appl Microb 32(1):49–55

Ikeda S, Kaneko T, Ohkubo T, Rallos LE (2009) Development of a bacterial cell enrichment method and its application to the community analysis in soybean stems. Microb Ecol 58:703–714

Ikeda S, Okubo T, Anda M, Nakashita H (2010) Community and genome-based views of plant-associated bacteria: plant–bacterial interactions in soybean and rice. Plant Cell Physiol 51:1398–1410

Ikenaga M, Muraoka Y, Toyota K, Kimura M (2002) Community structure of the microbiota associated with nodal roots of rice plants along with the growth stages: estimation by PCR-RFLP analysis. Biol Fertil Soils 36:397–340

Islam MT, Hashidoko Y, Deora A, Ito T, Tahara S (2005) Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne peronosporomycetes. Appl Environ Microbiol 71(7):3786–3796

Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH and temperature tolerant phosphate solubilizing bacteria in alkaline soils. Curr Microbiol 39:89–93

Johri BN, Sharma A, Virdi JS (2003) Rhizobacterial diversity in India and its influence on soil and plant health. Adv Biochem Eng Biotechnol 84:49–89

Joseph B, Mini Priya R (2011) Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Am J Biochem Mol Biol 1(3):291–309

Kaewkla O, Franco CMM (2013) Rational approaches to improving the isolation of endophytic actinobacteria from Australian native trees. Microb Ecol 65:384–393

Kämpfer P, McInroy JA, Glaeser SP (2015) *Chryseobacterium rhizoplanae* sp. nov., isolated from the rhizoplane environment. Antonie Van Leeuwenhoek 107(2):533–538

Kgomotso M, Maropola A, Ramond JB, Trindade M (2015) Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in *Sorghum bicolor* (L. Moench). J Microbiol Methods 112:104–117

Khan MU, Sessitsch A, Harris M, Fatima K, Imran A, Arslan M, Shabir G, Khan QM, Afzal M (2015) Cr-resistant rhizo and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils. Front Plant Sci 6(5):755

Kishore GK, Pande S, Podile AR (2005) Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut (*Arachis hypogaea* L.) Lett Appl Microbiol 40(4):260–268

Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front Plant Sci 5:216

Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PGL, van Veen JA (2002) Effects of aboveground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek 81:509–520

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean- associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Kuklinsky-Sobral J, Araujo WL, Mendes R, Pizzirani-Kleiner AA, Azevedo JL (2005) Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 273:91–99

Lau JA, Lennon JT (2011) Evolutionary ecology of plant-microbe interactions: soil microbial structure alters selection on plant traits. New Phytol 192:215–224

Lau JA, Lennon JT (2012) Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc Natl Acad Sci U S A 109:14058–14062

Lee HJ, Cho GY, Chung SH, Whang KS (2014) *Streptomyces panaciradicis* sp. nov., a β-glucosidase-producing bacterium isolated from ginseng rhizoplane. Int J Syst Evol Microbiol 64:3816–3820

Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40:238–246

Ma Y, Zhang C, Oliveira RS, Freitas H, Luo Y (2016) Bio augmentation with endophytic bacterium E6S homologous to *Achromobacter piechaudii* enhances Metal Rhizo accumulation in Host Sedum plumbizincicola. Front Plant Sci 7:75

Madhaiyan M, Poonguzhali S, Saravanan VS, Kwon SW (2014) *Rhodanobacter glycinis* sp. nov., a yellow pigmented gammaproteobacterium isolated from the rhizoplane of field-grown soybean. Int J Syst Evol Microbiol 64:2023–2028

Mano H, Morisaki H (2008) Endophytic bacteria in the rice plant. Microbes Environ 23:109–117

Manter DK, Kolodny EH, Hansen EM, Parke JL (2010) Virulence, sporulation, and elicitin production in three clonal lineages of *Phytophthora ramorum*. Physiol Mol Plant Pathol 74:317–322

Matsuzawa H, Tanaka Y, Tamaki H, Kamagata Y, Mori K (2010) Culture-dependent and independent analyses of the microbial communities inhabiting the giant duckweed (*Spirodela polyrrhiza*) rhizoplane and isolation of a variety of rarely cultivated organisms within the phylum Verrucomicrobia. Microbes Environ/JSME 25(4):302–308

Maurya MK, Singh R, Tomer A (2014) In vitro evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen. J Biopest 7(1):43–46

Mengoni A, Pini F, Shu WS, Huang LN, Bazzicalupo M (2009) Plant-by-plant variations of leaf-associated bacterial communities in the nickel-hyper accumulator *Alyssum bertolonii* Desv. Microb Ecol 58:660–667

Mercado-Blanco J (2015) Life of microbes inside the plant. In: Lugtenberg B (ed) Principles of plant- microbe interactions. Springer, Heidelberg, pp 25–32

Miliute I, Buzaite O, Stanys V (2015) Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste-Agriculture 102(4):465–478

Monteiro RA, Balsanelli E, Tuleski T, Faoro H, Cruz LM, Wassem R, de Baura VA, Tadra-Sfeir MZ, Weiss V, DaRocha WD, Muller-Santos M, Chubatsu LS, Huergo LF, Pedrosa FO, de Souza EM (2012) Genomic comparison of the endophyte *Herbaspirillum seropedicae* SmR1 and the phytopathogen *Herbaspirillum rubrisubalbicans* M1 by suppressive subtractive hybridization and partial genome sequencing. FEMS Microbiol Ecol 80:441–451

Muraoka Y, Hamakawa E, Toyota K, Kimura M (2000) Observation of microbial colonization on the surface of rice roots along with their development and degradation. Soil Sci Plant Nutr 46:491–502

Muratova A, Hubner T, Narula N, Wand H, Turkovskaya O, Kuschk P, Jahn R, Merbach W (2003) Rhizosphere microflora of plants used for the phytoremediation of bitumen-contaminated soil. Microbiol Res 158:151–161

Nair DN, Padmavathy S (2014) Impact of endophytic microorganisms on plants, environment and humans. Sci World J 2014: 11

Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396

Normander B, Prosser JI (2000) Bacterial origin and community composition in the barley phytosphere as a function of habitat and presowing conditions. Appl Environ Microbiol 66:4372–4377

Nunan N, Daniell TJ, Singh BK, Papert A, McNicol JW, Prosser JI (2005) Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. Appl Environ Microbiol 71(11):6784–6792

Ofek-Lalzar M, Sela N, Goldman-Voronov M, Green SJ, Hadar Y, Minz D (2014) Niche and host associated functional signatures of the root surface microbiome. Nat Commun 5:49–50

Owen NL, Hundley N (2004) Endophytes–the chemical synthesizers inside plants. Sci Prog 87(2):79–99

Prieto P, Schiliro E, Maldonado-González MM, Valderrama R, Barroso-Albarracín JB, Mercado-Blanco J (2011) Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. with biocontrol activity. Microb Ecol 62:435–445

Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81:537–547

Raja S, Subhashini P, Thangaradjou T (2016) Differential methods of localisation of fungal endophytes in the seagrasses. Mycology 7(3):112–123

Ravikumar M, Malani Devi S (2014) Occurrence of fungal flora in water, air, soil ecosystem and screening of fungal enzymes. Int J Adv Multidiscip Res 1(1):52–62

Reinhold-Hurek B, Bunger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hot spots for microbial activity. Annu Rev Phytopathol 53:403–424

Richter-Heitmann T, Eickhorst T, Knauth S, Friedrich MW, Schmidt H (2016) Evaluation of strategies to separate root-associated microbial communities: a crucial choice in rhizobiome research. Front Microbiol 7:773

Robinson RJ, Fraaije BA, Clark IM, Jackson RW, Hirsch PR, Mauchline TH (2016) Endophytic bacterial community composition in wheat (*Triticum aestivum*) is determined by plant tissue type, developmental stage and soil nutrient availability. Plant Soil 405:381–396

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19(8):827–837

Ryan PR, Delhaize E (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Mol Biol 52:527–560

Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. Mol. Plant Microbe Interact 28:212–217

Schmidt H, Eickhorst T (2014) Detection and quantification of native microbial populations on soil-grown rice roots by catalyzed reporter deposition fluorescence in situ hybridization. FEMS Microbiol Ecol 87:390–402

Sessitsch A, Hardoim P, Doring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T, Sarkar A, Bodrossy L, van Overbeek L, Brar D, van Elsas JD, Reinhold-Hurek B (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Interact 25:28–36

Singer MJ, Donald NM (2006) Soils: an introduction. Pearson Education, New Jersey

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Surette M, Sturz A, Lada R, Nowak J (2003) Bacterial endophytes in processing carrots (*Daucus carota* L. Var. sativus): their localization, population density, biodiversity and their effects on plant growth. Plant Soil 253:381–390

Sylvia D, Fuhrmann J, Hartel P, Zuberer D (2005) Principles and applications of soil microbiology. Pearson Education, New Jersey

Thomas P (2011) Intense association of non-culturable endophytic bacteria with antibiotic-cleansed in vitro watermelon and their activation in degenerating cultures. Plant Cell Rep 30:2313–2325

Tokala RK, Strap JL, Jung CM, Crawford DL (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol 68:2161–2171

Trujillo ME, Riesco R, Benito Pand Carro L (2015) Endophytic Actinobacteria and the interaction of *Micromonospora* and nitrogen nixing plants. Front Microbiol 6:1341

Turner TR, Ramakrishnan K, Walshaw J, Heavens D, Alston M, Swar-beck D, Osbourn A, Grant A, Poole PS (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME 7:2248–2258

Van Overbeek LS, Saikkonen K (2016) Impact of bacterial–fungal interactions on the colonization of the endosphere. Trends Plant Sci 21:3

Vendramin E, Gastaldo A, Tondello A, Baldan B, Villani M, Squartini A (2010) Identification of two fungal endophytes associated with the endangered orchid *Orchis militaris* L. J Microbiol Biotechnol 2:630–636

Villacieros M, Power B, Sanchez-Contreras M, Lloret J, Oruezábal RI, Martín M, Bonilla I, Whelan C, Dowling DN, Rivilla R (2003) Colonization behaviour of *Pseudomonas fluorescens* and *Sinorhizobium meliloti* in the alfalfa (*Medicago sativa*) rhizosphere. Plant Soil 251:47–54

Visioli G, Vamerali T, Mattarozzi M, Dramis L, Sanangelantoni AM (2015) Combined endophytic inoculants enhance nickel phytoextraction from serpentine soil in the hyperaccumulator Noccaea caerulescens. Front Plant Sci 6:638

Wagner MR et al (2014) Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild *Arabidopsis* relative. Ecol Lett 17:717–726

Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. Mol Plant-Microbe Interact 25:139–150

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Zhang X, Gao J, Cao Y, Sheirdi RA, Wang X, Xhang L (2015) Rhizobium oryzicola sp. nov., potential plant-growth-promoting endophytic bacteria isolated from rice roots. Int J Syst Evol Microbiol 65:2931–2936

Zinniel DK, Lambercht P, Beth Harris N, Feng Z, Kuczmarski D, Highley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68:2198–2208

Microbial Functions of the Rhizosphere

10

G.P. Brahmaprakash, Pramod Kumar Sahu, G. Lavanya, Sneha S. Nair, Vijaykumar K. Gangaraddi, and Amrita Gupta

Abstract

The rhizosphere is part of the soil surrounding the plant roots or being influenced by the plant roots. The exudates released from roots make it a site for complex biochemical activity. Microorganisms make up one of the dynamic parts of this rhizosphere, and affect soil and plant growth by various means. However, our absolute dependency on chemical fertilizers and other agrochemicals, although enhancing crop production to the desired levels required to feed the growing world population, has not shown sufficient concern for sustainability, leading to two serious problems, ecological imbalance and resource limitation. An ecological disturbance has been created through polluting soil and water, putting toxic agrochemicals into the food chain, threatening human and animal health, and developing resistance in pests. On the other hand, resources are diminishing as vital nutrients like phosphorus are limited and very soon there will be an extreme shortage of these nutrients because excessive consumption will make them no longer available. Therefore, balancing plant needs through microbe-mediated sources is becoming an urgent priority. The rhizosphere microflora have many beneficial effects on plant growth and health promotion. They can be successfully employed to partly substitute agrochemicals in the long term for sustainable farming. Understanding the roles of these microbes therefore becomes imperative for enhancing quality and quantity of agricultural products. In the quest to improve productivity, management of rhizosphere dynamics provides an important tool.

G.P. Brahmaprakash (✉) • G. Lavanya • S.S. Nair • V.K. Gangaraddi
Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangaluru 560 065, India
e-mail: gpbrahmaprakash@yahoo.co.in

P.K. Sahu • A. Gupta
ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhajan 275 103, Uttar Pradesh, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_10

Inoculation of microorganisms, adjustment of soil, nutrient management, genetic engineering-based approaches, etc. all represent ways of managing the rhizosphere for enhancing crop production.

Keywords
Rhizosphere • Nitrogen fixers • Phosphorus solubilizers • Abiotic stress • Biocontrol • Bioformulation

10.1 Introduction: The Rhizosphere and Its Composition

The growth and development of a plant is largely influenced by the soil environment, especially surrounding the roots. It is a microenvironment provided by the plant, where major contributors are the microbes. This region is specifically referred to as the 'rhizosphere,' and represents a realm of greater nutritional activity, increased gas exchange, and enhanced release of root exudates, all of which collectively contribute to plant growth. As plants grow through soil, they release amino acids, sugars, and organic acids into soil that supply food for the microbes. In return, the microorganisms provide nutrients in available forms and other benefits to the plants. All this activity makes the rhizosphere an extremely dynamic soil environment. It is thus important to understand the composition, ecology, and interactions of rhizosphere.

10.1.1 Structure of the Rhizosphere

The term rhizosphere was first defined by the German scientist Hiltner (1904), and is derived from the Greek word *Rhiza* meaning roots. It is an area near to plant roots inhabited by a diverse class of microflora influenced by the chemicals released from the plant roots (Ahemad and Khan 2012). Today the rhizosphere is being redefined to include three distinct zones, viz., the endorhizosphere, the rhizoplane, and the ectorhizosphere. The endorhizosphere includes root the cortex and the endodermis, which are occupied by endophytic bacteria. The zone directly adjacent to the root surface is called the rhizoplane, and includes the epidermis and mucilage; the ectorhizosphere extends from the rhizoplane out to the soil. The term endorhizosphere was useful to identify and illustrate the importance of those microorganisms that exist in the internal portion of the root; however, if the rhizosphere is soil based, then the term endorhizosphere may be appropriate as it identifies the region of the root and not the rhizosphere. The ectorhizosphere is the actual portion of soil under the influence of plant roots. To avoid confusion between the endorhizosphere, rhizoplane, and ectorhizosphere, the terms root, rhizoplane, and rhizosphere, respectively, are used currently.

10.1.2 The Rhizosphere Environment

10.1.2.1 Physical Changes

The soil's physical properties such as temperature, moisture, and soil structure greatly influence the nutrient uptake by plants. The rhizosphere extension is affected majorly by the soil's physical properties as it affects root growth and transfer of ionic and molecular compounds (Nye 1981; Hinsinger 1998). Plant roots have been shown to increase the stability of surrounding aggregates in their rhizosphere region due to the large arbuscular mycorrhizal (AM) fungal population that encourages aggregate formation in rhizosphere soils. It has a high water-holding capacity, which further improves the soil's physical properties such as bulk density, porosity, pore size distribution, etc.

10.1.2.2 Chemical Changes

The interaction between plant roots and soil in the rhizosphere causes several chemical changes. These interactions change the pattern of root exudation, pH, nutrients, redox potential, and rhizodeposition, amongst other things, of the soil, which significantly affects nutrient solubility and thus its uptake. Apart from providing support and absorption of water and nutrients, plant roots are also involved in releasing organic and inorganic compounds into the rhizosphere. The availability of nutrients and rhizosphere microflora is affected by these chemicals, which are secreted out from roots (Neumann and Romheld 2001). They provide defense against pathogenic attack, keep the soil moist around the roots, mineralize nutrients, and inhibit the growth of competing plant species.

10.1.2.3 Biological Interactions in the Rhizosphere

The rhizosphere is a centre of intense biological activity due to nutrient-rich exudates. Some soil microorganisms interact with specific plants. These interactions can be pathogenic or beneficial. The beneficial interactions include mycorrhizae, legume nodulators, associative and free living dinitrogen fixers, nutrient solubilizers, and antimicrobial compound producers. Rhizosphere microorganisms also produce vitamins, antibiotics, plant hormones, communication molecules, etc. that encourage plant growth and alleviate abiotic stress. The rhizosphere is also one of the major sites that contribute to entry of endophytes into plant roots. The sites near to lateral root emergence and wounded parts of roots are important sites of endophyte entry into the plant. This later turns into a complex plant-endophyte interaction, which benefits the plants in several ways, including nutrient acquisition, phytohormone production, induction of host resistance, alleviation of abiotic stresses, production of secondary metabolites, etc. (Compant et al. 2005).

10.2 Microbes Associated with the Plant Rhizosphere

The nature of microbial population in the rhizosphere is related directly or indirectly to the root exudates released by the plant. Populations in the root zone are composed of both microflora (bacteria, fungi, and algae) and the micro- and the meso-fauna (nematodes, protozoa, mites, and insects). Since rhizosphere soil is a zone of greater microbial activity, it is essential to qualitatively and quantitatively analyze the microbial population, their interaction with the root interface, and their involvement in plant health.

10.2.1 Soil Microflora

10.2.1.1 Bacteria

Various bacterial genera are involved in complex rhizosphere activities and are the most abundant microorganisms in cultivated soil (Ahemad and Khan 2012). Their population is estimated to be between 10^6 and 10^8 cells per cubic centimeter of soil. The number of bacteria varies from a couple of million to a few billion cells per gram of soil. The highest number of bacteria occurs in the plough depth of cultivable soil at a depth of up to ~30 cm. In deeper layers their numbers gradually reduce. The rhizosphere harbors a high percentage of motile bacteria, namely amylolytic, proteolytic, ammonifying, denitrifying, and cellulose decomposing bacteria. Soil bacteria are broadly divided into autochthonous and zymogenous bacteria. Autochthonous bacteria are native microflora of a soil and zymogenous bacteria are foreign microflora that grow after addition of organic matter to the field.

According to Bergey's Manual of Systematic Bacteriology, soil bacteria predominantly belong to Pseudomonadales, Eubacteriales, and Actinomycetales from the class Schizomycetes. The most common soil bacteria belong to the genera *Arthrobacter, Pseudomonas, Clostridium, Achromobacter, Sarcina, Enterobacter,* etc., and bacteria from Myxobacteria belonging to the genera *Micrococcus, Chondrococcus, Archangium, Polyangium,* and *Cyptophaga* are also common in the soil. *Arthrobacter* is the most common autochthonous bacteria in soil, and makes up 2–60 % of the whole population of soil microflora; they are characterized by the tendency to form branching and coccus forms. *Pseudomonas* are one of the most common groups of zymogenous bacteria in soil; they attack a wide variety of substrates including hydrocarbons, oils, humic acids, and many of the synthetic pesticides. The pseudomonads apparently function best during periods of root exudate release. Many species produce diffusible fluorescent pigments, fluorescein-linked siderophores that have a great affinity for Fe^{3+}.

If an initial advantage over competing microorganisms by application to seed is given, *Azotobacter* persists in the soil. *Azospirillum,* another nitrogen fixer, is commonly associated with roots of tropical grasses (Rueda et al. 2016). The status of *Rhizobium* is difficult to assess as they are effective only in the legume rhizosphere and are mostly suppressed by microbial antagonism and competition in a non-legume rhizosphere. Actinomycetes are another group of bacteria in soil and are

best known for their ability to produce antibiotics and for inhibition of root-borne pathogens. Many bacteria are found to degrade xenobiotics and sequester heavy metals from the rhizosphere (Ahemad and Malik 2011; Ahemad 2012).

10.2.1.2 Fungi

Fungi belong to a group of eukaryotic organisms that are the absolute heterotrophs, mainly belonging to aerobes or a fermenting group of organisms. The main mass of fungi is found in the upper 20- to 30-cm layer. The combined mass of fungi in the upper layers is almost identical to that of bacteria and in forest soils it may even be greater. On an average it is between 0.001 and 1.0 billion fungi (about 1.5 tons/ha).

The rhizosphere contains both pathogenic and symbiotic fungi but their predominance of a specific community depends on many factors related to plants and soil. In particular, root exudates have been deemed an important factor when selecting for specific rhizosphere fungi (Buée et al. 2009). The most common soil fungi are the genera *Penicillium, Aspergillus, Trichoderma, Verticillium, Fusarium, Rhizopus, Mucor, Zygorhynchus, Chaetomium,* etc. Fungal associates of ectomycorrhizae (Agaricales, Gasteromycetes) and endomycorrhizae (*Gigaspora*, *Acaulospora*, *Glomus*, and *Sclerocystis*) are also of equal importance with regard to phosphorus uptake and suppression of pathogens (Lugtenberg and Kamilova 2009).

Both bacteria and fungi contribute to the soil structure by creating humus. Humus is a vital component of soil, and affects soil structure, sorption, and the organic compounds in soil. Humus is largely responsible for the creation of a crumbly and spongy texture of the soil by producing mucous capsules and their form of growth.

10.2.2 Microfauna

The significance of bacterial grazing by protozoa has been established in the rhizosphere by plant microcosm experiments. There have been reports of increased shoot biomass and shoot nitrogen in the presence of protozoa and nematode grazers. The microfaunal stimulation of nitrogen mineralization via the microbial loop was considered as one of the major mechanisms behind this occurrence (Clarholm 1985; Griffiths 1994; Zwart et al. 1994).

10.2.2.1 Protozoa

Humid soils support development of protozoans. They can reach populations of anywhere from a few hundred to several million individuals in a gram of dry soil. Soil contains 0.1–0.5 tons/ha mass of protozoa. They are mainly rhizopods (*Amoebae*) and flagellates with a smaller number of ciliates. The bacteria form a major source of food supply for the protozoans but they are selective in the type of bacteria they ingest. Therefore they exert a selective influence on the composition of bacterial population. Naked *Amoebae* make the most important bacterial grazer group in soil due to their high biomass, turnover, and specialized feeding modes. They form a biofilm that is also attached to soil and root surfaces, and thus have access to most of the bacteria in soil. With the aid of their pseudopodia, amoebae

can reach bacterial colonies in soil pores and even inside roots that are inaccessible to other predators (Darbyshire and Greaves 1973), and they may still continue grazing in tiny water films when other protozoa or nematodes are restricted by the decreased water potential in soil.

10.2.2.2 Nematodes

Environmetal conditions often determine the parasitic nature of nematodes. Plant parasitic forms are mostly endoparasites (*Heterodera*) or ectoparasites (*Tylenchus* and *Dorylaminus*). Nematodes are abundant in the upper 30 cm of soil and are influenced by soil texture, structure, temperature, moisture, etc.

As mentioned, the rhizosphere is part of the soil adjacent to plant roots. The biochemical activity in this zone is under the influence of plant roots. It is therefore necessary to understand the ecological interactions in the rhizosphere to gain greater benefits of plant growth and health from the microbial sources.

10.3 Effect of the Rhizosphere on Microbes

10.3.1 Effects

The rhizosphere is enriched with a variety of microorganisms, and their abundance generally reduces as the distance from the root increases. Measurement of the effect of the rhizosphere on a particular organism or the rhizosphere effect (RS ratio) has been classically defined as the ratio of activity/unit volume of rhizosphere soil and the activity/unit weight of non-rhizosphere soil. This relationship can provide an approximate estimation of how strongly a rhizosphere affects a particular population. An RS ratio >1 indicates selective stimulation, an RS ratio equal to 1 indicates no rhizosphere effect, and a RS ratio <1 indicates inhibition of activity in the rhizosphere. The RS ratio can also help in determining rhizosphere competency of various plant microbe interactions.

The rhizosphere effect in three plant species under periglacial conditions was studied. In this study, three plants (*Helianthemum nummularium*, *Dryas octopetala*, and *Silene acaulis*) were assessed for the changes in physical, chemical, and biological properties between the rhizosphere and bulk soil. All three plants have shown variable rhizosphere effects. *Helianthemum* showed a strong rhizosphere effect and synergism between roots and microbial community of rhizosphere. *Dryas* was found to be affected by nutrients in the rhizosphere but did not shown any specific microbial community associated with it. In the case of *Silene*, no difference was observed in the microbial community of bulk soil and rhizosphere (Massaccesi et al. 2015). A few of the aromatic and medicinal plants secrete phenolics and other antimicrobial compounds in the rhizosphere, and have an RS ratio <1.

To a soil microorganism, the rhizosphere is like a lush oasis in the desert. In comparison to the near starvation condition of bulk soil, the rhizosphere is a place where nutrients are bountiful. Therefore the rhizosphere has been considered to be an altered zone of microbial diversity, increased microbial activity, and complex

interactions among microorganisms and plants. Because roots are underground, the rhizosphere activity has been largely overlooked and it is only now that we are starting to unravel the complex interactions that occur. The rhizosphere has been called the last frontier in agricultural science and much attention has been given to the roots as the "hidden half" for their huge hidden potential (Veeger et al. 1981; Waisel and Eshel 2002; Kottke and Kovacs 2013).

Although the rhizosphere can be perceived to be a kitchen, it is also a safe home for soil microorganisms. The nature of the rhizosphere is still not completely unraveled (Stolp 1988; Sahu et al. 2016), although potentiality is well understood, but the composition is highly dynamic and complex. Since organic compounds secreted by roots are subject to microbial attack, the nature and composition of root exudates in the natural environment is often difficult. The rhizosphere effect comes into play when there is an enhancement in the growth of soil microorganisms resulting from physical and chemical alteration of the soil and the contribution of the excretion of organic debris from the root within the rhizosphere.

Plants remain perpetually in contact with a community of soil biota (Corey et al. 2008), which is made up of various microorganisms ranging from pathogenic to symbiotic. It has been reported by quantification of CO_2 from the rhizosphere of ^{14}C-labelled plants that about 40% of photosynthates from plant parts are deposited in the rhizosphere through the root system within an hour of its synthesis (Kumar et al. 2006; Nihorimbere et al. 2011). Deposition of those photosynthates is influenced by several factors, e.g., plant age (Singh and Mukerji 2006) and various biotic and abiotic stresses (Ratnayale et al. 1978; Kumar et al. 2006). The role of environmental factors as playing a more vital role in both the qualitative and quantitative compositions of root exudates than plant species has been exaggerated (Singh and Mukerji 2006). This would make one wonder why a plant would spend that amount of energy on the rhizosphere? What benefit does it gain? Is it self-sustainable and economically sound enough to compete and withstand adversity in the ecosystem? This brings the plant-microbe interaction concept into discussion, and this section of this chapter provides insight into root exudates and their role in influencing a dynamistic rhizosphere effect on microbes.

10.3.2 Compounds Secreted in the Rhizosphere

Healthy roots exude various organic compounds (Rovira et al. 1978; Jones and Darrah 1995; Bais et al. 2006) including more than 100,000 different low-molecular-weight secondary metabolites (Bais et al. 2004; Feth et al. 2014), called root exudates. Root exudates are carbonaceous substances containing a wide range of amino acids, water-soluble sugars, organic acids, inorganic ions/gaseous molecules, and various vitamins and enzymes (Uren 2001; Kumar et al. 2006). To date our knowledge on root exudates is based on the application of techniques from other fields. The complexity of root exudates is slowly being unravelled, as chromatographic techniques enable researchers to discover the multitude of carbonaceous substances released from roots grown under axenic or sterile conditions. A list of important root exudates and their functions is presented in Table 10.1.

Table 10.1 Elucidation of important root exudates and their functions in the rhizosphere (Paul and Clark 1996; Kumar et al. 2006; Vranova et al. 2013)

Exudates	Specific compound in root exudates identified	Possible functions in the rhizosphere
Amino acids and phytosiderophores	α- and β-alanine proline	Nutrient source
	Asparagine, valine, threonine, aspartate, tryptophan, cystein, ornithine, cystine, histidine, glutamate, arginine, glycine, homoserine, isoleucine, phenylalanine, leucine, α-aminobutyric acid, lysine α-aminoadipic acid, methionine, serine, homoserine	Chemo-attractant signals to microbes
		Chelaters of poorly soluble mineral nutrients
Phenolics	Daidzein, 4′,7-dihydroxyflavanone	Chemo-attractant signals to microbes
	Genistein, 4′,7-dihydroxyflavone	Microbial growth promoters
	Coumetrol, 4,4′-dihydroxy-2′-methoxychalcone	*Nod* gene inducers in *rhizobia*
	Eriodictyol, 4′-7-dihydroxyflavone	*Nod* gene inhibiters in *rhizobia*
		Resistance inducers against phytoalexins
	3,5,7,3′-tetrahydroxy-4′methoxyflavone	Act as chelaters
	Liquiritigenin, luteolin	Nutrient source
	Isoliquiritigenin, 7,3′-dihydroxy-4′-methoxyflavone umbelliferone, (+)- and (−)- catechin	Phytoalexin against soil pathogens
	Citric, glutaric, oxalic, malonic	Nutrient source
Organic acids	Malic, aldonic, fumaric, erythronic	Chemo-attractant signals to microbes
	Succinic, ferulic, acetic, butanoic	Chelaters of poorly soluble mineral nutrients
	Butyric, syringic, valeric, rosmarinic, lactic, glycolic	Acidifiers of soils
	Trans-cinnamic, piscidic, formic	Detoxifiers of Al
	Aconitic, pyruvic	*Nod* gene inducers
Vitamins	Biotin, thiamin, ribose, niacin	Promoters of plant and microbial growth
Purines	Adenine, guanine, cytidine, uridine	Nutrient source
Enzymes and proteins	Acid/alkaline, phosphatase amylase, invertase, protease	Catalysts for phosphorus release from organic molecules; biocatalyst for organic matter transformation in soil

10.4 Beneficial Role of Rhizosphere Microbes on Plants

Many resources have been poured into research on plant growth-promoting rhizobacteria (PGPR) in terms of understanding and utilizing microbial potential for plant performance. Understanding their mode of action will help in making microbe-based commercial products for growth promotion. Despite many hindrances, the microbial inoculant industry has been grown to new heights in both the public and the private sectors. This indicates how tremendous a potential microbes have in plant productivity.

Microbes are important in the utilization of poorly available nutrients by plants, e.g., iron. The world's current population of around seven billion is predicted to increase to around ten billion in the next 50 years, which will require that agricultural productivity be increased within the next few decades to adequately feed all these individuals (Glick 2014).

The beneficial role of microbes in the rhizosphere can be manifested by direct plant growth promotion, indirectly providing protection from phytopathogens and fortifying the plant's tolerance to certain abiotic stresses under sub-optimal environmental conditions (Penrose and Glick 2003; Kang et al. 2014). Microbial formulations are also important in soil stability as they help in soil aggregation (Van Veen et al. 1997).

The rhizosphere is a site of complex physical, chemical, and biochemical interaction, all of which affect the plant growth. Below is a brief list of the effects of this interaction (Ahemad and Malik 2011; Ahemad and Khan 2012; Brahmaprakash and Sahu 2012; Nehra and Choudhary 2015; Sahu and Brahmaprakash 2016):

- Dinitrogen fixation
- P-mobilization and solubilization
- Nutrient uptake in deficient soils
- Improvement of water uptake
- Production of plant growth regulators
- Promoting seed germination and early plant growth
- Improvement in soil structure
- Competing with plant pathogens
- Induced systemic resistance
- Systemic-acquired resistance
- Induced systemic tolerance
- Overall biomass enhancement
- Remediation of problematic soils

The efficiency of plant fertilizers was found to be enhanced by plant growth-promoting microbes and arbuscular mycorrhizal fungi (Adesemoye and Kloepper 2009). Biodegradation of 1, 4-dichlorobenzene by rhizosphere bacteria of *Jatropha curcas* has been reported (Pant et al. 2016). There are many such effects that microbes exert in the rhizosphere that are directly or indirectly beneficial for the plants.

10.4.1 Nitrogen Nutrition

Nitrogen is the most important mineral nutrient in plants. Despite being the most abundant element on earth (N_2 form), nitrogen is a limiting element for plant growth, because it is primarily taken in the form of nitrate ions by plants. Biological nitrogen is a process through which microbes convert atmospheric dinitrogen into plant-available forms (Biswas and Gresshoff 2014). In India, biofertilizers have been introduced for soybean crops as Indian soils were lacking in rhizobia, which nodulates the soybean crop. The effects of inoculation were fantastic and able to meet a substantial portion of the N_2 requirement. Encouraged by the results with soybean, inoculant technology was extended to all other legumes, and then to cereals. The photograph shown in Fig. 10.1 was taken during early 1970s, and shows the response of *Rhizobium japonicum* in an experimental farm at the University of Agricultural Science, GKVK, Bengaluru in soybean. Yellowing (a nitrogen-deficiency symptom) is visible in un-inoculated border rows, and other parts of the field inoculated by *Rhizobium japonicum* are lush and green (a nitrogen-sufficiency sign). This positive response has not only been obtained for soybean crops, but also for other legume crops (Brahmaprakash and Sahu 2012).

Plants have different levels of ecological interactions with dinitrogen fixers that benefit nitrogen nutrition. These interactions can be symbiotic or asymbiotic, and create a bridge between plants and microbes. Efficient nitrogen nutrition through these microbes also helps to reduce loss of nitrogen by volatilization, leaching, and denitrification (Nepolean et al. 2012). With nitrogen fixation, a greater understanding has been gained with regard to the transport of photosynthates to *Rhizobium* and the presence of dicarboxylates transporters LjALMT4 in nodules of *Lotus japonicus* (Takanashi et al. 2016).

Fig. 10.1 Response of a first inoculation of *Rhizobium japonicum* in the 1960s at the experimental farm of the University of Agricultural Science, GKVK, Bengaluru in soybeans (Adapted from: Brahmaprakash and Sahu 2012)

10.4.1.1 Symbiosis Between Legumes and Rhizobium

One of the most common inoculation techniques in the true sense of artificial inoculation of microbial agents has been used for the benefit of legumes. Since the time when Hellriegel and Wilfarth experimentally proved the fixation of N_2 in a legume nodule by *Rhizobium*, the inoculation technology has spread all round the globe (Brahmaprakash and Sahu 2012). There are different species of *Rhizobia that* nodulate different legume crops (cross-inoculation group). Using these cross-inoculation groups, *Rhizobium* was initially classified based on the ability to nodulate different legumes (Fred et al. 1932), and then based on the growth rate for slow- and fast-growing rhizobia (Quispel 1974); it is now based on molecular taxonomy using 16s rRNA sequence into ten genera, some of them are phylogentically outside the traditional rhizobia but they do carry *nod* genes, which encode for *Nod* factors (Raychaudhuri et al. 2007).

After infection, the *symbiosis* begins in a new tissue called a nodule, which contains bacteroids. Nodule formation is the result of a molecular dialogue exchange between a legume and a *Rhizobium*. The atmospheric nitrogen is fixed in the *Rhizobium* by the dinitrogenase enzyme complex and transferred to the plant, and in turn gets nutrients and shelter from the plants.

10.4.1.2 Symbiosis Between *Frankia* and *Casuarina*

Several genera of angiosperms such as *Casuarina, Alnus, Myrica, Coriaria, Discaria,*and *Hippophae*, develop symbiosis with *Frankia*, which is a filamentous, spore-forming actinobacteria. Actinobacteria form nodules with hundreds of plant species over 25 genera and eight dicotyledons families. The tree species with actionrhizal symbiosis are important components in agroforestry systems for improving N_2 economy and stabilizing eroded soils (Brahmaprakash and Sahu 2012). These are known to enhance the fertility of soil in temperate areas as legume species do in tropical areas, as the actinorhizal plants can grow in waste lands that are nitrogen poor and have eroded slopes, as well as producing trees of commercial importance.

The nodules formed in actinorhizal symbiosis establish clusters of modified roots with the *Frankia*-infected cells in the cortex. These nodules first appear as a swelling, which later develops into lobes at the apices. They develop vesicles that are sites for nitrogen fixation. The extent of biological nitrogen fixation in *Frankia* is about 90 kg N_2/ha/year in the plant *Coriaria arborea* (Silvester 1975).

10.4.1.3 Asymbiotic Nitrogen Fixation

Bacteria like *Azotobacter*, *Derxia,* and *Beijerinkia* asymbiotically fix dinitrogen in the rhizosphere of certain crops. *Azotobacter* is a commonly used plant growth promoter in all non-leguminous plants like rice, cotton, and vegetable crops (Esitken et al. 2009). It produces a large amount of slime, which helps in soil aggregation. One of the most common species, *A. chroococcum*, fixes 10 mg N_2/g of carbon source supplied *in vitro* (Thomas 1993). It is known to produce growth hormones like indole acetic acid (IAA) and gibberellic acid (Barat et al. 2016), and also shows fungistatic activity. *A. chroococcum* is recommended for many cereals such as maize, wheat, pearl millet, sorghum, etc. (Gupta 2010). Apart from enhancing yield,

it improves the post-harvest quality of wheat (Abbasdokht 2008) and solubilizes phosphorus (Kumara Swamy et al. 2010). Co-inoculation of *Azotobacter* and *Azospirillum* has also been reported to enhance growth and yield of strawberries in hydroponic systems (Rueda et al. 2016). Enhancement in plant yield parameters through inoculation of *Azotobacter* has been reported in green leafy vegetables with different carriers. Inoculation has been shown to significantly increase plant height, number of leaves, shoot length, root length, number of roots, chlorophyll, and carotenoids (Maheswari and Kalaiyarasi 2015).

10.4.1.4 *Azospirillum* and a Free Living Nitrogen Fixation System

Azospirillum lipoferum is microaerophilic soil-inhabiting bacterium and is also found in the intercellular spaces of the root cortex of graminaceous plants. It fixes dinitrogen in non-leguminous plants like cereals, millets, oilseeds, cotton, etc. It is the most studied plant growth-promoting bacteria as it can colonize most of the agriculturally important crops (Bashan et al. 2004). Co-inoculation of *Azospirillum lipoferum* along with *Bacillus megaterium* (phosphate-solubilizing microorganisms, PSMs) on the growth of rice (*Oryza sativa* L.) was found to enhance plant growth parameters in seed and pot experiments (Ahilandeswari and Maheswari 2016). There are many other mechanisms involved in attainment of the effectiveness of *Azospirilum.* An increase in nitrite production also increases formation of lateral roots (Bashan and de Bashan 2005), IAA, and gibberellins for promoting root proliferation, mineralizes soil nutrients, sequesters iron, can survive in harsh environmental conditions, and favors a mycorrhiza-plant association (Bashan et al. 2004). The large group of free-living nitrogen fixers also involves several cyanobacteria such as *Anabaena, Nostoc, Cylindrospermum, Aulosira, Tolypothrix,* etc. These are photosynthetic bacteria that can fix atmospheric dinitrogen in specialized cells called heterocysts. They make up one of the most predominant and vital groups of bacteria on earth (Berry et al. 2008)**.**

10.4.2 Phosphorus Nutrition

Phosphorus (P) is one of the most important nutrient elements for growth and development of living beings and makes up to about 0.2% of plant dry weight. It is applied to plants in the form of phosphate fertilizers but most of the applied P is fixed in soil and thus becomes unavailable for plant uptake (Rashid et al. 2004). A few of the microorganisms present in soil dissolve the fixed forms of P into available forms by various mechanisms and make it available for plant uptake (Kang et al. 2002; Pradhan and Sukla 2005). These are called as P-solubilizers, and include *Pseudomonas, Bacillus*, *Acinetobacter, Rhizobium, Penicillium, Aspergillus,* etc. (Krishnaveni 2010). There is another class of microorganisms that enhances surface area of roots by colonizing on it and thus mobilizes the phosphorus from distant parts where roots cannot reach; these are called P-mobilizers. Mycorrhiza is a class of P-mobilizers that contain several fungal genera like *Glomus, Gigaspora, Entrophospora Scutellospora, Acaulospora,* etc.

There is a large amount of fixed phosphorus in soil. Microbe-mediated solubilization is of great benefit in enhancing efficiency of nutrients, crop yield, and judicious use of phosphorus, which is available in limited quantities. There are several mechanisms by which microbes solubilize phosphorus, for instance production of various organic acids and acid phosphatases (Rashid et al. 2004; Chen et al. 2006) are important. The phosphate-solubilizing bacterial (PSB) genera *Bacillus*, *Pseudomonas, Rhizobium* etc., (Wani and Lee 2002) and fungi like *Penicillium* and *Aspergillus* are potential P-solubilizers (Wakelin et al. 2004).

Application of P-solubilizing microorganisms is an established practice and there are many reports of success with their use in enhancing availability of native P from the soil and P from phosphatic rock by co-inoculation of AM fungi and P-solubilizing bacteria (Goenadi et al. 2000; Cabello et al. 2005). Other reports include: *Pseudomonas* and *Bacillus* sp. enhanced P nutrition in *Zea mays* in nutrient-deficient soils (Egamberdiyeva 2007); improvement of biological nitrogen fixation apart from P solubilization (Ponmurugan and Gopi 2006); rock phosphate bio-activation and seed treatment by PSMs was found to enhance the uptake of P in cowpea and ragi (Kumara Swamy et al. 2010); higher germination rates and other parameters in artichoke (*cynara Scolymus)* through application of *Pseudomonas putida, Azospirillum*, and *Azotobacter* with P-solubilizing bacteria (Jahanian et al. 2012); P-solubilizing bacteria improved growth performance and uptake of P in mung bean plants (Walpola and Yoon 2013); promotion of phytate mineralization in soil as a result of AM fungus and PSB interaction in the hyphosphere (Zhang et al. 2014).

The decreasing availability of land for agriculture is a major issue in crop production; in the last two decades, agricultural land shrinkage has been nearly 2.76 million hectares (Abbasdokht and Gholami 2010). The required production of food grain for 1.4 billion for the Indian population in 2025 is 300 million tones, which will require 30 million tonnes of major chemical fertilizers. Similarly, about 15 million tonnes of fertilizers will be required for other crops. Of the total need, the P_2O_5 requirement will be 11–13 million tonnes (Tiwari 2001). To meet this huge demand in the near future, it is now imperative to pool resources for advanced research into P-solubilization by using genomic tools, proteomics, metabolic regulation, improvement in ecological competency, application of microbial products instead of microbes themself, etc., so that the huge potential of tiny microbes can be harnessed in an efficient way.

Mycorrhiza represents a symbiotic association between roots of higher plants and fungi. In this interaction, the fungal partner gets its requirement of carbon and in turn provides various nutrients like P, Cu, Zn, Ca, and water absorption to the host plant. Mycorrhiza is associated with almost all agricultural crops. The fungal partner belongs to one of the genera *Glomus, Gigaspora, Entrophospora, Scutellospora, Archaeospora, Acaulospora, Paraglomus, Sclerocysts*, *Endogone,* etc. They are important parts of the P-cycle. Apart from providing the P need of plants, mycorrhiza also help to establish better absorption of water (Mahdi et al. 2010) and induction of host defense responses (Kasiamdar et al. 2001).

10.4.3 Secretion of Plant Growth Hormones

The term "hormone" is derived from Greek, meaning to set in motion, and represents a *chemical messenger*. Plant hormones (also referred as phytohormones) are signalling molecules devised from naturally occurring organic substances produced within a plant at extremely low concentrations to carry out physiological processes. The major phytohormones include auxins, gibberellins, cytokinins, ethylene, and abscisic acid, and are often called the five classical hormones because they not only regulate cellular processes in targeted cells but are also involved in determining the formation of flowers, leaves, stems, and development and ripening of fruit.

Even though plants lack glands, unlike animals, that produce and secrete hormones, each plant cell is capable of producing hormones that affect gene expression and cellular division, which results in considerable changes in the phenotype such as in determining the flowering time, the sex of flowers, formation and senescence of leaves and fruits, and overall tissue growth, thus shaping a plant.

10.4.3.1 Auxin

Auxin is produced in the stem, root, and bud tip, and includes the cell elongation process and inhibition of growth of lateral buds (to maintain apical dominance). The microbial production of auxin has been well known for some time (Spaepen and Vanderleyden 2011), and it has been speculated that about 80% of the rhizobacterial population is capable of synthesizing IAA (Khalid et al. 2005; Spaepen and Vanderleyden 2011). The first successful isolation of IAA from the fungus *Rhizopus suinus* was carried out by K.V. Thimann (Baca and Elmerich 2007). Auxin production has been well documented in bacteria, and a plant operates a multiple pathway for biosynthesis of auxin, which has also been observed in bacteria. It is also well documented that plants operate down-regulation of auxin signalling as part of their defence against plant pathogenic bacteria. The plant growth-promoting bacteria *Azospirillum* sp. also produce IAA to enhance the immunity of plant against plant pathogens (Spaepen et al. 2007).

10.4.3.2 Gibberellins

Gibberellins (GAs) are plant hormones that are primarily associated with seed germination and leaf and stem growth (King and Evans 2003). They also control plant growth by regulating the degradation of growth-repressing DELLA proteins (Pieterse et al. 2012). Many plants produce GAs endogenously and also through microbes, as a causative agent such as *Gibberella fujikuroi*, which is involved in the bakanae effect in maize, rice, and other plants. In bacterial species *Azospirillum brasilense* and *Azospirillum lipoferum,* GAs production promotes shoot elongation, and root hair density, and are also they involved in reversing dwarfism in maize and rice (Baca and Elmerich 2007).

10.4.3.3 Cytokinins

Cytokinins (CKs) are another class of hormones primarily involved in promoting cell growth, differentiation, and cytokinesis (cell division) in plant roots and shoots.

The CKs are often linked to a plant's response to biotrophic pathogens to alter a host's physiology (Walters and McRoberts 2006; Pieterse et al. 2012). The microbial production of CKs is well documented. Inoculation of *Bacillus subtilis* in lettuce plants increased the CK concentration in both shoots and roots (Arkhipova et al. 2005). Ortíz-Castro et al. (2008) showed that cytokinin receptors play a complimentary role in plant growth promotion by *Bacillus megaterium*. The CKs have an anti-auxin and anti-gibberellin properties, hence the correct concentration of auxin and gibberellin is properly maintained. The CKs also play a role in conservation of water in plants in water-deficient situations.

10.4.3.4 Ethylene

Ethylene (ET) is a gaseous phytohormone. It is more of growth inhibitor, like abscisic acid. The role of ET has been established in fruit maturation and ripening. It is responsible for cell enlargement and it shows a geotropism disposition (growing towards earth). ET is an important constituent of a defence pathway that manifests during a pathogen attack and functions as an important modulator of plant immunity (Broekaert et al. 2006). Once ET starts accumulating inside the plant, it leads to aging. It is not helpful when a plant is facing stresses, which hinder the growth as well as the yield. Hence many microbes are involved in regulating ET concentration with the help of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) by cleaving 1-aminocyclopropane-1-carboxylate (ACC), which is the immediate precursor of ET into α-ketobutyrate and ammonia (Glick 2014; Ali et al. 2014; Gamalero and Glick 2015).

10.4.3.5 Abscisic Acid

Abscisic acid (ABA) is an isoprenoid phytohormone and performs many specific functions related to plant growth (Taylor et al. 2000). Its name is based on its role in the abscission of plant leaves. ABA inhibits seed germination, photosynthesis, fruit ripening, and kinetin biosynthesis. ABA is also involved in establishing dormancy and regulation of stress responses like adaptation to drought, low temperature and salinity. These activities are regulated by the combined activity of interconnected ABA-dependent and ABA-independent signalling pathways (Shinozaki et al. 2003). Koga et al. (2004) stated that the application of ABA increased the susceptibility of rice plants to *Magnaporthe grisea*.

10.4.4 Alleviation of Abiotic Stress by Rhizosphere Microflora

Over the past few decades, abiotic stress has become an important area of research, mainly focusing on suitable mechanisms to alleviate stress in plants. Our understanding of alleviation of abiotic stress has taken huge shape with the knowledge of adaptation strategies of plants to various climatic, edaphic, and physiographic factors that limit plant growth substantially. Plants adopt some strategies to cope with abiotic stresses, such as stress tolerance and stress avoidance. The plants themselves may not confront the abiotic stress successfully, so it have to be genetically

modified through down- or up-regulation of gene expression or it has to utilize its rhizosphere microbial friends to help to withstand harsh environmental stress. In this subsection of the chapter, certain rhizospheric microbial mechanisms are discussed in brief.

Climatic factors influencing abiotic stresses include drought, extreme temperature (both hot and cold), and excessive precipitation. The edaphic factors mostly are soil related, like soil salinity, soil acidity, soil fertility as a whole, and the presence of heavy metals, organic pollutants, and their level of toxicity. Physiographic parameters such as physical structure of soil/land, slope or steepness are key factors in abiotic stresses. All these parameters are natural havocs threats and impact on crop production and food safety problems.

Many microbes possess genes that are responsible for the expression of multimeric enzymes with a monomeric subunit (Glick 2005). ACCD (1-aminocycloprop ane-1-carboxylate deaminase) cleaves ACC, an immediate precursor for ethylene into α-ketobutyrate and ammonia (Penrose and Glick 2003). By converting ACC into α-ketobutyrate and ammonia, it reduces the possibility of ethylene accumulation in the plant. The production of salicylic acids and Jasmonic acids induces systemic tolerance in the plant against abiotic stress. When a plant is exposed to the stress of drought, generation of reactive oxygen species (ROS) is accelerated due to water scarcity, resulting desiccation and dehydration, which ultimately leads to death of the cell. In order to avoid these problems, microbes possessing antioxidant strategies are initiated, and microbial production of exopolysaccharides help in proper aggregation of soil particles resulting in maintenance of the soil water status. These all are the important possible mechanisms for alleviating abiotic stresses.

The yield of crop plants is reduced in saline soils (Shahbaz and Ashraf 2013), and the soil salinity is expected to reach 50% of arable land by 2050 (Jamil et al. 2011). Therefore, microbe-mediated alleviation of salt stress is imperative to enhance yield in salt-affected soils. Enhanced plant growth has been reported in salt stress conditions by rhizobacteria-induced tolerance in wheat (Tiwari et al. 2011). An array of studies has shown microbial potential as a tool to alleviate stress from soil salinity (Yao et al. 2010). Erratic and reduced rainfall is another serious concern for agriculture. In the face of water deprivation, many plant growth-promoting rhizobacteria possess huge potential to modulate the physiological response of plants and help them to survive (Marasco et al. 2012).

10.4.5 Biocontrol Activity

Plants make up most of the earth's living environment. They grow and produce well as long as soil provides them with an able environment. However, pathogenic microorganisms in soil, for example bacteria, fungi, viruses, protozoa, and nematodes, and unfavorable natural conditions cause sickness in plants, leading to diminished yield. This has prompted the use of chemicals to control plant diseases, usually referred to as pesticides. With the perpetually growing unsafe impacts of harmful

pesticides on nature, a requirement for a feasible eco-friendly administration practice became necessary. Hence today there is global enthusiasm for the advancement and utilization of biocontrol agents and the need to decrease the use of chemicals for pest control.

Biocontrol or biological control is a term that refers to the use of living organisms in order to suppress pathogens. DeBach (1964) defined biological control as "the action of parasites, predators, or pathogens in maintaining another organism's population density at a longer average than would occur in their absence." The organism that suppresses the pathogen is a biocontrol agent. In the twentieth century it was realized that soils harbor microorganisms that can suppress disease caused by soil-borne pathogens.

A few of the organisms, for example *Penicillium*, restrain the development of other fungi and bacteria. In 1930s, scientists infected plants with mild strains of virus to prevent or delay the infection by a more severe strain (cross-protection). Biological control of plant diseases with antagonistic microorganisms came into practice in the 1960s with the use of non-pathogenic fungal spores of *Phleviopsis gigantea* on pines against the fungus *Heterobasidion annosum.* In the 1980s scientists introduced viral genes into host plants through genetic engineering, which prevented or delayed the infection of the plant by the virus. Another recent encouraging method for biocontrol uses pathogens or chemicals that cause tiny lesions in plants that will activate their defense against subsequent infections by similar pathogens (systemically acquired resistance). Feng et al. (2013) studied the effect of endophytic bacterial communities in tomato plants with differential resistance to *Ralstonia solanacearum* wherein the number of endophyte species with the ability to antagonise *R. solanacearum* in resistant tomato plants was more than that in susceptible plants. The isolated bacterial species showed high similarity to *Sphingomonas yanoikuya, Bacillus megaterium, Serratia marcescens, Pseudomonas pseudoalcaligenes, Paenibacillus polymyxa, B. cereus and B. pumilus* in partial rRNA sequencing. Similar studies were conducted by Achari and Ramesh (2014) on eggplants, chillis, and *Solanum torvum*. Diversity, plant growth, and plant health promotion traits were studied from 167 xylem-residing bacteria. Suppression of *R. solanacearum* was reported by these isolates through production of volatile and diffusible antagonistic compounds.

The mechanism of biocontrol can be divided into three types, direct antagonism, mixed pathogen antagonism, and indirect antagonism (Pal and McSpadden 2006).

10.4.5.1 Direct Antagonism

In direct antagonism, the biocontrol agent (BCA) uses hyperparasitism or predation as the mechanism. In hyperparasitism, the biocontrol agent directly attacks the pathogen and kills it. These BCAs can be obligate bacterial pathogens, hypoviruses, facultative parasites, or predators. *Trichoderma harzianum*, the most frequently studied mycoparasite, occurs in soils, rotting wood, and many other environments. When it grows over other fungi it dissolves its host's hyphae with extracellular enzymes such as glucanases and chitinases. Other widely studied mycoparasites

include *Coniothgrium minitans* and *Sporidesmium sclerotiorum*, which are antagonists of sclerotial fungi and *Gliocladium* spp., which parasitize a range of soil-borne pathogens.

Bacteria occurring in the rhizosphere are also known to parasitize pathogens. A recent study on the mycoparasitic nature of *Bionectria* sp. strain 6.21 conducted by Melo et al. (2014) revealed the parasitic nature of *Bionectria* sp. on *Rhizoctonia solani* and *Pythium aphanidermatum*. The obtained results indicated that this biocontrol agent has both antibiotic and mycoparasitic properties. Likewise, *Ampelomyces quisqualis* is one of the successful mycoparasites for powdery mildew fungus.

10.4.5.2 Mixed-Pathogen Antagonism

10.4.5.2.1 Antibiotics

Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. When grown in pure culture, most micro-organisms produce secondary metabolites. These compounds are generally not essential intermediaries to the primary metabolism. They have unusual structures and are toxic to other microorganisms. Many antibiotics produced by biocontrol bacteria exhibit broad-spectrum activity. For example, pyrrolnitrin produced by *Burkholderia* and *Pseudomonas* spp. has activity against a wide range of basidiomycetes, deuteromycetes, and ascomycetes, including the plant pathogens *Rhizoctonia solani*, *Botrytis cinerea*, *Verticillium dahliae*, and *Sclerotinia sclerotiorum* (Raaijmakers et al. 2002). Similarly, 2,4-diacetylphloroglucinol (2,4-DAPG), produced by *Pseudomonas* spp., exhibits antimicrobial activities (Weller et al. 2007). Some of the antibiotics are listed in Table 10.2.

Table 10.2 Biocontrol agent (BCA) antibiotics

Antibiotic	Strain	Pathogen	Disease	Reference
Fengycin	*Bacillus subtilis* strain NCD-2	*Rhizoctonia solani*	Cotton damping-off	Guo et al. (2014)
Iturin A, fengycin macrolactin, bacillaene and difficidin	*B. amyloliquefaciens* GA1	*B. cinerea*	Post harvest infection	Arias et al. (2009)
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomyces cochlioides*	Damping off	Islam et al. (2010)
2, 4-diacetyl-phloroglucinol	*Pseudomonasv fluorescens* F113	*Pythium* spp.	Damping off	Shanahan et al. (1992)
Herbicolin	*Pantoea agglomerans* C9-1	*Erwinia amylovora*	Fire blight	Sandra et al. (2001)

10.4.5.2.2 Lytic Enzymes

Extracellular hydrolytic enzymes produced by microbes may also play a role in suppression of plant pathogenic fungi. Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA. For example, control of soil-borne pathogens of the groundnut, mainly *Sclerotium rolfsii* by *Trichoderma viride*, is mediated through chitinase production (Parmar et al. 2015) where as *Trichoderma harzianum* was shown to lyse 68% of the cell wall of *Phomopsis vexans* (Phomopsis blight) using f -1, 3 glucanase and chitinase within 48 h (Ghosh et al. 2015).

Other microbial by-products may also contribute to pathogen suppression. Hydrogen cyanide (HCN) is highly toxic to aerobic microbes as it blocks the cytochrome oxidase pathway even at very low concentrations. Suppression of several root pathogens was reported by HCN-producing fluorescent pseudomonads. In a recent study, *Pseudomonas fluorescens* Pf1 strain was shown to produce an iron-chelating agent (siderophore), volatiles (HCN), and antibiotic (fluorescein and pyocyanin) inhibiting *Macrophomina phaseolina* (coleus root rot).

10.4.5.3 Indirect Antagonism

10.4.5.3.1 Competition

Competition occurs when two or more organisms require the same resource for growth and survival. The use of this resource by one organism reduces the amount available to the other. This leads to a deficiency in the already available resources leading to competition between the organisms. The rhizosphere is a region of intense microbial activity. Biocontrol by competition occurs once the biocontrol agent reduces the availability of a particular nutrient element and thereby limits the growth of the pathogen. Biocontrol agents have a more efficient uptake or utilizing system for the substance than do pathogens. Biocontrol by competition for important micronutrients has also been revealed. Kloepper et al. (1980) demonstrated the importance of siderophore production for the first time as a biological control mechanism of *Erwinia carotovora* by several plant-growth-promoting *Pseudomonas fluorescens* strains.

10.4.5.3.2 Induction of Host Resistance

Induction of host defenses can be local and/or systemic in nature, depending on the type, source, and amount of stimuli. The inducible resistance in plants to an array of pathogens is called systemic acquired resistance (SAR). Salicylic acid (SA) is key compound in SAR and is frequently produced after pathogen infection; it leads to the expression of pathogenesis-related (PR) proteins. Some of these PRs are 1,3-glucanases and chitinases capable of hydrolyzing fungal cell walls. SAR may also be induced by inoculating plants either with a necrogenic pathogen or a non-pathogen or with certain natural or synthetic chemical compounds like benzothiazole (BTH).

A second pathway, referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of

Table 10.3 Induced host resistance in bacteria

Strain	Plant	Determinant	Type	Reference
Bacillus fortis IAGS 162	Fusarium wilt of tomato	Phenyl acetic acid	ISR	Akram et al. (2016)
Pseudomonas fluorescens WCS374r	*Pseudomonas syringae* pv. *tomato* in *Arabidopsis*	Iron-regulated	ISR	Djavaheri et al. (2009)
Pseudomonas fluorescens CHA0	Tobacco	Siderophore	SAR	Maurhofer et al. (1994)
Bacillus amyloliquefaciens LJ02	Cucurbit powdery mildew	Antibiotics and siderophores	ISR	Li et al. (2015)
Pseudomonas putida strains	*Arabidopsis*	Lipopolysaccharide	ISR	Meziane et al. (2005)

some nonpathogenic rhizobacteria. The protection mediated by ISR is considerably less than that of SAR (Van Loon 2000) and it is more or less dependent on the plant genotype (Bloemberg and Lugtenberg 2001). However, ISR and SAR together could provide significantly better protection than either of them alone. This indicates their additive roles in resistance towards plant pathogens (Van Wees et al. 2000). Some examples of ISR- and SAR-mediated pathogen suppression are listed in Table 10.3.

With the advent of good agricultural practices, it is unlikely that BCAs will completely replace chemical pesticides, as BCAs have a slow action and the world population continues to increase at a rapid rate with a need for greater and faster food production. However, BCAs could be developed as a component of integrated disease management programs.

10.5 Application of Rhizosphere Microflora for Enhancing Crop Productivity: Bioformulations

A bioformulation is a mixture of an active ingredient with a formulated product with inactive or inert substances. Here the active ingredient is a live microbe or spore or any other latent form (Hynes and Boyetchko 2006). The term biofertilizer encompasses formulations that contain microorganisms and/or a biological product that can fix atmospheric nitrogen, enhance the solubility of soil nutrients, and/or have the potential to enhance the yield of crop plants (Kumar 2014). These microbes help to develop an eco-friendly control strategy for plant diseases as a biocontrol agent (Heydari and Gharedaghli 2007). According to the Bureau of Indian Standards (BIS), carrier-based biofertilizer should contain 5×10^7 CFU/g in solid based and 1×10^8 CFU/ml in liquid biofertilizers (Yadav 2009; Anandaraj and Delapierre 2010; Sahu and Brahmaprakash 2016). A biofertilizer formulation is the form in which microbes are delivered to plants. It contains potential microorganisms, a carrier material, and suitable additives. A good bioformulation will protect bacterial cells from harsh environmental conditions and help to attain a good physiological state and sufficient production.

Application of plant-beneficial microbes is achieved in the form of biofertilizers. Biofertilizers are preparations containing beneficial microorganisms that enhance plant growth (Brahmaprakash and Sahu 2012). An inoculant or formulation is the means of transporting these beneficial microbes from the place of their manufacture to the field to enhance plant growth (Tittabutr et al. 2007). In India, the term "biofertilizer" refers to fertilizers that meet a crop's nutritional requirements through microbiological means; in other countries the term "microbial inoculants" is used (Brahmaprakash and Sahu 2012).

10.5.1 Types of Carrier Material

A carrier material is an inert substance that supports the cells and ensures that the cells are easily established in and around the plant and provides help in enhancing plant growth and biocontrol activity. Various types of materials are used as carriers. A superior carrier material makes a good quality inoculant. A list of the constituents of a superior quality carrier material for microbial inoculants is given below (Mishra and Dahich 2010; Bazilah et al. 2011; Sahu and Brahmaprakash 2016):-

- High water-holding and water-retention capacity
- No heat of wetting
- Nearly sterile, chemically and physically uniform
- Non-toxic in nature, easily biodegradable and non-polluting
- Nearly neutral pH
- Supports growth and survival of bacteria
- Amenable to nutrient supplementation
- Rapid release of bacteria in soil
- Manageable in mixing, curing, and packaging operation
- Available in powder or granular form in adequate quantities and at a reasonable cost.

The kind of carrier utilized defines the physical form of the biofertilizer. Dry inoculants are mainly produced using several soil materials (e.g., peat, coal, clays, inorganic soil, etc.), organic materials (e.g., composts, soybean meal, wheat bran, sawdust, etc.), or inert materials (e.g., vermiculite, perlite, kaolin, bentonite, silicates) (Smith 1992) and additives such as gums, silica gel, methyl cellulose, and starch that protect the cells from harsh environmental conditions (Hynes and Boyetchko 2006) are used.

10.5.2 Types of Bioformulations

There are many variations available as biofertilizer formulations. The major types are:

- Solid carrier-based formulation
- Liquid formulation

- Polymer-entrapped formulation
- Fluidized bed dried formulation.

Solid formulations include granules, microgranules, wettable powders, and dust (Guijarro et al. 2007; Swapna et al. 2016). Liquid inoculants are based on culture broth, mineral oil, organic oil, or on oil-in-water emulsions. In a polymer-entrapped formulation, the mass multiplied inoculum is mixed with polymer and then chemical solidification is carried out (Jung et al. 1982). This provides uniform beads with entrapped live cells inside. Alginate is usually preferred for making beads. It slowly releases the entrapped microbes into the environment and protects them from harsh conditions. Reducing contamination in the formulation is one of the serious concerns in biofertilizer quality. In this regard fluidized bed dried formulations of beneficial microorganisms are of importanceas they have resulted in decreased contamination and increased survival of inoculants (Sahu et al. 2013; Lavanya et al. 2015).

Among the solid types of bioformulations, granules are dry particles containing an active ingredient, a binder and a carrier, and are classified as coarse particles of 100–1000 μm in size, and microgrnaules of 100–60 μm in size, which should be non-dusty, free flowing, and should disintegrate in the soil to release the active ingredient. Granules are safer and are more oriented toward increased shelf life (O'Callaghan and Gerard 2005), e.g., wheat meal granule, corn meal bait, corn starch granules, cotton seed flour granules, alginate, semolina wheat flour granules, etc. Wettable powders are the oldest type of bioformulations, and consist of 50–80% technical powder, 15–45% filler, 1–10% dispersant, and 3–5% surfactant (Brar et al. 2006). These powders are readily miscible with water and can be easily added to a liquid carrier like water just before application. They have a longer shelf life of 18 months and more, e.g., wheat bran-sand mixtures, sawdust-sand-molasses mixture, corn cob-sand-molasses mixture, sawdust-sand-molasses mixture, organic cakes, farmyard manure, inert charcoal, and flyash can be used prepare powder formulations (Khan et al. 2007).

Liquid formulations are aqueous suspensions consisting of biomass suspensions in water, oils, or a combination of the two (Schisler et al. 2004; Brahmaprakash et al. 2007; Velineni and Brahmaprakash 2011). They contain 10–40% of microorganisms, 1–3% suspender ingredient, 1–5% dispersant, 3–8% surfactant, and 35–65% carrier liquid (oil/water) (Brar et al. 2006). The form and concentration of osmolytes used affects the survival of microorganisms in the formulation (Sridhar et al. 2004; Girisha et al. 2006a, b; Dayamani 2010; Dayamani and Brahmaprakash 2014a, b). Liquid formulations maintain a higher number of microorganisms for a long time, which adds to their effectiveness (Navi 2004; Girisha et al. 2006a, b; Bhaskara 2011). Oil dispersion is a stable suspension of an active ingredient in water-immiscible solvent or oil. Soybean oil and other oil-based formulations have been shown to have greater efficacy in foliar spray and are considered effective in enhancing the activity of entomopathogens (Feng et al. 2004). Liquid inoculants have been reported to have a shelf life of 18–24 months (Sharma et al. 2010). Addition of trehalose (15 mM) and PVP (2.5%) has been reported to enhance the

shelf life of a liquid bioformulation of *Azospirillum* and PSB (Surendra Gopal and Baby 2016).

Different technologies are being developed to provide a good bioformulation to replace chemical pesticides and fertilizers. Nanotechnology is one of the novel avenues for development of the next generation bioformulation. It employs inorganic or organic nanoparticles with dimensions of 100 nm or less (Auffan et al. 2009). The integration of microbial cells with nanotechnology will lead to a useful hybrid system that can have numerous applications in many fields like agriculture (Bailey et al. 2010).

Cost-effectiveness is a major concern with bioformulations. The cost of abiofertilizer should not put economic pressure on the end users, the farmers (Xavier et al. 2004). A spray of PGPR containing *Bacillus cereus* and *Pseudomonas rhodesiae* on tomato, cauliflower, chili, and brinjal plants resulted in increased shoot height, early fruiting, and increased total biomass of plants (Kalita et al. 2015). Suman et al. (2016) reported on a hydrogel-based bioformulation containing *Azotobacter chroococcum, Pseudomonas fluorescence*, and *Trichoderma viride* for harnessing their potential to support growth, stable shelf life, and bio efficacy. Shelf life and other parameters were compared among lignite-, liquid-, and hydrogel-based carriers. After 90 days, the population of *Azotobacter chroococcum* was 1.2×10^7, 1.4×10^8 and 3.5×10^9 CFU/ml; *Pseudomonas fluorescence* was 2.2×10^7, 2.4×10^8 and 4.5×10^9 CFU/ml; and *Trichoderma viride* 1.4×10^6, 2.8×10^7 and 2.5×10^8 CFU/ml, respectively, from the lignite-, liquid-, and hydrogel-based carriers. Hydrogel-based bioinoculants enhanced growth in wheat seeds as compared to liquid- and lignite-based bioinoculants.

Rhizosphere microflora can enhance soil fertility, nutrient availability, nutrient uptake, and plant health (Adesemoye et al. 2009; Berg 2014). A liquid inoculant formulation of *Rhodopseudomonas palustris* strain PS3 improves soil quality and promotes plant growth (Lee et al. 2016). Rhizosphere inoculants also help to enhance nutrient uptake efficiency from applied fertilizer in the soil (Wong et al. 2014). The plant growth promotion ability of a liquid inoculant formulation of *Rhodopseudomonas palustris* strain PS3 was tested with different additives. In Chinese cabbage up to 40% yield enhancement over control was observed (Lee et al. 2016). In a paddy rhizosphere, *Rhodopseudomonas palustris* was reported as decomposing phytotoxins like hydrogen sulfide (Kornochalert et al. 2013; Idi et al. 2014).

Microbial inoculants are now becoming an important component of sustainable agricultural practice (Das et al. 2013). Rhizosphere microbes like *Pseudomonas* spp., *Azospirillum* spp., *Azotobacter* spp., and *Rhizobium* spp. are successful inoculants (Bashan et al. 2013; Dayamani and Brahmaprakash 2014a, b).

The difficulty of mismatch of performance of microbial inoculants from the laboratory/green house to the field (Stephens and Rask 2000) arises as a result of poor quality of formulation, carrier material, poor compatibility, poor rhizosphere competence, etc. (Bhattacharyya and Jha 2012; Dayamani and Brahmaprakash 2014a, b; Sahu and Brahmaprakash 2016). A successful bioformulation is one that supplies microbes in sufficient numbers and appropriate physiological form (Brahmaprakash and Sahu 2012; Sahu and Brahmaprakash 2016). An ideal formulation should have

the following criteria (Xavier et al. 2004; Pandya and Saraf 2010; Herrmann and Lesueur 2013; Sahu and Brahmaprakash 2016):

- Stability of the microorganisms during production, storage, and distribution
- Maintainence of a higher number of viable cells and enhancement of the activity of the organisms in the field
- Easy to handle and use
- Should not have adverse effects on the environment
- Should help to improve soil properties
- Release of bioinoculants in entrapped formulation should not be too fast or too slow
- Must be able to be applied by using standard agrochemical machinery
- Must be cost-effective and commercially viable

10.5.3 Mode of Application

There are three common ways of using biofertilizers:

1. Seed treatment
2. Root/seedling dipping
3. Soil application.

10.5.3.1 Seed Treatment

Seed treatment is the most commonly used method for different types of inoculants, and is an effective and economic method. For 10 kg of normal-size seeds, 200 g of inoculant is used, and for larger size seeds, 400–500 g of inoculants is used. The bag is opened and the seed is dried in shade for 20–30 min. The inoculant is mixed with seeds by adding a sticking agent jaggery (200 g) solution in a bucket and the microbial inoculant can be mixed directly by hand. Treated seeds have to be shade-dried and should be used for further sowing. Seed treatment can be carried out using *Rhizobium*, *Azotobacter, Azospirillum*, and with PSMs. Seed treatment can be accomplished using a consortium of compatible microorganisms. The seeds should be coated first with *Rhizobium, Azotobacter*, or *Azospirillum*. A PSM inoculant can be coated as the outer layer after a layer of other bacteria. This method will maintain a higher number of each bacterium, which is needed for better results.

10.5.3.2 Root Dipping

This method is useful when crops are being transplanted. It is also ideal for vegetable crops. *Azospirillum*/PSMs/*Pseudomonas* can be used for root dipping. Inoculant suspension in water at a ratio of 1:10 is prepared and the roots of seedlings are dipped into this suspension for 10–15 min. The seedlings are then removed and transplanted as early as possible.

10.5.3.3 Soil Application

Soil application can be done by mixing the inoculum with FYM/compost. For every 100 kg of FYM or compost, 2–3 kg of inoculant is thoroughly mixed and sprinkled with water. The resulting mix has to be covered with a wet gunny bag to retain the moisture and has to be left for 1–2 days. The result will be an inoculants-enriched mixture, which can then be used for application. This enriched compost has to be dispersed onto the field or can be used for soil application in rows or during leveling of soil.

Table 10.4 Recommended dose of liquid biofertilizers and their application method

Crop	Recommended biofertilizer	Application method	Quantity to be used
Field crops	*Rhizobium*	Seed treatment	200 ml/acre
Pulses			
Chickpea, pea, groundnut, soybean, bean, lentil, lucern, berseem, green gram, black gram, cowpea, pigeon pea			
Cereals wheat, oat, barley	*Azotobacter/Azospirillum*	Seed treatment	200 ml/acre
Rice	*Azospirillum*	Seed treatment	200 ml/acre
Oil seeds: mustard, seasum, linseeds, sunflower, castor	*Azotobacter*	Seed treatment	200 ml/acre
Millets: pearl millets, finger millets, kodo millet	*Azotobacter*	Seed treatment	200 ml/acre
Maize and sorghum	*Azospirillum*	Seed treatment	200 ml/acre
Forage crops and grasses: bermuda grass, sudan grass, napier grass, para grass, star grass, etc.	*Azotobacter*	Seed treatment	200 ml/acre
Other miscellaneaous plantation crops tobacco	*Azotobacter*	Seedling treatment	500 ml/acre
Tea, coffee	*Azotobacter*	Soil treatment	400 ml/acre
Rubber, coconuts	*Azotobacter*	Soil treatment	2–3 ml/ plant
Agro-forestry/fruit plants all fruit/agro-forestry (herbs, shrubs, annuals, and perennial) plants for fuel, wood fodder, fruits, gum, spice, leaves, flowers, nuts and seeds purpose	*Azotobacter*	Soil treatment	2–3 ml/ plant at nursery
Leguminous plants/trees	*Rhizobium*	Soil treatment	1–2 ml/ plant

Doses recommended when count of inoculum is 1×10^8 cells/ml. Doses will be ten times more because the given nitrogen fixers and phosphate solubilizers can be applied on all crops at a rate of 200 ml/acre

10.5.3.4 Dosage of Liquid Biofertilizers in Different Crops

Recommended liquid biofertilizers, their application method and quantity to be applied in different crops are shown in Table 10.4 (Harimuraleedharan et al. 2010; Vora et al. 2008).

10.6 Contribution of Rhizosphere Microflora to Modern Agriculture: A Future Perspective

Roots, the "hidden half" of a plant, are gaining greater attention of agro researchers for harnessing the potential hidden underground. Rhizosphere engineering can bring nutritional benefit by providing nutrients and enhancing efficiency of nutrient use of applied fertilizers. In conditions of water scarcity, rhizosphere microflora can enhance water use efficiency to produce a greater crop-per-drop concept. Induced systemic resistance for disease resistance and induced systemic tolerance for alleviation of abiotic stresses are two major areas of sustainable plant health promotion where rhizosphere microflora play a key role. Investigations need to be better targeted in order to acquire greater economical and more sustainable crop production by using rhizosphere microflora.

Acknowledgements The Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangaluru, India is gratefully acknowledged for continuous support and guidance.

References

Abbasdokht H (2008) The study of *Azotobacter chroococum* inoculation on yield and postharvest quality of wheat (*Triticum aestivum*). In: International meeting on soil fertility land management and agroclimatology, pp 885–889

Abbasdokht H, Gholami A (2010) The effect of seed inoculation (*Pseudomonas putida*+ *Bacillus lentus*) and different levels of fertilizers on yield and yield components of wheat (*Triticum aestivum* L.) cultivars. World Acad Sci Eng Technol 68:979–983

Achari GA, Ramesh R (2014) Diversity, biocontrol, and plant growth promoting abilities of xylem residing bacteria from solanaceous crops. Int J Microbiol 2014.: Article ID 296521:14

Adesemoye AO, Kloepper JW (2009) Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85(1):1–2

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929. doi:10.1007/s00248-009-9531-y

Ahemad M (2012) Implications of bacterial resistance against heavy metals in bioremediation: a review. IIOABJ 3:39–46

Ahemad M, Khan MS (2012) Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica compestris*) rhizosphere. Chemosphere 86(9):945–950

Ahemad M, Malik A (2011) Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater. Bacteriol J 2:12–12

Ahilandeswari K, Maheswari NU (2016) Co–inoculation of *Azospirillum lipoferum* and phosphate solubilizing microorganisms on the growth of rice (*Oryza sativa* L.) Int J Pure Appl Biosci 4:317–320

Akram W, Anjum T, Ali B (2016) Phenylacetic acid is ISR determinant produced by *Bacillus fortis* IAGS162, which involves extensive re-modulation in metabolomics of tomato to protect against *Fusarium* wilt. Front Plant Sci 7:498

Ali SZ, Sandhya V, Rao LV (2014) Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas* sp. Ann Microbiol 64(2):493–502

Anandaraj B, Delapierre LR (2010) Studies on influence of bioinoculants (*Pseudomonas fluorescens, Rhizobium* sp., *Bacillus megaterium*) in green gram. J Biosci Technol 1:95–99

Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, Fickers P (2009) *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microb Cell Factories 8(1):1

Arkhipova TN, Veselov SU, Melentiev AI, Martynenko EV, Kudoyarova GR (2005) Ability of bacterium Bacillus subtilis to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 272(1–2):201–209

Auffan M, Rose, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR et al (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 4(10):634–641

Baca BE, Elmerich C (2007) Microbial production of plant hormones. In: Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Springer, Dordrecht, pp 113–143

Bailey KL, Boyetchko SM, Angle TL et al (2010) Social and economic drivers shaping the future of biological control: a Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biol Control 52(3):221–229

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway? Trends Plant Sci 9:26–32

Bais HP et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Ann Rev Plant Biol 57:233–266

Barat SP, Gupta A, Singh D, Srivastav A (2016) Production of liquid biofertilizer by using *Azotobacter* species and their effect on plant growth. Int J Curr Microbiol App Sci 5(7):654–659

Bashan Y, de Bashan LE (2005) Plant growth-promoting. Encycl Soils Environ 1:103–115

Bashan Y, Holguin G, de-Bashan LE (2004) *Azospirillum* plant relationships: physiological, molecular, agricultural, and environmental advances. Can J Microbiol 50:521–577

Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP (2013) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33. doi:10.1007/s11104-013-1956-x

Bazilah ABI, Sariah M, Abidin MAZ et al (2011) Influence of carrier materials and storage temperature on survivability of Rhizobial inoculants. Asian J Plant Sci 10:331–337

Berg G (2014) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berry JP, Gantar M, Perez MH, Berry G, Noriega FG (2008) Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. Mar Drugs 6:117–146

Bhaskara V (2011) Comparative performance of liquid and lignite formulations of *Azotobacter chroococcum* and *Bacillus megaterium* (PSB) on aerobic rice (*Oryza sativa* L.). MSc thesis, University of Agricultural Sciences, Bengaluru, India

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350. doi:10.1007/s11274-011-0979-9

Biswas B, Gresshoff PM (2014) The role of symbiotic nitrogen fixation in sustainable production of biofuels. Int J Mol Sci 15:7380–7397

Bloemberg GV, Lugtenberg BJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Brahmaprakash GP, Sahu PK (2012) Biofertilizers for sustainability. J Indian Inst Sci 92(1):37–62
Brahmaprakash GP, Girisha HC, Navi V, Laxmipathy R, Hegde SV (2007) Liquid *Rhizobium* inoculant formulations to enhance biological nitrogen fixation in food legumes. J Food Legum 20:75–79
Brar SK, Verma M, Tyagi RD, Valero JR et al (2006) Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. Process Biochem 41:323–342
Broekaert WF, Delauré SL, De Bolle MF, Cammue BP (2006) The role of ethylene in host-pathogen interactions. Annu Rev Phytopathol 44:393–416
Buée M, De Boer W, Martin F, Van Overbeek L, Jurkevitch E (2009) The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant Soil 321(1–2):189–212
Cabello M, Irrazabal G, Bucsinszky AM, Saparrat M, Schalamuck S (2005) Effect of an arbuscular mycorrhizal fungus, *G. mosseae* and a rock-phosphate-solubilizing fungus, *P. thomii* in *Mentha piperita* growth in a soil less medium. J Basic Microbiol 45:182–189
Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41
Clarholm M (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol Biochem 17:181–187
Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Barka EA (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71(4):1685–1693
Corey D, Amanda K, Joy B et al (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744
Darbyshire JF, Greaves MP (1973) Bacteria and protozoa in the rhizosphere. Pestic Sci 4:349–360
Das AJ, Kumar M, Kumar R (2013) Plant growth promoting rhizobacteria (PGPR): an alternative of chemical fertilizer for sustainable, environment friendly agriculture. Res J Agric For Sci 1:21–23
Dayamani KJ (2010) Formulation and determination of effectiveness of liquid inoculants of plant growth promoting rhizobacteria'. PhD thesis, University of Agricultural Sciences, Bengaluru, India
Dayamani KJ, Brahmaprakash GP (2014a) Influence of form and concentration of the osmolyte in liquid inoculants of plant growth promoting bacteria. Int J Sci Res Publ 4(7):1–6
Dayamani KJ, Brahmaprakash GP (2014b) Influence of form and concentration of the osmolytes in liquid inoculants formulations of plant growth promoting bacteria. Int J Sci Res Publ 4:1–6
DeBach P (ed) (1964) The scope of biological control. In: Biological control of insect pests and weeds. Chapman and Hall Ltd, London, pp 3–20
Djavaheri M, Blanco JM, Van Loon LC, Bakker PAHM (2009) Analysis of determinants of *Pseudomonas fluorescens* WCS374r involved in induced systemic resistance in *Arabidopsis thaliana*. Biol Control Fungal Bacterial Plant Pathog 43:109–111
Egamberdiyeva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36(2):184–189
Esitken A, Yildiz H, Ercisli S, Donmez M, Turan M, Gunes A (2009) Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Sci Hortic 124(1):62–66
Feng MG, Pu XY, Ying SH, Wang YG (2004) Field trials of an oil based emulsifiable formulation of *Beauveria bassiana* conidia and low application rates of imidacloprid for control of false use leafhopper *Empoasca vitis* on tea in S. china. Crop Prot 23:489–496
Feng H, Li Y, LiU Q (2013) Endophytic bacterial communities in tomato plants with differential resistance to *Ralstonia solanacearum*. Afr J Microbiol Res 7(15):1311–1318
Fred EB, Baldwin IL, McCoy E (1932) Root nodule bacteria and leguminous plants. Univ. Wisconsin, Madison
Gamalero E, Glick BR (2015) Bacterial modulation of plant ethylene levels. Plant Physiol 169(1):13–22

Ghosh SK, Pal S, Banerjee S, Chakraborty N (2015) In vitro study of lysis of cell wall preparation from *Phomopsis vexans* by lytic enzyme from some biocontrol agents. Int J Curr Microbiol App Sci 4:153–157

Girisha HC, Brahmaprakash GP, Mallesha BC (2006a) Effect of osmoprotectant (PVP-40) on survival of *Rhizobium* in different inoculants formulation and nitrogen fixation in cowpea. Geobios 33:151–156

Girisha HC, Brahmaprakash GP, Manjunath A (2006b) Liquid inoculant technology: a boon to pulse production. Biofertilizer Newsl 14(1):3–8

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251(1):1–7

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39

Goenadi DH, Siswanto, Sugiarto Y (2000) Bioactivation of poorly soluble phosphate rocks with a phosphate solubilizing fungus. Soil Sci Soc Am J 64:927–932

Griffiths BS (1994) Soil nutrient flow. In: Darbyshire JF (ed) Soil protozoa. CAB International, Wallingford, pp 65–91

Guijarro B, Melgarejo P, Decal A (2007) Effect of stabilizers on the shelf life of Penicillium frequentans conidia and their efficacy as a bioagent against peach brown rot. J Food Microbiol 113:117–124

Guo QG, Dong WX, Li SZ, Lu XY, Wang PP, Zhang XY (2014) Fengycin produced by *Bacillus subtilis* NCD-2 plays a major role in biocontrol of cotton seedling damping-off disease. Microbiol Res 169:533–540

Gupta P (2010) Studies on shelf–life of fly–ash based *Azotobacter chroococcu*m formulation and its bio–efficacy in wheat. Res J Agric Biol Sci 6(3):280–282

Harimuraleedharan, Seshadri S, Perumal K (2010) Booklet on biofertilizer (phosphobacteria). Shri AMM Murugappa Chettiar Research centre, Taramani

Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97:8859–8873. doi:10.1007/s00253-013-5228-8

Heydari A, Gharedaghli A (2007) Integrated pest management on cotton in Asia and North Africa. INCANA Press, Tehran

Hiltner L (1904) Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb DLG 98:59–78. (Originals not seen)

Hinsinger P (1998) How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. Adv Agron 64:225–265

Hynes RK, Boyetchko SM (2006) Research initiatives in the act science of biopesticides formulation. Soil Boil Biochem 38:845–849

Idi A, Md Nor MH, Abdul Wahab MF, Ibrahim Z (2014) Photosynthetic bacteria: an eco-friendly and cheap tool for bioremediation. Rev Environ Sci Biotechnol 14:271–285

Islam TM, Hashidoko Y, Deora A, Ito T, Tahara S (2010) Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne peronosporomycetes. Appl Environ Microbiol 71:3786–3796

Jahanian A, Chaichi MR, Rezaei K, Rezayazdi K, Khavazi K (2012) The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (*Cynara scolymus*). Int J Agric Crop Sci 4:923–929

Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. Crit Rev Plant Sci 30(5):435–458

Jones DL, Darrah PR (1995) Influx and efflux of organic acids across the soil-root interface of *Zea mays* L. and its implications in rhizosphere C flow. Plant Soil 173:103–109

Jung G, Mugnier J, Diem HG et al (1982) Polymer entrapped *Rhizobium* as an inoculant for legume. Plant Soil 65:219–231

Kalita M, Bharadwaz M, Dey T, Gogoi K, Dowarah P, Unni BG, Ozah D, Saikia I (2015) Developing novel bacterial based bioformulation having PGPR properties for enhanced production of agricultural crops. Indian J Exp Biol 53(1):56–60

Kang SC, Ha CG, Lee TG, Maheshwari DK (2002) Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102. Curr Sci 82:439–442

Kang SM, Khan AL, Waqas M et al (2014) Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus. J Plant Interact 9(1):673–682

Kasiamdar RS, Smith SE, Smith FA, Scott ES (2001) Influence of the mycorrhizal fungus, *Glomus coronatum*, and soil phosphorus on infection and disease caused by binucleate *Rhizoctonia* and *Rhizoctonia solani* on mung bean (*Vigna radiata*). Plant Soil 238:235–244

Khalid A, Tahir S, Arshad M, Zahir ZA (2005) Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. Soil Res 42(8):921–926

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate solubilizing microorganisms in sustainable agriculture- a review. Agron Sustain Dev 27:29–43

King RW, Evans LT (2003) Gibberellins and flowering of grasses and cereals: prizing open the lid of the "florigen" black box. Ann Rev Plant Biol 54(1):307–328

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) *Pseudomonas* siderophores: a mechanism explaining disease suppression in soils. Curr Microbiol 4:317–320

Koga H, Dohi K, Mori M (2004) Abscisic acid and low temperatures suppress the whole plant-specific resistance reaction of rice plants to the infection of *Magnaporthe grisea*. Physiol Mol Plant Pathol 65(1):3–9

Takanashi K, Sasaki T, Kan T, Saida Y, Sugiyama A, Yamamoto Y, Yazaki K (2016) A dicarboxylate transporter, LjALMT4, mainly expressed in nodules of *Lotus japonicas*. MPMI 29(7):584–592

Kornochalert N, Kantachote D, Chaiprapat S, Techkarnjanaruk S (2013) Use of *Rhodopseudomonas palustris* P1 stimulated growth by fermented pineapple extract to treat latex rubber sheet wastewater to obtain single cell protein. Ann Microbiol 64:1021–1032

Kottke I, Kovacs GM (2013) Mycorrhizae- Rhizosphere determinant of plant communities: what can we learn from tropics? In: Eshel A, Beeckman T (eds) Plant roots: the hidden half, 4th edn. CRC Press, Taylor and Francis Group, Hoboken. pp 40; 1–10

Krishnaveni MS (2010) Studies on phosphate solubilizing bacteria (PSB) in rhizosphere and non-rhizosphere soils in different varieties of foxtail millet (*Setaria italica*). Int J Agric Food Sci Tech 1(1):23–39

Kumar V (2014) Characterization, bio-formulation development and shelf-life studies of locally isolated bio-fertilizer strains. Octa J Env Res 2(1):32–37

Kumar R, Pandey S, Pandey A (2006) Plant roots and carbon sequestration. Curr Sci 91(7):885–890

Kumara Swamy CA, Raghunandan BL, Chandrashekhar M, Brahmaprakash GP (2010) Bioactivation of rock phosphate vis- a- vis seed treatment with phosphorus solubilizing microbes (PSM) in enhancing P nutrition in cowpea and ragi. Indian J Sci Technol 3(7):689–692

Lavanya G, Sahu PK, Manikanta DS, Brahmaprakash GP (2015) Effect of fluid bed dried formulation in comparison with lignite formulation of microbial consortium on finger millet (*Eleucine coracana* Gaertn.) J Pure Appl Microbiol 9(2):193–199

Lee SK, Lur HS, Lo KJ, Cheng KC, Chuang CC, Tang SJ, Yang ZW, Liu CT (2016) Evaluation of the effects of different liquid inoculant formulations on the survival and plant-growth-promoting efficiency of *Rhodopseudomonas palustris* strain PS3. Appl Microbial Biotechnol 6:1–1

Li Y, Gu Y, Li J, Xu M, Wei Q, Wang Y (2015) Biocontrol agent *Bacillus amyloliquefaciens* LJ02 induces systemic resistance against cucurbits powdery mildew. Front Microbiol 6:1–15

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting Rhizobacteria. Annu Rev Microbiol 63:541–556

Mahdi SS, Hassan GI, Samoon SA, Rather HA, Dar Showkat A, Zehra B (2010) Bio-fertilizers in organic agriculture. J Phytology 2(10):42–54

Maheswari NU, Kalaiyarasi M (2015) Comparative study of liquid biofertilizer and carrier based biofertilizer on green leafy vegetables. Int J Pharm Sci Rev Res 33(1.) 42):229–232

Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, Zocchi G, Daffonchio D (2012) A drought resistance promoting microbiome is selected by root system under desert farming. PLoS One 7:e48479

Massaccesi L, Benucci GMN, Gigliotti G, Cocco C, Corti G, Agnelli A (2015) Rhizosphere effect of three plant species of environment under periglacial conditions (Majella Massif, central Italy). Soil Biol Biochem 89:184–195

Maurhofer M, Hase C, Meuwly P, Métraux JP, Defago G (1994) Induction of systemic resistance to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the *gac*A gene and of pyoverdine production. Phytopathology 84:139–146

Melo ISD, Valente AMMP, Kavamura VN, Vilela ESD, Faull JL (2014) Mycoparasitic nature of *Bionectria* sp. strain 6.21. J Plant Protect Res 54(4):327–333

Meziane H, Van der Sluis I, Van Loon LC, Hofte M, Bakker PAHM (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol Plant Pathol 6:177–185

Mishra BK, Dahich SK (2010) Methodology of nitrogen biofertilizer production. J Adv Dev Res 1:3–6

Navi V (2004) Development of liquid inoculant formulations for *Bradyrhizobium* sp. (Arachis), *Azospirillum lipoferum* and *Azotobacter chroococcum*. PhD thesis, University of Agricultural Sciences, Bengaluru, India

Nehra V, Choudhary M (2015) A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. J Appl Nat Sci 7(1):540–556

Nepolean P, Jayanthi R, Vidhya PR, Balamurugan A, Kuberan T, Beulah T, Premkumar R (2012) Role of biofertilizers in increasing tea productivity. Asian Pac J Trop Biomed 2:1443–1445

Neumann G, Romheld V (2001) The release of root exudates as affected by the plants physiological status. In: Pinto R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York, pp 41–93

Nihorimbere V, Ongena M, Smargiassi M, Thonart P (2011) Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol Agron Soc Environ 15(2):327

Nye PH (1981) Changes of pH across the rhizosphere induced by roots. Plant Soil 61:7–26

O'Callaghan M, Gerard EM (2005) Establishment of *Serratia entomophila* in soil from a granular formulation. In: New Zealand plant protection, vol 58. Proceedings of a conference, Wellington, New Zealand, 9–11 August 2005. New Zealand Plant Protection Society, pp 122–125

Ortíz-Castro R, Valencia-Cantero E, López-Bucio J (2008) Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signal Behav 3(4):263–265

Pal KK, McSpadden GB (2006) Biological control of plant pathogens. Plant Health Instr. doi:10.1094/PHI-A-2006-1117-02

Pandya U, Saraf M (2010) Application of fungi as a biocontrol agent and their biofertilizer potential in agriculture. J Adv Dev Res 1(1):90–99

Pant R, Pandey P, Kotoky R (2016) Rhizosphere mediated biodegradation of 1,4-dichlorobenzene by plant growth promoting rhizobacteria of *Jatropha curcas*. Ecol Eng 94:50–56

Parmar HJ, Bodar NP, Lakhani HN, Patel SV, Umrania VV, Hassan MM (2015) Production of lytic enzymes by *Trichoderma* strains during in vitro antagonism with *Sclerotium rolfsii,* the causal agent of stem rot of groundnut. Afr J Microbiol Res 9(6):365–372

Paul E, Clark F (1996) Soil microbiology and biochemistry, 2nd edn. Academic, San Diego

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase containing plant growth promoting rhizobacteria. Physiol Plant 118(1):10–15

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Ponmurugan P, Gopi C (2006) Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J Agron 5:600–604

Pradhan N, Sukla LB (2005) Solubilization of inorganic phosphate by fungi isolated from agriculture soil. Afr J Biotechnol 5:850–854

Quispel A (1974) Biology of nitrogen fixation. North Holland Press, Amsterdam, p 748

Raaijmakers JM, Vlami M, De Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81:537–547
Rashid M, Khalil S, Ayub N, Alam S, Latif F (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pak J Biol Sci 7(2):187–196
Ratnayale M, Leonard R, Menge A (1978) Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal infection. New Phytol 81:543–552
Raychaudhuri S, Yadav AK, Raychaudhuri M (2007) Changing face of *Rhizobium* taxonomy. Biofertilizer Newsl 15(1):3–10
Rovira AA, Foster RC, Martin JK (1978) Origin nature and nomenclature of the organic materials in the rhizosphere. In: Harley JL, Scott Russel R (eds) The soil root interface. Academic, London, pp 1–4
Rueda D, Valencia G, Soria N, Rueda BB, Manjunatha B, Kundapur RR, Selvanayagam M (2016) Effect of *Azospirillum* spp. and *Azotobacter* spp. on the growth and yield of strawberry (Fragaria vesca) in hydroponic system under different nitrogen levels. J Appl Pharma Sci 6(01):048–054
Sahu PK, Brahmaprakash GP (2016) Formulations of biofertilizers–approaches and advances. In: Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 179–198
Sahu PK, Lavanya G, Brahmaprakash GP (2013) Fluid bed dried microbial inoculants formulation with improved survival and reduced contamination level. J Soil Biol Ecol 33(1and2):81–94
Sahu PK, Sharma L, Gupta A, Renu (2016) Rhizospheric and endophytic beneficial microorganisms: treasure for biological control of plant pathogens. In: Santra S, Mallick A (eds) Recent biotechnological applications in India. ENVIS centre on Environmental Biotechnology, University of Kalyani, West Bengal, pp 50–63
Sandra AI, Wright CH, Zumoff LS, Steven VB (2001) *Pantoea agglomerans* strain EH318 produces two antibiotics that inhibit *Erwinia amylovora* in vitro. Appl Environ Microbiol 67:282–292
Schisler DA, Slininger PJ, Behle RW, Jackson MA (2004) Formulation of spp. for biological control of Plant diseases. Phytopathology 94(11):1267–1271
Shahbaz M, Ashraf M (2013) Improving salinity tolerance in cereals. Crit Rev Plant Sci 32:237–249
Shanahan P, O'Sullivan DJ, Simpson P, Glennon JD, O'Gara F (1992) Isolation of 2,4-Diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. Appl Environ Microbiol 58:353–358
Sharma MP, Sharma SK, Dwivedi A (2010) Liquid biofertilizer application in soybean and regulatory mechanisms. Agriculture Today, pp 44–45
Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6(5):410–417
Silvester WB (1975) Ecological and economical significance of the non- legume symbioses'. In: Newton WE, Nyman CJ (eds) 1st Int. symposium on nitrogen fixation. Washington State Univ. Press, Washington, DC, pp 489–586
Singh G, Mukerji KG (2006) Root exudates as determinant of rhizospheric microbial biodiversity. In: Microbial activity in the Rhizoshere. Springer, Berlin, pp 39–53
Smith RS (1992) Legume inoculant formulation and application. Can J Microbiol 38(6):485–492
Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3(4):a001438
Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signalling. FEMS Microbiol Rev 31(4):425–448
Sridhar V, Brahmaprakash GP, Hegde SV (2004) Development of a liquid inoculant using osmoprotectants for phosphate solubilizing bacteria. Karnataka J Agric Sci 17:251–257
Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crop Res 65:249–258. doi:10.1016/s0378-4290(99)00090-8
Stolp H (1988) Microbial ecology: organisms, habitats, activities. Cambridge University Press, New York
Suman A, Verma P, Yadav AN, Srinivasamurthy R, Singh A, Prasanna R (2016) Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (*Triticum aestivum* L.) Int J Curr Microbiol App Sci 5(3):890–901

Surendra Gopal K, Baby A (2016) Enhanced shelf life of *Azospirillum* and PSB through addition of chemical additives in liquid formulations. Int J Sci Environ Technol 5(4):2023–2029
Swapna G, Divya M, Brahmaprakash GP (2016) Survival of microbial consortium in granular formulations, degradation and release of microorganisms in soil. Ann Plant Sci 5(5):1348–1352
Taylor IB, Burbidge A, Thompson AJ (2000) Control of abscisic acid synthesis. J Exp Bot 51(350):1563–1574
Thomas GV (1993) Biological nitrogen fixation by asymbiotic and non leguminous symbiotic systems. In: Thampan PK (ed) Organics in soil health and crop production. Peekey Tree Crops Development Foundation, Cochia, pp 105–124
Tittabutr P, Payakapong W, Teaumroong N, Singleton PW, Boonkerd N (2007) Growth, survival and field performance of bradyrhizobial liquid inoculant formulations with polymeric additives. Sci Asia 33:69–77
Tiwari KN (2001) Phosphorus needs of Indian soils and crops. Better Crop Int 15(2):6
Tiwari S, Singh P, Tiwari R, Meena KK, Yandigeri M, Singh DP, Arora DK (2011) Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. Biol Fertil Soils 47(8):907–916
Uren NC (2001) Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere. Biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York, pp 19–40
Van Loon LC (2000) Systemic induced resistance. In: Slusarenko AJ, Fraser RSS, Van Loon LC (eds) Mechanisms of resistance to plant diseases. Kluwer Academic Publishers, Dordrecht, pp 521–574
Van Veen JA, van Overbeek LS, van Elsas JD (1997) Fate and activity of microorganisms introduced into soil. MMBR 61(2):121–135
Van Wees SCM, De Swart EAM, Van Pelt JA, Van Loon LC, Pieterse CMJ (2000) Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 97:8711–8716
Veeger C, Haaker H, Laane, C (1981) Energy transduction and nitrogen fixation. In: Current perspectives in nitrogen fixation. Proceedings of 4th International Symposium on Nitrogen Fixation, Canberra, pp 101–104
Velineni S, Brahmaprakash GP (2011) Survival and phosphate solubilizing ability of *Bacillus megaterium* in liquid inoculants under high temperature and desiccation stress. J Agric Sci Technol 13:795–802
Vora MS, Shelat HN, Vyas RV (2008) Handbook of biofertilizers and microbial pesticides, 1st publication. Satish Serial Publishing House, India, pp 13–15
Vranova V, Rejsek K, Formanek P (2013) Aliphatic, cyclic, and aromatic organic acids, vitamins, and carbohydrates in soil. Sci World J 15. http:// dx.doi.org/10.1155/2013/524239. Article ID 524239
Waisel Y, Eshel A (2002) Functional diversity of various constituents of a single root system. In: Waisel Y, Eshel, Kafkafi U (eds) Plant roots: the hidden half, 3rd edn. Marcel Dekker, New York, pp 157–174
Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* sp. closely associated with wheat roots. Biol Fertil Soils 40:36–43
Walpola B, Yoon MH (2013) Phosphate solubilizing bacteria: assessment of their effect on growth promotion and phosphorous uptake of mung bean (*Vigna radiata* [L.] R. Wilczek). Chil J Agric Res 73(3):275–281
Walters DR, McRoberts N (2006) Plants and biotrophs: a pivotal role for cytokinins? Trends Plant Sci 11(12):581–586
Wani SP, Lee KK (2002) Population dynamics of nitrogen fixing bacteria associated with pearl millet (*P. americanum* L.). In: Biotechnology of nitrogen fixation in the tropics. University of Pertanian, Malaysia, pp 21–30
Weller DM, Landa BB, Mavrodi OV, Schroeder KL, De La Fuente L (2007) Role of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in the defense of plant roots. Plant Biol 9:4–20

Wong WT, Tseng CH, Hsu SH, Lur HS, Mo CW, Huang CN, Hsu SC, Lee KT, Liu CT (2014) Promoting effects of a single *Rhodopseudomonas palustris* inoculant on plant growth by *Brassica rapa chinensis* under low fertilizer input. Microbes Environ 29:303–313. doi:10.1264/jsme2.ME14056

Xavier IJ, Holloway G, Leggett M (2004) Development of rhizobial inoculant formulations. In: Proceedings of the greatplains inoculant forum. Plant Management Network, Saskatoon

Yadav AK (2009) Glimpses of fertilizer (control) order, 1985 for biofertilizers (amendment, November 2009), National center for organic farming, Department of Agriculture and cooperation, Government of India. Biofertilizer Newslett 17(2):11–14

Yao L, Wu Z, Zheng Y, Kaleem I, Li C (2010) Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. Eur J Soil Biol 46:49–54

Feth el Zahar H, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground. Soil Biol Biochem 77:69–80

Zhang L, Fan J, Ding X, He X, Zhang F, Feng G (2014) Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. Soil Biol Biochem 74:177–183

Zwart KB, Kuikman PJ, van Veen JA (1994) Rhizosphere protozoa: their significance in nutrient dynamics. In: Darbyshire JF (ed) Soil protozoa. CAB International, Wallingford, pp 93–122

Rhizosphere Signaling Cascades: Fundamentals and Determinants

11

Utkarsh M. Bitla, Ajay M. Sorty, Kamlesh K. Meena, and Narendra P. Singh

Abstract

Molecular interactions among the plants and microbes represent an important microecological phenomenon. The cross talk involves multiple ecological aspects like exchange of metabolites, signaling and chemotaxis, etc. These bilateral interactions are crucial for the health and development of both the plant and colonizing microbes. The signal molecules play major role as inducers of different pathways that contribute indispensable role for the survival of the participants under adverse circumstances and development of symbiotic associations as well. Though the recent high-throughput techniques have generated considerable data regarding the molecular exchanges happening in the rhizosphere microbes and the host, our current knowledge in this area is still in infancy. It is thus critical to get deeper insights of such interactions so as to develop next-generation strategies relating to the sustainable agriculture under the changing climate scenario. We describe herewith the major aspects concerning the contributors and their role in rhizosphere signaling cascades and the consequent post-signaling responses given by the host and the colonizing microbes.

Keywords

Rhizosphere • Bacteria • Host–microbe interaction • Chemotaxis • Signaling

U.M. Bitla • A.M. Sorty • K.K. Meena (✉) • N.P. Singh
School of Edaphic Stress Management, ICAR-National Institute of Abiotic Stress Management, Baramati, 413 115 Pune, India
e-mail: kkmeenamicro@gmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_11

11.1 Introduction

Microbial interactions are omnipresent. Microbial communities in soil represent the pool of biological diversity (Berendsen et al. 2012). In plants also the microbes comprise a core portion of the phyllosphere ecosystem. However, the major fractions of the microbes interacting with plants are mostly from rhizosphere or endophyte origin that depends on their habitat inside or outside the plant. These organisms can promote the seed germination, improve the uptake of nutrients, and enhance the growth and development of plants under the influence of different abiotic and biotic stressors (Ryan et al. 2008; Grover et al. 2013; Meena et al. 2017). The rhizosphere-colonizing microbial communities principally comprise bacteria, fungi, protozoa, algae, and actinomycetes; however, bacteria are the major copious microbes present in the soil. Microbial interactions play key role in the survival of microorganisms by developing homeostasis between microbial community and the surrounding environment. Microbes respond to the environmental stimuli by carrying out metabolic exchange. Majority of the biologically active metabolites produced by fungi and bacteria can potentially serve as bio-fertilizers, biopesticides, phytostimulators, and plant strengtheners (Berg 2009). On the other hand, plants also exudate number of organic compounds like sugars, vitamins, amino acids, etc. which serve either as nutrients or signaling molecules for colonization. Responding to the plant signals, the microbial population produces an array of biologically active molecules, including microbial derivatives of phytohormones and other volatile compounds having the capability to regulate plant growth and enhance the plants' immunity (Ortíz-Castro et al. 2009). The exudates secreted by plant roots permit development of microbial communities and increase microbial metabolic turnover in the rhizosphere region. In addition to the function as chemoattractant, the root exudates also serve as sources of carbon and nitrogen. The chemical composition of root exudates is species dependent in addition to the influence of environmental conditions, nutrient availability, soil chemical characteristics, weather conditions, etc. (Mimmo et al. 2011). Flavonoids are the major signaling molecules known to participate in plant–microbe interactions. The beneficial microbial communities generally interact with plant by two strategies: first being the plant growth promotion by producing phytohormones, VOCs, cytokinins, siderophores, etc. and, second one, to work as biocontrol agents against phytopathogens. Both the strategies result in improved health of plants, enhanced immunity against phytopathogens, and efficient tolerance to biotic and abiotic stresses.

11.2 Molecular Signaling in Plants and Microbes

Each entity develops a system of signaling cascades to intellect the habitat and other organisms and to launch specific cellular, molecular, and developmental oscillations. The significance of the interaction among plant and specific microbial communities is well known. The biological communication between plants and microorganisms involves diverse signal molecules originating from both

allies. Plant root exudates during different developmental stages of the plant induce comprehensive signaling cascades between the plant and soil microbes. Microorganisms produce signal molecules to synchronize their gene expression in response to changes in cell density (Atkinson and Williams 2009). During the past several decades, researchers made their energies to understand the molecular mechanisms of signaling in the rhizosphere environment (Guttman et al. 2014), but still we have limited information about the signaling pathways in the rhizosphere. Phytohormones predominantly regulate the whole plants' developmental process. Microorganisms also produce auxins and cytokinins that act as central protagonist in plants' overall development. Some microbial interactions are also governed by auxins where they serve the role of signaling molecules at a particular stage of microbial growth. Phenolic compounds, the secondary metabolites secreted by the plants, also contribute in the interactive signaling cascades involved in the development of legume-rhizobia symbioses and arbuscular mycorrhizal symbioses; moreover the former also act as active mediators in plant defense response (Mandal et al. 2010). Plants secrete number of secondary metabolites in rhizosphere like phenylpropanoids, glucosinolates, flavonoids, and terpenes (Dixon and Paiva 1995; Bressan et al. 2009; Moore et al. 2014). Flavonoids are capable to mimic the quorum-sensing molecules in bacteria, thus impelling the overall metabolism of bacteria (Hassan and Mathesius 2012). Strigolactones are terpenoid lactones that serve as derivatives of carotenoid metabolism; they also act as major signaling molecules in plant–arbuscular mycorrhizal symbiosis (Bonfante and Genre 2015). These molecules also serve as key regulators of developmental adaptations in plants under changing environmental conditions. Moreover, they can also stimulate the nodulation process during the root-*Rhizobium* symbiosis (Soto et al. 2010; Foo and Davies 2011).

11.3 Defense

Plant pathogenic microbes and insects are the most persistent threat in agriculture, representing one of the major causes of yield loss. Farmers mostly depend on agrochemicals to protect plants from the pathogens. However, the increased use of agrochemicals pesticides and insecticides has ultimately resulted in the development of another serious threat of the agrochemical resistance. Beneficial microorganisms associated with plants also protect plants from pathogens, both directly and indirectly. The major phenomena include production of toxins and antibiotics, competition with pathogens for nutrition, suppression of their growth, and development of ISR against pathogens and abiotic stresses. Studies concerning the action mechanisms of PGPB open new gateways to develop the strategies to improve the overall efficacy of biocontrol agents (Walsh et al. 2001; Morrissey et al. 2002, 2004). The biomolecules like antibiotics, chromosomal and/or ribosomal production of antimicrobial peptides, organic acids, proteinaceous exotoxins, and lytic enzymes are generally produced for defense purpose (Subramanian and Smith 2015). *Pseudomonas* hinders the growth of virulent bacteria by secreting antibiotics after inhabiting the

root systems. The signaling pathways involved in the antibiotic synthesis are yet to be explored keenly; however, it might be similar to the other two-component signaling systems that induce antibiotic synthesis (Whipps 2001). Owing to this property of bacteria, their use as biocontrol agents has been extensively studied worldwide principally to control the plant pathogens from agricultural crops. Similarly, yeasts from the genera *Pichia* and *Candida* and filamentous fungi from the genera *Trichoderma*, *Gliocladium*, and *Ulocladium* also have biocontrol potential due to their ability to produce fungicidal and bactericidal compounds (Jacometti et al. 2010).

11.4 Induced Resistance

The plants defend through biochemical responses and resistance mechanisms against pathogens. Induction of resistance in plants against phytopathogens can be of either localized and/systemic nature. Localized induced immune response in plants is often associated with systemic acquired resistance (SAR), which requires distant communication and involvement of signal molecules. Some of these signal molecules in plants have been characterized in recent years (Shah and Zeier 2013). Jasmonic acid and salicylic acid serve as important coordinators in the complex signaling pathways involved between plant-beneficial microbes and plant–pathogenic microbe-insect interactions (Robert-Seilaniantz et al. 2011; Pieterse et al. 2012). They also work as the signal molecules to regulate symbiosis and mediate induced systemic resistance (ISR) in plants by nonpathogenic, beneficial microbes (De Vleesschauwer et al. 2009; Zamioudis and Pieterse 2012). Elicitors produced by ISR-inducing PGPR are generally lipopolysaccharides (LPS), salicylic acid and pyoverdine, which are iron-regulated metabolites (De Vleesschauwer and Hofte 2009; Van Loon et al. 1998). *Paenibacillus polymyxa*, an ISR-inducing bacterial strain, successfully enhanced JA-responsive *Atvsp*, SA-responsive *Pr1*, and ET-responsive *Hel* gene expression (Timmusk and Wagner 1999). Many ISR elicitors have been identified in the previous studies, e.g., ethylene signaling pathway, volatile organic compounds (VOCs) like 2R,3R-butanediol produced by *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a (Ryu et al. 2004). *Paenibacillus polymyxa* emitted C13 compounds that elicited ISR in *A. thaliana* against *Erwinia carotovora* (Lee et al. 2012). The siderophore pyoverdine, LPS-containing cell walls, and flagella of *P. putida* WCS358 elicit ISR in *Arabidopsis* when applied exogenously to the roots (Meziane et al. 2005). *T. harzianum* and *P. fluorescens*, alone and in combination, increase the induced systemic resistance in rice plants against sheath blight disease caused by *R. solani* (Singh et al. 2016). Biocontrol agents are considered as potential alternatives of chemical agents in sustainable agriculture (Postma et al. 2003; Welbaum et al. 2004).

Beneficial microbes also elicit the systemic acquired resistance in plants; ISR pathways in plant are regulated by microbes mainly via SA-independent mechanisms. Studies have shown that SA-dependent form of ISR in plants induced by numerous PGPR resembles with the pathogen-induced SAR (Pieterse et al. 2000).

The PGPR strain *Pseudomonas aeruginosa* 7NSK2–SA-producing mutant was shown to improve the disease resistance in wild-type bean and tomato (Audenaert et al. 2002; De Meyer et al. 1999). Wild-type PGPR *Paenibacillus alvei* K165 and *P. fluorescens* SS101 also showed the development of SA-dependent SAR (Tjamos et al. 2005; Van de Mortel et al. 2012). Efficiency of biocontrol agents is mainly reliant on the capability and efficacy of the microbes to colonize the root surface (Ahmad and Baker 1987); this could be resolved better when one considers the significance of endophytic microbes to elicit defense responses against phytopathogens. The process involves an array of signaling cascades, e.g., SA- independent pathway induced by *Azospirillum* sp. B510 (Yasuda et al. 2009).

11.5 Endophytes

Endophytes are the class of microorganisms inherent and colonized inside the healthy plants without causing any disease or symptoms. Endophytes are mostly beneficial for plants' growth and development; they are also emerging as effective biocontrol agents in agriculture (Fig. 11.1). The endophytic organisms having biocontrol ability colonize the plant and induce numerous modifications in cell wall structure like development of structural obstacles by deposition of pectin, callose,

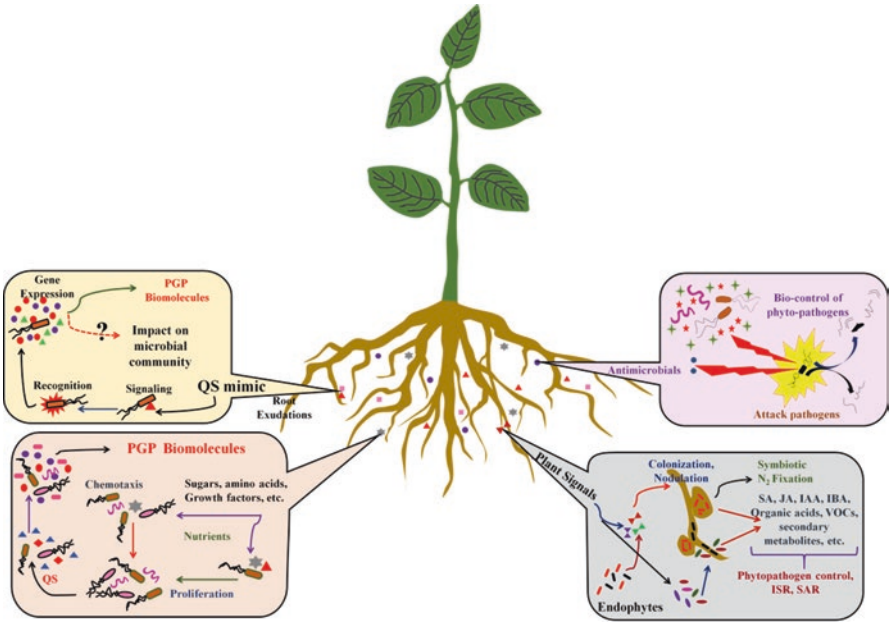


Fig. 11.1 An overview of signaling cascades in the rhizosphere. The plant root exudations elicit different amendments in the cumulative expression of various traits in the responding fraction of the microbial community. In addition to the induction of ISR and IST, the combined action of root exudations and microbial metabolic products may effectively manage the pathogenic microbes

cellulose, and phenolic compounds at the site potentially attacked by pathogens (Benhamou et al. 1998, 2000). Endophytes are also important sources of antibiotics and antifungal agents that inhibit and kill diverse types of pathogenic microbes. *Cryptosporiopsis* cf. *quercine*, an endophyte isolated from *Tripterygium wilfordii* produced antifungal agent cryptoc and in that exhibited inhibitory action against different pathogenic fungal endophytes like *Sclerotinia sclerotiorum*, *Botrytis cinerea* (Strobel et al. 1999). *Muscodor albus*, a fungal endophyte of *Cinnamomum zeylanicum*, produces volatile compounds that inhibit and kill broad range of plant pathogenic bacteria and fungi (Strobel et al. 2001). Rhizobia produce nod factors which serve as chemical signals during nodulation; the nodules act as symbiotic organs on the host plant roots (Fisher and Long 1992). Plant roots exudate flavonoids and strigolactones which play a key role of signaling in plant–microbe interactions, mycorrhizal formation, and establishment of legume–rhizobia symbiosis (Steinkellner et al. 2007). Flavonoids are the predominant controlling molecules in the nod gene expression in rhizobia and legume-rhizobia symbiosis (Kondorsi and Schultze 1998). Highly specific classes of flavonoids have been also shown to enhance rhizobial chemotaxis, bacterial growth, and endophytic association of *Azospirillum brasilense, Serratia* spp., and *Azorhizobium caulinodans* in wheat and rice (Bais et al. 2006; Balachandar et al. 2006; Webster et al. 1998). However, the information related to the majority of the roles contributed by flavonoids during the interaction of plants with other colonizing microbial groups is quite limited.

11.6 Siderophores

Siderophores are low-molecular-weight organic compounds of microbial origin, produced under the iron-starved conditions (Ahmed and Holmstrom 2014). These molecules can be easily detected in the late log phase of growth of siderophore-producing bacteria; however, the production initiates along with the recognition of the iron-starved environment. Siderophores are metal chelating agents that chelate the insoluble ferric iron (Fe^{+3}) from different habitats (Nagoba and Vedpathak 2011). Around 500 types of different siderophores have been recognized so far, with 270 of them characterized to the structural level (Boukhalfa et al. 2003). Microbes produce broad range of siderophores which specifically sequester Fe^{+++} from the surroundings due to high affinity. Bacterial siderophores are categorized into two major class, viz., catecholetes and hydroxymates (Matzanke 1991). Some other classes such as carboxylates and carboxymates are also known. A major fraction of plant growth-promoting microbial population from the rhizosphere and rhizoplane actively produce siderophores (Supanekar et al. 2013a, b; Supanekar and Sorty 2013; Sorty et al. 2016). Iron acquisition mechanism of the majority of soil microbes, including less dominant factions of the community, mainly comprises of siderophore production. The siderophore production by PGPB causes reduction in the available ferric iron content in the rhizosphere, thereby inhibiting the growth of other microorganisms, including phytopathogens in vicinity of the roots, and also decreases nutrient competition between the microbial communities (Jing et al.

2007). The siderophore-mediated biocontrol of phytopathogens also probably induces the host resistance mechanism (Meziane et al. 2005). Recent studies have focused on the role of siderophores in plant–pathogen interactions; siderophores can also trigger the ISR in plants (Aznar and Dellagi 2015). For instance, micromolar concentrations of siderophore pseudobactin when applied to roots successfully induced ISR in *Arabidopsis* (Meziane et al. 2005). The pyoverdine, a siderophore secreted by *Pseudomonas aeruginosa*, regulates the production of virulence factors like exotoxin A, an endoprotease, and pyoverdine itself. These factors are categorized among the major determinants of pathogenicity (Lamont et al. 2002). Quorum-sensing signals, viz., 2-heptyl-3-hydroxy-4 (1H)-quinolone (PQS), 2-heptyl-4-quinolone (HHQ), have been shown to facilitate the siderophore-mediated iron uptake in *Pseudomonas aeruginosa* (Diggle et al. 2007). This also necessitates the requisite of selective inducers for smooth operation of siderophore-mediated iron uptake systems in microbes.

The soil harbors surplus quantity of iron than is needful for the plants; however, the biological availability of the same has been the area of concern as plants are unable to intake the native form of iron present in the soil. Plants use different mechanisms to fulfill the iron requirement, including secretion of phenolic compounds and acids, and some monocots also synthesize phyto-siderophores and corresponding transporters (Morrissey and Guerinot 2009). These approaches, most of the time, are inadequate to fulfill the plant's iron requirement. The plant-associated microbes have been shown to contribute significant role in the supplement of iron under starvation conditions (Masalha et al. 2000); however, the operative mechanisms are yet unknown. It is thus clear that microbial activity in the soil devotes indispensable contribution to favor the iron uptake in plants. Plants also manipulate the associative microbial community depending upon their iron-nutritional status; this is mainly achieved by altering the root exudations (Yang and Crowley 2000). Another significance of siderophore production in the rhizosphere region is the enhancement of microbial colonization, which otherwise become difficult under iron-starved conditions where the surface hydrophobicity of the microbial cells decreases significantly due to insufficient iron which ultimately inhibits the development of biofilm (Simoes et al. 2007). Both the production of siderophores and binding with Fe^{+3} are highly pH-dependent processes (Supanekar and Sorty 2013; Supanekar et al. 2013a, b). The soil chemistry therefore acts as an important determinant of siderophore-mediated iron uptake.

11.7 Microbial Volatile Organic Compounds (VOCs)

The VOCs emitted by microbes contribute important role in positive plant–microbe interactions. Bacterial VOCs can bring significant improvement in ISR and plant growth promotion without physical contact. Bacterial volatiles belong to various chemical classes, including alkenes, alcohols, ketones, benzenoids, terpenes, esters, acids, pyrazines, etc.; the composition of bacterial volatiles may vary with cultivation conditions and the substrate composition of the medium (Cleason 2006; Blom

et al. 2011; Groenhagen et al. 2013; Garbeva et al. 2014). The characteristic mobility of VOCs through air spaces and fluids enables them to act as ideal mediators in both short- and long-distance intra- and intercellular signaling and the signaling between host and colonizing microbes as well (Effmert et al. 2012). Bacterial emission of volatiles may help plants to regulate defense signaling pathways, with SA, JA, and ET signaling pathways, thus protecting plants from various types of pathogens. Ethylene, the first gaseous hormone revealed in nature, serves as defense response activator in plants (Bleeker and Kende 2000). The strains of plant growth-promoting rhizobacteria enhanced the growth of *Arabidopsis thaliana* seedlings by releasing a blend of volatile chemicals (Ryu et al. 2003). The PGPR strains also found to elicit ISR via chemical volatile emissions under in vitro conditions (Kai et al. 2009). The VOCs have been also shown to alter the plant physiology and chemical composition of the root exudates (Goh et al. 2013). Plants' biological processes like root branching, auxin distribution, leaf cell expansion, and photosynthesis were found triggered by volatiles emitted by *B. subtilis* GB0 (Zhang et al. 2007, 2008). Rhizospheric bacterial strains modulate the plant growth and root system architecture by VOC emission (Gutierrez-Luna et al. 2010). The current knowledge regarding VOC-mediated communication between the plant and microbes is still in infancy; however, the number of active VOC reports has been increasing with number of microbial strains (Ryu et al. 2003, 2004; Blom et al. 2011). Very few bacterial VOCs have been identified for their accountability toward the positive and negative impacts on plants, owing to the complexity involved in their route of action. So far over 300 candidate bacterial VOCs have been structurally identified (Schulz and Dickschat 2007). The VOCs also enhance the growth and development of plants; for instance, *A. thaliana* grown in the presence of VOCs emitted by *Trichoderma* effectively increases plant size, biomass, and chlorophyll content (Lee et al. 2016).

11.8 Phytohormones

Phytohormones play an important role as growth regulators in the development of a plant. The production of phytohormones by many soil- and plant-associated microbes has been well documented. *Rhizobium* sp., *Azotobacter* sp., *Acetobacter* sp., and *Herbaspirillum* sp. produce microbial derivatives of phytohormones like auxins, GA, and cytokinins (Atzorn et al. 1988; Salmeron et al. 1990; Bastian et al. 1998). The production of auxins and biological nitrogen fixation are the major required traits for PGP microbes. These further trigger the development of root system, thereby helping the plant for increased uptake of water and nutrients (Aloni et al. 2006; Hernandez et al. 2009). Most of the auxin-producing bacteria produce IAA (Arkhipova et al. 2005; Joo et al. 2004). ACC deaminase-producing bacteria degrade 1-aminocyclopropane-1-carboyclic acid (ACC) which is a precursor of ethylene in plants. Thus, by lowering the endogenous levels of ACC, these bacteria involve in the enhancement of the root growth (Glick 2005). The ethylene is recognized as a stress hormone; however, the ACC deaminase production property of the

bacteria results in a significant decrease in the ethylene levels in plants, thereby mitigating the abiotic and biotic stress (Saleem et al. 2007). Bacterial endophytes with highly induced ACC deaminase activity can be excellent plant growth promoters, as they alleviate the abiotic stresses by efficiently blocking synthesis of ethylene (Cheng et al. 2007). Rhizobia can produce auxins (Bianco and Defez 2010) and cytokinins (Phillips and Torrey 1972); it was suggested that these two hormones are involved in the initiation of nodule formation and in bacterial symbiotic signals such as nitrogen fixation. IAA is a natural auxin which also acts as a signal molecule in microorganisms. It also has the ability to influence the gene expression in microbes; thus IAA plays the role of reciprocal signal molecule in plant–microbe interaction (Spaepen and Vanderleyden 2011) (Fig. 11.1). IAA also contributes integral role in the plants' adaptation to salinity stress (Fahad et al. 2015). GAs also act as signal molecules in the host plant; for instance, *Azospirillum and Bacillus* sp. produce GAs which trigger the growth and development in plants (Bottini et al. 2004; Gutiérrez-Manero et al. 2001).

11.9 Inter-microbial Signaling: Quorum Sensing

Though the bacteria are unicellular organisms, they have an inherent ability to coordinate activities and work in groups for survival in the complex environment. Intracellular communication between the bacteria gives the positive outcomes (Lazdunski et al. 2004). Quorum sensing is based on the production and secretion of signal molecules called autoinducers. These are low-molecular-weight signal molecules, which regulate a number of microbial processes at the gene-expression level. Quorum sensing in bacteria also plays an important role in growth promotion and the development of the plants (Persello-Cartieaux et al. 2003). Expression of phenotypic characters in Gram-negative bacteria such as biofilm formation, production of virulence factor, exoenzymes, bioluminescence, and antibiotic production is often regulated by cell density-dependent intercellular communication-QS networks (Waters and Bassler 2005; Ng and Bassler 2009). Gram-negative bacteria secrete autoinducers, which are diffusible signal molecules like N-acyl-homoserine lactone (AHL) which tightly regulates the response to cell density; the regulatory system is typically dependent on two proteins: AHL synthase, member of *Lux*l family proteins, and AHL receptor protein, member of *Lux*R family proteins (Eberl 1999). Some Gram-negative bacteria like *Stenotrophomonas maltophilia* and *Burkholderia* spp. synthesize another QS signal DSF (diffusible signal factor). The DSF acts as inter-kingdom signal which induces innate immune response in plants (Kakkar et al. 2015). The Gram-positive bacterial inhabitants in rhizosphere use peptides as QS signaling molecules (Bassler 2002; Monnet et al. 2014). Another type of quorum sensing is autoinducer 2 (AI-2), described in both Gram-positive and Gram-negative bacteria (Miller and Bassler 2001; Waters and Bassler 2005; Federle and Bassler 2003), and QS system specific for intraspecies signaling, thus also known as cross-species communication (Federle and Bassler 2003). In bean and tomato plants, *Serratia plymuthica* HROC48, producing OHC4-/OHC6- and

C4-/C6- homoserine lactones found to induce systemic protection against fungal leaf pathogen *Botrytis cinerea* (Liu et al. 2007; Pang et al. 2009).

Diverse bacterial species can produce the same AHL or AHL with similar structures. Acyl chain carries 4–14 carbon atoms which may also contain double bonds; it is evident that quorum sensing via AHL is most common in plant-associated bacterial communities than bacterial population from bulk soil (Elasri et al. 2001; d'Angelo-Picard et al. 2005). QS plays major roles in one or more stages of symbiosis; the cell density of *Rhizobium* sp. reaches threshold level around the roots before the onset of nodulation (Caetano-Anolles and Gresshoff 1991). The activity of nitrogen-cycling enzymes in rhizosphere is accompanied by bacterial QS behavior, suggesting that AHL signaling is a central point in nitrogen mineralization (DeAngelis et al. 2008; Lamers et al. 2012). Transgenic tomato plants *Las I* (long-chain AHL producers) and *Yen I* (short-chain AHL producers) alter the QS in PGPR *Burkholderia graminis* strains, to promote plant growth and salt stress tolerance (Barriuso et al. 2008). Many plants produce AHL mimic substances to induce the quorum-sensing signaling in plant-associated bacteria (Fig. 11.1). *Medicago truncatula*-produced AHL mimic to stimulate or inhibit the responses in QS of the correspondent bacteria; the QS mimic activity was found in both extracts of seedlings and exudates as well (Gao et al. 2003). Plant root exudate rosmarinic acid, a homoserine lactone (HSL) which mimics QS signals in *Pseudomonas* sp., thereby inducing premature quorum sensing (Corral-Lugo et al. 2016). Engineering beneficial plant-associated microbes and GM plants for the ability to produce beneficial enzymes to quench the bacterial signals may permit the construction of microbial communities directed against phytopathogen (Dong et al. 2001; Ryan et al. 2009). The QS NAHL is essential for the life cycle of plant pathogen P*ectobacterium*, some microorganisms interfere with this QS signal molecule, and researcher have worked on this concept by engineering plants to degrade NAHL signals, i.e., the *Solanum tuberosum* plants were transformed with bacterial lactonase gene *aiiA* from *Bacillus* sp., which enabled the degradation of NAHL molecules (Dong et al. 2000, 2001).

11.10 Conclusions and Recommendations

Over the past few decades, researchers have made significant progress regarding the information about microbial and plant signaling at molecular level and their involvement in plant–microbe signaling cascades. Though the involvement of plant root exudations like phenolic compounds, phytohormones, and organic acid indifferent signaling pathways and root colonization has been understood to significant extent, the major fraction of plant and microbial VOCs, hormones, and other secondary metabolites governing different signaling cascades and plant development are still awaiting the keen characterization. There is a significant gap in the area of triangular interactions between PGP microbes, plants, and plant pathogenic microbes which need to be probed deliberately so as to reveal their importance in agricultural and ecological aspects. The high-throughput, omic-based characterization and

interpretation of such molecular interactions represent challenge of the hour; moreover, it may also overcome the limitations concerning the inclusion of non-culturable microbes in this regard.

Acknowledgments The authors gratefully acknowledge the financial assistance from Indian Council of Agricultural Research (ICAR), Govt. of India, under Application of Microorganisms in Agriculture and Allied Sectors (AMAAS).

References

Ahmad JS, Baker R (1987) Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182–189

Ahmed E, Holmstrom SJM (2014) Siderophores in environmental research: roles and applications. Microb Biotechnol 7:196–208

Aloni R, Aloni E, Langhans M et al (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Arkhipova TN, Veselov SU, Melantiev AI et al (2005) Ability of bacterium *Bacillus* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 272:201–209

Atkinson S, Williams P (2009) Quorum sensing and social networking in the microbial world. J R Soc Interface 6:959–978

Atzorn R, Crozier A, Wheeler CT et al (1988) Production of gibberellins and indole-3-acetic acid by Rhizobium phaseoli in relation to nodulation of Phaseolus vulgaris roots. Planta 175:532–538

Audenaert K, Pattery T, Cornelis P et al (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. Mol Plant-Microbe Interact 15:1147–1156

Aznar A, Dellagi A (2015) New insights into the role of siderophores as triggers of plant immunity: what can we learn from animals? J Exp Bot 66:3001–3010. doi:10.1093/jxb/erv155

Bais HP, Weir TL, Perry LG (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. doi:10.1146/annurev.arplant.57.032905.105159

Balachandar D, Sandhiya GS, Sugitha TCK et al (2006) Flavonoids and growth hormones influence endophytic colonization and in planta nitrogen fixation by a diazotrophic *Serratia* sp in rice. World J Microbiol Biotechnol 22:707–712

Barriuso J, Solano BR, Fray RG et al (2008) Transgenic tomato plants alter quorum sensing in plant growth-promoting rhizobacteria. Plant Biotechnol J 6:442–452. doi:10.1111/j.1467-7652.2008.00331.x

Bassler BL (2002) Small talk. Cell-to-cell communication in bacteria. Cell 109:421–424. 15

Bastian F, Cohen A, Piccoli P, Luna V et al (1998) Production of indole 3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. Plant Growth Regul 24:7–11

Benhamou N, Kloepper JW, Tuzun S et al (1998) Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. Planta 204:153–168

Benhamou N, Gagné S, Quéré DL et al (2000) Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum*. Biochem Cell Biol 90:45–56

Berendsen RL, Pieterse CMJ, Bakker PAHM et al (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. doi:10.1016/j.tplants.2012.04.001

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Bianco C, Defez R (2010) Improvement of phosphate solubilization and *Medicago* plant yield by an indole-3-acetic acid-overproducing strain of *Sinorhizobium meliloti*. Appl Environ Microbiol 76:4626–4632. doi:10.1128/AEM.02756-09

Bleeker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plant. Annu Rev Cell Dev Biol 16:1–18

Blom D, Fabbri C, Connor EC et al (2011) Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environ Microbiol 13:3047–3058. PMID:21933319; http://dx.doi.org/10.1111/j.1462-2920.2011.02582.x

Bonfante P, Genre A (2015) Arbuscular mycorrhizal dialogues: do you speak 'plantish' or 'fungish'? Trends Plant Sci 20:150–154. doi:10.1016/j.tplants.2014.12.002

Bottini R, Cassán F, Piccoli P et al (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503

Boukhalfa H, Lack J, Reilly SD, Hersman L, Neu MP et al (2003) Siderophore production and facilitated uptake of iron and plutonium in P. putida. AIP Conf Proc 673:343–344

Bressan M, Roncato MA, Bellvert F et al (2009) Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. ISME J 3:1243–1257. doi:10.1038/ismej.2009.68

Caetano-Anolles G, Gresshoff PM (1991) Plant genetic control of nodulation. Annu Rev Microbiol 45:345–382

Cheng Z, Park E, Glick BRet al. (2007) 1-aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918

Cleason A (2006) Volatile organic compounds from microorganisms. Ph.D. thesis, Umeå University, Umeå

Corral-Lugo A, Daddaoua A, Ortega A et al (2016) Rosmarinic acid is a homo-serine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator. Sci Signal 409(9):ra1

d'Angelo-Picard C, Faure D, Penot I et al (2005) Diversity of N-acyl homoserine lactone-producing and -degrading bacteria in soil and tobacco rhizosphere. Environ Microbiol 7:1796–1808. doi:10.1111/j.1462- 2920.2005.00886.x

De Meyer G, Capieau K, Audenaert K et al (1999) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa*7NSK2 activate the systemic acquired resistance pathway in bean. Mol Plant-Microbe Interact 12:450–458

De Vleesschauwer D, Hofte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51:223–281

De Vleesschauwer D, Höfte M, Loon LCV et al (2009) Rhizobacteria induced systemic resistance. In: Van Loon LC (ed) Advances in botanical research. Academic, New York, pp 223–281

DeAngelis KM, Lindow SE, Firestone MK et al (2008) Bacterial quorum sensing and nitrogen cycling in rhizosphere soil. FEMS Microbiol Ecol 66:197–207. doi:10.1111/j.1574-6941.2008.00550.x

Diggle SP, Matthijs S, Wright VJ et al (2007) The *Pseudomonas aeruginosa* 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. Chem Biol 14:87–96. doi:10.1016/j.chembiol.2006.11.014

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097. doi:10.1105/tpc.7.7.1085

Dong YH, Xu JL, Li XZ et al (2000) AiiA, an enzyme that inactivates the acyl homoserine lactone quorum sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci U S A 97:3526–3353

Dong YH, Wang LH, Xu JL et al (2001) Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 411:813–817. doi:10.1038/35081101

Eberl L (1999) N-acyl-homoserine lactone mediated gene regulation in gram-negative bacteria. Syst Appl Microbiol 22:493–506. doi:10.1016/S0723-2020(99)80001-0

Effmert U, Kalderás J, Warnke R et al (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703

Elasri M, Delorme S, Lemanceau P et al (2001) Acyl homoserine lactone production is more common among plant-associated *Pseudomonas spp*. than among soil borne Pseudomonas spp. Appl Environ Microbiol 67:1198–1209. doi:10.1128/AEM.67.3.1198-1209.2001

Fahad S, Hussain S, Bano S et al (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res 22:4907–4921

Federle MJ, Bassler BL (2003) Interspecies communication in bacteria. J Clin Invest 112:1291–1299

Fisher RF, Long SR (1992) Rhizobium plant signal exchange. Nature 357:655–660

Foo E, Davies NW (2011) Strigolactones promote nodulation in pea. Planta 234:1073–1081

Gao M, Teplitski M, Robinson JB et al (2003) Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. Mol Plant-Microbe Interact 16:827–834. doi:10.1094/MPMI.2003.16.9.827

Garbeva P, Hordijk C, Gerards S et al (2014) Volatile-mediated interactions between phylogenetically different soil bacteria. Front Microbiol 5:289. doi:10.3389/fmicb.2014.00289

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 252:1–7

Goh C-H, Veliz Vallejos DF, Nicotra AB, Mathesius U (2013) The impact of beneficial plant-associated microbes on plant phenotypic plasticity. J Chem Ecol 39(7):826–839

Groenhagen U, Baumgartner R, Bailly A et al (2013) Production of bioactive volatiles by different *Burkholderia ambifaria* strains. J Chem Ecol 39:892–906. doi:10.1007/s10886-013-0315-y

Grover A, Mittal D, Negi M, Lavania D et al (2013) Generating high temperature tolerant transgenic plants: achievements and challenges. Plant Sci 20:38–47. doi:10.1016/j.plantsci.2013.01.005

Gutierrez-Luna FM, Lopez-Bucio J, tamirano-Hernandez J et al (2010) Plant growth-promoting rhizobacteria modulate root-system architecture in *Arabidopsis thaliana* through volatile organic compound emission. Symbiosis 51:75–83. doi:10.1007/s13199-010-0066-2

Gutiérrez-Manero FJ, Ramos B, Probanza A et al (2001) The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plant 111:206–211

Guttman DS, McHardy AC, Schulze-Lefert P et al (2014) Microbial genome-enabled insights into plant-microorganism interactions. Nat Rev Genet 15:797–813. doi:10.1038/nrg3748

Hassan S, Mathesius U (2012) The role of flavonoids in root–rhizosphere signalling: opportunities and challenges for improving plant – microbe interactions. J Exp Bot 63:3429–3444. doi:10.1093/jxb/err430

Hernandez JP, de-Bashana LE, Rodriguez DJ et al (2009) Growth promotion of the freshwater microalga *Chlorella vulgaris* by the nitrogen-fixing, plant growth-promoting bacterium *Bacillus pumilus* from arid zone soils. Eur J Soil Biol 45:88–93

Jacometti MA, Wratten SD, Walter M et al (2010) Review: alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. Aust J Grape Wine Res 16:154–172

Jing YD, He ZL, Yang XE et al (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ (Sci) 8(3):192–207

Joo GJ, Kim YM, Lee KIJ et al (2004) Growth promotion of red pepper seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus mycoides*, *Bacillus pumilus*. Biotechnol Lett 26:487–491

Kai M, Haustein M, Molina F et al (2009) Bacterial volatiles and their action potential. Appl Microbiol Biotechnol 81:1001–1012

Kakkar A, Nizampatnam NR, Kondreddy A et al (2015) *Xanthomonas campestris* cell–cell signalling molecule DSF (diffusible signal factor) elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan. J Exp Bot 66:6697–6714

Kondorsi A, Schultze M (1998) Regulation of symbiotic root nodule development. Annu Rev Genet 32:33–57

Lamers LP, van Diggelen JM, den Camp HJ et al (2012) Microbial transformations of nitrogen, sulfur, and iron dictate vegetation composition in wetlands: a review. Front Microbiol 3:156. doi:10.3389/fmicb.2012.00156

Lamont IL, Beare PA, Ochsner U et al (2002) Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. PNAS 99:7070–7077. doi:pnas.orgcgidoi 10.1073pnas.092016999

Lazdunski AM, Ventre I, Sturgis JN et al (2004) Regulatory circuits and communication in Gram negative bacteria. Nat Rev Microbiol 2:581–592

Lee B, Farag MA, Park HB et al (2012) Induced resistance by a long chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. PLoS One 7:e48744

Lee s, Yap M, Behringer G, Hung R et al (2016) Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. Fungal Biol Biotechnol 3:7. doi:10.1186/s40694-016-0025-7

Liu X, Bimerew M, Ma Y et al (2007) Quorum-sensing signaling is required for production of the antibiotic pyrrolnitrin in a rhizospheric biocontrol strain of *Serratia plymuthica*. FEMS Microbiol Lett 270:299–305. doi:10.1111/j.1574-6968.2007.00681.x

Mandal SM, Chakraborty D, Dey S et al (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5(4):359–368

Masalha J, Kosegarten H, Elmaci Ö et al (2000) The central role of microbial activity for iron acquisition in maize and sunflower. Biol Fertil Soils 30:433–439

Matzanke BF (1991) Structures, coordination chemistry and functions of microbial iron chelates. In: Winkelmann G (ed) CRC handbook of microbial iron chelates. CRC Press, Boca Raton, pp 15–64

Meena KK, Sorty AM, Bitla UM et al (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:868. doi:10.3389/fpls.2017.00172

Meziane H, Van Der Sluis I, Van Loon LC et al (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol Plant Pathol 6:177–185

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Mimmo T, Hann S, Jaitz L et al (2011) Time and substrate dependent exudation of carboxylates by *Lupinus albus L.* and *Brassica napus L.* Plant Physiol Biochem 49:1272–1278. doi:10.1016/j.plaphy.2011.08.012

Monnet V, Juillard V, Gardan R et al (2014) Peptide conversations in gram-positive bacteria. Crit Rev Microbiol 42:339–351. doi:http://dx.doi.org/10.3109/1040841X.2014.948804

Moore BD, Andrew RL, Kühlheim C et al (2014) Explaining intra specific diversity in plant secondary metabolites in an ecological context. New Phytol 201(733):750. doi:10.1111/nph.12526

Morrissey JP, Walsh UF, O'Donnell A, Moenne-Loccoz Y, O'Gara F et al (2002) Exploitation of genetically modified inoculants for industrial ecology applications. Antonie Van Leeuwenhoek 81:599–606

Morrissey JP, Dow JM, Mark GL et al (2004) Are microbes at the root of a solution to world food production? EMBO Rep 5:922–926

Morrissey J, Guerinot ML (2009) Iron uptake and transport in plants: the good, the bad, and the ionome. Chem Rev 109(10):4553–4567. doi:10.1021/cr900112r

Nagoba B, Vedpathak D (2011) Medical applications of siderophores. Eur J Gen Med 8:229–235

Ng WL, Bassler BL (2009) Bacterial quorum-sensing network architectures. Annu Rev Genet 43:197–222. doi:10.1146/annurev-genet-102108-134304

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J et al (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4:701–712

Pang Y, Liu X, Ma Y et al (2009) Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of *Serratia plymuthica* are dependent on N-acyl homoserine lactones. Eur J Plant Pathol 124:261–268. doi:10.1007/s10658- 008-9411-1

Persello-Cartieaux F, Nussaume L, Robaglia C et al (2003) Tales from the underground: molecular. Plant Cell Environ 26:189–199

Phillips DA, Torrey JG (1972) Studies on cytokinin production by *Rhizobium*. Plant Physiol 49:11–15. doi:10.1104/pp.49.1.11

Pieterse CMJ, Van Pelt JA, Ton J et al (2000) Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Physiol Mol Plant Pathol 57:123–134

Pieterse CM, Van Der Does D, Zamioudis C et al (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521. doi:10.1146/annurevcellbio-092910-154055

Postma J, Montanari M, van den Boogert PHJF et al (2003) Microbial enrichment to enhance the disease suppressive activity of compost. Eur J Soil Biol 39:157–163

Robert-Seilaniantz A, Grant M, Jones JDG et al (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49:317–343. doi:10.1146/annurevphyto-073009-114447

Ryan RP, Germaine K, Franks A et al (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol 278:1–9. doi:10.1111/j.1574-6968.2007.00918

Ryan PR, Dessaux Y, Thomashow LS et al (2009) Rhizosphere engineering and management for sustainable agriculture. Plant Soil 321:363–383. doi:10.1007/s11104-009-0001-6

Ryu CM, Farag MA, Hu CH et al (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci U S A 100:4927–4932

Ryu CM, Farag MA, Hu CH et al (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol 134:1017–1026. http://dx.doi.org/10.1104/pp.103.026583

Saleem M, Arshad M, Hussain S et al (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Salmeron V, Martinez-Toledo MV, Gonzalez-Lopez J et al (1990) Nitrogen fixation and production of auxins gibberellins and cytokinins by an *Azotobacter chroococcum* strain isolated from the root of *Zea mays* in the presence of insoluble phosphate. Chemosphere 20:417–422

Schulz S, Dickschat JS (2007) Bacterial volatiles: the smell of small organisms. Nat Prod Rep 24:814–842. PMID: 17653361; http://dx.doi.org/10.1039/b507392h

Shah J, Zeier J (2013) Long-distance communication and signal amplification in systemic acquired resistance. Front Plant Sci 4:30. doi:10.3389/fpls.2013.00030

Simões M, Simões LC, Cleto S, Machado I, Pereira MO, Vieira MJ (2007) Antimicrobial mechanisms ofortho - phthalaldehyde action. J Basic Microbiol 47(3):230–242

Singh UB, Malviya D, Wasiullah et al (2016) Bio-protective microbial agents from rhizosphere eco-systems trigger plant defense responses provide protection against sheath blight disease in rice (*Oryza sativa* L.) Microbiol Res 192:300–312

Sorty AM, Meena KK, Choudhary K, Bitla UM, Minhas PS, Krishnani KK (2016) Effect of plant growth promoting bacteria associated with halophytic weed (*Psoralea corylifolia* l) on germination and seedling growth of wheat under saline conditions. Appl Biochem Biotechnol 180(5):872–882

Soto MJ, Fernandez-Aparicio MN, Castellanos-Morales V et al (2010) First indications for the involvement of strigolactones on nodule formation in alfalfa (*Medicago sativa*). Soil Biol Biochem 42:383–385

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:a001438. doi:10.1101/cshperspect.a001438

Steinkellner S, Lendzemo V, Langer I et al (2007) Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant–fungus interactions. Molecules 12:1290–1306. doi:10.3390/12071290

Strobel GA, Miller RV, Miller C et al (1999) Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina*. Microbiology 145:1919–1926

Strobel GA, Dirksie E, Sears J, Markworth C et al (2001) Volatile antimicrobials from a novel endophytic fungus. Microbiology 147:2943–2950

Subramanian S, Smith DL (2015) Bacteriocins from the rhizosphere microbiome – from an agriculture perspective front. Plant Sci 6:909. doi:10.3389/fpls.2015.00909

Supanekar SV, Sorty AM (2013) Siderophoregenic Klebsiella pneumoniae SUP II from wheat (Triticum aestivum) rhizoplane. PARIPEX-Indian J Res 7:243–245

Supanekar S, Sorty A, Raut A (2013a) Study of catethol siderophore from a newly isolated Azotobacter sp. for its antimicrobial property. J Microbiol Biotechnol Food Sci 3:270–273

Supanekar SV, Sorty AM, Raut AA (2013b) Catechol siderophore produced by Klebsiella pneumoniae isolated from rhizosphere of *Saccharum Officinarum* L. Int J Sci Res 5:423–425

Timmusk S, Wagner EG (1999) The plant growth promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant Microbe Interact 12:951–959

Tjamos SE, Flemetakis E, Paplomatas EJ et al (2005) Induction of resistance to *Verticillium dahlia* in *Arabidopsis thaliana* by the biocontrol agent K-165 and pathogenesis-related proteins gene expression. Mol Plant-Microbe Interact 18:555–561

Van de Mortel JE, De Vos RCH, Dekkers E et al (2012) Metabolic and transcriptomic changes induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101. Plant Physiol 160:2173–2188

Van Loon LC, Bakker PAHM, Pieterse CMJ et al (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Walsh UF, Morrissey JP, O'Gara F et al (2001) *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Curr Opin Biotechnol 12:289–295

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Webster G, Jain V, Davey MR et al (1998) The flavonoid naringenin stimulates the intercellular colonization of wheat roots by *Azorhizobium caulinodans*. Plant Cell Environ 21:373–383

Welbaum G, Sturz AV, Dong Z, Nowak J et al (2004) Fertilizing soil microorganisms to improve productivity of agroecosystems. Crit Rev Plant Sci 23:175–193

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Yang C-H, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl Environ Microbiol 66(1):345–351

Yasuda M, Isawa T, Shinozaki S et al (2009) Effects of colonization of a bacterial endophyte, *Azospirillum sp.* B510, on disease resistance in rice. Biosci Biotechnol Biochem 73:2595–2599

Zamioudis C, Pieterse CM (2012) Modulation of host immunity by beneficial microbes. Mol Plant-Microbe Interact 25:139–150. doi:10.1094/MPMI-06-11- 0179

Zhang H, Kim MS, Krishnamachari V et al (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. Planta 226:839–851

Zhang H, Kim MS, Sun Y et al (2008) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mol Plant Microbe 21:737–744

Endophytic and Epiphytic Modes of Microbial Interactions and Benefits

12

Jay Kumar, Divya Singh, Paushali Ghosh, and Ashok Kumar

Abstract

Plants and microbes are the important components of ecosystem, and their interactions help in regulating the biogeochemical cycle in the environment. Plant-associated microorganisms include bacteria, fungi, viruses, and some algae. They may be endophytic and/or epiphytic depending upon their location on the host plants. These microbes use host plants for their growth, colonization, and proliferation; however, they offer a variety of benefits to the hosts. Colonization of microorganisms on host plants takes place through air, water, and insects, or they may also be present in germinating plant parts. Endophytic microbial interactions influence the internal part, while epiphytic microbial interactions influence the exterior surface of the plants. These microbes are not harmful to the plants; however, they secrete some beneficial substances which may help in plant growth promotion, resistance to pathogenic microbes, removal of harmful contaminants, and production of secondary metabolites. In such a way, microbes contribute in agricultural crop improvement, food safety, and industries. This chapter briefly deals with the ecology, interactions, and benefits of plant-microbe interaction, especially in the area of sustainable agriculture and crop improvement.

Keywords

Endophytes • Epiphytes • Bacteria • Colonization • Plants • Crop improvement

J. Kumar • D. Singh • P. Ghosh • A. Kumar (✉)
School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi 221 005, India
e-mail: kasokbt@rediffmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_12

12.1 Introduction

Microorganisms are found ubiquitously in the entire biosphere. They interact with their environmental factors and play a primary role in regulating the cycling of nutrients. Microbes also have an important role in nitrogen fixation, phosphate solubilization, carbon, methane, and sulfur metabolism, and control biogeochemical cycling (Delong 2009). Plants are one of the important habitats and support the growth of a wide variety of microorganisms. Microbes associated with plants have a strapping influence on growth and development of plants. They harbor microorganisms on their aerial parts as well as inside the tissues including vascular network and root tissues. Plant and microbial activities considerably modify the local environment and supply certain plentiful nutrients in comparison with the nutrient-limited terrestrial ecosystem (Walker et al. 2003). Plants are populated by microorganisms both below and above the ground. They interact with plants since plants offer a wide variety of habitats including phyllosphere (aerial part of the plant), rhizosphere (zone of interaction of root and soil), and the endosphere (internal parts of the plants) (Lindow et al. 2002; Lynch 1990).

Microorganisms colonizing on the aerial parts of the plants are generally called as epiphytic, and those which are found on the zone of influence of root system and soil constitute a group called rhizospheric, whereas microbes present inside the internal tissue including aerial and root tissue are considered as endophytes. Some of them are mutualistic symbionts with beneficial effects on their host such as enhanced growth, disease resistance, and tolerance to environmental stresses and are being used as microbial inoculants. Epiphytic microorganisms live on the surface of different plant parts such as leaves, roots, flowers, buds, seeds, and fruits. The term "phyllosphere" is used in microbiology to represent the total aboveground portions of plants as habitat for microorganisms (Newton et al. 2010).

Several researchers have documented that epiphytes and endophytes basically do not have any harmful effects on the plant; instead they induce the formation of some important chemicals such as hormones. Some microorganisms produce auxin which promotes the growth and plays an important role in the life cycle of plants (Fernandes et al. 2011). Interactive association of microbes with host plant may be harmful or beneficial for either the microbe or the plant depending upon various types of association classified as commensalism, mutualism, amensalism, competition, neutralism, synergism, or parasitism. Some of the microbes associated with plants may also simply grow saprophytically on the nutrients released by the host plant.

Each plant may be occupied by different types of microbes including bacteria, fungi, yeasts, archaea, and viruses (Preece and Dickinson 1971). Colonization of microbes on plants is not only limited to terrestrial plants, aquatic plants also have equal opportunity (Goulder and Baker 1991). Various parts of plant and tissues represent a variety of microenvironments for the colonization of microbes which influence the microbial diversity, density, population size, species organization, and activity of adherent microbes at different sites on the plants. Microenvironment is a very small and defined area in any habitat, which is separated from its nearby surroundings by different environmental factors such as the amount of sunlight

exposure, moisture content, and the range of temperatures where organisms occupy space for growth and reproduction. Therefore, plant parts colonized by microbial communities with a characteristic phylogenetic structure represent different habitats (Zhang et al. 2011a). Plant-microbe interaction and secretion of different types of bioactive compounds are reflective of the heterogeneity of the microorganisms. The trichomes, stomates, and veins of leaves provide physical and chemical heterogeneity in the phyllosphere that is exploited for the benefit of microbial epiphytes. The wax cuticle that often covers the leaf surface differs on the dorsal and ventral sides of leaves, and the ventral parts of the leaves have a tendency to accumulate more bacterial biomass as compared to dorsal surface (Krimm et al. 2005).

Microorganisms associated with plants have strong effects on host physiology and performance predicting that ecology and evolution of plants and animals can be inherent only in a holobiont (host plant and the organisms that live in or on it) environment. A better understanding of plant-associated microorganisms and their prospective to confer plant beneficial effects are significant not only for exploiting their ecological role and interaction with plants but also for the biotechnological interventions. Recent studies carried out on plant growth promotion by the microbes showed the production of a vast array of secondary metabolites and other bioactive compounds. These bioactive compounds and secondary metabolites may be beneficial to both the host plant and microorganisms (Kobayashi and Palumbo 2000; Hamayun et al. 2010).

A better knowledge of endophytic and epiphytic microbial interaction with host plants may help in elucidation of their function and potential role in developing sustainable system towards crop improvement. This may increase crop yield, remove contaminants, inhibit pathogen development, and fix nitrogen or beneficial substances (Rosenblueth and Martinez-Romero 2004). This chapter aims to provide an overview of the ecology and diversity of endophytes and epiphytes along with the nature of interactions with the host plant and potential benefits of plant-microbe interactions particularly in the area of phytoremediation and sustainable agriculture.

12.2 Ecology

12.2.1 Endophytic Microbes

Plants are one of the major and important sources of microorganisms. Different parts of growing plants including leaves, stems, flowers, buds, fruits, and roots represent a specific habitat for the microorganisms. These microorganisms include bacteria, fungi, and viruses, among them bacteria are the most common microbial resident of the plant. These microorganisms are found both as endophytes (within plant tissues) and epiphytes (on the plant surface) (Inacio et al. 2002).

The word “endophyte” is derived from two Greek words “endo” means inside and “phyton” means plant. Endophytic microbes can be defined as those microorganisms that colonize the internal tissue of the plant including vascular system

without any mark of infection or harmful effect on the host plant. An endophyte is an endosymbiont which includes bacteria (Kobayashi and Palumbo 2000), fungi (Stone et al. 2000), algae (Peters 1991), and viruses that usually colonize inside plant tissues. They are ubiquitous and have been reported from almost every plant studied so far. Isolation of endophytic bacteria has been reported from both monocot and dicot plants, ranging from woody trees, such as teak and pear, to herbaceous crop plants such as mustard and maize. Studies carried out suggest that majority of these microorganisms come from the soil and the main organ where endophytic bacteria get entry into plants is the root. Many candidate genes with unknown functions have been found to be differentially expressed during plant-microbe interactions (Mehta and Rosato 2005).

There are more than 352,000 species of plants present on the Earth. Among them, each individual plant is likely to be a host to one or more endophytic microorganisms (Strobel et al. 2004). Plant endophytes may be intercellular or intracellular depending upon their location in the plant tissue, i.e., they are present inside the cells or in the intracellular space, respectively.

Colonization of endophytes is almost similar to that of plant pathogenic microbes (Hallmann et al. 1997), and plant-microbe/endophyte interactions are often considered mutualistic. The microorganisms obtain nutrients and a safe niche to reside, whereas the host benefits from bacterial activities resulting in plant growth promotion, nutrient uptake, control of plant pathogens, induction of systemic resistance, and increased stress tolerance (Sturz et al. 2000). These events are induced and/or controlled by the production of phytohormones, N_2 fixation, P solubilization, and production of antibiotics and siderophore (Mitter et al. 2013).

Distribution of microorganisms inside the plant tissue is heterogeneous, and some of them get clumped together in their secreted mucilage or tend to absorb to particles including plant cell wall components (Fisher et al. 1992). Colonization of endophytes takes place through seeds, vegetative planting materials, rhizosphere soil, and phyllosphere. The initial process of colonization of plants by endophytes can take place through stomata, lenticels, germinating radicals, and emerging lateral roots except in seed-transmitted inoculums (Huang 1986). Since microbes are present in the seeds; they survive inside the tissue after germination of seeds; colonization of such endophytic microbes takes place inside the tissue; however, exact mechanism of colonization inside the seed is not well known. Extensive cytological work is needed to elucidate the specific site of colonization inside the seed (McInroy and Kloepper 1995). Some pathogens are also found inside the seeds (Schaad et al. 1995). Several plants are propagated vegetatively such as potatoes, sugarcane, etc., and can transmit the inoculums of endophytes to the next generation and would not require the infection process. There is a group of microorganisms which are able to survive in the root exudates and compete with others in the rhizosphere. Rosenblueth and Martínez-Romero (2004) have investigated some strains that were showing equal competition for rhizospheric colonization and inside tissues as endophyte of the plant root. For colonization inside the plant, bacteria enter into the tissue through cracks formed at the junction of lateral roots or at the elongation and differentiation zone of the roots.

Endophytes are different from parasites as they do not cause any harm to the host plant. However, there are reports which suggest that endophytes can act like parasites under certain conditions and vice versa (Muller and Krauss 2005; Schulz and Boyle 2005). In these conditions, the endophytes may cause disease, and thus "true endophytes" mean those whose colonization inside the plant tissue never results in visible disease symptoms (Mostert et al. 2000). As such, host-endophyte interactions can range from being symbiotic, mutualistic, commensalistic, to parasitic. Isolation of several bacterial and fungal endophytes has been reported from different parts/tissues of plant like roots, root nodules, leaves, and flowers. These endophytes manifest significant effects on growth and development of the respective host plants. The relationship of the endophytic partners with their host plant varies from each other. A better understanding of the interaction of endophytes with host plants and their function within the hosts is essential to identify the ecological significance of endophytes.

12.2.2 Epiphytic Microbes

Surface is a major habitat for microbes, and the number is always higher than the numbers present in other components of the biosphere. The greatest impossibility is in the approximation for the terrestrial subsurface because this estimate is based on only a few parameters. However, even for the terrestrial subsurface, two independent methods suggest that the number of prokaryotes is very high which may account for about $2.5–25 \times 10^{29}$ cells (Whitman et al. 1998). The term "epiphyte" is derived from two Greek words "epi" which means upon and "phyton" means plant. Plant surfaces are one of the important surfaces for growth and survival of microbes. Beattie and Lindow (1995) have given some of the definitions which describe the epiphytic bacteria. According to them these are the bacteria capable of growing on plant surface and can be removed by washing or killed by ultraviolet radiation or chemicals used for surface disinfection.

The main source of inoculums and colonization of all the epiphytic microbes takes place on the surface of all the aboveground plant parts. Microbes may arrive to surface or depart from the plant surface by the action of rain, wind, or insects. This analysis points toward the soil and air as important sources of leaf and root microbial inoculums (Hirano et al. 1996; Lilley et al. 1997). On arrival, these microorganisms are subjected to the challenges of the unfavorable conditions of new environment including the availability of water, exposure to solar UV radiation, and limited availability of nutrient resources (Sundin and Jacobs 1999). To cope up with these variable conditions and to survive the leaf environment, microorganisms have adopted different strategies such as the production of photoprotective pigments to protect against the adverse effects of UV radiation or the secretion of polysaccharides to prevent desiccation during water deficiency (Ophir and Gutnick 1994; Yu et al. 1999). There is an opportunity for millions of bacteria and fungi to colonize leaf surface each year. Till date very little information about colonization and

persistence of nonpathogenic microbes on this widespread habitat and their interactions with pathogenic microorganisms is available.

Colonization and population of microbes on the plants depend upon the factors such as environmental conditions, location of leaves, and the chemical composition of the cuticle. It also depends on the presence of veins, stomata, and surface appendages, including trichomes and hydathodes. These factors modify nutrient availability to the microbial population (Leveau and Lindow 2001). All these microbes are dependent on nutrient material secreted by the plants for growth and development. The main source of nutrients on the leaf surface is plant photoassimilates that diffuse hydrophobically through the cuticle lining the leaf epidermal cells (Van der Wal and Leveau 2011). An attempt was made by some researchers for analysis and characterization of potential leaf surface nutrient substrates in maize, leek, bean, tobacco, and poplar under greenhouse and field conditions. Various carbohydrates, lipids, and amino acids were found to be present on plant surface in varying quantities as detected by enzymatic assays and gas/liquid chromatography (Fiala et al. 1990; Mercier and Lindow 2000). Employing environmental metabolomics approaches and quantitative nuclear magnetic resonance (NMR) and imaging high-resolution mass spectrometry (IMS) techniques, Ryffel et al. (2016) have analyzed the metabolic interplay in *Arabidopsis thaliana* leaves upon colonization of microorganisms. Data showed sucrose, fructose, and glucose as dominant carbohydrates on leaves.

There are growing evidences for interactions of epiphytic microbial residents that may affect the health of natural plant populations and improve the quality and productivity of agriculturally important crops. Phyllospheric bacteria are known to stimulate plant growth and can either suppress or stimulate the colonization and infection of tissues by plant pathogenic microorganisms (Lindow and Brandl 2003; Rasche et al. 2006). The relationship of the epiphytic microbes with the host plant varies from plant to plant. In order to analyze the ecological significance of epiphytic microbes with their host plant, a proper understanding of the interaction is desirable.

12.3 Diversity of Microbes

A large number of microbes are present on plant parts, out of which a very small population can be cultured and grown in laboratories due to lack of proper nutrient supply and growth conditions required. Above constraints cause problems in isolation and culture of epiphytes, and, therefore, it is hard to elucidate a proper diversity of microorganisms on these plants. However, to observe microbial community structure, different researchers have employed various strategies. Some have employed culture-dependent strategies which give a detail of culturable microorganisms only, and others have used culture-independent strategy which provides a better understanding of the population size and diversity of microbes present in the habitat. Culture-dependent techniques include culture of microbes in the nutrient culture medium, diversity analysis, characterization, and identification of

microorganisms. The main limitation of culture-dependent techniques is that >98% of the microorganisms in any environment observed through a microscope cannot be cultured by standard microbial culturing techniques (Hugenholtz 2002). Therefore culture-dependent methods are not suitable to analyze the whole community and diversity of microorganisms in an ecological niche.

Culture-independent techniques include direct analysis of microbial community using biomolecules such as DNA, RNA, protein, and lipids (Ikeda et al. 2010). During the last two decades, the field of microbial ecology has made tremendous progress, and a number of molecular techniques have been developed for identification, characterization, phylogenetic analysis, and functional diversity of various microbes growing in diverse habitats. These techniques include whole-genome sequencing, metagenomics, metaproteomics, proteogenomics, metatranscriptomics, and DNA-DNA reassociation. These techniques can describe structural and functional diversity of microorganisms in a better way (Rastogi and Sani 2011). As plants inhabit both endophytic and epiphytic microorganisms, study of both types of microorganisms is essential for proper understanding of diversity, functional genomics, and mechanism of plant-microbe interaction.

12.3.1 Bacteria

Bacteria are the most abundant and diverse colonizer on leaves, with culturable counts ranging between 10^2 to 10^{12} cells per gram of leaf. The terrestrial plant leaf surface is a principal microbial habitat covering approximately 10^8 km^2 with an estimated 10^{26} bacterial cells (Lindow and Brandl 2003). Culture-dependent studies of sugar beet (*Beta vulgaris*) during the growing season showed above 78 bacterial species including 37 known bacterial genera (Thompson et al. 1993). Similar studies carried out on wheat showed 88 bacterial species belonging to 37 bacterial genera (Legard et al. 1994).

Endophytic bacteria are found in leaf, stem, and root of plants. A number of researchers have studied endophytic bacteria by using different plant parts independently. Bacterial diversity analysis of culturable endophytic bacteria from common bean (*Phaseolus vulgaris*) leaves showed the number of endophytic bacteria in the range of 4.5×10^2–2.8×10^3 CFU g^{-1} of fresh tissue weight. A total of 158 different isolates were successfully cultured. These isolates belonged to the *Proteobacteria* (36.7%), *Firmicutes* (32.9%), *Actinobacteria* (29.7%), and *Bacteroidetes* (0.6%). *Bacillus*, *Delftia*, *Methylobacterium*, *Microbacterium*, *Staphylococcus*, *Paenibacillus*, and *Stenotrophomonas* were common endophytic bacterial isolates (de Oliveira Costa et al. 2012). Arau'jo et al. (2002) investigated the diversity of endophytic bacteria in branches of citrus plants. They selected both healthy plants and plants infected with *Xylella fastidiosa*, a plant pathogenic bacterium which infects all the cultivars of *Citrus sinensis* and causes citrus variegated chlorosis. Additionally, above study showed that *Alcaligenes* sp.; *Bacillus cereus*; *Bacillus pumilus*; *Enterobacter cloacae*; *Burkholderia cepacia*; *Curtobacterium flaccumfaciens*; *Methylobacterium* sp. including *M. extorquens*, *M. fujisawaense*, *M.*

radiotolerans, *M. mesophilicum*, and *M. zatmanii*; *Nocardia* sp.; *Pantoea agglomerans*; *Streptomyces* sp.; and *Xanthomonas campestris* are the major endophytic bacteria of citrus plant. Investigation of endophytic bacterial analysis performed by Loh et al. (2013) from 1055 plants samples revealed 996 endophytic bacterial strains and characterization on the basis of 16S rRNA genes showed 27 genera belonging to 6 classes of bacteria. These bacteria included species of *Acinetobacter*, *Bacillus*, *Enterobacter*, *Brevibacterium*, *Klebsiella*, *Pantoea*, *Lysinibacillus*, *Pseudomonas*, *Burkholderia*, *Cloacibacterium*, *Cronobacter*, *Enterococcus*, *Erwinia*, *Exiguobacterium*, *Escherichia*, *Jeotgalicoccus*, *Lysinibacillus*, *Micrococcus*, *Paenibacillus*, *Pasteurella*, *Pectobacterium*, *Sporosarcina*, *Staphylococcus*, *Stenotrophomonas*, *Terribacillus*, and *Vibrio*.

Leaf surface area corresponds to a broad habitat for microorganisms and represents microbial communities, a large part of which are *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* and some fungal genera (Vorholt 2012). In general, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are the major colonizer of leaf surface, of which the last often dominates the phyllosphere community. A few bacterial genera, including *Pseudomonas*, *Sphingomonas*, *Bacillus*, *Methylobacterium*, *Massilia*, *Arthrobacter*, and *Pantoea*, appear to compose the center of phyllosphere communities. Analysis based on the DNA and protein samples obtained from the leaf surfaces of field-grown soybean, clover, and *Arabidopsis thaliana* revealed the presence of bacteria such as *Sphingomonas* sp. and *Methylobacterium* sp. (both belonging to the class of *Alphaproteobacteria*) in abundance (Delmotte et al. 2009). Kumar et al. (2016) studied the bacterial flora of rice phyllosphere and reported that *Enterobacter cloacae*, *Pantoea* sp., *Microbacterium* sp., and *Agrobacterium* sp. are common epiphytic bacteria. In an another study, analysis of olive and oleander leaf showed the presence of epiphytic community comprising *Pseudomonas* (33%), *Bacillus* (22%), and *Xanthomonas* (10%) in higher density and *Acinetobacter*, *Erwinia*, *Serratia*, *Lactobacillus*, *Corynebacterium*, *Flavobacterium*, and certain unidentified nitrogen fixers in lesser number (Lavermicocca et al. 1987).

12.3.2 Fungi

Fungi are mainly found as endophytes, and these endophytes may protect against pathogens and increase drought tolerance (Arnold et al. 2003; Schweitzer et al. 2006), although some epiphytic fungi are also reported. Few members are found as endophytic as well as epiphytic according to the conditions, inoculums, source, and time of residence. Studies on plant-associated fungi have demonstrated that fungal endophytes are widespread in all the major taxonomic groups of plants growing under different environmental conditions. Therefore, fungal endophytes make up an important contribution in the hyperdiversity of fungi (Rodriguez et al. 2009). Some endophytic fungi present in plants include the species of *Alternaria*, *Colletotrichum*, *Guignardia*, *Fusarium*, and *Aspergillus* (Xiong et al. 2013; Links et al. 2014; Zhang et al. 2014; Pérez et al. 2016). Endophytic fungal diversity analysis carried out from

roots, stems, and leaves of *Brassica napus* showed that most of the fungal species isolated and identified belong to *Ascomycota* (80%). *Alternaria alternata* was the dominant species and comprised 12.4% of the all isolates (Zhang et al. 2014). Xiong et al. (2013) have isolated 81 endophytic fungi from *Taxus media* and placed them into eight different groups on the basis of morphological and molecular characters. *Colletotrichum* (34.5%) and *Guignardia* (6.9%) were the most dominant fungal genera, whereas *Phomopsis*, *Glomerella*, *Gibberella*, *Nigrospora*, *Alternaria*, and *Phoma* were present in less number.

Epiphytic fungi are not as abundant on plant surfaces as compared to endophytic ones. Osono (2008) performed experiments with *Camellia japonica* and analyzed the fungal epiphytes of plant leaves in different seasons. Analysis showed 52 epiphytic fungi in different season's leaves and represented mostly by *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Pestalotiopsis* sp., *Cladosporium cladosporioides*, *Aureobasidium pullulans*, *Phoma* sp.1, and *Ramichloridium* sp. Similarly studies carried on banana fruit showed that *Acremonium strictum*, *Aspergillus caespitosus*, *Cylindrocarpon* sp., *Curvularia pallescens*, *Clonostachys byssicola*, *Plectosporium tabacinum*, *Penicillium oxalicum*, *Trichoderma harzianum*, *Ulocladium atrum*, *Verticillium tricorpus*, and *Verticillium* sp. are the important fungal epiphytes (Alvindia and Natsuaki 2008).

12.4 Microbial Interactions

Plant-microbe interaction is a mode of communication between plants and microbes which is initiated by the secretion of different signaling molecules. One of the important questions of communication pathways is how the plant distinguishes a microbial mutualist from pathogen. It has been reported that during the course of evolution, plants have evolved unique and sophisticated defense mechanism that involves innate immune system consisting of two classes of immune receptors that recognize the presence of nonself molecules both inside and outside of host cells (Jones and Dangl 2006). Encounter with nonself molecule evokes powerful immune responses which in turn prevents the multiplication of microbial pathogens. An increasing number of pattern recognition receptors have been identified on the plant cell surface during the past few decades (Boller and Felix 2009). As this class of immune receptors shows a vast range of microbe-associated molecular patterns (MAMPs), it is difficult to relate it with the colonization of the interior parts of root by soil-derived endophytic microbial communities. The interaction between plants and microbes leads to the activation of local and systemic defenses under controlled conditions by plant signaling hormones such as salicylic acid, jasmonic acid, and ethylene which depend upon the nature of the microbe (Koornneef and Pieterse 2008; Yi et al. 2014). There are two types of recognition patterns in plants: the first recognizes and responds to molecules which are common to many classes of microorganisms, referred to as pathogen-associated or microbe-associated molecular patterns (PAMPs/ MAMPs), and the second recognizes pathogen virulence factors (effector molecule) such as flagellin (Jones and Dangl 2006). Mechanism of

plant-microbial interaction is essential to understand the effects of interactions. Endophytic and epiphytic microorganisms have a different mechanism of interaction with plants due to the presence of different environmental conditions. On the basis of these variable conditions, brief detail of endophytic and epiphytic modes of interactions is presented under separate headings.

12.4.1 Bacterial Endophytes

Every plant studied so far has been found to be associated with at least one kind of endophytic microbe. Exploration of endophyte-plant interactions can prove beneficial for the promotion of plant health.

12.4.1.1 Entry Within the Host Plant

An endophyte enters the endosphere mostly through the soil and rhizosphere, or it may be present in inoculum. The sequence of events in the colonization of a plant by an endophytic bacterium is presumably similar, particularly in the early stages, to that observed for rhizospheric bacteria. In general, colonization of roots by the endophytic bacteria starts with the recognition of specific compounds that are released from the root tissue (Lugtenberg and Dekkers 1999). For instance, certain organic compounds including amino acids secreted by tomato roots were reported to function as chemoattractants for *P. fluorescens* strain WCS365. Once released, bacteria sense these molecules and respond to their surrounding environment via two-component sensor systems (Faure et al. 2009). In this two-component system, the first component typically constitutes of single protein with input and output transmembrane domains and lacks receiver domain, whereas the second component has only phosphotransfer histidine kinase. These two-component systems have been reported to be responsible for the recognition of root-exuded compounds which ultimately leads to active root colonization. Among two-component regulatory systems, GacS/GacA is present in some pseudomonads and enteric bacteria, wherein GacS functions as the sensor kinase to recognize unknown environmental signals and GacA acts like a transcriptional regulator. The latter activates synthesis of secondary metabolites and enzymes which in turn promote the fitness of host colonization (Heeb and Haas 2001). Involvement of one-component system like Nod factor has also been ascribed in recognition of environmental stimuli. The one- and two-component sensor/response systems together with other cross regulation systems allow bacteria to execute complex information processing and enable them to coordinate suitable responses in the active rhizosphere environment.

For successful interactions with the plant, many bacterial traits are required which help in responding to environmental stimuli (transcriptional regulator), communication (e.g., autoinducers), niche adaptation, adhesion, and plant colonization.

12.4.1.2 Transcriptional Regulators

Transcriptional regulators affect a number of physiological responses like transport processes, metabolism of sugars and amino acids, pilus synthesis, quorum sensing(QS), and motility (Korner et al. 2003; Molina-Henares et al. 2006; Maddocks and Oyston 2008).

The function of transcriptional regulators in root colonization by bacteria has been studied in detail by English et al. (2010). They introduced a transposon upstream of the *hns* gene in *Enterobacter cloacae* UW5, which enhanced gene expression when the strain was exposed to Canola roots. As a result, the *hns* transcript level increased up to twofold, and the mutant strain showed increased root colonization and outcompeted the wild-type strain in a direct competition assay. They reported that *hns* gene encodes the small histone-like protein H-NS that binds primarily to AT-rich sequences of DNA present in promoter sequences (English et al. 2010).

12.4.1.3 Autoinducers

Boyer et al. (2008) developed a mutant of the rice endophyte *Azospirillum lipoferum* B518 that constitutively expressed *AttM* lactonase which promoted the synthesis of proteins involved in transport and chemotaxis. This shows that QS in this strain is devoted to control functions related to root colonization.

Autoinducer molecules are essential for bacterial communication as they have an important role in endophytic colonization. This is supported from the report of Suarez-Moreno et al. (2010), wherein it was observed that QS mutant strains of *B. kururiensis* M130 failed to show effective root and aerial rice tissue colonization in comparison with the wild type.

12.4.1.4 Niche Adaptation and Adhesion

In order to survive and grow inside the plant tissue, bacteria must rapidly adapt their metabolism within the range of available nutrients. Matilla et al. (2007) conducted gene expression analyses of the root-colonizing bacterium *Pseudomonas putida* KT2440 and observed an upregulation of genes involved in stress adaptation and metabolism in the corn plant's rhizosphere. It was noted that certain upregulated genes were involved in the uptake of "readily available" compounds like amino acid, polyamines, dipeptides, and aromatic compounds as well as genes of enzymes related to stresses such as glutathione peroxidase and fatty acid cis-trans isomerase and detoxification of proteins such as putative efflux transporters. Likewise, survival of certain bacteria in the roots of plant growing in flooded ecosystems requires adaptation to anoxic conditions. In support of above notion, Brune et al. (2000) reported that under anoxic condition, rice plants make heterogeneous oxic/anoxic interfaces which allow rhizobacteria and endophytes to carry out fermentation processes. This process leads to the accumulation of lactic acid and ethanol in root tissues. It has been reported that ethanol acts as a carbon source for the endophytic bacterium *Azoarcus* sp. strain BH72, which is known to harbor ten genes in its genome encoding for alcohol dehydrogenases (Krause et al. 2006). Once the bacteria have entered upon root surface, its adhesion is mediated by cell surface

components, namely, polysaccharides, pili, and adhesins (Hori and Matsumoto 2010). These components are found to be involved in adhesion of bacteria.

12.4.1.5 Colonization

For colonization inside the plant tissue, an important process known as rhizodeposition comes into play which distinguishes rhizosphere microflora from soil biomes. In this, plants developmental processes and the secretory activities are entangled within the root system. Rhizodermis cells secrete a varied range of compounds, including organic acid, phytosiderophores, purines, nucleosides, sugars, vitamins, amino acids, and inorganic ions, and the root cap produces polysaccharide mucilage (Dakora and Phillips 2002). Rhizodeposition also involves the release of a specialized population of cell known as root cap border cells in the rhizosphere (Dennis et al. 2010). Root cap border cells are predominantly significant contributors to the "rhizospheric effect" because even after detachment from the root mass into soil, this cell population typically remains alive (Hawes et al. 2000).

Multiplication of soil microflora is thought to be fueled by the pooling of organic carbon and nitrogen by the roots as most commonly occurring soil bacteria are organotroph. Studies conducted on *Arabidopsis* species point towards the probable role of cell-derived proteoglycan and arabinogalactan proteins (AGPs) in the attachment of *Rhizobium* to root cells. Identification and characterization of an AGP from pea root exudates showing ability to induce biofilm formation by *R. leguminosarum* on an artificial glass surface provide a strong evidence for its role in bacterial attachment (Xie et al. 2012). Furthermore, Bulgarelli et al. (2012) demonstrated that soil type and bacteria present in the soil play important role on the composition of endophytic bacterial communities of root than the host genotype. Hence, a major but weak host genotype-dependent effect aids in the choice of *Arabidopsis* root-inhabiting bacterial communities.

Plant-microbe interactions are difficult to study at molecular level, but recent advancements in the field of molecular tools and techniques have made it possible to get an insight of the mechanism of interaction and find out the outcome of interactions. Autofluorescent protein (AFP) methods are now widely used for studying plant-microbe interactions and biofilm formation (Larrainzar et al. 2005). These techniques have been utilized to identify and count microorganisms in situ on plant surfaces and inside the plant (Gage et al.1996). One of such AFP strategies employs a marker system which codes for the green fluorescent protein (GFP). GFP is an advantageous AFP biomarker because it does not need any substrate or cofactor to fluoresce. ß-Glucuronidase (GUS) reporter system is also used for the visualization of endophyte colonization.

12.4.2 Fungal Endophytes

Like the bacterial counterpart, fungal endophytes can also have profound effect on plants health, and they also play essential role in shaping plants communities. There are numerous endosymbiotic fungi reported particularly in grasses that enhance

plant growth, and many of them have an additional feature as they produce alkaloid having insecticidal property (Wilkinson et al. 2000). In simple terms, many fungi live in symbiotic association with plant where the fungus provides the plant with extra nutrients or growth stimulators or suppresses disease, and in return, the plant provides a suitable habitat and photosynthates for the fungus.

On the basis of evolutionary relatedness, taxonomy, plant hosts, and ecological function, endophytic fungi have been classified into two groups, namely, (i) clavicipitaceous endophytes (C-endophytes also known as class 1 type), which are found as endophyte in some grasses, and, (ii) nonclavicipitaceous endophytes (NC-endophytes), which are found in tissues of ferns, conifers, angiosperms, and some nonvascular plants (Rodriguez et al. 2009). Till date, most of the work has been done only on C-endophytes (Clay and Schardl 2002). The reason behind this bias seems to be important agricultural impacts of C-endophytes and a lack of knowledge about the environmental consequences of NC-endophytes. C-endophytes of grasses include certain phylogenetically related species of clavicipitaceous endophyte that shows fastidious growth in culture and restricted to some cool- and warm-season grasses (Bischoff and White 2005). Generally these endophytes show systemic intercellular infections within plant shoots. C-endophytes transmit in a vertical manner, i.e., mother plants passing fungi on to progeny through seed infections (Saikkonen et al. 2002). These endophytes perform many vital functions such as increase in plant biomass, drought tolerance, and production of certain toxic chemicals which are harmful to animals and discourage herbivory (Clay 1988).

On the other hand, NC-endophytes are highly diverse and comprise at least three distinct functional classes namely 2, 3 and 4 according to life history and their ecological significance. Among the three classes, class 2 endophytes grow in both above- and belowground tissues of plants, whereas classes 3 and 4 endophytes mostly grow in aboveground tissues and roots, respectively. NC-endophytes have been reported and isolated from every lineage of land plants and all land ecosystems ranging from the tropics to the tundra (Arnold and Lutzoni 2007).

Henceforth, evolution of mutualisms can better be understood by extensive characterization of different endophyte-plant interactions. For example, the mode of transmission (vertical or horizontal) is thought to greatly influence the evolution and sustainability of mutualisms (Sachs et al. 2004). In case of vertically transmitted endophytes, the fitness of the two partners is connected, and the result of the interaction is predictable and mutualism is strongly selected. On the other hand, the horizontal mode of transmission brings opportunities for a variety of fungi showing symbiotic lifestyles for plant colonization. Therefore, horizontal transmission may reduce fitness linkages between specific species. As a result, class 1 and 2 endophytes are transmitted either vertically or horizontally, while class 3 and 4 endophytes are transmitted only horizontally.

12.4.3 Bacterial Epiphytes

In addition to microbial species isolated from the interior of plant tissues, there are a large number of microbes which are recovered from the surfaces of healthy-growing plants. Leaves comprise a large habitat for microorganisms; therefore, most of the work has been done and is available on the process of epiphytic bacterial colonization and interaction with leaves. It is estimated that the terrestrial leaf surface area that is probably colonized by microbes is about 6.4×10^8 km^2 (Morris and Kinkel 2002).

The interaction of epiphytic bacteria with plants is beneficial and important in a sense that these bacteria residing on the leaf surfaces or those used as foliar sprays are reported to inhibit plant pathogenic bacteria and fungi of global significance. *Erwinia herbicola*, the epiphytic bacterium colonizing the leaf surfaces of rice, was known to reduce the pH of the rice leaf and thus made it hard for the bacterial pathogen *(Xanthomonas oryzae* pv. *oryzae*) to grow (Hsieh and Buddenhagen 1974; Santhi et al. 1987). This ability of epiphytic bacteria makes it as strong biocontrol agent. Further, these epiphytes protect the plant from frost injury which is considered as a serious abiotic stress of crop plants in many areas of the temperate zone. Bacterial population on the leaf surface also limits supercooling in the plant parts in which they live by damaging ice formation at temperatures of −2 to −4 °C as the plants lack intrinsic ice nuclei activity at these temperatures as studied in *Zea mays* (Arny et al. 1976). The unfolding results from the studies on host-microbe interaction have provided the evidence for potential application and significance of such interaction.

12.4.4 Fungal Epiphytes

As reported by the researchers, most of the fungi associated with plants are endophytic, whereas very few are epiphytic in nature. The mechanism of plant and epiphytic fungal interaction is not well studied so far. Therefore, further studies are required for proper understanding of the interactions and its outcomes.

12.5 Benefits of Microbial Interactions

Interactions between microorganisms and host plant with their function are important to address the agroecological importance of endophytic and epiphytic microbes. The colonization and proliferation of plant growth promoting endophytes (PGPE) are well recognized for their importance in the enhancement of plant growth by providing a number of growth regulators/hormones, synthesis of 1-aminocycloprop ane-1-carboxylic acid (ACC) deaminase, assimilable essential nutrients and by reducing disease through suppressing growth of pathogens (Doty 2008; Aravind

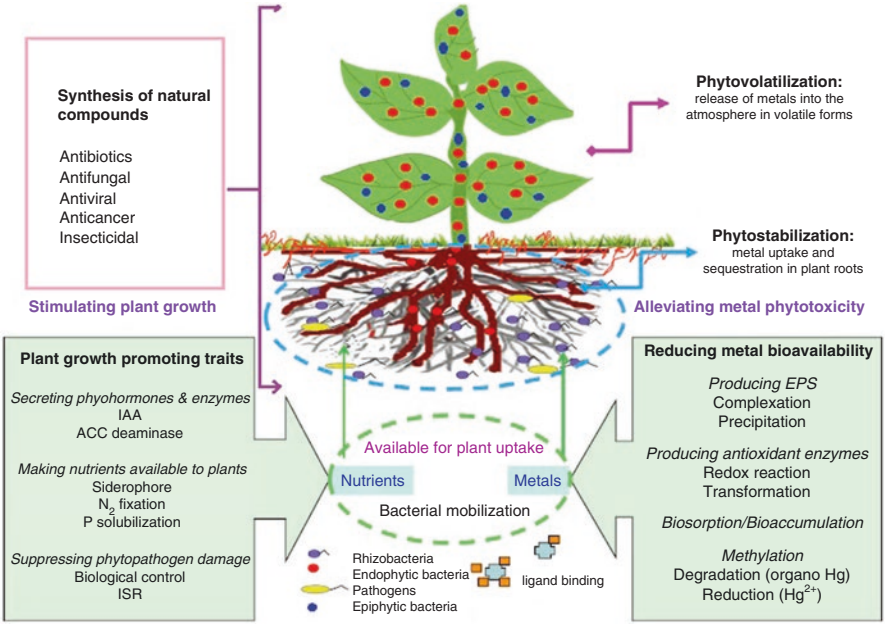


Fig. 12.1 Diagrammatic representation of plant growth-promoting activities and production of certain beneficial metabolites/compounds by plant-associated bacteria (modified after Ma et al. 2016). *Abbrev*.: IAA : indole-3-acetic acid, ACC : 1-aminocyclopropane-1-carboxylic acid, ISR: induced systemic resistance, EPS : extracellular polymeric substances

et al. 2010; Zhang et al. 2011b; Phetcharat and Duangpaeng 2012). Some of the beneficial aspects and outcomes of microbial interactions are described in the Fig. 12.1 (Ma et al. 2016). Interaction may be beneficial in other way since bioaugmentation with such endophytic and epiphytic microbes possessing multiple plant growth promoting traits including metal resistance/accumulation/detoxification/transformation/sequestration can diminish the metal phytotoxicity and alter the phyto-availability of heavy metals in contaminated soils (Rajkumar et al. 2009; Weyens et al. 2009; Ma et al. 2011). This makes these microbes and the host plants as a perfect choice for phytoremediation studies. Furthermore, there are many ecological factors which strongly influence the diversity of epiphytic growth and persistence at different spatial locations in different plant species and at different geographical locations (Hietz 2005; Cruz-Angón et al. 2009).

Microbes are reported to exert their beneficial effects in two possible ways:

(a) By colonization inside the plant tissues, they suppress pathogenic microbes by the production of antibiotics, niche occupation, or both.
(b) By colonization of the root cortex, they generate systemic plant defenses/resistances and enhance plant growth and crop performance.

12.5.1 Plant Growth Promotion

Plant growth-promoting microbes can affect plant growth directly or indirectly. The direct promotion of plant growth entails to provide the plants with a compound that is synthesized by the bacterium or facilitate the uptake of nutrients from the soil. The indirect promotion occurs when endophytes decrease or prevent the deleterious effects of pathogenic organisms.

12.5.1.1 Direct Plant Growth Promotion

There are many different ways through which endophyte can directly facilitate the plant growth promotion. These attributes include production of phytohormones, specific enzyme, siderophores, nitrogen fixation, and solubilization of minerals. They can alleviate metal toxicity and enhance plant development through one or more of the above mechanisms (Rajkumar et al. 2009; Pereira and Castro 2014). These bacteria have strong associative nitrogen-fixing ability, supply atmospheric nitrogen to their host plants, and enable plants to survive even in nitrogen-poor environment (Hurek and Reinhold-Hurek 2003; Monta~nez et al. 2012). Microbes can also serve as a sink for phosphorous by rapid microbial phosphorus mobilization even under phosphate-limiting condition. Thereby these endophytes become a source of phosphorus to the hosts upon its release from plant cells. This is probably due to its ability to produce extracellular phytase, which makes phytate-P, available for plant uptake. PGPE can also solubilize iron under conditions of iron deficiency by production of siderophores which form Fe-siderophore complex and make them readily available to plants through root-mediated chelator degradation (Rajkumar et al. 2009). Microbial endophytes are also able to supply essential vitamins and growth regulators to plants for enhancing nutrient accumulation and metabolism (Shi et al. 2009). The ability to produce the plant hormone indole-3-acetic acid (IAA) is most common among both epiphytic and endophytic bacteria (Costacurta et al. 1994; Patten and Glick 1996). IAA is involved in pathogenesis of several microorganisms including *Agrobacterium tumefaciens*, *A. rhizogenes*, *Pseudomonas syringae* pv. *syringae* (Fett et al. 1987; White and Zeigler 1991; Clark et al. 1993), and in other members, such as the genera of *Azospirillum*, *Rhizobium*, *Bradyrhizobium*, *Enterobacter*, *Xanthomonas*, and several *Pseudomonas* species. Besides role in pathogenesis, they may be beneficial for plant growth promotion and in enhancing the release of plant metabolites which bacteria can exploit for nutrients (Bar and Okon 1993; Glick 1995; Patten and Glick 1996).

12.5.1.2 Indirect Plant Growth Promotion

Several plant growth-promoting microbes are known to diminish the stress effects in plants by suppressing phytopathogenic damage either via biological control or induced systemic resistance (ISR) of plants against pathogens (Harish et al. 2008). Fungal-induced systemic resistance associated with the expression of pathogenesis related genes against phytopathogens has been reported by few researchers.

Fusarium solani, an endophytic fungus, isolated from root tissues of tomato plants elicited induced systemic resistance against the tomato foliar pathogen *Septoria lycopersici* and triggered pathogen-related (PR) genes, PR5 and PR7, expression in roots (Kavroulakis et al. 2007).

12.5.2 Improved Phytoremediation

Phytoremediation, one of the "soft" bioremediation techniques, is becoming an acceptable alternative for the treatment of contaminated sites and wastewater. Phytoremediation of organic compounds depends on the collective action of the plants and the associated microbes. While using plants and microbes in combination, the plant provides the habitat and nutrients to the associated bacteria, and, in return, the bacteria enhance the stress tolerance of plant by improving plant growth and detoxifying the plant environment by degrading the pollutant.

The exploitation of plant microbial relationship has overcome the limitations exhibited by the plants in polluted environment where the abundance of organic pollutants in soil generally suppresses plant development and eventually phytoremediation efficacy (Weyens et al. 2009; Glick 2010; Yousaf et al. 2011; Khan et al. 2013). Although both rhizospheric and endophytic bacteria have been extensively studied for their plant growth promotion and phytoremediation of polluted soil and water, it is pertinent to mention that the endophytic bacteria which colonize the plant interior and thereby interact more closely with their host plant are better equipped with pollutant degradation pathways and metabolic activities that can reduce both phytotoxicity and evapotranspiration of organic compounds (Sessitsch et al. 2004; Germaine et al. 2009).

Overall, the bacterial endophytes improve the process of phytoremediation by two distinct pathways, i.e., by the enhancement of metal tolerance in plants and growth together with alteration in the accumulation of metal from the metal polluted soils. Moreover, they can be engineered to enhance heavy metal resistance/sequestration system to degrade organic pollutants present in soil (Weyens et al. 2010). This is one of the potential approaches to enhance the biomass production and phytoremediation of co-contaminants, namely, toxic metals and organic pollutants present in the soil. In order to remove the metal stress, endophytic and epiphytic microbes have developed different types of regulatory mechanisms, through which they decrease the toxicity of metal ions. These mechanisms include either efflux exterior to the cell, conversion of complex forms to less toxic forms, sequestration on the cell surface or in the intracellular polymers, and adsorption/desorption or precipitation or biomethylation of metal ions (Rajkumar et al. 2013). Certain organic volatile pollutants may not be degraded by the plant but may be released through the stoma and can be degraded by microorganisms, which question the advantages of phytoremediation in such cases. Research is under progress to create genetically modified microbes which have high phytoremediation capacity.

12.5.3 Biocontrol Agents

Plant-associated microbes can also act as biocontrol agents, and their protective effect is probably based on the production of antibiotics and hydrolases that destroy the cell wall of phytopathogenic organisms. They can act as antagonists by inhibiting the growth of phytopathogens and at the same time can induce systemic resistance in plants. Diseases of fungal, bacterial, and viral origin and in some cases damages caused by insects and nematodes can be minimized by prior inoculation with endophytes (Kerry 2000; Berg and Hallmann 2006). Some fungal endophytes can protect host from different diseases caused by pathogenic microbes (Ganley et al. 2008; Mejía et al. 2008). Many fungal species produce secondary metabolites which have antifungal and antibacterial activity and strongly inhibit the growth of other microorganisms including pathogens (Gunatilaka 2006). They can produce multiple kinds of antibiotics including alkaloids, terpenoids, polypeptides, and aromatic compounds to which plant pathogens are sensitive too. Alkaloids strongly suppress growth of several microbes. For example, altersetin, a new alkaloid isolated from *Alternaria* sp., exhibits antibacterial activity against several pathogenic bacteria (Hellwig et al. 2002). The mechanism involved in this biocontrol efficiency is signal interference which is a new form of microbial antagonism. *Bacillus thuringiensis* most widely used biocontrol agent for insect control suppresses virulence of plant pathogenic bacterium, *Erwinia carotovora*, by quorum sensing through *N*-acyl homoserine lactones (AHLs), which are present in the quorum-sensing system of certain Gram-negative bacteria (Dong et al. 2003). AHLs take part in the regulation of different microbial biological processes, including production of antibiotics, virulence factor expression, and biofilm formation through cell to cell communication (de Kievit and Iglewski 2000; Whitehead et al. 2002).

12.5.4 Natural Products from Microbes

Natural products obtained from endophytic microbes have their potential role in the field of pharmaceutical, agroecological and scientific research.

12.5.4.1 Antibiotics

Cryptosporiopsis quercina, an endophyte isolated from *Tripterygium wilfordii*, a medicinal plant found in Eurasia, produces a unique antimycotic peptide, cryptocandin. Cryptocandin is known to be active against many fungal pathogens such as *Candida albicans* and *Trichophyton* sp. causing diseases in humans (Strobel et al. 1999). The ecomycins, produced by *Pseudomonas viridiflava*, associated with the leaves of many grass species and located on and within the tissues are also active against several human pathogenic fungi (Miller et al. 1998). Similarly, another group of antifungal compounds, pseudomycins produced by pseudomonads, are active against a variety of plant and human pathogenic fungi (Harrison et al. 1991; Ballio et al. 1994).

12.5.4.2 Antiviral Compounds

The discovery of compounds from microbes having antiviral activity is still in its infancy; however, some compounds are promising. Two novel human cytomegalovirus protease inhibitors named cytonic acids A and B have been purified from the solid-state fermentation of the *Scytonema* sp. (Guo et al. 2000). The main limitation in such discovery is probably related to the absence of suitable antiviral screening systems in most of the antiviral compound discovery programs.

12.5.4.3 Anticancer Agents

Paclitaxel, the world's first billion dollar anticancer drug represented to be the first anticancer agent, is produced by an endophytic fungus which is found in each of the world's yew (*Taxus*) species (Suffness 1995). Some of the most common endophytes of the world's yews are species of *Pestalotiopsis*, of which *P. microspora* is the most common (Strobel 2002). However, species of *P. microspora* isolated from bald cypress (*Taxodium distichum*) in South Carolina also produced paclitaxel (Li et al. 1996). This suggests that the distribution of paclitaxel-producing endophytic fungi is not confined to yew only. The explanation for wide distribution of fungi producing paclitaxel might be due to the fact that it is a type of fungicide and the microorganisms which show sensitivity to it are plant pathogens such as *Pythium* sp. and *Phytophthora* sp. (Young et al. 1992). These two organisms are the world's most destructive plant pathogens. In fact, their sensitivity toward the paclitaxel is due to the interaction with tubulin proteins similar to those reported in rapidly dividing human cancer cells (Schiff and Horowitz 1980).

12.5.4.4 Insecticidal Compounds

Several microbes are known to possess insecticidal properties. Nodulisporic acid, a novel indole diterpene analogue, exhibits potent insecticidal property against the larvae of blowfly. It works by activating insect glutamate-gated chloride channels. The first nodulisporic compounds were isolated from an endophyte, *Nodulisporium* sp., from the plant *Bontia daphnoides*. This discovery has resulted in an intensive search for more *Nodulisporium* sp. and/or other producers of more potent nodulisporic acid (Demain 2000). In an another study, *Muscodor vitigenus* (an endophytic fungus) isolated from a liana (*Paullina paullinioides*) showed synthesis of naphthalene as major product which is widely used as insect repellant. Naphthalene is the active ingredient in common mothballs which is mostly used for the storage of clothes and other articles. In a preliminary study, *M. vitigenus* showed promising results as an insect repellant with potent activity against the *Cephus cinctus* (wheat stem sawfly) (Daisy et al. 2002a, b).

12.5.5 Stimulation of Plant Secondary Metabolites Production

Plant secondary metabolites are a group of compounds, which do not play important role in basic life functions of organism but have a major role in the adaptation of organisms according to their environmental conditions (Bourgaud et al. 2001).

Among these compounds, plants produce phytoalexins, an antimicrobial molecule, which contain multiple components including flavonoid, terpenoid, etc. (Smith 1996). However, studies carried so far pertaining to the plant secondary metabolites production mediated by the fungal endophytes are still in the early stage. To this effect, Yong et al. (2009) reported that endophytic fungi *Fusarium* sp. E4 and E5 promoted the growth of *Euphorbia pekinensis*, resulting in increased production of terpenoids.

12.6 Conclusion

There are various studies available on the diversity and ecology of epiphytes and endophytes which give an idea of their diverse nature and habitat. However, our understanding of the mechanism of interaction between the host plant and microbe is very limited. It is significant to isolate and characterize microbial communities living inside and outside the plants so as to get a deep knowledge about how a microbe enters and colonizes a plant and what are the beneficial outcomes of such interaction. There is a general consensus that critical study on plant-microbe associations can not only enhance our knowledge of the mechanisms of interaction but can also result in better understanding of plant health. Expected outcome may have an important impact on sustainable agriculture for all types of crop. The benefits that endophytes usually offer include enhanced plant growth by production of phytohormones, increase in N budget by nitrogen fixation, resistance to environmental stresses, remediation of contaminated sites, and production of important agricultural, medicinal, and industrial compounds. Current research focuses on genetically engineered endophytes to facilitate enhanced bioremediation of highly contaminated sites and put into global use. Endophytes have also attracted special attention as potent biocontrol agent; a deeper understanding of the endophyte-host plant interaction at molecular level can enhance their use in agriculture and alleviate toxic effects for humans and animals. Availability of whole genome sequence and recent techniques with "omic" technologies provide the opportunity to search for genes which are differentially regulated during colonization of plant tissues on a global level. Some researchers have already reported the presence of genes responsible for the colonization and establishment of endophytic bacteria within plants. Research done so far has been mostly focused on endophytes, and very little information is available on epiphytic mode of interaction and its benefits. Future research should aim to have a detailed understanding of the ecology of plant-associated microbes and determining their successful colonization for their effective use in agriculture. Developing and/or discovering novel strain combinations in place of individual strains would be useful in promoting sustainable production of biomass and bioenergy crops. However, the future of endophytic biology is at stake due to the rapid diminishment of rainforests which holds the greatest resource for discovering novel microorganisms and their products. Henceforth, multistep processes are required to secure life-forms before they continue to be lost.

Acknowledgments JK is grateful to University Grants Commission (UGC), New Delhi, for the award of Junior Research Fellowship (23/06/2013 (I) EU-V). DS and PG are the recipient of DST-INSPIRE (DST/INSPIRE Fellowship/2014/296, IF140707) and Banaras Hindu University Research fellowship, respectively. Research in the area of PGPR is partly supported by a research grant sanctioned to AK by the Indian Council of Agricultural Research, Government of India, New Delhi (NBAIM/AMAAS/2014-17/PF/4).

References

Alvindia DG, Natsuaki KT (2008) Evaluation of fungal epiphytes isolated from banana fruit surfaces for biocontrol of banana crown rot disease. Crop Protect 27:1200–1207. doi:10.1016/j.cropro.2008.02.007

Araujo WL, Marcon J, Maccheroni W Jr, Van Elsas JD, Van Vuurde JW, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914. doi:10.1128/AEM.68.10.4906

Aravind R, Eapen SJ, Kumar A, Ramana KV (2010) Screening of endophytic bacteria and evaluation of selected isolates for suppression of burrowing nematode (*Radopholus similis* Thorne) using three varieties of black pepper (*Piper nigrum* L.). Crop Prot 29:318–324

Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88:541–549. doi: 10.1890/05-1459

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N et al (2003) Fungal endophytes limit pathogen damage in a tropical tree. Pros Natl Acad Sci USA 100:15649–15654

Arny DC, Lindow SE, Upper CD (1976) Frost sensitivity of *Zea mays* increased by application of *Pseudomonas syringae*. Nature 262:282–284

Ballio A, Bossa F, DiGiogio P, Ferranti P, Paci M, Pucci P et al (1994) Structure of the pseudomycins, new lipodepsipeptides produced by *Pseudomonas syringae* MSU 16H. FEBS Lett 355:96–100

Bar T, Okon Y (1993) Tryptophan conversion to indole-3-acetic acid via indole-3-acetamide in *Azospirillum brasilense* Sp7. Can J Microbiol 39:81–86

Beattie GA, Lindow SE (1995) The secret life of foliar bacterial pathogens on leaves. Annu Rev Phytopathol 33:145–172

Berg G, Hallmann J (2006) Control of plant pathogenic fungi with bacterial endophytes. In: BJE S, CJC B, Sieber TN (eds) Microbial root endophytes. Springer, Berlin, pp 53–69

Bischoff JF, White JF Jr (2005) Evolutionary development of the Clavicipitaceae. In: Dighton J, White JF, Oudemans P (eds) The fungal community: its organization and role in the ecosystem. Taylor and Francis, Boca Raton, pp 505–518

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci 16:839–851

Boyer M, Bally R, Perrotto S, Chaintreuil C, Wisniewski-Dye F (2008) A quorum-quenching approach to identify quorum-sensing-regulated functions in *Azospirillum lipoferum*. Res Microbiol 159:699–708

Brune A, Frenzel P, Cypionka H (2000) Life at the oxic-anoxic interface: microbial activities and adaptations. FEMS Microbiol Rev 24:691–710

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N et al (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488:91–95

Clark E, Manulis S, Ophir Y, Barash I, Gafni Y (1993) Cloning and characterization of *iaaM* and *iaaH* from *Erwinia herbicola* pathovar *gypsophilae*. Phytopathology 83:234–240

Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10–16

Clay K, Schardl CL (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160:S99–S127
Costacurta A, Kiejers V, Vanderleyden J (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilence* indole-3- pyruvate decarboxylase gene. Mol Gen Genet 243:463–472
Cruz-Angón A, Baena ML, Greenberg R (2009) The contribution of epiphytes to the abundance and species richness of canopy insects in a Mexican coffee plantation. J Trop Ecol 25:453–463
Daisy BH, Strobel GA, Castillo U, Ezra D, Sears J, Weaver D, Runyon JB (2002a) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. Microbiology 148:3737–3741
Daisy BH, Strobel GA, Ezra D, Castillo U, Baird G, Hess WM (2002b) *Muscodor vitigenus* anam. sp. nov., an endophyte from *Paullinia paullinioides*. Mycotaxon 84:39–50
Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low nutrient environments. Plant Soil 245:35–47
de Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun 68:4839–4849
de Oliveira Costa LE, de Queiroz MV, Borges AC, de Moraes CA, de Araújo EF (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). Braz J Microbiol 43:1562–1575. doi: 10.1590/S1517-838220120004000041
Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B et al (2009) Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc Natl Acad Sci U S A 106:16428–16433
Delong EF (2009) The microbial ocean from genomes to biomes. Nature 459:200–206. doi:10.1038/nature08059
Demain AL (2000) Microbial natural products: a past with a future. In: Wrigley SK, Hayes MA, Thomas R, Chrystal EJT, Nicholson N (eds) Biodiversity: new leads for pharmaceutical and agrochemical industries. The Royal Society of Chemistry, Cambridge, pp 3–16
Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327
Dong Y, Iniguez AL, Triplett EW (2003) Quantitative assessments of the host range and strain specificity of endophytic colonization by *Klebsiella pneumoniae* 342. Plant Soil 257:49–59
Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
English MM, Coulson TJD, Horsman SR, Patten CL (2010) Overexpression of *hns* in the plant growth-promoting bacterium *Enterobacter cloacae* UW5 increases root colonization. J Appl Microbiol 108:2180–2190
Faure D, Vereecke D, Leveau JHJ (2009) Molecular communication in the rhizosphere. Plant Soil 321:279–303
Fernandes R, Júnior G, Aparecida E, Pedrinho N, Cristina T, Castellane L (2011) Auxin-producing bacteria isolated from the roots of *Cattleya walkeriana*, an endangered Brazilian orchid, and their role in acclimatization. Rev Bras Ciênc Solo 35:729–737
Fett WF, Osman SF, Dunn MF (1987) Auxin production by plant pathogenic *Pseudomonads* and *Xanthomonads*. Appl Environ Microbiol 53:1839–1845
Fiala V, Glad C, Martin M, Jolivet E, Derridj S (1990) Occurrence of soluble carbohydrates on the phylloplane of maize (*Zea mays* L.): variations in relation to leaf heterogeneity and position on the plant. New Phytol 115:609–615
Fisher PJ, Petrini O, Lappin-Scott HM (1992) The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). New Phytol 122:299–305
Gage DJ, Bobo T, Long SR (1996) Use of green fluorescent protein to visualize early events of symbiosis between *Rhizobium meliloti* and alfalfa (*Medicago sativa*). J Bacteriol 178:7159–7166
Ganley RJ, Sniezko RA, Newcombe G (2008) Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. For Ecol Manag 255:2751–2760
Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296:226–234

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–114

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374

Goulder R, Baker JH (1991) Submerged leaf surfaces as a microbial habitat. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 60–86

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69:509–526

Guo B, Dai J, Ng S, Huan Y, Leong C, Ong W, Carte BK (2000) Cytonic acids A and B: novel tridepside inhibitors of hCMV protease from the endophytic fungus *Cytonaema* species. J Nat Prod 63:602–604

Hallmann J, Qualt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hamayun M, Khan SA, Khan AL, Rehman G, Kim YH et al (2010) Gibberellins production and plant growth promotion by pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.) Mycologia 102:989–995

Harish S, Kavino M, Kumar N, Saravanakumara D, Soorianathasundaramb K, Samiyappana R (2008) Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy topvirus. Appl Soil Ecol 39:187–200

Harrison L, Teplow D, Rinaldi M, Strobel GA (1991) Pseudomycins, a family of novel peptides from *Pseudomonas syringae*, possessing broad spectrum antifungal activity. J Gen Microbiol 137:2857–2865

Hawes MC, Gunawardena U, Miyasaka S, Zhao X (2000) The role of root border cells in plant defense. Trend Plant Sci 5:128–133

Heeb S, Haas D (2001) Regulatory roles of the *GacS*/*GacA* two-compound system in plant-associated and other Gram-negative bacteria. Mol Plant-Microb Interact 14:1351–1363

Hellwig V, Grothe T, Mayer-Bartschmid A, Endermann R, Geschke FU, Henkel T et al (2002) Altersetin, a new antibiotic from cultures of endophytic *Alternaria* spp. Taxonomy, fermentation, isolation, structure elucidation and biological activities. J Antibiot 55:881–892

Hietz P (2005) Conservation of vascular epiphyte diversity in Mexican coffee plantations. Conserv Biol 19:391–399

Hirano SS, Baker LS, Upper CD (1996) Raindrop momentum triggers growth of leaf-associated populations of *Pseudomonas syringae* on field-grown snap bean plants. Appl Environ Microbiol 62:2560–2566

Hori K, Matsumoto S (2010) Bacterial adhesion: from mechanism to control. Biochem Eng J 48:424-434

Hsieh SPY, Buddenhagen IW (1974) Suppressing effects of *Erwinia herbicola* on infection by *Xanthomonas oryzae* and on symptom development in rice. Phytopathology 64:1182–1185

Huang JS (1986) Ultrastructure of bacterial penetration in plants. Annu Rev Phytopathol 24:141–157

Hugenholtz P (2002) Exploring prokaryotic diversity in the genomic era. Genome Biol 3(2):reviews0003.1–reviews0003.8

Hurek T, Reinhold-Hurek B (2003) *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. J Biotechnol 106:169–178

Ikeda S, Okubo T, Anda M, Nakashita H, Yasuda M, Sato S et al (2010) Community- and genome-based views of plant-associated bacteria: plant-bacterial interactions in soybean and rice. Plant Cell Physiol 51:1398–1410

Inacio J, Pereira P, de Carvalho M, Fonseca A, Amaral-Collaco MT, Spencer-Martins I (2002) Estimation and diversity of phylloplane mycobiota on selected plants in a Mediterranean-type ecosystem in Portugal. Microb Ecol 44:344–353

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Kavroulakis NS, Zervakis GI, Ehaliotis C, Haralampidis K, Papadopoulou K (2007) Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. J Exp Bot 58:3853–3864

Kerry BR (2000) Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annu Rev Phytopathol 38:423–441
Khan S, Afzal M, Iqbal S, Khan QM (2013) Plant bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere 90:1317–1332
Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon CW, White JF (eds) Microbial endophytes. Marcel Dekker, New York, pp 199–233
Koornneef A, Pieterse CM (2008) Cross-talk in defense signaling. Plant Physiol 146:839–844
Korner H, Sofia HJ, Zumft WG (2003) Phylogeny of the bacterial superfamily of *Crp-Fnr* transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. FEMS Microbiol Rev 27:559–592
Krause A, Ramakumar A, Bartels D, Battistoni F, Bekel T, Boch J et al (2006) Complete genome of the mutualistic, N_2-fixing grass endophyte *Azoarcus* sp strain BH72. Nat Biotechnol 24:1385–1391
Krimm U, Abanda-Nkpwatt D, Schwab W, Schreiber L (2005) Epiphytic microorganisms on strawberry plants (*Fragaria ananassa* cv. *Elsanta*): identification of bacterial isolates and analysis of their interaction with leaf surfaces. FEMS Microbiol Ecol 53:483–492
Kumar J, Babele PK, Singh D, Kumar A (2016) UV-B radiation stress causes alterations in whole cell protein profile and expression of certain genes in the rice phyllospheric bacterium *Enterobacter cloacae*. Front Microbiol 7:1440. doi: 10.3389/fmicb.2016.01440
Larrainzar E, O'Gara F, Morrissey JP (2005) Applications of autofluorescent proteins for *in situ* studies in microbial ecology. Ann Rev Microbiol 59:257–277
Lavermicocca P, Surico G, Varvaro L, Babelegoto NM (1987) Plant hormone, cryogenic and antimicrobial activities of epiphytic bacteria of live and oleander. Phytopathol Mediterr 26:65–72
Legard DE, McQuilken MP, Whipps JM, Fenlon JS, Fermor TR, Thompson IP et al (1994) Studies of seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of a genetically modified microorganism. Agric Ecosyst Environ 50:87–101
Leveau JHJ, Lindow SE (2001) Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. Proc Natl Acad Sci U S A 98:3446–3453
Li JY, Strobel GA, Sidhu R, Hess WM, Ford E (1996) Endophytic taxol producing fungi from bald cypress *Taxodium distichum*. Microbiology 142:2223–2226
Lilley AK, Hails RS, Cory JS, Bailey MJ (1997) The dispersal and establishment of pseudomonad populations in the phyllosphere of sugar beet by phytophagous caterpillars. FEMS Microbiol Ecol 24:151–157
Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Appl Environ Microbiol 69:1875–1883. doi:10.1128/AEM.69.4.1875-1883
Lindow SE, Hecht-Poinar EI, Elliot VJ (eds) (2002) Phyllosphere microbiology. American Phytopathological Society, St. Paul, USA
Links MG, Demeke T, Gräfenhan T, Hill JE, Hemmingsen SM, Dumonceaux TJ (2014) Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on *Triticum* and *Brassica* seeds. New Phytol 202:542–553. doi:10.1111/nph.12693
Loh CY, Tan YY, Rohani R, Weber JF, Bhore SJ (2013) Diversity of endophytic bacteria in Malaysian plants as revealed by 16S rRNA encoding gene sequence based method of bacterial identification. J Young Pharm 5:95–107. doi: 10.1016/j.jyp.2013.07.001
Lugtenberg BJJ, Dekkers LC (1999) What makes *Pseudomonas* bacteria rhizosphere competent? Environ Microbiol 1:9–13
Lynch JM (1990) The rhizosphere. Wiley, New York
Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258
Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174:14–25. doi:10.1016/j.jenvman.2016.02.047
Maddocks SE, Oyston PCF (2008) Structure and function of the *LysR*-type transcriptional regulator (LTTR) family proteins. Microbiology 154:3609–3623. doi:10.1099/mic.0.2008/022772-0

Matilla MA, Espinosa-Urgel M, Rodriguez-Herva JJ, Ramos JL, Ramos-Gonzalez MI (2007) Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. Genome Biol 8:R179. doi:10.1186/gb-2007-8-9-r179

McInroy JA, Kloepper JW (1995) Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil 173:337–342

Mehta A, Rosato YB (2005) Identification of differentially expressed genes of *Xanthomonas axonopodis* pv. *citri* by representational difference analysis of cDNA. Genet Mol Biol 28:140–149

Mejía LC, Rojas EI, Maynard Z, Bael SV et al (2008) Endophytic fungi as biocontrol agents of *Thebroma cacao* pathogens. Biol Control 46:4–14

Mercier J, Lindow SE (2000) Role of leaf surface sugars in colonization of plants by bacterial epiphytes. Appl Environ Microbiol 66:369–374

Miller RV, Miller CM, Garton-Kinney D, Redgrave B, Sears J, Condron M et al (1998) Ecomycins, unique antimycotics from *Pseudomonas viridiflava*. J Appl Microbiol 84:937–944

Mitter B, Brader G, Afzal M, Compant S, Naveed M, Trognitz F, Sessitsch A (2013) Advances in elucidating beneficial interactions between plants, soil and bacteria. In: Donald LS (ed) Advances in agronomy. Academic Press, Cambridge, pp 381–445

Molina-Henares AJ, Krell T, Guazzaroni ME, Segura A, Ramos JL (2006) Members of the IclR family of bacterial transcriptional regulators function as activators and/or repressors. FEMS Microbiol Rev 30:157–186

Monta~nez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. Appl Soil Ecol 58:21–28

Morris CE, Kinkel LL (2002) Fifty years of phyllosphere microbiology: significant contributions to research in related fields. In: Lindow SE, Hecht-Poinar EI, Elliott V (eds) Phyllosphere microbiology. APS Press, St. Paul, pp 365–375

Mostert L, Crous PW, Petrini O (2000) Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. Sydowia 52:46–58

Muller CB, Krauss J (2005) Symbiosis between grasses and asexual fungal endophytes. Curr Opin Plant Biol 8:450–455

Newton AC, Gravouil C, Fountaine JM (2010) Managing the ecology of foliar pathogens: ecological tolerance in crops. Ann Appl Biol 157:343–359

Ophir T, Gutnick DL (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60:740–745

Osono (2008) Endophytic and epiphytic phyllosphere fungi of *Camellia japonica*: seasonal and leaf age-dependent variations. Mycologia 100:387–391. doi:10.3852/07-110R1

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Pereira SIA, Castro PML (2014) Diversity and characterization of culturable bacterial endophytes from *Zea mays* and their potential as plant growth promoting agents in metal-degraded soils. Environ Sci Pollut Res 21:14110–14123

Pérez ML, Collavino MM, Sansberro PA, Mroginski LA, Galdeano E (2016) Diversity of endophytic fungal and bacterial communities in *Ilex paraguariensis* grown under field conditions. World J Microbiol Biotechnol 32:61. doi:10.1007/s11274-016-2016-5

Peters AF (1991) Field and culture studies of *Streblonema macrocystis* sp. nov. (Ectocarpales, Phaeophyceae) from Chile, a sexual endophyte of giant kelp. Phycology 30:365–377

Phetcharat P, Duangpaeng A (2012) Screening of endophytic bacteria from organic rice tissue for indole acetic acid production. Procedia Eng 32:177–183

Preece TF, Dickinson CH (eds) (1971) Ecology of leaf surface micro-organisms. Academic Press, London/New York

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160

Rajkumar M, Ma Y, Freitas H (2013) Improvement of Ni phytostabilization by inoculation of Ni resistant *Bacillus megaterium* SR28C. J Environ Manag 128:973–980. doi:10.1016/j.jenvman.2013.07.001

Rasche F, Marco-Noales E, Velvis H, Leo S, van Overbeek LMM et al (2006) Structural characteristics and plant-beneficial effects of bacteria colonizing the shoots of field grown conventional and genetically modified T4-lysozyme producing potatoes. Plant Soil 289:123–140

Rastogi G, Sani RK (2011) Molecular techniques to assess microbial community structure, function and dynamics in the environment. Microb Microb Technol 29–57. doi:10.1007/978-1-4419-7931-5_2

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Rosenblueth M, Martinez-Romero E (2004) *Rhizobium etli* maize populations and their competitiveness for root colonization. Arch Microbiol 181:337–344

Ryffel F, Helfrich EJN, Kiefer P, Peyriga L, Portais JC, Piel J et al (2016) Metabolic footprint of epiphytic bacteria on *Arabidopsis thaliana* leaves. ISME J 10:632–643. doi:10.1038/ismej.2015.141

Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. Q Rev Biol 79:135–160

Saikkonen K, Ion D, Gyllenberg M (2002) The persistence of vertically transmitted fungi in grass metapopulations. Proc R Soc Lond B 269:1397–1403

Santhi DP, Unnamalai N, Gnanamanickam SS (1987) Epiphytic association of *Erwinia herbicola* with rice leaves infected by *Xanthomonas campestris* pv. *oryzae* and its interaction with the pathogen. Indian Phytopathol 40:327–332

Schaad NW, Cheong SS, Tamaki S, Hatziloukas E, Panopoulos NJ (1995) A combined biological and enzymatic amplification (BIO-PCR) technique to detect *Pseudomonas syringae* pv. *phaseolicola* in bean seed extracts. Phytopathology 85:243–246

Schiff PB, Horowitz SB (1980) Taxol stabilizes microtubules in mouse fibroblast cells. Proc Natl Acad Sci U S A 77:1561–1565

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686

Schweitzer JA, Bailey JK, Bangert RK, Hart SC, Whitham TG (2006) The role of plant genetics in determining above- and below-ground microbial communities. In: Bailey MJ, Lilley AK, PTN T-W, Spencer-Phillips PTN (eds) Microbial ecology of the aerial plant surface. CABI International, Wallingford, pp 107–119

Sessitsch A, Reiter B, Berg G (2004) Endophytic bacterial communities of field grown potato plants and their plant-growth-promoting and antagonistic abilities. Can J Microbiol 50:239–249

Shi YW, Lou K, Li C (2009) Promotion of plant growth by phytohormone producing endophytic microbes of sugar beet. Biol Fertil Soils 45:645–653

Smith CJ (1996) Accumulation of phytoalexins: defense mechanism and stimulus response system. New Phytol 32:1–45

Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: endophytism defined. In: Bacon CW, White JF (eds) Microbial endophytes. Marcel Dekker, New York, pp 3–30

Strobel GA (2002) Microbial gifts from rain forests. Can J Plant Pathol 24:14–20

Strobel GA, Miller RV, Miller C, Condron M, Teplow DB, Hess WM (1999) Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina*. Microbiology 145:1919–1926

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. CRC Crit Rev Plant Sci 19:1–30

Suarez-Moreno ZR, Devescovi G, Myers M, Hallack L, Mendonca-Previato L, Caballero-Mellado J et al (2010) Commonalities and differences in regulation of N-acyl homoserine lactone quorum sensing in the beneficial plant-associated *Burkholderia* species cluster. Appl Environ Microbiol 76:4302–4317

Suffness M (1995) Taxol, science and applications. CRC Press, Boca Raton

Sundin GW, Jacobs JL (1999) Ultraviolet radiation (UVR) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (*Arachis hypogeae* L.) Microb Ecol 38:27–38

Thompson 1P, Bailey MJ, Fenlon JS, Fermor TR, Lilley AK et al (1993) Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vdgaris*). Plant Soil 150:177–191

Van der Wal A, Leveau JH (2011) Modelling sugar diffusion across plant leaf cuticles: the effect of free water on substrate availability to phyllosphere bacteria. Environ Microbiol 13:792–797

Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–840

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132:44–51

Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598

Weyens N, Truyens S, Dupae J, Newman L, van der Lelie D, Carleer R et al (2010) Potential of the TCE-degrading endophyte *Pseudomonas putida* W619-TCE to improve plant growth and reduce TCE phytotoxicity and evapotranspiration in poplar cuttings. Environ Pollut 158:2915–2919. doi:10.1016/j.envpol.2010.06.004

White FF, Ziegler SF (1991) Cloning of the genes for indole acetic acid synthesis from *Pseudomonas syringae* pv. *syringae*. Mol Plant-Microbe Interact 4:207–210. doi:10.1094/MPMI-4-207

Whitehead NA, Byers JT, Commander P, Corbett MJ, Coulthurst SJ, Everson L et al (2002) The regulation of virulence in phytopathogenic *Erwinia* species: quorum sensing, antibiotics and ecological considerations. Antonie Van Leeuwenhoek 8:223–231

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci U S A 95:6578–6583

Wilkinson HH, Siegel MR, Blankenship JD, Mallory AC, Bush LP, Schardl CL (2000) Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. Mol Plant-Microbe Interact 13:1027–1033

Xie F, Williams A, Edwards A, Downie JA (2012) A plant arabinogalactan-like glycoprotein promotes a novel type of polar surface attachment by *Rhizobium leguminosarum*. Mol Plant-Microbe Interact 25:250–258

Xiong ZQ, Yang YY, Zhao N, Wang Y (2013) Diversity of endophytic fungi and screening of fungal paclitaxel producer from Anglojap yew, *Taxus x media*. BMC Microbiol 13:71

Yi SY, Shirasu K, Moon JS, Lee SG, Kwon SY (2014) The activated SA and JA signaling pathways have an influence on *flg*22-triggered oxidative burst and callose deposition. PLoS One 9(2):e88951

Yong YH, Dai CC, Gao FK, Yang QY, Zhao M (2009) Effects of endophytic fungi on growth and two kinds of terpenoids for *Euphorbia pekinensis*. Chin Tradit Herb Drugs 40:18–22

Young DH, Michelotti EJ, Sivendell CS, Krauss NE (1992) Antifungal properties of taxol and various analogues. Experientia 48:882–885

Yousaf S, Afzal M, Reichenauer TG, Brady CL, Sessitsch A (2011) Hydrocarbon degradation, plant colonization and gene expression of alkane degradation genes by endophytic *Enterobacter ludwigii* strains. Environ Pollut 159:2675–2683

Yu J, Peñaloza-Vázquez A, Chakrabarty AM, Bender CL (1999) Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of *Pseudomonas syringae* pv. *syringae*. Mol Microbiol 33:712–720

Zhang YF, He LY, Chen ZJ, Zhang WH, Wang QY, Qian M et al (2011a) Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape. J Hazard Matter 186:1720–1725

Zhang Q, Lambert G, Liao D, Kim H, Robin et al (2011b) Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. Science 333:1764–1767. doi: 10.1126/science.1208747

Zhang Q, Zhang J, Yang L, Zhang L, Jiang D, Chen W, Li G (2014) Diversity and biocontrol potential of endophytic fungi in *Brassica napus*. Biol Control 72:98–108. doi:10.1016/j.biocontrol.2014.02.018

Fascinating Fungal Endophytes Role and Possible Beneficial Applications: An Overview

13

N.M. Sudheep, Avinash Marwal, Nita Lakra, Khalid Anwar, and Saquib Mahmood

Abstract

Plants constitute immense and diverse niches for endophytic organisms, and their associations are well reported by many researchers. Certain microorganisms like endophytes prevail in the interior portion of plants, like roots, shoots, leaves, and stems, and do not harm the host plant. Fungi pose symbiotic relationship with plants, showing diversity in enrichment of resources and habitats. Even though these plant microbial interactions were reported from ancient years, an understanding of the mechanisms enabling these microorganisms to interact with host plants is still a dilemma. Unrevealing such unknown interaction pathways and signaling would be a crucial step in biotechnology which would probably lead to the production of different unique and novel compounds. Such compound may have the ultimate role in various applications in future biotechnology. Similarly, the potential of many isolated fungal endophytes has also not been studied well. Hence, an attempt has been made to coordinate the possibilities of usage of isolated endophytes in this chapter. Their uniqueness and specificity were studied with solid-state fermentation and submerged fermentation at a wide range of pH and temperature and few secondary metabolites and industrially important enzymes; its various applications and the common fungi used for such studies have also been discussed in this chapter.

N.M. Sudheep (✉)
Department of Plant Science, School of Biological Sciences, RST Campus, Central University of Kerala, Kasaragod, Kerala, India
e-mail: Sudheepnm@gmail.com

A. Marwal
Department of Biosciences, College of Arts, Science and Humanities, Mody University, Lakshmangarh, Sikar, Rajasthan, India

N. Lakra • K. Anwar • S. Mahmood
School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_13

Keywords
Endophyte • Plant-microbe interaction • Solid and submerged fermentations • Secondary metabolites • Enzymes • Applications

13.1 Introduction

Endophytic microorganisms are renowned for their ability to interact with the peripheral and internal tissues of plants more often through the process of symbiotic mutualisms. The endophytic concept was once exclusively used for the fungal-plant interactions, but at present the term extended to all the microbe-plant interactions (Wilson 1995). However, broadly the term endophyte applies only to such microorganisms which are proficient enough to reside inside plant tissues without imparting any sort of external disease symptoms on the plant. Endophytic fungi have a unique ecological niche and are considered essential for the survival, distribution, and most of the biological processes and functions in plants, such as nutrient intake, nutrient assimilation, plant defense, decomposition of leaf litter, seed germination, and so on (Thompson and Stewart 1981; Clay and Schardl 2002).

The history of endophyte concept dates back to the early 1886, when De Bary introduces his seminal work, "On some species of Sclerotia and the diseases they cause," which is published in *Botanical Magazine*. Even though various definitions have been put forwarded by many scientists, the definition proposed by Petrini (1991) that states that endophytes are "the organisms inhabiting plant organs that at some time in their life, can colonize internal plant tissues without causing apparent harm to the host" is accepted broadly.

In general, endophytes cause asymptomatic infections in any parts of healthy plants either by vertical transmission through host seed or vegetative propagules or by horizontal transmission. Among the microbes, endophytic fungi are extensively reported from almost all types of plants such as grasses, ferns, conifers, mosses, angiosperms, seaweeds, and epiphytic orchids (Clay 1988, Raps and Vidal 1998; Saikkonen et al. 1996; Tao et al. 2008; Sudheep and Sridhar 2011). Many reports uncover that the endophytic fungal symbiosis with the plants help them gain resistance to various pathogenic agents such as insects and other microbes (Saikkonen et al. 1996; Clay 1988). This is proven through the implanting techniques, where the endophytes isolated from a disease-resistant host plant when inserted in a disease-prone plant species increased its fitness against the disease. The specificity of the endophytic fungi to a host is still a topic of debate (Hyde et al. 2007; Tao et al. 2008). However, it is interesting to note that most of the isolated plant secondary metabolites have structural and functional similarities with endophytic allelochemicals and toxins (Cheplick and Clay 1988; Marquis 1992; Zhang et al. 2006; Singh et al. 2003). This throws challenges and opportunities in applied research in the fields of medicine, silviculture, agriculture, forestry, biological pesticide industry, etc. Apart from that, the plant endophyte association is an excellent model system that helps us to explore further our knowledge on the evolution of plant-microbe

symbioses. This paper discusses the general concepts on endophytes and their symbiosis with the host plants, common endophytic fungal species and enzymes, and possible industrial important applications.

13.2 Endophytes

Endophytes are inimitable group of microorganisms and its less understanding made them away from the active researchers interest in the past. At present the scenario has been changed, and many reports suggest that these are rich sources of natural products and chemically novel compounds and have proven their greater applications in the fields of medicine, agriculture, and various other industries (Li et al. 2008; Tan and Zou 2001). The proper perceptive of antagonistic or synergistic mechanisms through which endophytes exist and interact with its host plants could be reveled better in order to be simply predictive about the functions, roles, and biological communications. Such type of studies may be facilitated to solve several unanswered questions that still remain in the fields. Such type of studies may be facilitated to solve several unanswered questions that still remain in the fields; for instance different signaling, interactions through the manufacture of essential chemical compounds intern help us to find the product discovery processes. Furthermore, it would even be helpful to find out the mechanism of the fitness gain of both partners due to mutualistic interaction (Bacon 1993; Elmi and West 1995).

13.3 Host-Endophyte Interaction

The exact mode of interaction between host plants and endophytes is still unknown, or the available information is not enough to explain the complex relationship. In most cases, mutual relationship benefits the endophytic fungi in terms of supply of energy, nutrients, shelter, and most importantly protection from abiotic stress (such as light, temperature, and drought) and biotic stress (such as herbivore, insect or pathogen, and nematode attack). On the other hand, fungal endophytes indirectly promote plant growth by synthesizing special substances mainly secondary metabolites, hormones, and enzymes whenever necessary or in accordance with the particular signaling (Barz et al. 1988).

The endurance and conservation of endophytic communities of fungi can also be affected by the type of host proliferation or propagation methods. Normally, endophytic fungi could be distributed in the entire plant as it grows and would then probably return to the soil through decomposition of senesced or accidently damaged parts. Thus, vegetatively propagated plants may be a home for an enduring community of endophytic microorganisms that would further transmit to succeeding progeny or generations. Similarly, seeds could also be a source of endophytic microorganisms. The interaction remains asymptomatic as long as the endophytic potential and plant defense are balanced. The imbalanced condition of host plant and endophyte interaction would either result in disease in the host plant if the host is susceptible or the plant defense mechanism kills or removes the pathogenic

endophytic fungus (Schulz and Boyle 2005). In general status of the partners, virulence of the fungi or other endophytes, and the resistance or defense of the host plant determine the interaction as either healthy or unhealthy.

Endophytes have evolved a resistance mechanism by overcoming pathogenic invasion by synthesizing secondary metabolites belonging to several structural classes such as alkaloids, peptides, terpenoids, steroids, phenols, quinines, etc. (Tan and Zou 2001; Yu et al. 2010). A study by Taylor and Taylor (2000) reported that endophytic association evolved hundreds of millions of years ago during the evolutionary process of the plants on the earth while compiling the information on the fossilized plant. Furthermore, fossil studies also focus lights on coevolution of plants and endophytic fungi. The endophytic fungi therefore are adapted to the special microenvironments in the host plants and the surrounding soil through exhibiting genetic variations within the species. The possibilities of genetic material exchanges between the host plants and fungi or activation of some segments of host plant DNA or vice versa would probably result in the biosynthesis of several new phytochemicals or various biochemical defense mechanisms by the endophytic fungi and host (Zhang et al. 2006; Stierle et al. 1993).

13.4 Biological Role of Endophytes

Endophytes may facilitate plant growth directly or indirectly. The direct process is through the production of phytohormones (auxin or cytokinin) or the enzyme 1-aminocyclopropane-1-carboxylate deaminase, which is known to lower the plant's ethylene level. The indirect mechanism is through enhancing the plant's systemic resistance or preventing pathogenic infections through the production of secondary metabolites and siderophores. Endophytes also help the plants to acquire nutrients through nitrogen fixation and phosphate solubilization. The systemic growth of endophytes inside the plants helps the plants to overcome several physical stresses (Bacon 1993; Faeth and Fagan 2002).

13.5 Natural Products and Traditional Approaches in Medicine

Application of naturally derived products and traditional drug use has gone a long way in the applied studies related to agriculture and medicine. Naturally derived products have its importance due to its diverse nature in structure and the multiple actions of its active compounds against various diseases. Hence, medicinal plants therefore become the major targets to study the endophyte associations. Few study reports have shown that the bioactive compounds isolated from the medicinal plants and the associated endophytes have similarity in structures (Tan and Zou 2001). Since these microbes are easily isolatable, the pharmaceutical industries are now exploiting the endophytes for a large-scale production of an active compound useful for a medicine rather than depending on the endangered or endemic important

plants. Furthermore endophytes are believed to be potent sources for novel and natural compounds.

The discovery of potential anticancer drug "Taxol" (a tetracyclic lactam) is a well-known microtubule inhibitor isolated first from yew tree species (*Taxus brevifolia*). One of the breakthroughs in medical field had certain restriction in commercial production of the drug from the plant source alone due to its massive destruction for the drugs. Stierle et al. (1993) have later isolated and characterized a novel Taxol-producing fungus *Taxomyces andreanae*, an endophyte isolated from the same plant *Taxus brevifolia*. This was the platform for more researchers to explore endophyte-based drugs from the folk medicinal plants and their bioactive metabolites (Verma et al. 2007; Huang et al. 2008; de Siqueira 2011). Later, Taxol-producing endophytes have been isolated even from non-*Taxus* cypress species, *Wollemia* pines, and so on. Researches now indicate that the bioactive compounds that are originally isolated from plants are initiated by their mutualistic endophytes. *Muscodor vitigenus* is considered as a novel endophytic fungus isolated from *Paullinia paullinioides* which is capable of producing naphthalene, an insect repellent (Daisy et al. 2002). This finding gave an opportunity to explore for the endophytic microbes capable of producing repellents, vermicides, insecticides, antimicrobials, and antioxidants (Tan and Zou 2001; Strobel and Daisy 2003; Strobel et al. 2007).

Coffee berry borer is considered one of the main overwhelming pests against coffee across the globe. A study by Vega et al. (2008) found that the fungal entomopathogens such as *Beauveria bassiana* and *Clonostachys rosea* are pathogenic to coffee berry borer. The metabolite produced by such microorganism may have detrimental effects on pests. Currently, many plants, epiphytes, and orchids have been studied for their endophytic fungi, and almost all of them have been proven to be loaded with endophytic fungi (Sudheep and Sridhar 2011). Several novel and priceless bioactive compounds with innumerable functions such as antioxidant, antimicrobial, larvicidal, insecticidal, cytotoxic, anticancer, and growth regulator activities have been recorded from the endophytic fungi (Ten et al. 2004).

13.6 Application of Fungal Endophytes in Biotechnological Processes

Endophytes produce bioactive secondary metabolites with unique structure, such as alkaloids, flavonoids, phenolics, quinones, steroids, terpenoids, etc. (Tan and Zou 2001). Such bioactive metabolites show a large range of applications in agrochemical, antibiotic, antiparasitic, antioxidant, insecticidal, and anticancer agents (Strobel and Daisy 2003). Apart from that, endophytes produce various novel and known enzymes which have a crucial role in different biotechnological processes and applications (Pimentel et al. 2011).

13.7 Fermentation Technology

Fermentation has been generally used for manufacture of a broad variety of substances in bulk quantity that are advantageous in several industries. For the past few years, fermentation method has gained momentum due to their cost-effective production and other environmental advantages. Most of the older techniques have been now personalized further and sophisticated to increase the efficiency and the yield of fermentation industries (Cherry and Fidantsef 2003; Dutta et al. 2010). Fermentation technology is concerned with the large-scale culturing of microorganisms in fermenters and the recovery of valuable yield from the microbial cells or from the surrounding medium in released form. For an optimum fermentation, the fungus must behave normally and render unsurprising activity and perform consistency in growth rates and metabolite production. The basic requirement for any type of fermentation is a nutrient-rich medium which contains compatible sources of carbon, nitrogen, and added essential elements in suitable proportions. The critical part of a fermentation process is the identification and the use of a suitable microorganism which secretes a valuable product. Two general fermentation techniques commonly used in the industries are submerged fermentation (SmF) and solid-state fermentation (SSF) (Machado et al. 2004; Cherry and Fidantsef 2003; Subramaniyam and Vimala 2012).

13.8 Solid-State and Submerged Fermentations

Solid-state fermentation (SSF) is known as the fermentation process in which microorganisms grow on a solid substrate or without the presence of free liquid (Fig. 13.1). SSF has great importance and offers plentiful openings in processing of agro-industrial residues. This is partly because solid-state processes have lower energy requirements, produce less amount of water, and are environmentally friendly as they resolve the problem of solid waste disposal. Submerged

Fig. 13.1 Preparation of solid substrate fermentation on vegetable substrate

fermentation (SmF) involves fermentation of a substrate in the presence of a free liquid. The substrate is therefore dissolved or suspended in a large amount of water. In watery situation, fungi grow as pellets or as gratis mycelia, subject to the type of strain and culture conditions. Each form has its own characteristics, which will greatly affect the process yields. In recent years, many studies have been made in order to succeed improved productivity (Cherry and Fidantsef 2003; Dutta et al. 2010).

SSF technique is best suited for fungi that require less moisture content, but it cannot be used in case of bacterial fermentation processes since that require high water activity (Babu and Satyanarayana 1996). Purification and extraction of products are convenient in SSF, while SmF is chiefly used in the mining of secondary metabolites which work in liquid medium (Subramaniyam and Vimala 2012). SSF supersedes SmF in various aspects such as fewer requirements of space and energy, simplicity of media, and high product yields (Raimbault and Alazard 1980). Interestingly in another study, Oda et al. (2006) concluded that certain enzymes were selectively secreted under conditions of solid-state culture or in submerged culture, independent of the composition of the medium.

In recent years, researchers have started focusing on bioactive compounds originated from the endophytic fungi and improving the efficiency of some prospective candidates through genetic engineering in the microbial fermentation (Strobel et al. 2004). The pH and temperature in the fermentation medium are the important aspects that have profound influence on the production of the end product.

13.9 General Aspects on Enzymes

Cellulose is the most abundant linear polymer of polysaccharides of glucose residues with beta-1,4-glycosidic linkages. Cellulose is one of the major ingredients in the solid municipal waste; the majority of it comes from food processing, timber, paper, and sugarcane industries (Shoemaker et al. 1983). In cellulolytic system, cellulose is converted to multi-utility product glucose (Gupta et al. 2011). Eubacteria and fungi are the primary cellulolytic microorganisms followed by some anaerobic protozoa and slime molds. Synergistic interaction between cellulolytic and non-cellulolytic microorganisms present in the waste leads to complete degradation of cellulose, with the release of carbon dioxide and water under aerobic conditions and carbon dioxide, methane, and water under anaerobic conditions (Leschine 1995). Cellulase and its substantial usages have been used in different industrial processes such as bioethanol, agricultural, and plant waste management, pulp and paper industry (Buchert et al. 1996; Wang et al. 2004), textile industry (Gusakov et al. 2000), detergent industry, and food industry. Cellulase enzymes are even known for its great role in improving digestibility of animal feeds (Lewis et al. 1996).

Among the various commercialized enzymes, many of them are products of fermentation of filamentous fungi (Piccoli-valle et al. 2001). Filamentous fungi are particularly an interesting group of organisms due to their easiness of handling and cultivation, fast growth, and high production of extracellular enzymes of large

industrial potential (Singh et al. 2003). Fungal enzymes implicated in plant-related polysaccharide degradation belong to at least 35 families of glycoside hydrolase, constituting of carbohydrate esterase families (three in number), and six polysaccharide lyase families (Battaglia et al. 2011; Coutinho et al. 2009). Studies on cellulases and related polysaccharides are initiated in the early 1950s, and it has outstanding prospective to change lignocellulose, the most plentiful and unexplored renewable source of energy on earth, to glucose and soluble sugars (Reese and Mandels 1984; Coughlan 1985). However, the usages of cellulases and hemicellulases are initiated in the early 1980s, initially in animal feed followed by different food-related applications (Thomke et al. 1980; Voragen 1992). The rate and efficiency of biomass degradation by different fungi vary greatly. They produce different kinds of lytic enzymes in diverse amounts and concentrations. Most of the fungi have its own uniqueness in producing degradative enzymes, but in some case, in relation to the surrounding medium, fungi behave or act unusually by its capacity.

13.9.1 Factors Affecting Enzyme Production and Activity

The rate of production of enzymes can vary with certain basic factors such as temperature, pH, presence of supplements in the medium, and incubation period. For instance, *Aspergillus terreus* showed the best cellulose activity in pH 4–7 and at temperature approximately 40 °C (Naghavi et al. 2013). In *Penicillium chrysogenum*, the optimum pectinase activity was found to be highest at pH 6.5. Supplementation of sucrose to the production medium increased the pectinolytic activity of *P. chrysogenum*; however, the production rate was highly repressed in the presence of starch. Of the different nitrogen sources used, ammonium persulfate has enhanced the production of *P. chrysogenum* pectinase (Banu et al. 2010). Enzyme production has also been found to be influenced by the incubation period. According to Green et al. (1989), α-amylase production peak was obtained in 4-day-old culture that declined gradually to 7-day-old culture. Similarly, α-amylase activity from *Aspergillus niger* (Hernandez et al. 2006) and *Aspergillus oryzae* (Tiwari et al. 2007) was obtained at 3 days of cultivation. This may be because of the denaturation of the enzymes since the third day of culture due to the production of other compounds in the culture medium (Ramachandran et al. 2004).

The presence of carbon, nitrogen sources, and mineral nutrients such as phosphorous, potassium, magnesium, and calcium is essential for the growth of fungi as well as enzyme production (Hughes and Poole 1991). The optimum growth conditions for enzyme production by *A. oryzae* were pH 5.0 and 35 °C. The alpha amylase production was found to be tolerant to a wide range of initial pH values (4.0–10) and temperature (25–42 °C).

13.9.2 Fungal Species and Enzyme Production

Filamentous fungi are remarkably significant organisms having an effect on the lives of many other higher-order organisms and communities. *Aspergillus*, *Penicillium*, *Fusarium*, etc. are included in higher filamentous fungi group, whereas *Mucor*, *Rhizopus*, etc. are included in lower filamentous fungi group. The commercially exploited fungi by various industries include *Alternaria* sp., *Trichoderma* sp., *Penicillium* sp., and *Fusarium* sp. (Schuster and Schmoll 2010; Kang et al. 2004).

13.9.2.1 *Aspergillus*

As with fungi in general, *Aspergillus* taxonomy is complex and ever evolving. The genus is easily identified by its character, conidiophore. *Aspergillus* is also well known for its opportunistic pathogenic activities on animals and human beings; for example, aspergillosis disease incidence becomes common in the immune-susceptible populations. The genus *Aspergillus* produces a large number of traditional fermented foods which includes soy sauce, soybean paste, and rice wine since the ancient years (Bennett 2001). The species such as *A. versicolor*, *A. oryzae*, *A. tamari*, *A. niger*, *A. tamarii*, *A. awamori*, and *A. oryzae* have been used widely in the manufacture of bioactive compounds and hydrolytic enzymes. *A. niger* (Fig. 13.2a), another saprobic filamentous fungi, has been used to produce citric acid in the food production units, beverage industry, and pharmaceutical industry. *A. niger* is also an excellent producer of a wide spectrum of extracellular enzymes. *Aspergillus* species are considered as the primary agents of decomposition because of its ability to produce *cellulase* enzymes (Oxenboll 1994).

On the other hand, different kinds of mycotoxins are produced by many species of *Aspergillus*. *A. flavus* (Fig. 13.2b) and *A. parasiticus* that generate aflatoxins are considered detrimental to humans and animals (Horn et al. 1995). *A. niger* has the skill to manufacture secondary metabolites such as ochratoxin, another deleterious toxin having nephrotoxic, genotoxic, teratogenic, and carcinogenic actions. *Aspergillus niger* derived enzymes are extensively used in manufacturing for the reason that this strain is considered having a generally recognized as safe (GRAS) status so that most of the metabolites formed by this strain can be used safely. This fungal strain is a good producer of pectinase enzymes.

El-Safey and Ammar (2004) purified and characterized α-amylase isolated from *Aspergillus flavus* var. *columnaris*. Findings reveal that the α-amylase activity increases with the concentration of the enzyme. The most favorable substrate concentration (starch) and incubation temperature for this bioactivity are 0.2% (w/v) and 35 °C, respectively. Additionally, purified α-amylase enzyme has the maximum activity at pH 6.2 and after an incubation period of 30 h.

13.9.2.2 *Penicillium*

Penicillium is a genus of ascomycete anamorphic fungi having greater value for food and drug production (Fig. 13.2c). Numerous species of the genus *Penicillium* play a crucial role in the manufacture of cheese and meat. In addition to their greater role in food industry, species of *Penicillium* are also known producers of various

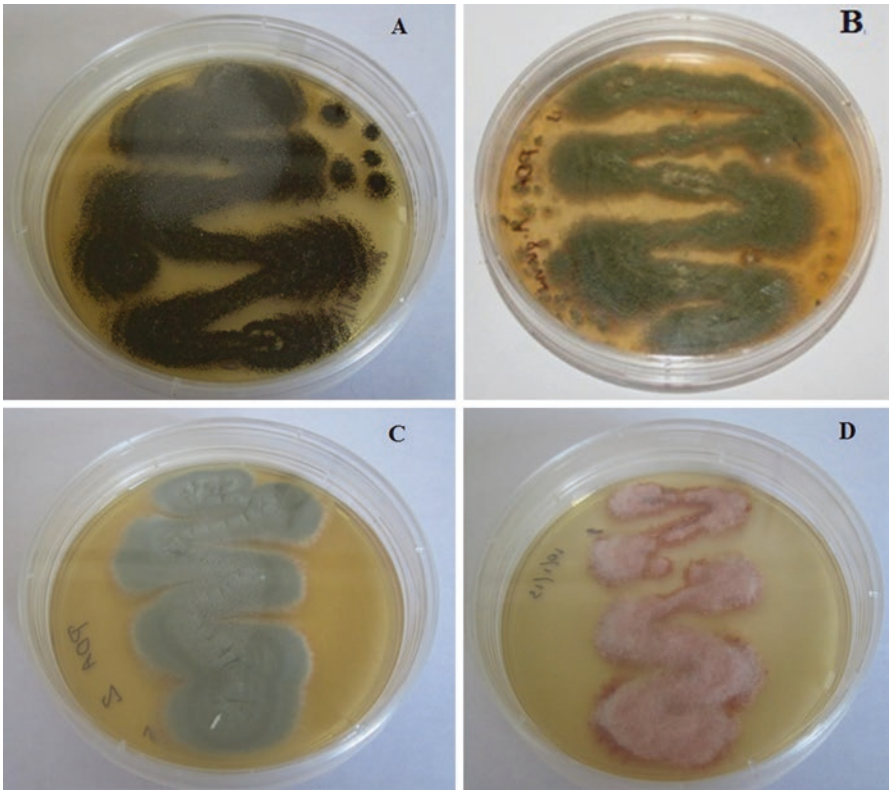


Fig. 13.2 Fungal isolates on PDA media: (**a**) *A. niger*, (**b**) *A. flavus*, (**c**) *Penicillium* sp., and (**d**) *Fusarium* sp.

important enzymes and macromolecules such as gluconic, citric, and tartaric acids, as well as several pectinases, lipases, amylases, cellulases, and proteases (Leitao 2009).

A number of *Penicillium* species, such as *P. purpurogenum*, *P. funiculosum*, and *P. emersonii* (*Talaromyces*), are extraordinary producers of cellulases, hemicellulases, and pectinases. Commercially, *P. funiculosum* has been used to generate a cocktail of enzymes which could degrade composite agricultural residues like arabinoxylan, cellulose, hemicellulose, and protease in it. Pectinase enzyme from *P. chrysogenum* has been characterized by submerged fermentation. In their study, enzyme production by *Penicillium chrysogenum* was found higher at pH 6.5 and at a temperature of 35 °C using sucrose and ammonium persulfate as carbon source and nitrogen source, respectively.

13.9.2.3 *Fusarium*

Fusarium is a filamentous fungus. It is considered as fungal-plant pathogens which cause significant crop losses and contamination of grain by mycotoxins (Fig. 13.2d).

F. graminearum is the contributory agent of some important plant diseases. Many other *Fusarium* strains are used in fermentation processes, including production of single-cell protein permitted for human consumption, and some of these strains may have the capacity for the production of enzymes. *Fusarium oxysporum* produces many enzymes which act upon cellulose and pectic components of the cell wall (Apel-Birkhold and Walton 1996). Production of xylanolytic and cellulolytic enzymes *by Fusarium oxysporum* beneath solid-state culture (SSC) on corn stover substrate was enhanced by optimization of the type of nitrogen source, initial moisture, temperature, and pH of the culture medium (Nwagu et al. 2012).

13.9.2.4 *Alternaria*

Alternaria is an ascomycete fungal genus and found to be a common allergen in human beings and a serious plant pathogen. Wipusaree et al. (2011) characterized a xylanase from the endophytic fungus *Alternaria alternate*. In this study, they have accomplished optimal xylanase production after 4 days of culture with 2% (w/v) rice bran and 0.1% (w/v) ammonium sulfate as the carbon and nitrogen source, respectively.

13.9.3 Common Fungal Enzymes and Application

Extracellular enzymes of the organisms are used for industrial purposes. The isolation and characterization of these enzymes are crucial steps in biotechnology. To date, enzymologists have turned their attention to fungi as a source of enzymes because such enzymes are cheaper and quick in their action (Zambare et al. 2011; Anitha and Palanivelu 2013).

Different fungal and bacterial strains have extensively been used for the commercial enzyme production. Fungi are excellent producers of extracellular enzymes and probably all classes of industrially important enzymes. Cellulase is one of the major enzymes used in industries. Filamentous fungi are capable to exude big amount of extracellular protein that qualifies them in industrial enzyme production. However, the cost of substrates used for the cellulase production using fungi and rather slow growth rate of fungi are often projected as limitations to depend on fungi always. Besides bacteria have more growth rate in contrast to fungi which make them a potential candidate in cellulase production. However, the lack of FPase activity in bacterial cellulose limits their use also in some industries. However, bacteria can be easily genetically engineered to enhance cellulase production (Mahadevan et al. 2008; Escovar-Kousen 2004). The enzymes produced by different fungal species are mainly hydrolytic in nature. Among these enzymes, cellulolytic enzymes require great importance because of its massive applicability in various biological and industrial processes.

13.9.3.1 Cellulase

Cellulase consists of three-enzyme system which has soluble extracellular natural enzymes, 1,4-β-endoglucanase, 1,4-β-exoglucanase, and β-1,4-glucosidase.

Endoglucanase triggers the random cleavage of β-1,4-glycosidic bonds present in the chain form of cellulose, whereas exoglucanase helps in the cleavage of the non-reducing end of a cellulose chain, and β-1,4-glucosidase enzyme translates glucosidase hydrolyse cellobiose and soluble dextrin to glucose.

Interestingly, it is reported that a cocktail of the abovementioned three enzymes is essential for the absolute hydrolysis of glucose (Ryu and Mandels 1980). Cellulase enzyme has its importance due to its specific role and application potential in lignocellulosic conversion (Ilyas et al. 2011). Wood, grass, agricultural wastes, and solid municipal wastes are the main sources of lignocelluloses in nature. Irrespective of the substrate, lignocellulosic materials consist of cellulose (a homopolymer of glucose), hemicellulose (a heteropolymer of hexoses and pentoses), and lignin (an amorphous polymer of phenylpropanoid units).

13.9.3.2 Amylases

It is a group of extracellular enzymes which degrades starch. Amylase hydrolyzes the bonds between the adjacent glucose units in the starch molecule. Amylases have application in food industry especially for baking, in dairy industry, and in detergent, fermentation, and paper industries. Microbial amylases are used in biological hydrolysis of starch due to its effectiveness in consistency, easiness of handling, and optimization of desired environment, and chemical catalysts are no longer used (Mamo and Gessesse 1999) (Fig. 13.3).

13.9.3.3 Xylanases

Xylan is a multifaceted polysaccharide consisting of xylose residues which are joined by β-1,4-glycosidic bonds. The main chain of xylan is composed of β-xylopyranose residues. Xylan is the major hemicellulosic polysaccharide present in cell walls of plants signifying up to 30–35% of the total dry weight. Xylanases are widely used in various industries; for instance, it is used to pre-bleach the pulps for paper manufacture; improve the nutritional qualities of wheat; clarify the juices and wines; extract juices, oils, spices, and pigments; and adjust the cereal flours to enhance the texture and volume of the bread (Gilbert and Hazlewood 1993) (Fig. 13.4).

13.9.4 Enzyme Assay

Enzyme assays are essential tools for enzyme engineering, where they present the functional foundation for identifying and selecting novel enzymes, generally by screening a large group of microorganism collection from different sources or by several modern techniques such as genetic recombination methods and creation of enzyme mutants. Enzyme assays are vital for the study of enzyme kinetics and enzyme inhibition (Escovar-Kousen 2004). Continuous assays include spectrophotometry, fluorometry, calorimetry, chemiluminescent, and light scattering studies. Discontinuous assays include radiometric and chromatographic assays. In the case of spectrophotometric analysis, one pursues the course of the reaction by computing

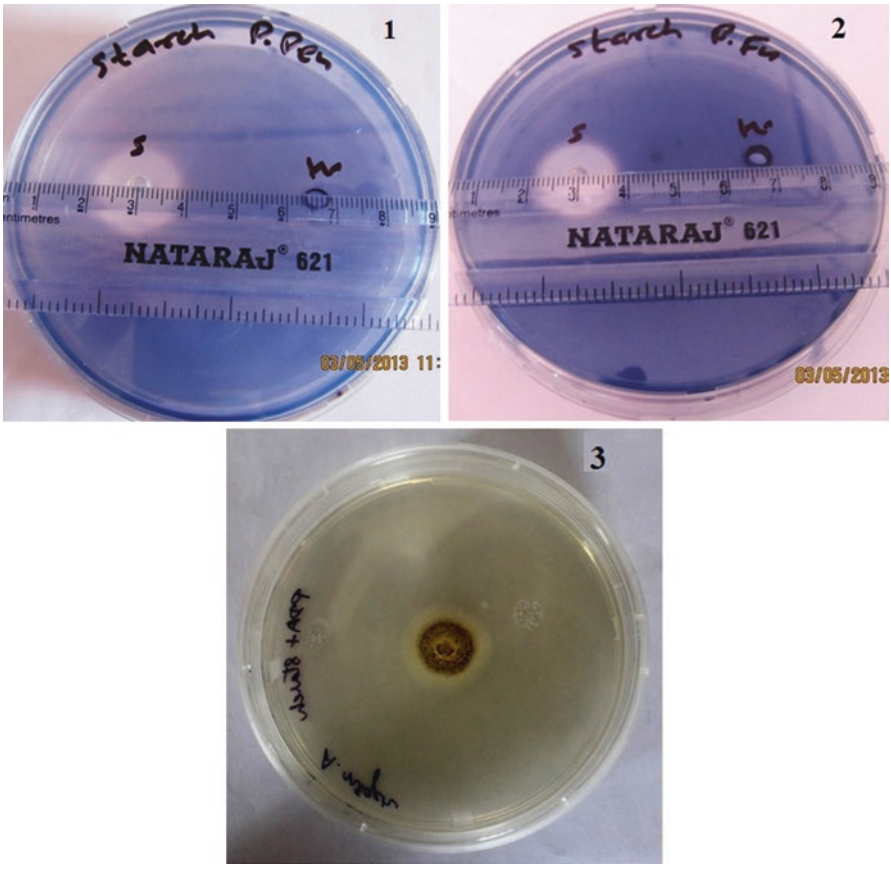


Fig. 13.3 Enzyme activity screening of fungal isolates; zone of clearance on starch plate (amylase activity by *Penicillium* sp., *Fusarium* sp., *A. niger*)

the change in the amount of light absorbed in the assay solution. If this light is in the visible region, one can actually see a change in the color of the assay. UV light is frequently used, as the general coenzymes NADH and NADPH absorb UV light in their reduced forms, but not in their oxidized forms. An oxidoreductase using NADH as a substrate could therefore be assayed by following the decrease in UV absorbance at a wavelength of 340 nm as it consumes the coenzyme (Bergmeyer 1974).

13.9.4.1 Assay Methods for Different Enzymes

Cellulase generates glucose via carboxymethyl cellulose which is identified calorimetrically with alkaline copper reagent (Robyt and Whelan 1972). Xylanase activity can be known by measuring the amount of reducing sugar using dinitrosalicylic acid (DNS) method where D-xylose acts as a standard and xylan as substrate (Kumar et al. 2010) (Fig. 13.5a, b).

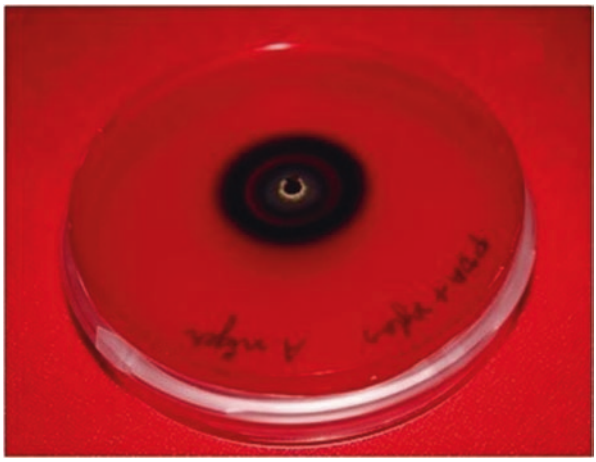


Fig. 13.4 Enzyme activity screening of fungal isolates; zone of clearance on xylan plate (xylanase activity by *A. niger*)

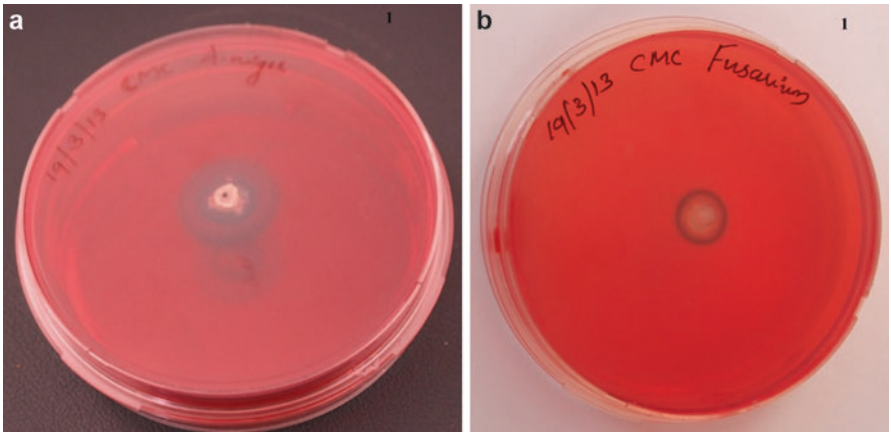


Fig. 13.5 Enzyme activity screening of fungal isolates; zone of clearance on CMC plate (**a** cellulase activity by *A. niger*; **b** cellulase activity by *Fusarium* sp.)

The hydrolysis of pectic acid is done by pectinase which liberated D-galacturonic acid. Alkaline copper reagent is used to identify D-galacturonic acid (Rexova-Benkova 1973). Moreover, the protease activity is characterized by the hydrolysis of casein (dimethyl). The generated amino acids were characterized by 2,4,6-trinitrobenzene sulfonic acid (Lin et al. 1969).

13.10 Conclusion

Host plant resistance against pathogens by the action of endophytes has been revealed by many researchers. Hence, obviously, endophyte is able to produce many metabolites of industrial importance. The available information from research and

lab experiences focus further on the good antimicrobial activity of isolated endophytes. Moreover, changes in pH and temperature have also given significant enzyme production by the isolated endophytes (Pandey 2003; Sato and Sudo 1999). There are opportunities to increase the enzyme production by altering the physical condition, media, nutrient ratio, etc. Once the optimum pH and temperature for the making of a specific enzyme is understood, one could alter the ratio or addition of minerals and nutrients for an optimum yield of enzymes. But if not properly conducted, altering the substrate or carbon source may sometimes affect the proper functioning of fungi leading to the minimal production of enzymes. Solid-state fermentation would be an ideal and efficient technique that researchers are interested in. Plate method is a simple and quick method to establish the enzymatic activity of a given microorganism by studying the clear zone around the inoculated microorganism (Gusakov et al. 2000; Hernandez et al. 2006). Thus, it helps to study in detail about the effect of different parameters on the productivity of industrially important enzymes. Almost all the enzymes have its great potential in industry. Due to their microbial nature, it is often easy to genetically engineer them to optimize the production of the enzymes from a given microorganism. Interestingly, reports are available that gibberellic acid and many other hormones are produced during the submerged and solid-state fermentation by endophytic fungi.

Such reports confirm the greater role of endophytic fungi as a plant growth promoter. Natural product chemistry would be greatly advantaged from the inventory of novel compounds from the natural sources. Studies indicate that endophytic microorganisms, particularly fungi, have greater potential to contribute to the world of natural product chemistry, agriculture, and several commercial industries (Coughlan 1985; Oxenboll 1994; Tan and Zou 2001; Pimentel et al. 2011). Endophytic fungi, since they live in host plant, produce compounds of similar nature of what is produced by the plants. In the past few decades, scientists focused generally on the exploration of endophytic fungal diversity, associations between endophytic fungi and their host plants, etc. Recently, researchers have started focusing on researches leading to the production of secondary metabolites, enzymes, and their possible applications. Similarly, information on the entophytic community associated with the endemic and IUCN red listed medicinal plants will help us to frame a way forward for the commercial synthesis of bioactive compounds with limited exploitation of the plant from the wild. Seed dormancy is another critical process in the plant recruitment in stressful environments (Copland 1981; Latch 1983). Dormancy may be perhaps imposed due to the seed coat or due to the changes in growth inhibitor and promoter ratio. Reports suggest that colonization of fungal endophytes can break the dormancy of the seeds by working as a growth promoter and seed coat decomposer. Even though there has been an increasing concern for endophytic fungi which act as a basis of novel bioactive compounds potentially useful in medicine, agriculture, and industry, still the precise ecological role is poorly understood or less known.

Acknowledgments The authors are grateful to Central University of Kerala for providing lab facilities and permission to carry out this study at the Department of Plant Science, School of

Biological Sciences. NMS is indebted to the DST-SERB, Delhi, for the award of research fellowship under Fast Track Young Scientist Scheme.

References

Anitha TS, Palanivelu P (2013) Purification and characterization of an extracellular keratinolytic protease from a new isolate of *Aspergillus parasiticus*. Protein Expr Purif 88:214–220

Apel-Birkhold PC, Walton JD (1996) Cloning, disruption, and expression of two endo β-1, 4 xylanase genes, XYL2 and XYL3, from *Cochliobolus carbonum*. Appl Environ Microbiol 62:4129–4135

Babu KR, Satyanarayana T (1996) Production of bacterial enzymes by solid state fermentation. J Sci Ind Res 55:464–467

Bacon CW (1993) Abiotic stress tolerances (moisture, nutrients) and photo-synthesis in endophyte infected all fescue. Agric Ecosyst Environ 44:123–141

Banu R, Devi MK, Gnanaprabhal GR, Pradeep BV, Palaniswamy M (2010) Production and characterization of pectinase enzyme from *Penicillium chrysogenum*. Indian J Sci Technol 3:4

Bary D, Morphologie A, der Pilze P (1886) Flechten and Myxomyceten, *Holfmeister's* Handbook of Physiological Botany, vol 2. Leipzig, Germany

Barz W, Daniel S, Hinderer W, Jaques U, Kessmann H, Koster J, Tiemann K (1988) In: Pais M, Mavituna F, Novais J (eds) Plant cell biotechnology. Springer (NATO ASI series), Berlin, pp 211–213

Battaglia E, Benoit I, Van Den Brink J, Wiebenga A, Coutinho PM, Henrissat B, De Vries RP (2011) Carbohydrate active enzymes from the zygomycete fungus *Rhizopus oryzae*: a highly specialized approach to carbohydrate degradation depicted at genome level. BMC Genomics 12:38

Bennett JW (2001) Aspergillus and koji*:* history, practice and molecular biology. Soc Ind Biol News 51:65–71

Bergmeyer HU (1974) Methods of enzymatic analysis 4. Academic, New York, pp 2066–2072. ISBN:0-89573-236-X

Buchert J, Carlsson G, Viikari L, Strom G (1996) Surface characterisation of unbleached kraft pulps by enzymatic peeling and ESCA. Holzforschung 50:69–74

Cheplick GP, Clay K (1988) Acquired chemical defenses in grasses; the role of fungal endophytes. Oikos 52:309–318

Cherry JR, Fidantsef AL (2003) Directed evolution of industrial enzymes: an update. Curr Opin Biotechnol 14:438–443

Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10–16

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160:S99–S127

Copland LO (ed) (1981) Rules for testing seeds. J Seed Technol 6:1–125

Coughlan M (1985) Cellulases: production, properties and applications. Biochem Soc Trans 13:405–406

Coutinho PM, Andersen MR, Kolenova K, vanKuyk PA, Benoit I, Gruben BS, Trejo-Aguilar B, Visser H, Solingen P, Pakula T, Seiboth B, Battaglia E, Aguilar-Osorio G, Jong JF, Ohm RA, Aguilar M, Henrissat B, Nielsen J, Stalbrand H, Vries RP (2009) Post-genomic insights into the plant polysaccharide degradation potential of *Aspergillus nidulans* and comparison to *Aspergillus niger* and *Aspergillus oryzae*. Fungal Genet Biol 46(Suppl 1):S161–S169

Daisy HB, Strobel GA, Castillo U, Ezra D, Sears J, Weaver DK, Runyon JB (2002) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. Microbiology 148:3737–3741

De Siqueira VM, Conti R, de Araújo JM, Souza-Motta CM (2011) Endophytic fungi from the medicinal plant *Lippia sidoides* Cham. and their antimicrobial activity. Symbiosis 53:89–95

Dutta A, Dowe N, Ibsen KN, Schell DJ, Aden A (2010) An economic comparison of different fermentation configurations to convert corn stover to ethanol using *Z. mobilis* and Saccharomyces. Biotechnol Prog 26:64–72

Elmi AA, West CP (1995) Endophytic infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. New Phytol 131:61–67

El-Safey Ammar MS (2004) Purification and characterization of α-amylase isolated from *Aspergillus flavus* var. *columnaris*. e. m. Ass Univ Bull Environ Res 7:1

Escovar-Kousen JM, Wilson D, Irwin D (2004) Integration of computer modeling and initial studies of site-directed mutagenesis to improve cellulase activity on Cel9A from *Thermobifida fusca*. Appl Biochem Biotechnol 113–116:287–297

Faeth SH, Fagan WF (2002) Fungal endophytes: common host plant symbionts but uncommon mutualists. Integr Comp Biol 42:360–368

Gilbert HL, Hazlewood GP (1993) Bacterial cellulase and xylanase. J Gen Microbiol 139:187–194

Green F, Clausen CA, Highley TL (1989) Adaptation of the Nelson Somogyi reducing-sugar assay to a microassay using microtiter plates. Anal Biochem 182:197–199

Gupta P, Samant K, Sahu A (2011) Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Int J Microbiol. doi:10.1155/2012/58925

Gusakov AV, Sinitsyn AP, Berlin AG, Markov AV, Ankudimova NV (2000) Surface hydrophobic amino acid residues in cellulase molecules as a structural factor responsible for their high denim-washing performance. Enzym Microb Technol 27:664–671

Hernandez MS, Rodriguez MR, Guerra NP, Roses RP (2006) Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. J Food Eng 73:93–100

Horn BW, Greene RL, Dorner JW (1995) Effect of corn and peanut cultivation on soil populations of *Aspergillus flavus* and *A. parasiticus* in southwestern Georgia. Appl Environ Microbiol 61:2472–2475

Huang WY, Cai WZ, Hyde KD, Corke H, Sun M (2008) Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Divers 33:61–75

Hughes MN, Poole RK (1991) Metal speciation and microbial growth – the hard and soft facts. J Gen Microbiol 137:725–734

Hyde KD, Bussaban B, Paulus B, Crous PW, Lee S, Mckenzie EHC, Pathotita W, Lumyong S (2007) Biodiversity of saprobic fungi. Biodivers Conserv 16:17–35

Ilyas U, Majeed A, Hussain K, Nawaz K, Ahmed S, Nadeem M (2011) Solid state fermentation of *Vigna mungo* for Cellulase production by *Aspergillus niger*. World Appl Sci J 12(8):1172–1178

Kang SW, Park YS, Lee JS, Hong SI, Kim SW (2004) Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour Technol 91:153–156

Kumar G, Karthik L, Rao KVB (2010) Antimicrobial activity of latex of *Calotropis gigantea* against pathogenic microorganisms – an in vitro study. Pharmacol Online 3:155–163

Latch GCM (1983) Incidence of endophytes in seed lines and their control with fungicides. Proc N Z Grassl Assoc 44:251–253

Leitao AL (2009) Review potential of Penicillium species in the bio remediation field. Int J Environ Res Public Health 6:1393–1417

Leschine SB (1995) Cellulose degradation in anaerobic environments. Annu Rev Microbiol 49:399–426

Lewis GE, Hunt CW, Sanchez WK, Treacher R, Pritchard GT, Feng P (1996) Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J Anim Sci 74:3020–3028

Li J, Zhao GZ, Chen HH, Wang HB, Qin S, Zhu WY, Xu LH, Jiang CL, Li WJ (2008) Anti tumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. Lett Appl Microbiol 47:574–580

Lin YC et al (1969) Action of proteolytic enzymes on N, N-dimethyl proteins. Basis for a microassay for proteolytic enzymes. J Biol Chem 244:789–793

Machado CM, Oishi BO, Pandey A, Soccol CR (2004) Kinetics of *Gibberella fujikuroi* growth and gibberellic acid production by solid state fermentation in a packed-bed column bioreactor. Biotechnol Prog 20:1449–1453

Mahadevan SA, Wi SG, Lee DS, Bae HJ (2008) Site-directed mutagenesis and CBM engineering of Cel5A (*Thermotoga maritima*). FEMS Microbiol Lett 287(2):205–211

Mamo G, Gessesse A (1999) Effect of cultivation conditions on growth and alpha production. Biotechnol Tech 11:447–450

Marquis RJ (1992) The selective impact of herbivores. Plant resistance to herbivores and pathogens. Ecology, evolution, and genetics, RS Fritz, EL Simms, 301–325. Chicago: Univ. Chicago Press

Naghavi N, Emtiazi G, Karimian N (2013) Partial purification and immobilization of cellulose enzymes from the fungus *Aspergillus terreus* isolated from rotten wood. J Life Sci Technol 1:1

Nwagu KE, Ominyi MC, Nwoba GE (2012) Isolation, screening and measurement of amylase and cellulase activities of some microorganisms. Cont J Biol Sci 5(1):37–41

Oda K, Kakizono D, Yamada O, Lefuji H, Akita O, Iwashita K (2006) Proteomic analysis of extra cellular proteins of *Aspergillus oryzae* grown under submerged and solid state culture conditions. Appl Environ Microbiol 72:3448–3457

Oxenboll K (1994) In: Powell KA, Renwick A, Peberdy JF (eds) *Aspergillus* enzymes and industrial uses: in the genus *Aspergillus* from taxonomy and genetics to industrial application. Plenum Press, New York, pp 147–154

Pandey A (2003) Solid state fermentation. Biochem Eng J 13(2/3):81–84

Petrini O (1991) Fungal endophytes in tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 179–197

Piccoli-Valle RH, Silva DO, Passos VJF, Passos LMF (2001) Production of pectin lyase by *Penicillium griseoroseum* in bioreactors in the absence of inducer. Braz J Microbiol 32(2):135–140

Pimentel MR, Molina G, Dionisio AP, Junior MRM, Pastore GM (2011) The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Int 1:11

Raimbault M, Alazard D (1980) Culture method to study fungal growth in solid fermentation. Eur J Appl Microbiol Biotechnol 9:199–209

Ramachandran S, Patel AK, Nampoothiri KM, Chandran S, Szakacs G, Soccol CR, Pandey A (2004) Alpha amylase from a fungal culture grown on oil cakes and its properties. Braz Arch Biol Technol 47:309–317

Raps A, Vidal S (1998) Indirect effects of an unspecialized endophytic fungus on specialized plant-herbivorous insect interactions. Oecologia 114:541–547

Reese ET, Mandels M (1984) Rolling with the time: production and applications of *Trichoderma reesei* cellulase. Ann Rep Ferment Process 7:1–20

Rexova-Benkova L (1973) The size of the substrate-binding site of an *Aspergillus niger* extracellular endopolygalacturonase. Eur J Biochem 39:109–115

Robyt JF, Whelan WJ (1972) Reducing value methods for maltodextrins: 1. Chain length dependence of alkaline 3,5-dinitrosalicylate and chain length independence of alkaline copper. Anal Biochem 45:510–516

Ryu DD, Mandels M (1980) Cellulases: biosynthesis and applications. Enzym Microb Technol 2:91–101

Saikkonen K, Helander M, Ranta H, Neuvo-nen S, Virtanen T et al (1996) Endophyte-mediated interactions between woody plants and insect herbivores? Entomol Exp Appl 80:269–271

Sato K, Sudo S (1999) Small scale solid state fermentations- manual of industrial. Microbiol Biotechnol 6(1):60–79

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686

Schuster A, Schmoll M (2010) Biology and biotechnology of *Trichoderma*. Appl Microbiol Biotechnol 87:787–799

Shoemaker S, Schweickrt V, Ladner M, Gelfand D, Kwok S, Myambo K, Innis M (1983) Molecular cloning of exo cellobiohydrolase I derived from *Trichoderma reesei* strain L27. Bio Technol 1:691–696

Singh HP, Batish DR, Kohli RK (2003) Allelopathic interactions and chemicals: new possibilities for sustainable weed management. Crit Rev Plant Sci 22:239–311

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214–216

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol Biol Rev 67:491–502

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Strobel GA, Kluck K, Hess WM, Sears J, Ezra D, Vargas PN (2007) *Muscodor albus* E-6, an endophyte of *Guazuma ulmifolia*, making volatile antibiotics: isolation, characterization and experimental establishment in the host plant. Microbiology 153:2613–2620

Subramaniyam R, Vimala RIJSN (2012) Solid state and submerged fermentation for the product of bioactive substances: a comparative study. 3(3):480–486

Sudheep NM, Sridhar KR (2011) Non-mycorrhizal endophytic fungi in two orchids of Kaiga forest (western Ghats), India. J For Res 23(3):453–460

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Tao G, Liu ZY, Hyde KD, Yu ZN (2008) Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (orchidaceae). Fungal Divers 33:101–122

Taylor TN, Taylor EL (2000) The rhynie chert ecosystem: a model for understanding fungal interactions. In: Bacon CW, White JF (eds) Microbial endophytes. Marcel Decker, New York, pp 31–48

Ten LN, Im WT, Kim MK, Kang MS, Lee ST (2004) Development of a plate technique for screening of polysaccharide degrading microorganisms by using a mixture of insoluble chromogenic substrates. J Microbiol Methods 56(3):375–382

Thomke S, Rundgreen M, Hesselman K (1980) The effect of feeding high-viscosity barley to pigs. In: Proceedings of the 31st meeting of the European Association of Animal Production, Commission on Animal Production, Munich, Germany, p 5

Thompson K, Stewart AJ (1981) The measurement and meaning of reproductive effort in plants. Am Nat 117:205–211

Tiwari AK, Kumar A, Raheman H (2007) Biodiesel production from Jatropha (*Jatropha curcas*) with high free fatty acids: an optimized process. Biomass Bioenergy 31:569–575

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. Biol Control 46:72–82

Verma VC, Gond SK, Kumar A, Kharwar RN, Strobel G (2007) The endophytic *Mycoflora* of bark, leaf, and stem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). Microb Ecol 54:119–125

Voragen AGJ (1992) Tailor-made enzymes in fruit juice processing. Fruit Process 7:98–102

Wang HT et al (2004) Effect of inoculating flower stalks and vegetable waste with ligno cellulolytic micro organisms on composting process. J Environ Sci Health 39:871–887

Wilson D (1995) Endophyte – the evolution of a term, and clarification of its use and definition. Oikos 73:274–276

Wipusaree N, Sihanonth P, Piapukiew J, Sangvanich P, Karnchanatat A (2011) Purification and characterization of a xylanase from the endophytic fungus *Alternaria alternata* isolated from the Thai medicinal plant, *Croton oblongifolius* Roxb. Afr J Microbiol Res 5(31):5697–5712

Yu H, Zhang L, Li L, Sun P, Qin L et al (2010) Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165:437–449

Zambare V, Nilegaonkar S, Kanekar P (2011) A novel extracellular protease from *Pseudomonas aeruginosa* MCM B-327: enzyme production and its partial characterization. New Biotechnol 28:173–181

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Potential of Fungal Endophytes in Plant Growth and Disease Management

14

Kanika Chowdhary and Satyawati Sharma

Abstract

The historical background, various investigations, successful results and projected mode of activity for overall plant development and disease management conferred by fungal endophytes to their host plants have been elaboratively discussed. *Piriformospora indica*, a root endophyte, is the well-described plant promoter in literature. 2H-Pyran-2-one, 5,6-dihydro-6-pentyl, an antifungal metabolite with high activity towards *S. sclerotiorum*, was produced by endophytic *Macrophomina phaseolina*, while solanapyrone derivatives C and phomalactone produced by *Nigrospora* sp. YB-141 showed inhibition against plant pathogenic fungus *Botrytis cinerea.* The present chapter summarises various findings on fungal endophytes and their functional attributes with respect to enhancement in overall plant maturity and improvement recovered from indigenous plant of medicinal value *Ocimum sanctum* found widely in India till date.

Keywords

Endophytic fungi • *Ocimum sanctum* • Biopesticides • Indigenous plants • Antifungal activity

14.1 Introduction

In their entire lifecycle, plants being multicellular organisms constitute a myriad of multitrophic and synergistic interactions with other microorganisms, i.e. fungi, bacteria, virus, algae and protista. These endophytically residing microbes are

K. Chowdhary • S. Sharma (✉)
Centre for Rural Development and Technology, Indian Institute of Technology-Delhi, Hauz Khas, New Delhi 110016, India
e-mail: satyawatis@hotmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_14

associated with coordination of vital growth, development, overall fitness and defence pathways of plants (Hardoim et al. 2015). Studies and advanced research conducted on the given aspect have evolved the new area of research called 'plant microbiome' wherein collective genomes of both plants and their associated microorganisms respond to the surrounding environment concomitantly with each other (Rosenberg et al. 2009). Complex microbial networks inhabiting in plant ecosystem consist of multiple interdependent components that interplaying in various modes, i.e. symbiotic, synergistic, commensalistic, ammensalistic and parasitic modes. These networks on the whole impact soil fertility, plant health and each of its participating individual either directly or indirectly. Understanding these microbe-microbe/plant-microbe interactions is vital to understand the holistic consequences of these interactions for plant physiology and performance and to further explore them for different biotechnological aspects.

14.2 Endophyte: Its History and Definition

Glomeromycota is the foremost example of early plant-fungus symbiosis which dates back to colonisation of terrestial plants. Heinrich Friedrich Link, a German botanist, in 1809 firstly described endophytes as *Entophytae*, a peculiar type of fungi inhabiting in a partial parasitic mode inside plants. Many investigators have proposed definition of endophytes based on their studies and experience (Link 1809; Carroll 1986; Wilson 1995; Brown et al. 1998; Aly 2011) in the past decades. The most cited definition was contrived by Orlando Petrini in 1991 explaining endophytes as 'microorganisms colonising plants internally during a particular or whole time of their without causing any perceptible disease in the host' (Petrini 1991). But nevertheless, this explanation has certain shortcomings. At first, it holds true or is more appropriate for cultivated endophytes. Apart from that, it is widely described in the literature that latency lifestyle of plant pathogenic microbes could have direct relationship to endophytic state microbes (Reiter et al. 2002). Moreover, in culture-independent protocol, assessment of pathogenic state of the individual remains elusive whether latent or active during the course of isolation. Therefore, it can be concluded that the term 'endophyte' is more aptly suited for 'habitat-explicit' rather than 'functional-explicit' microbes capable of colonising internal plant segments (Coombs and Franco 2003).

Bioactivities expressed by endophytic microorganisms outnumber that of epiphytes, soil isolates and phytopathogenic counterparts collectively as documented in the literature (2002), for instance, in the case of rugulosin and taxol (plant-protecting) metabolites. Endophytes have been recognised to produce metabolites in extremely low quantities, maybe because they are required for highly localised, stressful defensive condition in small quantities to deter pathogen temporarily (Miller et al. 2002; Kusari et al. 2015).

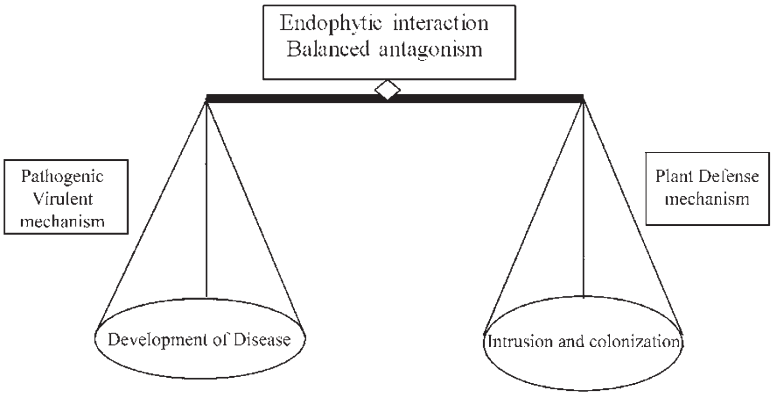


Fig. 14.1 Schematic depiction of balanced antagonism hypothesis (Modified and adapted from Schulz et al. 1999)

The earliest cited publication on fungal asymptomatic colonisation was reported inside *Lolium temulentum* embryo prior to seed maturation by fungus *Neotyphodium occultans* (Freeman 1904). Schulz in 1999 (Fig. 14.1) described a asymptomatic existence of endophytes as balanced antagonism, stating that both endophytic and pathogenic microbes initiate the defensive reaction inside the inhabited plant tissues upon their intrusion; nonetheless pathogen leads to disease development, while endophyte maintains the defence reaction to its infection and colonisation inside host plant (Schulz and Boyle 2005).

14.3 Production of Metabolites Mimetic to Host Plant

The competence to synthesise chemically mimetic metabolites by endophytes as of their inhabiting plant has been hypothesised to arise because of the substantive genetic exchange between endophyte and its host (Stierle et al. 1993; Zhang et al. 2006). For instance, anti-cancerous plant metabolite was secreted by *Alternaria* sp. recovered from *Podophyllum hexandrum* as endophytically (Yang et al. 2003). Likewise, camptothecin-producing endophytic fungus *Entrophospora infrequens* was obtained from *Nothapodytes foetida*. Furthermore, vincristine was purified from extracts of *Fusarium oxysporum* residing in *C. roseus* (Zhang et al., 2000). *Shirala* sp. synthesised huperzine A cholinesterase inhibitor skilfully recovered from *Huperzia serrata* (Wang et al., 2011). Reportedly, piperine presence in extracts of endophytic *Periconia* sp. reclaimed from *Piper longum* was confirmed by LC-MS/MS study (Verma et al. 2011). The success achieved by scientists at hitting upon the production of 'host plant similar metabolites' reiterates the need and enthusiasm their investigation contains. They hold the promise of serving as a substitute resource of plant-based bioactive compounds.

14.4 Diversity and Colonisation of Endophytes

Fungal endophytes occur both intercellularly and intracellularly in plants growing in diverse geographical areas such as Antarctic and Arctic continents, marine flora, geothermal soils, cold and hot deserts, rainforests, marshy swamps and grasslands. Also, their isolation has been reported from diverse host range such as algae, pteridophytes, gymnosperms, angiosperms, sponges and bryophytes (Kharwar et al. 2011). Reportedly, endophytically occurring fungi are present practically in all plant segments, i.e. leaf, stem, root, inflorescence, fruit and seed (Firakova et al. 2007). Out of the 420,000 different plants on earth, the investigated ones have depicted successful endophytic colonisation (Vuorela 2004; Aly et al. 2011). Numerous studies have reported that distribution of endophytic fungi in the host plant is not homogenous and the specificity for certain organs and tissues has been observed. A preference of endophytes for specific tissues in certain plants seems to exist while maintaining colonisation in almost all the plant organs. Mycobiota isolated from various locations has been greater in the aerial plant parts than in the underground organs (Sánchez Márquez et al. 2012). Based on colonisation characteristics and their extensive studies on endophytes, Rodriguez et al. (2009) categorised endophytic colonisation pattern into four groups. They categorised clavicipitaceous endophytes as type 1 fungal endophytes. Fungi occurring in above- and belowground plant tissues such as aerial tissues, rhizosphere and endorhiza either horizontally or vertically disseminated were put in a type 2 fungal endophytes. Type 3 endophytes mostly belonged either to *Ascomycota* or *Basidiomycota*, occurring in aerial tissues of hosts, and are generally horizontally disseminated. Type 4 endophytes comprise of DSE (mycorrhizal fungi), residing chiefly inside inter- and/or intracellular cortical cell layers (Rodriguez et al. 2009). However, a noteworthy group amongst fungal endophytic isolates is mycelia sterilia, fungi which are non-sporulating. Apart from this, Schulz and Boyle (2005) grouped the fungal endophytes into three ecological groups: mycorrhizal, balansicaeous and non-balansicaeous endophytes.

14.5 Plant Maturation and Protection by Fungal Endophytes

The interrelationship that exists between host plant and its endophyte is considered as ‘balanced antagonism’ – a cohabitation in which host plant gains resistance against pathogenic organisms and phytophagous insects and its overall growth or biomass quality improves. In most cases, various bioactive metabolites have been involved (Kumar and Kaushik 2013; Chowdhary et al. 2012). The molecular, physiological and biochemical mechanisms that trigger and regulate these relations are subjects of intense research.

The numerous fitness benefitting factors conferred by microbes inhabiting inside host plants in endophytic state have been thoroughly investigated by various extensive studies. These benefitting attributes hold a huge promise in sustainable agriculture and disease management of plants (Kaul et al. 2012; Kumar and Kaushik 2013).

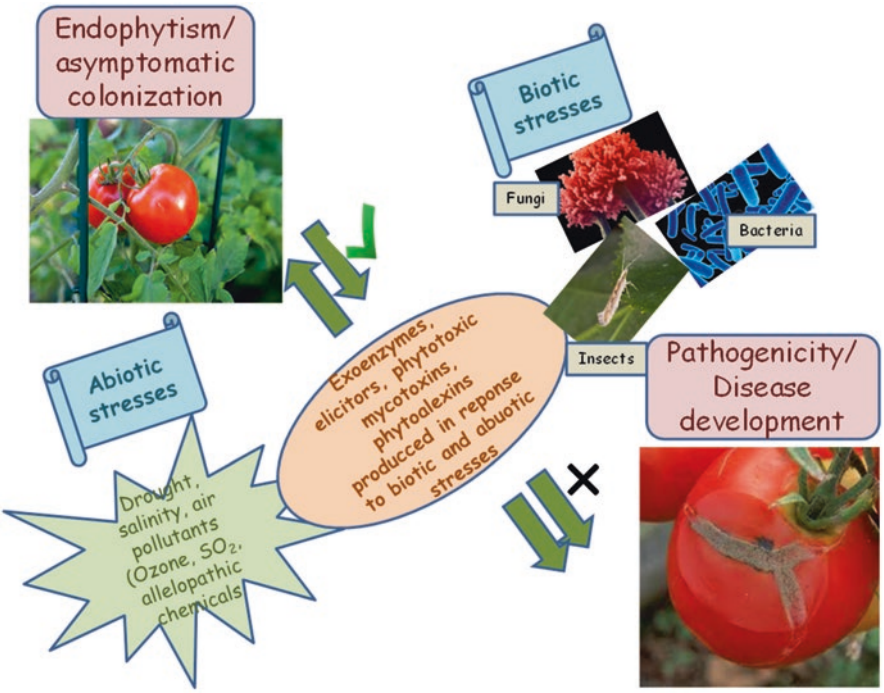


Fig. 14.2 Illustration and comparison of endophytic colonisation and pathogenic disease development in plants

As for the secondary metabolite production, there are significant numbers of compounds related to plant protection (Fig. 14.2, Table 14.1).

Protective role of fungal endophytes against lepidopteran, dipteran and coleopteran insect pests has been well cited (Bing and Lewis 1991; Latch 1993; Cherry et al. 2004; Jallow et al. 2008; Vega 2008). In conifers, it has been reported that fungus endophyte *Phialocephala scopiformis*, which endophytically lives in *Picea glauca* (Pinaceae), produces the toxin rugulosin which has an antifeeding activity against the spruce budworm *Choristoneura fumiferana* (Lep. Tortricidae) (Rohlfs and Churchill 2011; Larkin et al. 2012).

The production of antioxidant compounds by plants, i.e. phenol-based compound (Huang et al. 2007) derivatives of benzofurans such as isobenzofuranone and isobenzofurans and sugars like mannitol, is accredited to the presence of reactive oxygen species synthesised by endophytes (Richardson et al. 1992; Strobel 2002; Harper et al. 2003). Subsequent increase in antioxidant activity enhances the stress tolerance/defence mechanism of the plant (Huang et al. 2007; White and Torres 2010; Aly et al. 2011) including extreme temperatures (up to 65 °C) as in the case of *Curvularia protuberate* found in *Dichanthelium lanuginosum* from Yellowstone National Park soil (Redman et al. 2002; Loro et al. 2012).

Table 14.1 Different plant growth-promoting and disease-controlling activities of selective endophytic fungi

S. No.	Endophytic fungi	Bioactivities/fitness extended to host plant	References
1	*P. indica*	Plant productivity sustained in biotic stress in *L. esculeutum*	Andrade-Linares et al. (2013)
2	*P. indica*	Improved seed germination and yield in biodiesel crops in *Jatropha* and *Populus*	Varma et al. (2013)
3	*P. indica*	Antioxidant ability increased in *Brassica campestris*	Sun et al. (2010)
4	*P. indica*	Salinity stress tolerance in tobacco	Trivedi et al. (2014a, b)
5	*Phialocephala scopiformis*	Insecticidal metabolite rugulosin against spruce budworm *Choristoneura fumiferana*	Larkin et al. (2012)
6	*Curvularia protuberate*	Generates ROS for extreme temperature survival of host plant	Loro et al. (2012)
7	*Aspergillus flavus*	Insecticidal metabolites	Senthilkumar et al. (2014)
8	*Nigrospora* sp.	Nematicidal metabolites	Amin (2015)
9	*Chaetomium globosum*	Nematicidal metabolites	Hu et al. (2013)
10	*Botryosphaeria* sp.	Nematicidal metabolites	Chen et al. (2015)
11	*Microsphaeropsis* sp.	Phytoremediation	Xiao et al. (2010)
12	*Penicillium* sp.	Antifungal metabolites	Oliveira et al. (2009)
13	*Microbotryum violaceum*	Antifungal metabolites	Hussain et al. (2009)
14	*Nigrospora* sp.	Antifungal metabolites	Wu et al. (2008)
15	*Fusarium solani*	Tolerance towards biotic stress by SAR	Kavroulakis et al. (2007)
16	*Colletotrichum* sp.	Plant growth promotion	Lu et al. (2000)
17	*Muscodor albus*	Mycofumigation	Lacey et al. (2001)
18	*Phoma* sp.	VOC active against phytopathogens	Strobel (2011)
19	*Taxomyces andreanae*	Taxol producing endophytic fungi	Stierle et al. (1993)
20	*Trichoderma* sp.	Camptothecin-producing endophytic fungi	Pu et al. (2013)

Endophytic *A. flavus* and *N. sphaerica* isolated from leaves of *Tectona grandis* L. were screened for insecticidal metabolites by GC-MS methodology towards defoliators, i.e. *H. puera*, *A. fabriciella* and *E. narcissus*. Bioactive metabolites were duroquinone, amylmetacresol, lauric acid, tetradecanoic acid, pentadecanoic acid and myristic acid found responsible for insecticidal activity (Senthilkumar et al. 2014). Extracts of root endophytic fungi *Nigrospora* sp. exhibited nematicidal activity at dosages of 100, 50 and 25% towards root-knot nematode *Meloidogyne* spp. (Amin 2015), while another endophyte *Chaetomium globosum* NK102 significantly repelled juveniles (J2s) of *M. incognita* with 99.8% at 300 µg ChA/mL. The bioactive metabolite was identified as chaetoglobosin A in this study (Hu et al. 2013).

Endophytic fungus *Botryosphaeria* sp. isolated from *Huperzia serrata* produced antinematicidal compound botryosphaerin H (Chen et al. 2015).

Piriformospora indica, a root endophyte, has been promoted as plant protector, plant growth regulator and fertiliser in both agricultural and nonagricultural crops. *P. indica* confers enormous benefits to plants such as tolerance against environmental stresses, superior fitness by increasing biomass and growth performance during regular and stressful conditions (Schafer et al. 2007). *Piriformospora indica*-infected barley plants showed higher biomass when compared with non-infected plants at salt stress condition (Waller et al. 2005). Salinity stress tolerance was conferred by *P. indica* in tobacco by triggering formation of cyclophilin A-like protein (Trivedi et al. 2014a, b). Reportedly, *P. indica* sustained growth under drought by inducing inherent antioxidant mechanism through CAS protein in *Brassica campestris* sub sp. (Sun et al. 2010). *P. indica* enhances plant tolerance against water depletion and salinity, a crucial feature that might come into play owing to its natural desert origin (Waller et al. 2005; Baltruschat et al. 2008).

A plethora of research has been cited and chiefly underway on utilising the potential of endophytes which have been found resistant to heavy metals, can degrade organic contaminants and can provide assistance in phytoremediation of soils. Not only these isolates aid in phytoremediation but also enhance plant development, decrease metal phytotoxicity and stimulate effective metal movement and absorption within plant tissues. For the phytoremediation of organic toxins, endophytes secrete a protein cascade of enzymes to degrade unwanted organic metabolites and reduce both the phytotoxicity and evapotranspiration of volatile undesired toxin metabolites. For instance, *Microsphaeropsis* sp. established as an endophyte in cadmium hyperaccumulator variety of *Solanum nigrum* L. exhibited biosorption capacity of 247.5 mg/g therefore developing it as biosorption technology for the detoxification of cadmium (Xiao et al. 2010). Similarly, *Phomopsis liquidambari* decomposed an allelochemical 4-hydroxybenzoic acid into *cis*,*cis*-muconic acid, thereby alleviating its negative impacts in soil profile (Chen et al. 2011).

14.6 Antiphytopathogenic Secondary Metabolites

An elaborate body of literature on endophytic fungi has reportedly demonstrated antifungal metabolites exhibiting inhibition towards phytopathogenic microbes at highly significant dosages, thereby holding a gigantic potential to be developed into an agrochemical product.

Endophytic *Penicillium* synthesised orcinol, 4-hydroxymellein and 8-methoxymellein which exhibited inhibitory action at a highest detection limit of 10.0 μg against *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*, respectively (Oliveira et al. 2009). Polyketide metabolites, namely, 5-methoxy-7-hydroxyphthalide and (3*R*,4R)-*cis*-4-hydroxymellein, were produced from an unidentified ascomycete, recovered from *Melilotus dentatus*, capable of depicting 7 mm and 8 mm as radius of zone of inhibition against *Microbotryum violaceum* (Hussain et al. 2009). Likewise, three solanapyrone derivative C and phomalactone

produced by *Nigrospora* sp. YB-141 showed antifungal activities against plant pathogenic fungus *Botrytis cinerea* with the highest MIC value of 250 mcg/ml (Wu et al. 2008).

14.7 Plant Protection Mechanism by Fungal Endophytes

The ability of fungal endophytes to protect host plant from diseases and damages inflicted by pathogenic organisms stirred the research towards unravelling the underlying mechanism (Vega et al. 2008; Tian et al. 2008; Mejia et al. 2008). Gao et al. (2010) reviewed and proposed broadly three different ways of defensive interactions between endophytic fungi and pathogens in plants (Fig. 14.3) (Gao et al. 2010). In direct effect inhibition, protection is primarily localised and conferred by antibiosis (antibacterial, antifungal secondary metabolites or lytic enzymes) to endophyte-inflicted plant segments (Arnold et al. 2003). Indirect inhibition inherent plant defence (SAR and ISR) pathways are elicited. For instance, endophytic *Fusarium solani* educed systemic resistance proteins (PR5 and PR7) towards tomato leaf pathogen *Septoria lycopersici* in root tissues (Kavroulakis et al. 2007). Furthermore, endophytic microbes have been known to promote host plant growth and physiology. *Colletotrichum* sp., an endophyte of *A. aninua*, regulated plant processes by producing indole acetic acid (Lu et al. 2000). The third way of pathogen suppression is by way of ecological niche occupation. Endophytes colonise host tissues faster than corresponding pathogens leading to depletion of resources (Pal and Gardener 2006).

Case Study on Endophytes of *Ocimum sanctum*

In the present chapter, we have earnestly compiled various studies conducted in *O. sanctum* till date regarding biodiversity and bioactivities of fungal endophytes recovered from them (Table 14.2)

In the author's 2015 published study (Chowdhary and Kaushik 2015), fungal endophytes inhabited inside *Ocimum sanctum*, widely known as Tulsi/Tulasi in Hindi, were scrutinised for their diversity and bioactivity towards broad-spectrum phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Botrytis cinerea*

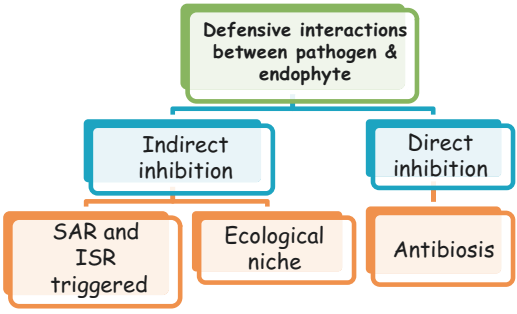


Fig. 14.3 Schematic diagram of mode of action conferred by endophytes for plant protection

Table 14.2 Endophytic fungi and their respective bioactivities recovered from *O. sanctum*

S. No.	Endophytic fungi	Bioactivities/fitness extended to host plant	References
1	*Macrophomina phaseolina*	Antifungal metabolite 2H-pyran-2-one and 5,6-dihydro-6-pentyl	Chowdhary and Kaushik (2015)
2	*Aspergillus* sp.	Antioxidant activities	Sharma and Kumar (2013)
3	*Aspergillus terreus*	Antioxidant activities	Sharma and Kumar (2013)
4	*Aspergillus versicolor*	Antioxidant activities	Sharma and Kumar (2013)
5	*Nigrospora oryzae*	Antimicrobial activity of extracts	Monali and Bodhankar (2014)
6	Unidentified fungi	Production of urease, lipase, laccase, chitinase and cellulase	Yadav et al. (2015)
7	*Aspergillus fumigatus*	Antimicrobial activity of extracts	Bhagat et al. (2012)
8	*Diaporthe* sp.	Inhibition of α-glucosidase	Pavithra et al. (2016)
9	*Colletotrichum gloeosporioides*	Inhibition of α-glucosidase	Pavithra et al. (2016)
10	*Paecilomyces variotii*	Antihepatotoxicity and antioxidant agent	Shukla et al. (2012)

and *Sclerotinia sclerotiorum*. For the study, host plants from three varied geographical locations, namely, Hyderabad, Mukteshwar and Delhi, in different sampling times within 2 years (2010 and 2011) were collected from India. In total 90 fungal isolates having representation of 17 genera were isolated. Fungal endophytes were molecularly identified based on rDNA ITS sequence analysis. The biodiversity data so obtained was studied and explained by utilising mathematical indices, i.e. Shannon diversity index, Menhinick's index, Camargo's index, Jaccard's similarity index, etc., along with principal component analysis and cluster analysis. Dendrogram created by cluster analysis established an interrelationship connecting a number of species and average temperature of the plant collection site during sampling. Increased abundance of *Penicillium* sp., *A. tenuissima*, *M. phaseolina* and *A. alternata* in leaf tissues indicates towards tissue preference. Inclination for a particular tissue has been a highly reported characteristic of fungal endophytes indicating both surviving strategy and substrate preference. As per reported in the literature, plant pathogens go into a phase of milder virulence for the period of elevated temperature and less humidity. This aspect has been supported by the presence of eight phytopathogens, viz. *D. phaseolorum*, *C. coarctatum*, *F. verticillioides*, *B. maydis*, *Hypoxylon* sp., *R. bataticola* and *A. tenuissima*, in sampling of 2011 as explained by PCA. Likewise, well-known phytopathogens such as *B. maydis*, *F. verticillioides*, *C. coarctatum*, *R. bataticola*, *Diaporthe phaseolorum*, *A. alternata*, *Hypoxylon* sp. and *Alternaria tenuissima* were reported as endophyte only during 2011 in this study. Bi-plot created by principal component analysis indicated towards selectivity of tissue of fewer fungal endophytes.

With respect to bioprospection, all the fungal isolates were analysed for preliminary inhibitory activity with the aid of dual culture/confrontation bioassay against economically notable plant pathogens. Nearly one-fourth of the fungal isolates were considered as potential candidates, which were subjected to mass multiplication on rice grain medium. The ethyl acetate crude extract of potent fungal isolates was further examined for antifungal efficacy with the aid of (biometric agar dilution method). Fungal extracts were also tested for bioactive metabolites. Terpenoid-positive crude extracts were in advance partitioned between n-hexane and 90% methanol. Finally non-polar (hexane) concentrate of *M. phaseolina* isolated from Hyderabad in 2010 exhibited the best inhibition against *S. sclerotiorum* having IC_{50} value of 0.38 mg/ml. This led to its GC-MS chromatography investigation revealing occurrence of aliphatic constituents such as 9-oleic acid, hexadecanoic acid, linoleic acid and octadecanoic acid amongst others. These non-polar metabolites have been previously documented having inhibition against fungi in former studies (Liu et al. 2008). 9,12-Octadecadienoic acid and hexadecanoic acid purified from *Tinospora crispa* concentrate were examined to be highly active against *C. albicans*. Oleic acid isolated from *L. cristata* was reportedly be extremely efficient in inhibiting plant pathogens *Colletotrichum falcatum*, *Fusarium oxysporum* and *Rhizoctonia solani* (Nuryanti 2015), while a study explained the antifungal activity of linolenic and linoleic acids against various phytopathogenic fungi, i.e. *Rhizoctonia solani* (Abubacker and Devi, 2014). Furthermore, from the extract of *M. phaseolina*, 2H-pyran-2-one, 5,6-dihydro-6-pentyl and palmitic acid and methyl ester were identified in GC-MS chromatography. When their fungicidal activity was evaluated, it exhibited high activity with IC_{50} value of >0.5 towards *S. sclerotiorum* signifying their noteworthy part in bioactivity.

While another study carried out on fungal endophytes harboured inside *O. sanctum* collected from Andhra Pradesh documented their remarkable antioxidant activities, ethyl acetate extracts of certain potent endophytes (*Aspergillus* sp., *A. terreus*, *A. versicolor* and mycelia sterilia) cultured on Czapek-Dox Broth were evaluated for radical scavenging activities against DPPH, reducing power assay (RP) and FRAP along with total phenolic content and total flavonoid content. The highest total phenolic and flavonoid content was reported from mycelia sterilia as 18.13 mgGAE/100 mL culture and 5.33 mgRE/100 mL culture, respectively (Sharma and Kumar 2013).

Similarly, Monali and Bodhankar in 2014 reported significant antimicrobial potential of ethyl acetate extract of endophyte *Nigrospora oryzae* isolated from leaf tissues of *O. sanctum.* The crude extract gave best results against *B. subtilis*, *E. coli*, *S. typhimurium*, *K. pneumonia*e, *S. aureus* and *B. cereus*.

Likewise, Banerjee et al. in 2009 explored endophytic mycobiota of *O. sanctum* amongst other indigenous plants to analyse their biodiversity and host specificity. *Tubercularia* sp., *Hymenula* sp., *Curvularia* sp. and *Trichoderma* sp. were found to be dominant species in *O. sanctum*.

Moreover, in another piece of work on *O. sanctum*, researchers evaluated antifungal activity of fungal endophytes against plant pathogens together with production capability of extracellular enzymes. The plant pathogens assessed under study

were *Helminthosporium maydis*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Colletotrichum falcatum*, while enzymes targeted were cellulase, amylase, pectinase, chitinase, lipase, laccase and urease. Six unidentified fungi were positive for urease production (Yadav et al. 2015).

Additionally, an elaborative investigation of fungal endophytes of *O. sanctum* was conducted to screen their cytotoxic and antimicrobial potential. Reportedly, 31 fungal endophytes were obtained from *O. sanctum* collected from Amritsar, India. Endophytic isolate *Aspergillus fumigatus* exhibited antimicrobial activity against *Salmonella typhii*, *Escherichia coli and Mycobacterium smegmatis*. Extract of *Cladosporium cladosporioides* at concentration of 30 μg/ml isolated as an endophyte from leaf tissue of *O. sanctum* was found to be most active (50% inhibition) against human cancer cell lines, i.e. breast (MCF-7), prostate (PC-3 and DU-145) and colon (colo-205 and HCT-15) (Bhagat et al. 2012).

In addition, endophytologists collected *O. sanctum* from Himachal Pradesh together with four other indigenous plants to explore them for biodiversity of their fungal endophytes. It was reported that the highest diversity and richness was contained in *O. sanctum* (Gautam 2014).

However, a first of its own kind of study was carried out on extracts of endophytic fungi as potential enzyme inhibitors isolated from *O. sanctum.* Extracts of *Diaporthe* sp. and *Colletotrichum gloeosporioides* exhibited remarkable inhibition of α-glucosidase with IC_{50} values of 29.51 and 31.26 μg/mL, respectively, whereas *Alternaria tenuissima* and *Trichoderma* sp. showed the highest suppression pancreatic α-amylase enzyme in-vitro with an IC_{50} value of 27.34 and 40.73 μg/mL, respectively (Pavithra et al. 2016).

Nevertheless, a recent study explored the extracts of endophytic fungus identified as *Paecilomyces variotii* Bain, recovered from roots of *O. sanctum* as an antihepatotoxicity and antioxidant agent. The extract reported the IC_{50} values of 71.83 μg/ml for DPPH. Assessment for in vivo CCl_4-induced hepatotoxicity results showed that the extract reversed the actions by restoring serum transaminase, bilirubin, triglycerides and protein level to normal values as compared to treated group (Shukla et al. 2012).

14.8 Conclusions and Future Prospects

Endophytic fungi are biomarkers of highly potential natural products which can be used in sustainable agriculture. Research areas in which attention should be focused are composition and formation of endophytic assemblages. The lead compounds can be tapped from traditional plants having medicinal values occurring in novel geographical location since 'novel biodiversity could lead to novel chemistry'. However, those endophytic fungi which have already been exhibited relevant bioactivity from *O. sanctum* must further be explored for scaling up the bioactive metabolite production. Biopesticide formulations derived from microbes are the best alternative solutions to chemical synthetic pesticides because of their specificity to

insect pests and pathogens, their biodegradable nature and their potential for commercialisation. Therefore novel microbial biopesticides are urgently needed.

The consequences of investigating endophyte-plant interrelation bring into light a diversity of information which contains specific aspects depending on genotypic and phenotypic and microecosystem interrelationships between species.

Acknowledgement Kanika Chowdhary would like to acknowledge the financial assistance provided by DST-NPDF scheme (grant no. PDF/2016/000317) and CRDT, IIT Delhi, for providing infrastructural support.

References

Abubacker MN, Devi PK (2014) In vitro antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propylester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence. Asian Pac J Trop Biomed 4:S661–S664

Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90:1829–1845

Amin N (2015) Nematicidal activity of root exudates of sengon plant inoculated with endophytic fungi *Nigrospora* sp. to control of root-knot nematode *Meloidogyne* spp. J Chem Pharm Res 7(3):307–310

Andrade-Linares DR, Müller A, Fakhro A, Schwarz D, Franken P (2013) Impact of *Piriformospora indica* on tomato. Soil Biol 33:107–117

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. PNAS USA 100:15649–15654

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schäfer P, Schwarczinger I, Zuccaro A, Skoczowski A (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol 180:501–510

Banerjee D, Manna S, Mahapatra S, Pati BR (2009) Fungal endophytes in three medicinal plants of Lamiaceae. Acta Microbiol Immunol Hung 56(3):243–250. doi:10.1556/AMicr.56.2009.3.4

Bhagat J, Kaur A, Sharma M, Saxena AK, Chadha BS (2012) Molecular and functional characterization of endophytic fungi from traditional medicinal plants. World J Microbiol Biotechnol 28(3):963–971. doi:10.1007/s11274-011-0894-0

Bing LA, Lewis LC (1991) Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. Environ Entomol 20:1207–1211

Brown KB, Hyde KD, Guest DJ (1998) Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Divers 1:27–51

Carroll GE (1986) The biology of the endophytism in plants with particular reference to woody perennials. In: Fokkema NJ, van den Heuvel J (eds) The microbiology of the phyllosphere. Cambridge University Press, Cambridge, pp 205–222

Chen Y, Peng Y, Dai CC, Ju Q (2011) Biodegradation of 4-hydroxybenzoic acid by *Phomopsis liquidambari*. Appl Soil Ecol 51:102–110

Chen YM, Yang YH, Li XN, Zou C, Zhao PJ (2015) Diterpenoids from the endophytic fungus *Botryosphaeria* sp. P483 of the Chinese herbal medicine *Huperzia serrata*. Molecules 20(9):16924–16932. doi:10.3390/molecules200916924

Cherry AJ, Banito A, Djegui D, Lomer C (2004) Suppression of the stem borer *Sesamia calamistis* (Lepidoptera: Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. Agric Entomol 7:171–181

Chowdhary K, Kaushik N (2015) Fungal endophyte diversity and bioactivity in the Indian medicinal plant *Ocimum sanctum* Linn. PLoS One 10(11):e0141444. doi:10.1371/journal.pone.014144

Chowdhary K, Kaushik N, Coloma AG, Raimundo CM (2012) Endophytic fungi and their metabolites isolated from Indian medicinal plant. Phytochem Rev 11:467–485. doi:10.1007/s11101-012-9264

Coombs JT, Franco CMM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608. http://dx.doi.org/10.1128/AEM.69.9.5603-5608.2003

Firakova S, Sturdikova M, Muckova M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants. Biology 62:251–257

Freeman EM (1904) The seed fungus of *Lolium temulentum* L. Darnel. Phil Trans R Soc Land B 196:1–2

Gao FK, Dai CC, Liu XZ (2010) Mechanisms of fungal endophytes in plant protection against pathogens. Afr J Microbiol Res 4(13):1346–1351

Gautam A (2014) Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India. Archives Phytopathol Plant Prot 47(5). doi:10.1080/03235408.2013.813678

Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev. doi:10.1128/MMBR.00050-14

Harper JK, Arif AM, Ford EJ, Strobel GA Jr, Porco JA, Tomer DP, Oneill KL, Heider EM, Grant DM (2003) Pestacin: a 1,3-dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. Tetrahedron 59:2471–2476

Hu Y, Zhang W, Zhang P, Ruan W, Zhu X (2013) Nematicidal activity of chaetoglobosin a produced by *Chaetomium globosum* NK102 against *Meloidogyne incognita*. J Agric Food Chem 61:41–46

Huang WY, Cai YZ, Hyde KD, Corke H, Sun M (2007) Endophytic fungi from *Nerium oleander* L (Apocynaceae):main constituents and antioxidant activity. World J Microbiol Biotechnol 23:1253–1263

Hussain H, Krohn K, Draeger S, Meier K, Schulz B (2009) Bioactive chemical constituents of a sterile endophytic fungus from *Meliotus denatus*. Rec Nat Prod 3(2):114–117

Jallow MFA, Dugassa-Gobena D, Vidal S (2008) Influence of an endophytic fungus on host plant selection by a polyphagous moth via volatile spectrum changes. Arthropod–Plant Integr 2:53–62

Kaul S, Gupta S, Ahmed M, Dhar M (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Phytochem Rev 11:487–505

Kavroulakis NS, Zervakis GI, Ehaliotis C, Haralampidis K, Papadopoulou KK (2007) Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. J Exp Bot 58:3853–3864

Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep 28:1208–1288

Kumar S, Kaushik N (2013) Endophytic fungi isolated from oil-seed crop Jatropha curcas produces oil and exhibit antifungal activity. PLoS One 8(2):e56202. doi:10.1371/journal.Pone.0056202PMID: 23409154

Kusari S, Singh S, Jayabaskaran C (2015) Rethinking production of Taxol® using endophyte biotechnology. http://dx.doi.org/10.1016/j.tibtech.2014.03.011

Lacey LA, Horton DR, Jones DC, Headrick HL, Neven LG (2009) Efficacy of the biofumigant fungus *Muscodor albus* (Ascomycota: Xylariales) for control of codling moth (Lepidoptera: Tortricidae) in simulated storage conditions. J Econ Entomol 102(1):43–49

Larkin BG, Hunt LS, Ramsey PW (2012) Foliar nutrients shape fungal endophyte communities in Western white pine (*Pinus monticola*) with implications for white-tailed deer herbivory. Fungal Ecol 5(2):252–260

Latch GCM (1993) Physiological interactions of endophytic fungi and their hosts: biotic stress tolerance imparted to grasses by endophytes. Agric Ecosyst Environ 44:143–156

Link HF (1809) Observationes in ordines plantarum naturales, dissertatioprima, complectens anandrarumordinesEpiphytas, Mucedines, Gastromycoset Fungos. DerGesellschaftNaturforschenderFreundezuBerlin, Berlin

Liu S, Ruan W, Li J, Xu H, Wang J, Gao Y et al (2008) Biological control of phytopathogenic fungi by fatty acids. Mycopathologia 166(2):93–102. doi:10.1007/s11046-008-9124-1PMID:18443921 84

Loro M, Valero-Jiménez CA, Nozawa S, Márquez LM (2012) Diversity and composition of fungal endophytes in semiarid Northwest Venezuela. J Arid Environ 85:46–55

Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Plant Sci 151:67–73

Mejıa LC, Rojas EI, Maynard Z, Bael SV, Elizabeth Arnold A, Hebbar P, Samuels GJ, Robbins N, Herre EA (2008) Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biol Control 46:4–14

Miller JD, MacKenzie S, Foto M, Adams GW, Findlay JA (2002) Needles of white spruce inoculated with rugulosin producing endophytes contain rugulosin reducing spruce budworm growth rate. Mycol Res 106:471–479

Monali, Bodhankar (2014) Antimicrobial activity of endophytic fungi isolated from Ocimum sanctum Linn. Am J Cont Sci Res 1(3) ISSN 2349-4425

Nuryanti WH (2015) Screening of volatile compounds of Brotowali (Tinospora Crispa) and antifungal activity against Candida albicans. Int J Pharma Phytochem Res 7(1);132–136. 85

Oliveira CM, Silva GH, Regasini LO, Zanardi LM, Evangelista AH, Young MC, Bolzani VS, Araujo AR (2009) Bioactive metabolites produced by *Penicillium* sp.1 and sp.2, two endophytes associated with *Alibertia macrophylla*. Z Naturforsch C 64(11–12):824–830

Pal KK, Gardener BM (2006) Biological control of plant pathogens. Plant Health Instr. doi:10.1094/PHI-A-2006–1117–02

Pavithra N, Sathish L, Suneel Kumar A, Venkatarathanamma V, Pushpalatha H, Bhanuprakash RG, Ananda K (2016) In-vitro studies on α-amylase, α-glucosidase and aldose reductase inhibitors found in endophytic fungi isolated from Ocimum sanctum. Curr Enzym Inhib 10(2):129–136

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 179–197. http://dx.doi.org/10.1007/978-1-4612-3168-4_9

Pu X, Qu X, Chen F, Bao J, Zhang G, Luo Y (2013) Camptothecin-producing endophytic fungus *Trichoderma atroviride* LY357: isolation, identification, and fermentation conditions optimization for camptothecin production. Appl Microbiol Biotechnol 97(21):9365–9375. doi:10.1007/s00253-013-5163-8

Redman RS, Sheehan KB, Stout TG, Rodríguez RJ, Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298:1581

Reiter B, Pfeifer U, Schwab H, Sessitsch A (2002) Response of endophytic bacterial communities in potato plants to infection with Erwinia carotovora subsp. atroseptica. Appl Environ Microbiol 68:2261–2268. http://dx.doi.org/10.1128/AEM.68.5.2261-2268.2002

Richardson MD, Chapman GW, Hoveland CS, Bacon CW (1992) Sugar alcohols in endophyte-infected tall fescue. Crop Sci 32:1060–1061

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330. http: //dx.doi.org/10.1111/j.1469-8137.2009.02773.x

Rohlfs M, Churchill ACL (2011) Fungal secondary metabolites as modulators of interactions with insects and other arthropods. Fungal Genet Biol 48:23–34

Rosenberg E, Sharon G, Zilber-Rosenberg I (2009) The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. Environ Microbiol 11:2959–2962. http://dx.doi.org/10.1111/j.1462-2920.2009.01995.x

Sánchez Márquez S, Bills GF, Herrero N, Zabalgogeazcoa I (2012) Non-systemic fungal endophytes of grasses. Fungal Ecol 5:289–297

Schafer P, Khatabi B, Kogel KH (2007) Root cell death and systemic effects of *Piriformospora indica*: a study on mutualism. FEMS Microbiol Lett 275:1–7

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–687

Schulz S, Keatinge JDH, Wells GJ (1999) Productivity and residual effects of legumes in rice-based cropping systems in a warm-temperate environment. Field Crop Res 61:37–49

Senthilkumar M, Anandham R, Madhaiyan M, Venkateswaran V, Tongmin S (2011) Endophytic bacteria: perspectives and applications in agricultural crop production. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer Verlag, Berlin, pp 61–96

Senthilkumar N, Murugesan S, Babu DS (2014) Metabolite profiling of the extracts of endophytic fungi of entomopathogenic significance, *Aspergillus flavus and Nigrospora sphaerica* isolated from tropical tree species of India, *Tectona grandis* L. J Agric Life Sci 1(1):108–114

Sharma, Kumar (2013) Isolation, characterization and antioxidant potential of endophytic fungi of Ocimum sanctum Linn. Indian J Appl Res 3(7) ISSN-2249-555X

Shukla ST, Kulkarni VH, Habbu PV, Jagadeesh KS, Patil BS, Smita DM (2012) Hepatoprotective and antioxidant activities of crude fractions of endophytic fungi of *O. sanctum* Linn in rats. Orient Pharma Exp Med 12:81–91

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae* and endophytic fungus of Pacific yew. Science 260:214–221

Strobel GA (2002) Rainforest endophytes and bioactive products. Crit Rev Biotechnol 22(4):315–333

Strobel G, Singh SK, Riyaz-Ul-Hassan S, Mitchell AM, Geary B, Sears J (2011) An endophytic/pathogenic Phoma sp. from creosote bush producing biologically active volatile compounds having fuel potential. FEMS Microbiol Lett 320(2):87–94. doi:10.1111/j.1574-6968.2011.02297.x

Sun C, Johnson JM, Cai D, Sherameti I, Oelmüller R, Lou B (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:10091017

Tian P, Nan Z, Li C (2008) Effect of the endophyte *Neotyphodium lolii* on susceptibility and host physiological response of perennial ryegrass to fungal pathogens. Eur J Plant Pathol 122:593–602

Trivedi DK, Ansari MW, Tuteja N (2014a) Response of PiCypA tobacco T2 transgenic matured plant to potential tolerance to salinity stress. Plant Signal Behav 8:e27538

Trivedi DK, Ansari MW, Dutta T, Singh P, Tuteja N (2014b) Molecular characterization of cyclophilin A-like protein from *Piriformospora indica* for its potential role to abiotic stress tolerance in E. coli. BMC Res Notes 23(6):555

Varma A, Bajaj R, Agarwal A, Asthana S, Rajpal K, Das A, Prasad R, Kharkwal AC (2013) Memoirs of 'Rootonic'-the magic fungus. Amity University Press, Noida

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. Biol Control 46:7282

Verma VC, Lobkovsky E, Gange AC, Singh SK, Prakash S (2011) Piperine production by endophytic fungus Periconia sp. isolated from *Piper longum* L. J Ant 64:427–431

Vuorela H (2004) Natural products in the process of finding new drug candidates. Curr Med Chem 11:1375–1389

Waller F, Baltruschat H, Achatz B, Becker K, Fischer M, Fodor J, Heier T, Huckelhoven R, Neumann C (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance and higher yield. Proc Natl Acad Sci U S A 102(38):13386–13391

Wang Y, Zeng QG, Zhang ZB, Yan RM, Wang LY, Zhu D (2011) Isolation and characterization of endophytic huperzine A producing fungi from *Huperzia serrata*. J Ind Microbiol Biotechnol 38:1267–1278

White JF Jr, Torres MS (2010) Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? Physiol Plant 138(4):440–446

Wilson D (1995) Endophyte-the evolution of a term, a clarification of its use and definition. Oikos 73:274–276

Wu SH, Chen YW, Shao SC, Wang LD, Yu Y, Li ZY, Yang LY, Li SL, Huang R (2008) Two new solanapyronean alogues from the endophytic fungus *Nigrospora* sp. YB-141 of *Azadirachta indica*. Chem Biodivers 6(1):79–85

Xiao X, Luo SL, Zeng G, Wei W, Wan Y, Chen L, Guo H, Cao Z, Yang L, Chen J, Xi Q (2010) Biosorption of cadmium by endophytic fungus (EF) *Microsphaeropsis* sp. LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L. Bioresour Technol 101(6):1668–1674

Yadav R, Singh AV, Joshi S, Kumar M (2015) Antifungal and enzyme activity of endophytic fungi isolated from *O. sanctum* and *Aloe vera*. Afr J Microbiol Res 9(29):1783–1788

Yang X, Guo S, Zhang L, Shao H (2003) Selection of producing podophyllotoxin endophytic fungi from podophyllin plant. Nat Prod Res Devpt 15:419–422

Zhang L, Guo B, Li H, Zeng S, Shao H, Go S, Wei R (2000) Preliminary study on the isolation of endophytic fungus of *Catharanthus roseus* and its fermentation to produce products of therapeutic value. Chin Trad Herbal Drugs 31:805–807

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5):753–771

Endophytes: Role and Functions in Crop Health

15

P. Kishore Varma, S. Uppala, Kiran Pavuluri,
K. Jaya Chandra, M.M. Chapala, and K. Vijay Krishna Kumar

Abstract

Plant-microbe interactions is an important concept, and the significance of these interactions on sustainable agriculture is enormous. These interactions can be neutral, commensal, mutualistic, saprophytic, or harmful. Endophytes are beneficial microbes that reside and establish symbiotic relationships with the plants. These beneficial microbes are of either bacterial, fungal, or actinomycete origin. A wide array of beneficial effects are reported with endophytic associations in plants that include bioremediation, herbivory, induced resistance, plant growth promotion, and pest and disease management. Nomenclature of endophytes is generally according to the plant tissue it harbors, such as endophytes of root, shoot, leaf, seed, etc. Our review presents different bacterial and fungal endophytes in plants and their role in improving crop health. The plant growth-promoting (PGP) activities of these endophytes such as production of growth hormones like indoleacetic acid (IAA), gibberellic acid (GA), and cytokinins and phosphate solubilization in different crops by specific endophytes are discussed in detail. Further, specific antagonistic activities of endophytes like induced systemic resistance; production of salicylic acid, siderophores, HCN, cell wall-degrading enzymes, and antimicrobial metabolites including antibiotics; and direct antagonism against different plant pathogens are thoroughly discussed.

P.K. Varma • K.J. Chandra • K.V.K. Kumar (✉)
Acharya N. G. Ranga Agricultural University, Regional Agricultural Research Station, Anakapalle 531001, Andhra Pradesh, India
e-mail: kotamvk@gmail.com

S. Uppala
Texas A & M AgriLife Research Centre, 1509 Aggie Drive, Beaumont, TX 77713, USA

K. Pavuluri
Sirius Minerals Plc 7-10 Manor Court, Manor Garth, Scarborough, YO11 3TU, UK

M.M. Chapala
Rice Tec, Alvin, TX 77511, USA

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_15

The preferences of endophytes to different plant parts; their different niches such as roots, shoots, leaves, and whole plants; and specific antagonistic and PGP activities are elaborated in detail. Other beneficial activities such as herbivory, bioremediation, biodegradation, and biofertilization including nitrogen fixation are also discussed briefly. Finally, we have also discussed the scope and future prospects of endophytes in improving soil and crop health.

Keywords
Endophytes • Induced systemic resistance • Plant growth promotion • Plant disease management

15.1 Introduction

Plant-microbe interactions is an extensively studied area and date back to the nineteenth century. The spectrum of plant-microbe interactions is highly complex, comprising of phylogenetically diverse microbial species (Hirsch 2004) as plants are constantly interacting with a range of microbes both in the rhizosphere and within the plant itself (Badri et al. 2009; Evangelisti et al. 2014). Most of the plant-microbe interaction research in the past has focused on the ancient symbiosis between plants and arbuscular mycorrhizae (Parniske 2008), nitrogen fixation by rhizobia within the nodules of legume roots (Oldroyd et al. 2011) and pathogenesis, and management of plant diseases by natural antagonistic microorganisms (Heydari et al. 2004; Sang et al. 2013; Uppala et al. 2007). However, the role of endophytes that reside in plants is yet to be explored to its fullest potential. Endophytic microorganisms and their role in crop health are now attracting great interest from researchers. Though different definitions are proposed, generally, endophytes are defined as microorganisms that live within the host plants for at least part of their life and do not cause any apparent symptoms of diseases. These microbes establish intimate association within their host plants, establishing a community in endosphere, and are present in different plant parts (Hardoim et al. 2015).

Their association with plants can be neutral, commensal, beneficial, saprophytic, or pathogenic in nature. The impact of endophytes include bioremediation (Lumactud et al. 2016), herbivory (Parisi et al. 2014), synthesis of bioactive metabolites against plant pathogens (Aravind et al. 2009), induced systemic resistance (Uppala et al. 2010a) besides improving biometric characteristics/yield attributes (Govindarajan et al. 2008; Uppala et al. 2010b), and disease control (Ziedan 2006; Uppala et al. 2010c). However, the nature/type of endophytes residing in plants varies and diversifies based on the habitat of cultivation. Different niches/origins of endophytes include roots (rhizosphere), stem (caulosphere), soil zone surrounding belowground plant stems (laimosphere), leaves (phyllosphere), flowers (anthosphere), fruits (carposphere), seeds (spermosphere), etc. (Compant et al. 2016).

Both climatic and edaphic factors dictate the nature of endophytes in plants and their contribution to agriculture. In order to reap the benefits of endophytes in agricultural crops to its fullest potential, it is a prerequisite to understand the commonly associated microbial symbionts in a particular crop, ambient growth conditions for their successful establishment in the plant physiological system, their role in plant growth promotion, disease control, and their ability to produce any bioactive metabolites. The overall goal of agricultural researchers in this area is to identify potential endophytes that not only are potential endosymbionts in a particular crop but also thrive well under varied climate conditions that crop is cultivated. For inundative release into crop soils and their establishment in crop, critical insight on these aspects is mandatory. A particular endophyte in a particular plant can be prevalent either in roots, shoots, stalks, leaves, or seeds. The nomenclature also goes according to the place/zone/tissue it harbors inside a plant such as root endophytes, shoot endophytes, leaf endophytes, etc. Further, a root endophyte though prevalent in root zone may also exist in other plant parts or can trigger beneficial impacts on plant health at distant locations of its niche. Endophytes are of either bacterial, fungal, or actinomycete origin in plants. Tables 15.1 and 15.2, respectively, show the details of some of the fungal and bacterial endophytes reported in various crop species.

15.2 Bacterial Endophytes

Bacterial endophytes are widely present in agricultural crops and include *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans* (Dobereiner et al. 1995), *Serratia* spp. (Gyaneshwar et al. 2001; McInroy and Kloepper 1995), *Bacillus* spp. (Aravind et al. 2009; Forchetti et al. 2010; Kumar et al. 2016; McInroy and Kloepper 1995; Paul et al. 2013; Szilagyi-Zecchin et al. 2014; UmaMaheswari et al. 2013; Uppala et al. 2007), *Enterobacter* spp. (McInroy and Kloepper 1995; Szilagyi-Zecchin et al. 2014), *Agrobacterium radiobacter*, *Burkholderia gladioli*, *B. solanacearum* (McInroy and Kloepper 1995), *Pseudomonas putida* (Aravind et al. 2009), *P. fluorescens* (Ramesh et al. 2009; Uppala et al. 2007), *Achromobacter xylosoxidans* (Forchetti et al. 2010); *P. aeruginosa* (Paul et al. 2013), *Micrococcus* spp., and *Flavobacterium* spp. (UmaMaheswari et al. 2013). The details of some of the reported bacterial endophytes in various crops are given in Table 15.1.

The activities of these bacterial endophytes range from nitrogen fixation (Dobereiner et al. 1995; Gyaneshwar et al. 2001; Szilagyi-Zecchin et al. 2014) to PGP activities such as production of indoleacetic acid (Khan et al. 2016), phosphate solubilization (Kumar et al. 2016), production of siderophores as in eggplant by *Pseudomonas fluorescens* (Ramesh et al. 2009), abiotic stress tolerance as in sunflower where *Achromobacter xylosoxidans* and *Bacillus pumilus* are reported to promote growth under water stress (Forchetti et al. 2010), etc. Other than IAA, these bacterial endophytes were also reported to produce other plant growth hormones such as gibberellic acid (GA) and cytokinins (UmaMaheswari et al. 2013). Besides PGP activities, the anti-plant pathogenic activities of these bacterial endophytes are also well documented. For example, the fungal pathogens of corn such as *Fusarium*

Table 15.1 Details of prevalent bacterial endophytes/endosymbionts in agricultural crops

S. No.	Crop	Endophytes	Activity	References
1	Sugarcane (*Saccharum officinarum*)	*Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans*	Nitrogen fixation	Dobereiner et al. (1995)
2	Rice (*Oryza sativa* L.)	*Serratia marcescens*	Nitrogen fixation	Gyaneshwar et al. (2001)
3	Corn (*Zea mays* L.)	*Bacillus* spp.	Nitrogen fixation, production of IAA, siderophores, lytic enzymes. Antagonistic to the pathogenic fungi *Fusarium verticillioides*, *Colletotrichum graminicola*, *Bipolaris maydis*, and *Cercospora zeae-maydis*	Szilagyi-Zecchin et al. (2014)
		Enterobacter spp.	Nitrogen fixation	Szilagyi-Zecchin et al. (2014)
4	Sweet corn (*Zea mays* L.)	*Burkholderia pickettii*, *Enterobacter* spp., *Bacillus megaterium*, *Burkholderia gladioli*, *Burkholderia solanacearum*, *Enterobacter cloacae*	Not studied	McInroy and Kloepper (1995)
5	Cotton (*Gossypium hirsutum* L.)	*Agrobacterium radiobacter*, *Serratia* spp., *Burkholderia solanacearum*, *Bacillus megaterium*, *Bacillus pumilus*, *Acinetobacter baumannii*, *Arthrobacter* spp.	Not studied	McInroy and Kloepper (1995)
6	Wild and ancient maize (*Zea* spp.)	*Burkholderia gladioli*	Antagonistic to *Sclerotinia homoeocarpa*	Shehata et al. (2016)
7	Turmeric (*Curcuma longa* L.)	*Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus* sp., *Bacillus pumilus*, *Pseudomonas putida*, *Clavibacter michiganensis*	IAA production, phosphate solubilization antagonism against *Escherichia coli*, *Klebsiella pneumoniae*, and some of the fungi like *Fusarium solani* and *Alternaria alternata*	Kumar et al. (2016)

(continued)

Table 15.1 (continued)

S. No.	Crop	Endophytes	Activity	References
8	Black pepper (*Piper nigrum* L.)	*P. aeruginosa*, *P. putida*, *Bacillus megaterium*	Antagonistic to *Phytophthora capsici*, the causal agent of foot rot of black pepper	Aravind et al. (2009)
9	Banana (*Musa* spp.)	*B. amyloliquefaciens*, *B. subtilis subsp. subtilis*, *B. thuringiensis*	Antagonistic activity against *Fusarium oxysporum* f.sp *cubense* and *Colletotrichum graminicola*	Souja et al. (2014)
10	Sunflower (*Helianthus annuus* L.)	*Achromobacter xylosoxidans*, *Bacillus pumilus*	Enhances growth of sunflower seedlings under water stress. Salicylic acid production. Inhibits growth of pathogenic fungi	Forchetti et al. (2010)
11	Peanut (*Arachis hypogaea* L.)	*B. amyloliquefaciens*	Antibiosis against *Aspergillus flavus*	Sobolev et al. (2013)
12	Chilies (*Capsicum annuum* L.)	*P. fluorescens* EBS 20	Antibiosis against *Pythium aphanidermatum*	Muthukumar et al. (2010)
		Bacillus tequilensis, *Burkholderia cepacia*, *Pseudomonas aeruginosa*	Antagonistic activity against *Botrytis cinerea*, *Colletotrichum acutatum*, *Fusarium oxysporum*, and *Phytophthora capsici*	Paul et al. (2013)
14	Eggplant (*Solanum melongena* L.)	*Pseudomonas fluorescens*	Antibiosis against *Ralstonia solanacearum*, production of IAA and siderophores	Ramesh et al. (2009)
15	Tropical legumes:	*Bacillus* spp., *Micrococcus* spp., *Serratia* spp., *Pseudomonas* spp., *Flavobacterium* spp.	Production of phytohormones, viz., IAA, GA, and cytokinins	UmaMaheswari et al. (2013)
	Red gram (*Cajanus cajan* L.)			
	Black gram (*Vigna mungo* L.)			
	Green gram (*V. radiata* L.)			
	Cowpea (*V. unguiculata* L.)			
	Chickpea (*Cicer arietinum* L.)			

(continued)

Table 15.1 (continued)

S. No.	Crop	Endophytes	Activity	References
16	Leafy vegetable Amaranth (*Amaranthus* spp.)	*Bacillus* spp.	Production of induced systemic resistance enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase	Uppala et al. (2010a)
		Pseudomonas spp.		
			Promoted plant biometrics	Uppala et al. (2010b)
			Antagonistic activity against *Rhizoctonia solani*	Uppala et al. (2010c)

verticillioides, *Colletotrichum graminicola*, *Bipolaris maydis*, and *Cercospora zeae-maydis* are antagonized by the endophyte *Bacillus* spp. (Szilagyi-Zecchin et al. 2014). Similarly, the endophyte harboring wild and ancient maize is antagonistic to its fungal pathogen, *Sclerotinia homoeocarpa* (Shehata et al. 2016). Other important examples of endophytes having antagonistic activity are *Bacillus* spp., *Pseudomonas putida*, and *Clavibacter michiganensis* against *Fusarium solani* and *Alternaria alternata* in *Curcuma longa* (Kumar et al. 2016). Similarly, the plant growth-promoting rhizobacteria (PGPR), *P. fluorescens*, an endophyte in eggplant, is antagonistic to *Ralstonia solanacearum* (Ramesh et al. 2009). In banana, the endophytic species of *Bacillus* such as *B. amyloliquefaciens*, *B. subtilis* subsp. *subtilis*, and *B. thuringiensis* are antagonistic to fungal pathogens such as *Fusarium oxysporum* f.sp. *cubense* and *Colletotrichum graminicola* (Souja et al. 2014). Sobolev et al. (2013) reported antibiosis by the endophytic bacterium, *Bacillus amyloliquefaciens*, in peanut.

15.3 Fungal Endophytes

Research on fungal endophytes in various plants has progressed significantly. Fungal species that were majorly reported as endophytes in agricultural crops include *Trichoderma* spp. (Romao-Dumaresq et al. 2012; Uppala et al. 2007), *Epicoccum nigrum* (Favaro et al. 2012), *Penicillium* spp., *Xylaria*, *Alternaria*, *Cladosporium*, *Fusarium* spp. (Paul et al. 2012), *Scolecobasidium humicola* (Mahmoud and Narisawa 2013), *Fusarium oxysporum* (Kim et al. 2007), *Chaetomium globosum*, *Cladosporium cladosporioides* (Naik et al. 2009), *Aspergillus*, *Curvularia*, *Gilmaniella*, *Arthrobotrys foliicola* (Zakaria et al. 2010), *Acremonium zeae*, *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *Colletotrichum graminicola*, *Fusarium verticillioides*, *Saccharomyces cerevisiae*¸ *Trichoderma koningii* (Orole and Adejumo 2011), and others. Entomopathogens such as

Table 15.2 Details of some of the reported prevalent fungal endophytes/endosymbionts in agricultural crops

S. No.	Crop	Endophytes	Activity	References
1	Sugarcane (*Saccharum officinarum* L.)	*Trichoderma virens*	Antagonistic against pineapple disease pathogen, *Ceratocystis paradoxa*, owing to the production of endochitinases	Romao-Dumaresq et al. (2012)
2	Sugarcane (*Saccharum officinarum* L.)	*Aspergillus niger*, *Trichoderma atroviride*, *Alternaria* sp., *Annulohypoxylon stygium*, *Talaromyces wortmannii*	Excellent producers of hydrolytic enzymes (hemicellulases and related enzymes) to be used as part of blends to decompose sugarcane biomass at industrial level	Robl et al. (2013)
3	Sugarcane (*Saccharum officinarum* L.)	*Epicoccum nigrum*	*E. nigrum* was capable of increasing the root system biomass and producing compounds that inhibit the in vitro growth of sugarcane pathogens *Fusarium verticillioides*, *Colletotrichum falcatum*, *Ceratocystis paradoxa*, and *Xanthomonas albilineans*	Favaro et al. (2012)
			The fungus is capable of producing a natural product, epilactone	Araujo et al. (2012)
4	Chili pepper (*Capsicum annuum* L.)	*Penicillium* in seedling stage, *Fusarium* in flowering stage, and *Colletotrichum* followed by *Fusarium*, Alternaria, and *Xylaria* in fruiting stage were predominant, and *Alternaria*, *Cladosporium*, and *Fusarium* were common in all growth stages	Antimicrobial activity against three major pathogens (*Phytophthora capsici*, *Colletotrichum acutatum*, and *Fusarium oxysporum*) of chili pepper	Paul et al. (2012)
5	Tomato (Solanum lycopersicum) and Chinese cabbage (*Brassica campestris*)	*Scolecobasidium humicola*	Improve plant growth under organic nitrogen conditions	Mahmoud and Narisawa (2013)

(continued)

Table 15.2 (continued)

S. No.	Crop	Endophytes	Activity	References
6	Vegetable plants	*Fusarium oxysporum*	Most potent in vivo anti-oomycete activity against tomato late blight and in vitro anti-oomycete activity against several oomycete pathogens	Kim et al. (2007)
7	Rice (*Oryza sativa* L.)	*Chaetomium globosum*	Production of biologically active compounds; some have antagonistic properties against fungal pathogens	Naik et al. (2009)
		Penicillium chrysogenum,		
		Fusarium oxysporum		
		Cladosporium cladosporioides		
8	Rice (*Oryza sativa* L.)	*Fusarium*,	Not studied	Zakaria et al. (2010)
		Aspergillus,		
		Curvularia,		
		Penicillium,		
		Gilmaniella		
		Arthrobotrys foliicola		
9	Maize (*Zea mays* L.)	*Acremonium zeae*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum graminicola*, *Fusarium verticillioides*, *Saccharomyces cerevisiae*, and	Not studied	Orole and Adejumo (2011)
		Trichoderma koningii		
10	Tobacco (*N. tabacum*)	*Beauveria bassiana*	Entomopathogen	Russo et al. (2015)
	Corn (*Zea mays*)			
	Wheat (*Triticum aestivum*)			
	Soybean (*Glycine max*)			
11	Cotton (*Gossypium hirsutum*)	*Drechslerella dactyloides*,	Antagonists against plant pathogens	Ek-Ramos et al. (2013)
		Exserohilum rostratum		
		Alternaria tenuissima		
		Epicoccum nigrum, *Acremonium alternatum*		
		Cladosporium cladosporioides, *Chaetomium globosum*		
		Paecilomyces sp.		

(continued)

Table 15.2 (continued)

S. No.	Crop	Endophytes	Activity	References
12	Cotton (*Gossypium hirsutum*)	*Beauveria bassiana*, *Lecanicillium lecanii*, *Paecilomyces* spp.	Cotton aphid reproduction was significantly reduced on endophytic colonization	Sword et al. (2012)
13	Transgenic cotton (*Gossypium* spp.)	*Phoma archeri*	Not studied	Vieira et al. (2011)
		P. destructiva		
14	Black pepper (*Piper nigrum* L.)	*Annulohypoxylon nitens*	Antagonism against *Phytophthora capsici* and *Radopholus similis*	Sreeja et al. (2016)
		Daldinia eschscholzii, *Fusarium* spp.		
		Ceriporia lacerata		
		Diaporthe spp. and *Phomopsis* spp.		
15	Common bean (*Phaseolus vulgaris*)	*Aureobasidium pullulans*	Highest colonization in first true leaves of seedlings	Parsa et al. (2016)
16	Leafy vegetable Amaranth (Amaranthus spp.)	*Trichoderma harzianum*	Production of enzymes related to induced systemic resistance such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia lyase	Uppala et al. (2010a)
			Promoted amaranth plant biometrics	Uppala et al. (2010b)
			Antagonistic activity against amaranth leaf blight pathogen *Rhizoctonia solani*	Uppala et al. (2010c)

Beauveria bassiana and *Paecilomyces* spp. were also reported as endophytes in cotton and tobacco (Ek-Ramos et al. 2013; Sword et al. 2012). The details of some of the reported fungal endophytes in various crops are given in Table 15.2.

Both antagonistic and PGP activities are reported by these fungal endophytes in different plants. For example, the endophyte *T. virens* produces endochitinases against *Ceratocystis paradoxa*, the causal agent of pineapple disease on sugarcane (Romao-Dumaresq et al. 2012). Anti-oomycete activity was reported by *Fusarium oxysporum* strain EF119 against tomato late blight pathogen and others in vegetables (Kim et al. 2007). Similarly, the growth of fungal pathogens of sugarcane such as *Fusarium verticillioides*, *Colletotrichum falcatum*, *Ceratocystis paradoxa*, and *Xanthomonas albilineans* is effectively controlled under *in vitro* conditions as described by Favaro et al. (2012). Production of biologically active compounds by fungal endophytes for controlling plant diseases was reported in rice (Naik et al. 2009) and cotton (Ek-Ramos et al. 2013). In black pepper, antagonism was reported on *Phytophthora capsici* by fungal endophytes. Nematicidal properties by fungal

endophytes were also reported. For example, in black pepper, the burrowing nematode, *Radopholus similis*, was antagonized by fungal endophytes (Table 15.2). Important PGP activities include enhancement of root system biomass as in sugarcane by *E. nigrum* (Favaro et al. 2012) and plant growth in tomato (Mahmoud and Narisawa 2013). Industrial uses of certain endophytes are also reported as in sugarcane by Robl et al. (2013) where endophytes produced hydrolytic enzymes such as hemicellulases and others for degradation of sugarcane biomass.

15.4 Examples of Specific Activities of Endophytes

Other important roles include herbivory, bioremediation, biodegradation, biofertilization, etc. (Table 15.3). Herbivory is a well-manifested mechanism exhibited by endophytes that protect plant species from herbivores. Several direct and indirect effects of alkaloids produced by endophytes are witnessed. For example, the endophyte, *Neotyphodium occultans* when present in neighboring *Lolium multiflorum* has reduced the aphid infestation in *Trifolium repens* plants. However, no reduction in aphid infestation on *Trifolium* was observed when *Lolium* was not grown as a symbiont. This phenomenon can be described as association protection of non-host plants due to changes in host-volatile compounds which is an indirect effect (Parisi et al. 2014). Direct effects of alkaloids by endophytes in host plants are a common phenomenon as in Fescue grass (by the endophytes *Neotyphodium* spp. and *Epichloe* spp.), wherein the host plant leaves are protected from herbivores by the production of alkaloid, loline, produced by mutualistic fungal endophytes (Roberts and Lindow 2014).

Endophyte-mediated induction of resistance to plant diseases is also reported. In sunflower, resistance to stem rot caused by *Sclerotium rolfsii* is reported with the endophytes *Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5 (Waqas et al. 2015). Antibiotic-mediated resistance is also commonly noticed in certain cases. The antibiotics like Taxol by *Pestalotiopsis microspora* in *Taxus wallichiana* (Strobel et al. 1996), ecomycins B and C in *Lactuca sativa* by *Pseudomonas viridiflava* EB 273 (Miller et al. 1998), and trichodermin in garlic by *Trichoderma brevicompactum* (Shentu et al. 2014) are effective against specific plant pathogens. Besides production of antibiotics, HCN is another antimicrobial compound that is produced by certain endophytes in crops. For example, *Bacillus* produces HCN in avocado and black grapes (Prasad and Dagar 2014). Similarly, *Pseudomonas putida* produces HCN that has antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*; and antifungal activity against *Pythium ultimum* (Kumar et al. 2015). Pathogen-related enzymes such as lipase, cellulose, protease, amylase, chitinase, and pectinases are also produced by these endophytes (Sharma et al. 2015; Quecine et al. 2008). Plant growth-promoting activities by endophytes are well established as is evident in rice by *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Micrococcus* spp. (Mbai et al. 2013). PGP activities of endophytes are attributed to the production of iron-chelating agents, siderophores as in rice (*Enterobacter* spp. and *Burkholderia* spp.) (Souza et al. 2013), indoleacetic acid (IAA) and other growth hormones as in

Table 15.3 Examples of various activities of endophytes in various crops

Crop	Microbe	PGP/antagonistic activity	References
Annual ryegrass (*Lolium multiflorum*)	*Neotyphodium occultans*	Herbivory	Parisi et al. (2014)
Fescue grass (*Festuca arundinacea*)	*Neotyphodium* spp., *Epichloe* spp.	Herbivory	Roberts and Lindow (2014)
Sunflower (*Helianthus annuus* L.)	*Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5	Disease resistance	Waqas et al. (2015)
Himalayan yew (*Taxus wallichiana*)	*Pestalotiopsis microspore*	Antibiosis	Strobel et al. (1996)
Lettuce (*Lactuca sativa*)	*Pseudomonas viridiflava EB 273*	Antibiosis	Miller et al. (1998)
Perennial onion (*Allium fistulosa*)	*Streptomyces* spp.	Antibiosis	Igarashi et al. (2002)
Garlic (*Allium sativum* L.)	*Trichoderma brevicompactum*	Antibiosis	Shentu et al. (2014)
Avocado (*Persea* sp.), black grapes (*Vitis vinifera*)	*Bacillus* spp.	HCN production	Prasad and Dagar (2014)
Dicot legume (*Cassia tora* L.)	*A. tumefaciens*, *P. putida*, *Pseudomonas* sp.	HCN production	Kumar et al. (2015)
Saffron (*Crocus sativus* L.)	*B. licheniformis*	–	Sharma et al. (2015)
Citrus (*Citrus* sp.) *and Soybean* (*Glycine max*)	*Streptomyces* spp.	–	Quecine et al. (2008)
Rice (*Oryza sativa* L.)	*Pseudomonas*, *Bacillus*, *Enterobacter*, and *Micrococcus*	PGP activity	Mbai et al. (2013)
Rice (*Oryza sativa* L.)	*Enterobacter* spp.	Siderophores	Souza et al. (2013)
	Burkholderia spp.		
Cashew (*Anacardium occidentale* L.)	*Staphylococcus saprophyticus*, *Escherichia coli* and *Shigella flexneri*	Growth hormones	Lins et al. (2014)
Echinacea (*Echinacea* SP.)	*Pseudomonas stutzeri* P3	Growth hormones	Lata et al. (2006)
Tomato (*Solanum lycopersicum* L.)	*Rhizobium* spp., *Rhodococcus* spp., *Agrobacterium* spp.	Growth hormones	Abbamondi et al. (2016)
Jamaican pepper (*Piper hispidum Sw.*)	*Phoma herbarum*	–	Orlandelli et al. (2015)

(continued)

Table 15.3 (continued)

Crop	Microbe	PGP/antagonistic activity	References
Common yarrow (*Achillea millefolium*), *Canada goldenrod* (*Solidago canadensis*), large hop trefoil, (*Trifolium aureum*), and cocksfoot or orchard grass (*Dactylis glomerata*)	*Microbacterium foliorum*	Bioremediation	Lumactud et al. (2016)
Chinese ladder brake (*Pteris vittata L.*)	*Bacillus pumilus*	Bioremediation	Srivastava et al. (2016)
Banana (*Musa* spp.)	*Rahnella* spp. and *Pseudomonas* spp.	Biofertilizers	Ngamau et al. (2012)
Rice (*Oryza sativa* L.)	*Burkholderia cepacia* (CS5), *Citrobacter* sp. (CR9), *Citrobacter* spp. (SS5), *Citrobacter* sp. (SS6), *Bacillus amyloliquefaciens* (25R14), *B. amyloliquefaciens* (SR1), and *B. thuringiensis* (25R2)	N-fixing diazotrophs	Hongrittipun et al. (2014)
Green gram (*Vigna radiata* L.)	*Azotobacter* spp.	–	Aung et al. (2011)
Sugarcane	*Novosphingobium sediminicola* and *Ochrobactrum intermedium*	–	Muangthong et al. (2015)

cashew (*Staphylococcus saprophyticus* and *Escherichia coli*) (Lins et al. 2014) and in *Echinacea by Pseudomonas stutzeri* (Lata et al. 2006), etc. Enzymes known to induce systemic resistance such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) were reported from endophytic fungi and bacteria isolated from leafy vegetable amaranth (Uppala et al. 2010a). Bioremediation activities were also reported by several endophytes. For example, degradative capabilities for octanol, toluene, naphthalene, kerosene or motor oil, and arsenic biodegradation were demonstrated by *Microbacterium foliorum* and *Bacillus pumilus* (Lumactud et al. 2016; Srivastava et al. 2016) as is evident in crops like *Pteris vittata*, *Achillea millefolium*, *Solidago canadensis*, *Trifolium aureum*, and *Dactylis glomerata*. Further, other major activities of endophytes include their role as biofertilizers as evident in banana (*Rahnella* spp. and *Pseudomonas* spp.) (Ngamau et al. 2012) and corn (*Azotobacter vinelandii*, *B. subtilis*, and *Enterobacter cloacae*). The examples of various activities of endophytes in various plants are presented in Table 15.3.

15.5 Niches of Endophytes in Plants: A Case Study on Sugarcane

Sugarcane (*Saccharum officinarum* L), a bamboo family crop, which is indigenous to India, has multifarious uses such as sugar, khandsari, gur, etc. The crop also generates employment through industries of sugar, petroleum, and paper industry. The crop residue is also used as a fodder. Several plant diseases affect sugarcane cultivation globally, thereby causing huge economic losses. Significant among them are red rot (*Colletotrichum falcatum*), wilt (*Fusarium sacchari*), whip smut (*Ustilago sacchari*), mosaic (sugarcane mosaic virus, SCMV), yellow leaf disease (YLD), sugarcane yellow leaf virus (SCYLV), leaf scald (*Xanthomonas albilineans*), etc. Currently available disease control practices including chemical control and host plant resistance are not able to generate satisfactory reductions in these pathogen inocula. So there is an urgent need to identify potential alternatives. These alternatives also need to be technically feasible, economically viable, and easily adoptable in farmers' fields, of course with least harm to the environment. In this direction, use of biological control agents such as beneficial bacteria, fungi, and actinomycetes is gaining significance for managing these diseases. The efficacy of these biocontrol agents in promoting plant growth and yield is also well established (Kumar et al. 2012). Among fungal biocontrol agents, species of *Trichoderma* (Martinez et al. 1998; Singh et al. 2008; Talukder et al. 2007; Yadav et al. 2008) and *Gliocladium* (Guevara 1990; Mahalingam et al. 2011) are significant disease suppressors. Among bacterial strains, plant growth-promoting rhizobacteria (PGPR) have wide applications, and of them, species of *Bacillus* and *Pseudomonas* are prominently used. Actinomycete strains are also known for their potential biocontrol capabilities in various crops (Jacob et al. 2016).

Success of a biocontrol agent depends on rapidity of its establishment in its niche, aggressiveness in crop microclimate (rhizosphere, rhizoplane, spermosphere/spermoplane, and phylloplane), thereby exhibiting its antagonistic traits on plant pathogens. For successful deployment of a candidate bioagent to field, it is prerequisite to evaluate its PGP traits and disease-suppressing abilities. However, inundative release of these microbes often may not be sufficient to reap desired benefits under field conditions, especially in crop soils where pathogen inocula are multifold in contrast to experimental conditions. Precisely at this juncture, the role of endophytes/endosymbionts in plant disease management assumes significance.

Research on endophytes on sugarcane has well been documented earlier. Endophytes of fungal, bacterial, and actinomycete origin are reported in different plant sites. The details of various endophytic genera are given in Table 15.4. *Streptomyces* sp. is the common actinomycetes that are prevalent in sugarcane (Sinma et al. 2015). Different endophytes are reported to colonize various plant tissues in sugarcane. For example, the roots of sugarcane are reported to harbor *Streptomyces* (Bhosale and Kadam 2015), *Herbaspirillum*, *Bacillus* spp. (Silva et al. 2015), *Enterobacter oryzae* LT7 (Tam and Diep 2014), *Gluconacetobacter diazotrophicus* (Prabudoss 2011), *Epicoccum nigrum* P16 (Favaro et al. 2012), *Burkholderia* spp. (Mendes et al. 2007; Omarjee et al. 2004), and *Trichoderma*

Table 15.4 Some of the reported fungal and bacterial endophytes of sugarcane along with their associated plant parts

Endophytic genera	Plant site	PGP/antagonistic activity	References
Streptomyces spp.	Roots	Siderophores	Bhosale and Kadam (2015)
Streptomyces spp.	Root, leaf and stem		Sinma et al. (2015)
Acetobacter diazotrophicus	Shoot	IAA	Patil et al. (2011)
Herbaspirillum spp. and *Bacillus* spp.	Roots	P solubilization	Silva et al. (2015)
Burkholderia vietnamiensis, *Klebsiella pneumonia*	Root, leaf, and shoots	N fixation	Govindarajan et al. (2007)
Novosphingobium sediminicola and *Ochrobactrum intermedium*	Shoot	–	Muangthong et al. (2015)
Enterobacter oryzae , *Achromobacter xylosoxidans*, *Achromobacter insolitus*, *and Pantoea agglomerans*	Roots and shoots	–	Tam and Diep (2014)
Gluconacetobacter diazotrophicus	Root, shoot and leaf	–	Prabudoss (2011)
Burkholderia spp. against *Ustilago* and *Fusarium*	Stalk	Antibiosis	Antwerpen et al. (2002)
Epicoccum nigrum against *Ceratocystis paradoxa*, *Fusarium verticillioides*, *Colletotrichum falcatum,* and *Xanthomonas albilineans*	Leaves and roots	–	Favaro et al. (2012)
Burkholderia cepacia against *Fusarium moniliforme*	Roots and stems	–	Mendes et al. (2007)
Burkholderia spp. (chitinases)	Shoot	Cell wall-degrading enzymes	Omarjee et al. (2004)
Baccharis dracunculifolia (*cellulases*)	Whole plant	–	Onofre et al. (2014)
Trichoderma virens (*endochitinases*)	Roots	–	Romao-Dumaresq et al. (2012)

virens (Romao-Dumaresq et al. 2012). Other than roots, shoots of sugarcane are also niches of endophytes such as *Streptomyces* spp. (Sinma et al. 2015), *Acetobacter diazotrophicus* (Patil et al. 2011), *Burkholderia vietnamiensis*, *Klebsiella pneumonia* (Govindarajan et al. 2007), *Novosphingobium sediminicola*, and *Ochrobactrum intermedium* (Muangthong et al. 2015). The leaves of sugarcane are harbored by endophytes such as *Streptomyces* spp. (Sinma et al. 2015), *Burkholderia vietnamiensis* (AY973820), *Klebsiella pneumonia* (Govindarajan et al. 2007), *Gluconacetobacter diazotrophicus* (Prabudoss 2011), *Epicoccum nigrum* P16 (Favaro et al. 2012), etc. Endophytes such as *Burkholderia dracunculifolia* were reported in whole sugarcane plant (Onofre et al. 2014).

The beneficial effects of endophytes in sugarcane are also well established. Varied activities relating to PGP are reported. For example, *Streptomyces* spp. has been associated with production of iron-chelating agents, siderophores (Bhosale and Kadam 2015). Further, *Herbaspirillum* spp. and *Bacillus* spp. that are found in roots of sugarcane were found to solubilize organic phosphorus in soil and make it available to plants (Silva et al. 2015). Diazotrophic strains of *Burkholderia vietnamiensis* and *Klebsiella pneumonia* isolated from sugarcane are reported to show nitrogen-fixing abilities (Govindarajan et al. 2007). Specific antagonistic activities of these endophytes in sugarcane targeting specific plant pathogens are also reported. For example, *Ustilago* (whip smut) and *Fusarium* (wilt) pathogens were inhibited by *Burkholderia* spp. through antibiosis-mediated mechanisms (Antwerpen et al. 2002). Further, these *Burkholderia* spp. are also known to produce cell wall-degrading enzymes (CWDE) of pathogens such as chitinases and cellulases (Omarjee et al. 2004; Onofre et al. 2014). Similarly, the *Epicoccum nigrum* (P 16) was antagonistic to *Ceratocystis paradoxa* (pineapple disease), *Fusarium verticillioides* (Pokkah boeng), *Colletotrichum falcatum* (red rot), and *Xanthomonas albilineans* (Leaf scald) (Favaro et al. 2012).

15.6 Conclusion and Future Prospects

Detection of an elite endophyte or consortium of endophytes that are constantly associated with crops and play vital role in improving crop health is essential. Further, these endophytes must be characterized for specific PGP and antagonistic activities including antibiotic production against specific plant pathogens. Besides biochemical characterization, the rapidity of establishment of these endophytes in specific niches of plants and/or whole plant as the case may be also examined before identifying them as candidates for biocontrol against economically important plant diseases.

References

Abbamondi GR, Tommonaro G, Weyens N, Thijs S, Sillen W, Gkorezis P, Iodice C, Rangel W, Nicolaus B, Vangronsveld J (2016) Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. Chem Biol Technol Agric 3:1. doi:10.1186/s40538-015-0051-3

Antwerpen TV, Rutherford RS, Vogel JL (2002) Assessment of sugarcane endophytic bacteria and rhizospheric *Burkholderia* species as antifungal agents. Proc S Afr Surg Technol Assoc 76:301–304

Araujo FD, Favaro LC, Araujo WL, Oliveira FL, Aparicio R, Marsaioli AJ (2012) Epilactone – natural product isolated from the sugarcane endophytic fungus, *Epicoccum nigrum*. Eur J Org Chem 27(3):5225–5230

Aravind R, Kumar A, Eapen SJ, Ramana KV (2009) Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: isolation, identification and evaluation against *Phytophthora capsici*. Lett Appl Microbiol 48(1):58–64

Aung TN, Nourmohammadi S, Sunitha EM, Myint M (2011) Isolation of endophytic bacteria from greengram and study on their plant growth-promoting activities. Int J Appl Biol Pharm Technol 2(3):525–537

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. Curr Opin Biotechnol 20:642–650

Bhosale HJ, Kadam TA (2015) Genetic diversity and a comparative account on plant growth promoting characteristics of actinimycetes in roots and rhizosphere of *Saccharum officinarum*. Int J Curr Microbiol App Sci 4:230–244

Compant S, Saikkonen K, Mitter B, Campisano A, Mercado-Blanco J (2016) Editorial special issue: soil, plant and endophytes. Plant Soil 405(1):1–11

Dobereiner J, Baldani VLD, Reis VM (1995) Endophytic occurrence of diazotrophic bacteria in non-leguminous crops. In: *Azospirillum* VI and related microorganisms, volume 37 of the series NATO ASI series. Springer, Berlin, pp 3–14

Ek-Ramos MJ, Zhou W, Valencia CU, Antwi JB, Kalns LL, Morgan GD, Kerns DL, Sword GA (2013) Spatial and temporal variation in fungal endophyte communities isolated from cultivated cotton (*Gossypium hirsutum*). PLoS One 8(6):e66049

Evangelisti E, Rey T, Schornack S (2014) Cross-interference of plant development and plant–microbe interactions. Curr Opin Plant Biol 20:118–126. doi:10.1016/j.pbi.2014.05.014

Fávaro LC, Sebastianes FL, Araújo WL (2012) *Epicoccum nigrum* P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. PLoS One 7(6):e36826

Forchetti G, Masciarelli O, Izaguirre MJ, Alemano S, Alvarez D, Abdala G (2010) Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid and inhibit growth of pathogenic fungi. Curr Microbiol 61(6):485–493

Govindarajan M, Kwon SW, Weon H (2007) Isolation, molecular characterization and growth-promoting activities of endophytic sugarcane diazotroph *Klebsiella* sp. GR9. World J Microbiol Biotechnol 23(7):997–1006

Govindarajan M, Balandreau J, Kwon S-W, Weon H-Y, Lakshminarasimhan C (2008) Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microbiol Ecol 55:21–37

Guevara MM (1990) Evaluation of Gliocladium spp. in the control of pineapple disease of sugarcane caused by *Ceratocystis paradoxa* (Dade) Moreau. Philipp Sugar Quart 1(4):20–28

Gyaneshwar P, James EK, Natarajan M, Reddy PM, Reinhold-Hurek B, Jagdish KL (2001) Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. J Bacteriol 183(8):2634–2645

Hardoim PR, van Overbeek LS, Berg G, Pirtilla AM, Compant S, Campisano A, Doring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Heydari A, Fattahi H, Zamanizadeh HR, Zadeh NH, Naraghi L (2004) Investigation on the possibility of using bacterial antagonists for biological control of cotton seedling damping-off in greenhouse. Appl Entomol Phytopathol 72:51–68

Hirsch AM (2004) Plant–microbe symbioses: a continuum from commensalism to parasitism. Symbiosis 37:345–363

Hongrittipun P, Youpensuk S, Rerkasem B (2014) Screening of nitrogen fixing endophytic bacteria in *Oryza sativa* L. J Agric Sci 6(6):66–74

Igarashi Y, Ogawa M, Sato Y, Saito N, Yoshida R, Kunoh H (2002) Fistupyrone, a novel inhibitor of the infection of Chinese cabbage by *Alternaria brassicicola*, from *Streptomyces* sp. J Antibiot 53(10):1117–1122

Jacob S, Sajjalaguddam RR, Kumar KVK, Varshney RK, Sudini H (2016) Assessing the prospects of *Streptomyces* sp. RP1A-12 in managing groundnut stem rot disease caused by *Sclerotium rolfsii* Sacc. J Gen Plant Pathol 82(2):96–104

Khan AL, Halo BA, Elyassi A, Ali S, Al-Hosni K, Hussain J, Al-Harrasi A, Lee IJ (2016) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64

Kim HY, Ghoi GJ, Lee HB, Lee SW, Lim HK, Jang KS, Son SW, Lee SO, Cho KY, Sung ND, Kim JC (2007) Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. Lett Appl Microbiol 44(3):332–337

Kumar KVK, Yellareddygari SKR, Reddy MS, Kloepper JW, Lawrence KS, Zhou XG, Sudini H, Groth DE, Krishnam Raju S, Miller ME (2012) Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. Rice Sci 19(1):55–63

Kumar V, Kumar A, Pandey KD, Roy BK (2015) Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. Ann Microbiol 65(3):1391–1399

Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD (2016) Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech 6(1):60

Lata H, Li XC, Silva B, Moares RM, Halda-Alija L (2006) Identification of IAA-producing endophytic bacteria from micropropagated *Echinacea* plants using 16S rRNA sequencing. Plant Cell Tissue Organ Cult 85(3):353–359

Lins MRCR, Fontes JM, Vasconcelos NM, Santos DMS, Ferreira OE, Azevedo JC, Araujo JM, Lima GMS (2014) Plant growth-promoting potential of endophytic bacteria isolated from cashew leaves. Afr J Biotechnol 13(33):3360–3365

Lumactud R, Shen SY, Lau M, Fulthorpe R (2016) Bacterial endophytes isolated from plants in natural oil sleep soils with chronic hydrocarbon contamination. Front Microbiol 7:755

Mahalingam R, Ambikapathy V, Panneerselvam A (2011) Biocontrol measures of pineapple disease in sugarcane. Eur J Exp Biol 1(2):64–67

Mahmoud RS, Narisawa K (2013) A new fungal endophyte, *Scolecobasidium humicola*, promotes tomato growth under organic nitrogen conditions. PLoS One 8(11):e78746

Martinez B, Gonzales R, Balance C (1998) Antagonism of *Trichoderma* spp. strains on some sugarcane pathogens. Fitopathologia 33:207–211

Mbai FN, Magiri EN, Matiru VN, Nganga J, Nyambati VCS (2013) Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenyan Basmati rice. Am Int J Contemp Res 3(4):25–40

McInroy JA, Kloepper JW (1995) Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil 173(2):337–342

Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JS (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl Environ Microbiol 73(22):7259–7267

Miller CM, Miller RV, Garton-Kenny D, Redgrave B, Sears J, Condron MM, Teplow DB, Strobel GA et al (1998) Ecomycins, unique antimycotics from *Pseudomonas viridiflava*. J Appl Microbiol 84(6):937–944

Muangthong A, Youpensuk S, Rerkasem B (2015) Isolation and characterization of endophytic nitrogen fixing bacteria in sugarcane. Trop Life Sci Res 26(1):41–51

Muthukumar A, Nakkeeran S, Eswaran A, Sangeetha G (2010) In vitro efficacy of bacterial endophytes against the chilli damping-off pathogen *Pythium aphanidermatum*. Phytopathol Mediterr 49(2):179–186

Naik BS, Shashikala J, Krishnamurthy YL (2009) Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. Microbiol Res 164(3):290–296

Ngamau N, Matiru., Viviene, N., Tani, A., and Muthuri, C. W. (2012) Isolation and identification of endophytic bacteria of bananas (Musa spp.) in Kenya and their potential as biofertilizers for sustainable banana production. Afr J Microbiol Res 6:6414–6422

Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144

Omarjee J, Antwerpen TV, Balandreau J, Kuniata L, Rutherford S (2004) Isolation and characterization of some endophytic bacteria from Papua New Guinea sugarcane. Proc S Afr Surg Technol Assoc 78:189–194

Onofre SB, Bonfante T, Santos ZMQ, Moura MC, Cardoso AF (2014) Cellulase production by endophytic strains of *Trichoderma reesei* from *Baccharis dracunculifolia* D.C. (Asteraceae). Adv Microbiol 4:275–283

Orlandelli RC, Almeida TT, Alberto RN, Polonio JC, Azevedo JC, Pamphile JA (2015) Antifungal and proteolytic activities of endophytic fungi isolated from *Piper hispidum* Sw. Braz J Microbiol 46(2):359–366

Orole OO, Adejumo TO (2011) Bacterial and fungal endophytes associated with grains and roots of maize. J Ecol Nat Environ 3(9):298–303

Parisi PAG, Grimoldi AA, Omacini M (2014) Endophytic fungi of grasses protect other plants from aphid herbivory. Fungal Ecol 9:61–64

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6(10):763–775

Parsa S, Garcia-Lemos AM, Castillo K, Oritz V, Lopez-Lavalle LAB, Braun J, Vega FE (2016) Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. Fungal Biol 120(5):783–790

Patil NB, Gajbhiye M, Ahiwale SS, Gunjal AB, Kapadnis BP (2011) Optimization of indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from sugarcane. Int J Environ Res 2(1):307–314

Paul NC, Deng JX, Sang HK, Choi YP, Yu SH (2012) Distribution and antifungal activity of endophytic fungi in different growth stages of chilli pepper (*Capsicum annuum* L.) in Korea. Plant Pathol J 28(1):10–19

Paul NC, Ji S,H, Deng JX, Yu SH (2013) Assemblages of endophytic bacteria in chilli pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens in vitro. Plant Omics 6(6):441–448

Prabudoss V (2011) A real multi beneficial endophytic diazotroph *Gluconacetobacter diazotrophicus* for sugarcane. Int J Curr Res 3(6):103–106

Prasad MP, Dagar S (2014) Identification and characterization of endophytic bacteria from fruits like avocado and black grapes. Int J Curr Microbiol App Sci 3(8):937–947

Quecine MC, Araujo WL, Marcon J, Gai CS, Azevedo JL, Pizzirani-Kleiner AA (2008) Chitinolytic activity of endophytic *Streptomyces* and potential for biocontrol. Lett Appl Microbiol 47(6):486–491

Ramesh R, Joshi AA, Ghanekar MP (2009) Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the egg plant (*Solanum melongena* L.) World J Microbiol Biotechnol 5(1):47–55

Roberts E, Lindow S (2014) Loline alkaloid production by fungal endophytes of *Fescue* species select for particular epiphytic bacterial microflora. ISME J 8:359–368

Robl D, Delabona PDS, Megel CM, Rojas JD, Costa PDS, Pimentel ID, Vincente VA, Pradella JGDC, Padilla G (2013) The capability of endophytic fungi for production of hemicellulases and related enzymes. BMC Biotechnol 13:94

Romao-Dumaresq A, Araujo WL, Talbot NJ, Thornton CR (2012) RNA interference of endochitinases in the sugarcane endophyte *Trichoderma virens* 223 reduces its fitness as a biocontrol agent of pineapple disease. PLoS One 7(10):e47888

Russo ML, Pelizza SA, Cabello MN, Stenglien SA, Scorsetti AC (2015) Endophytic colonization of tobacco, corn, wheat and soybeans by the fungal entomopathogen, *Beauveria bassiana* (Ascomycota, Hypocreales). Biocontrol Sci Tech 25(4):475–480

Sang MK, Shrestha A, Kim D, Park K, Pak CH, Kim KD (2013) Biocontrol of phytophthora blight and anthracnose in pepper by sequentially selected antagonistic rhizobacteria against *Phytophthora capsici*. Plant Pathol J 29(2):154–167

Sharma T, Kaul S, Dhar MK (2015) Diversity of culturable bacterial endophytes of saffron in Kashmir, India. Spring 4:61

Shehata HR, Lyons EM, Jordan KS, Raizada MN (2016) Bacterial endophytes from wild and ancient maize are able to suppress the fungal pathogen *Sclerotinia homoeocarpa*. J Appl Microbiol 120(3):756–769

Shentu X, Zhan X, Ma Z, Yu X, Zhang C (2014) Antifungal activity of metabolites of the endophytic fungus *Trichoderma brevicompactum* from garlic. Braz J Microbiol 45(1):248–254

Silva JM, Santos TMC, Albuquerque LS, Montaldo YC, Oliveria JUL, Silva SGM, Nascimento MS, Teixeria RRO (2015) Potential of endophytic bacteria (*Herbaspirillum* spp. and *Bacillus* spp.) to promote sugarcane growth. Aust J Crop Sci 9(8):754–760

Singh V, Joshi BB, Awasthi SK, Srivastava SN (2008) Eco-friendly management of red rot disease of sugarcane with *Trichoderma* strains. Sugar Tech 10(2):156–161

Sinma K, Nurak T, Khucharoenphaisan K (2015) Potentiality of endophytic actinomycetes isolated from sugarcane. KMITL Sci Technol J 15(2):88–97

Sobolev VS, Orner VA, Arias RS (2013) Distribution of bacterial endophytes in peanut seeds obtained from axenic and control plant material under field conditions. Plant Soil 37(1):367–376

Souja A, Cruz JC, Sousa NR, Procopio AR, Silva GF (2014) Endophytic bacteria from banana cultivars and their antifungal activity. Genet Mol Res 13(4):8661–8670

Souza R, Beneduzi A, Ambrosini A, Costa PB, Meyer J, Vargas LK, Schoenfeld R, Passaglia LMP (2013) The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. Plant Soil 366(1):585–603

Sreeja K, Anandaraj M, Bhai RS (2016) In vitro evaluation of fungal endophytes of black pepper against *Phytophthora capsici* and *Radopholus similis*. J Spices Aromat Crops 25(2):113–122

Srivastava S, Singh M, Paul AK (2016) Arsenic biodegradation and bioactive potential of endophytic bacterium *Bacillus pumilus* isolated from *Pteris vittata* L. Int J Adv Biotechnol Res 7(1):77–92

Strobel G, Yang X, Sears J, Kramer R, Sidhu RS, Hess WM (1996) Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallichiana*. Microbiology 142(2):435–440

Sword G, Ek-Ramos MJ, Lopez DC, Kalns L, Zhou W, Valencia C (2012) Fungal endophytes and their potential for biocontrol in cotton. In: Entomological society of America annual meeting 2012

Szilagyi-Zecchin VJ, Ikeda AC, Hungria M, Adamoski D, Kava-Cordeiro V, Glienke C, Galli-Terasawa LV (2014) Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. AMB Express 4:26

Talukder MI, Begum F, Azad MMK (2007) Management of pineapple disease of sugarcane through biological means. J Agric Rural Dev 5:79–83

Tam HM, Diep CN (2014) Isolation, characterization and identification of endophytic bacteria in sugarcane (*Saccharum* spp. L.) cultivated on soils of the Dong Nai Province, Southeast of Vietnam. Am J Life Sci 2(6):361–368

UmaMaheswari T, Anbukkarasi K, Hemalatha T, Chendrayan K (2013) Studies on phytohormone producing ability of indigenous endophytic bacteria isolated from tropical legume crops. Int J Curr Microbiol App Sci 2(6):127–136

Uppala S (2007) Potentiality of endophytic microorganisms in the managment of leaf blight disease of amaranth. Masters Thesis. Kerala Agricultural University. doi:10.13140/RG.2.2.33389.90083

Uppala S, Beena S, Chapala M, Bowen KL (2010a) Role of endophytes in inducing systemic resistance against leaf blight disease of amaranth. In: Reddy MS, Desai S, Sayyed RZ, Rao VK, Sarma YR, Reddy BC, Reddy KRK, Podile AR, Kloepper JW (eds) Plant growth promotion by Rhizobacteria for sustainable agriculture. Scientific Publishers, Jodhpur, pp 516–523. doi:10.13140/RG.2.1.2898.1289

Uppala S, Beena S, Chapala M, Bowen KL (2010b) Amaranth endophytes and their role in plant growth promotion. In: Reddy MS, Desai S, Sayyed RZ, Rao VK, Sarma YR, Reddy BC, Reddy KRK, Podile AR, Kloepper JW (eds) Plant growth promotion by Rhizobacteria for sustainable agriculture. Scientific Publishers, Jodhpur, pp 531–537. doi:10.13140/RG.2.1.3291.3441

Uppala S, Beena S, Chapala M, Bowen KL (2010c) Bioefficacy of endophytes in the management of leaf blight disease of amaranth. In: Reddy MS, Desai S, Sayyed RZ, Rao VK, Sarma YR, Reddy BC, Reddy KRK, Podile AR, Kloepper JW (eds) Plant growth promotion by Rhizobacteria for sustainable agriculture. Scientific Publishers, Jodhpur, pp 524–530. doi:10.13140/RG.2.1.2767.0564

Vieira PD, Motta CM, Lima D, Torres JB, Quecine MC, Azevedo JC, de Oliveira NT (2011) Endophytic fungi associated with transgenic and non-transgenic cotton. Mycology 2(2):91–97

Waqas M, Khan AL, Hamayun M, Shahzad R, Kang SM, Kim JG, Lee IJ (2015) Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. J Plant Interact 10(1):280–287

Yadav RL, Singh V, Srivastava SN, Lal RJ, Awasthi SK, Joshi BB (2008) Use of *Trichoderma harzianum* for the control of red rot disease of sugarcane. Sugarcane Int 26(4):28–33

Zakaria L, Yaakop AS, Salleh B, Zakaria M (2010) Endophytic fungi from paddy. Trop Life Sci Res 21(1):101–107

Ziedan EHE (2006) Manipulating endophytic bacteria for biological control to soil borne diseases of peanut. J Appl Sci Res 2(8):497–502

Quorum Sensing in Plant Growth-Promoting Rhizobacteria and Its Impact on Plant-Microbe Interaction

16

Mohd. Musheer Altaf, Mohd. Sajjad Ahmad Khan, Hussein Hasan Abulreesh, and Iqbal Ahmad

Abstract

Quorum sensing is a widespread mechanism in enormous number of bacteria for regulating various gene expression in a cell density-dependent manner through production and recognition of small molecules known as autoinducer. Diverse kinds of quorum-sensing networks are found in different bacterial species. Among various signal molecules, acyl homoserine lactone (AHL) signal molecules are the most and widely studied in bacteria. A number of simple to advanced techniques are being used to identify and characterize signal molecules. Production of signal molecules in a number of rhizospheric bacteria is documented. Rhizosphere is an active atmosphere where microbe-microbe and microbe-plant interaction is highest due to rich availability of nutrients provided in the form of root exudates. Several ecological and interdependent key characters of bacteria, like antibiotic, siderophore, or enzyme secretion, virulence factors of phytopathogens, as well as plant-microbe communications, are coordinated through quorum sensing (QS). In this chapter, we have provided brief fundamental aspects of quorum sensing and then addressed the recent trends on the significance of quorum sensing and signal molecules in microbe-microbe and microbe-plant interactions in the rhizosphere with special reference to plant growth-promoting rhizobacteria and plant health.

M.M. Altaf • I. Ahmad (✉)
Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh 202002, India
e-mail: iqbalahmad8@yahoo.co.in

M.S.A. Khan
Department of Biology, College of Medicine, Imam Abdulrahman Bin-Faisal University, Dammam 31451, Kingdom of Saudi Arabia

H.H. Abulreesh
Department of Biology, Faculty of Sciences, Umm Al-Qura University, Makkah 21955, Kingdom of Saudi Arabia

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_16

Keywords
Quorum sensing • AHLs • PGPR • Plant-microbe interaction • Rhizosphere signaling

16.1 Introduction

Since ages, scientist believed that the single cell prokaryotic bacterium lacking true nucleus is not capable of establishing a fundamental form of community attitude as a consequence of chemical conversation between the members of a community. Interdependent behavior by means of autoinducer compounds was first discovered in bacteria which are living in symbiotic association with a marine squid (Kaplan and Greenberg 1985; Verma and Miyashiro 2013). The fundamental part of this molecular conversation, termed as "quorum sensing" (QS), and the signaling molecules implicated were established through an extremely basic test: via adding together a formally habituated supernatant of a heavily developed bacterial culture to a fresh, low concentration culture, the characteristics of the high density culture were conferred (Eberhard 1972; Waters and Bassler 2005). The signaling compounds implicated in this conversation are called as "autoinducers," as they were derived from within the bacterial cell and controlling their individual expression. The signaling compound can be perceived and reimported into these cells, consequently permitting the whole inhabitants to react to altering situation/necessities once a significant volume (equivalent to a particular cell density) or "quorum," i.e., the minimum number of bacterial cell accumulated in a given volume to make the "decision" to switch on gene expression of QS-regulated genes, is achieved as described by Ahmad et al. (2011).

The marine bacterium *Vibrio fischeri* was the first bacterium to be examined for quorum sensing. As a communication compound, N-(3-oxo)-hexanoyl-L-homoserine lactone (3oxoC6-HSL) was recognized to regulate bioluminescence as a readily assessable result of supportive action. Currently, numerous chemical signaling compounds of bacterial origin have been recognized. AHL served as a universal signal molecule within Gram-negative bacteria (Galloway et al. 2011). Molecules of AHL are created by *LuxI* homologues, and comprise, clearly with *LuxR* homologues, a transcriptional regulator. AHL comprises a conserved homoserine lactone ring with an uneven N-acyl chain (Ahmad et al. 2008). Bacteria belonging to both Gram-positive and Gram-negative groups use QS messaging pathways to control a different group of physiological behavior of bacterial cells which includes symbiosis, competence, virulence, antibiotic production, conjugation, motility, sporulation, and biofilm formation (Rutherford and Bassler 2012).

Universally, Gram-negative bacteria utilize acylated homoserine lactones as autoinducers, and Gram-positive bacteria exploit processed oligopeptides for interaction (Miller and Bassler 2001). Commonly studied autoinducer signals are N-acyl homoserine lactones (von Bodman et al. 2003), although half a dozen of other molecules, including diketopiperazines, 4-hydroxy-2-alkylquinolines (HAQs), and

autoinducer-3 (AI-3) in various Gram-negative bacteria (Jimenez et al. 2012), furanosyl borate diester in *Vibrio harveyi* (Chen et al. 2002), and c-butyrolactone in *Streptomyces*, have also been involved in quantity-based signaling (Yamada and Nihira 1998). While quorum-sensing peptides (QSPs) are especially reported from Gram-positive bacteria (Wynendaele et al. 2013), autoinducer-2 (AI-2) has been reported from both Gram-positive and Gram-negative bacteria (Pereira et al. 2013). Recently, Papenfort and Bassler (2016) have reviewed these aspects in much detail.

Various procedures and protocols used for finding and depiction of signal molecules are described by several authors as compiled by Rumbaugh (2011). Many simple techniques such as bioassays and chemical techniques such as thin-layer chromatography (TLC) and chromatographic and spectroscopic methods are regularly employed for recognition and classification of signal molecules (Gonzalez and Keshavan 2006; Kendall and Sperandio 2007). Fascinatingly, secretion of quorum-sensing interfering (QSI) molecules by eukaryotic microbes has created huge curiosity within the researchers because such molecules are capable of influencing the bacterial signaling system positively or negatively. In contrast, production of structural homologues to the many QS signal compounds has resulted in the improvement of additional QSI molecules that can be employed to manage pathogenic bacteria. Additionally, the construction of transgenic plants to facilitate the expression of bacterial QS genes until now is an effective approach to meddle with bacterial activities (Fray 2002; Hartmann and Schikora 2012).

The rhizosphere comprises an elevated amount of AHL-secreting bacteria in comparison to bulk soil, signifying their position in colonization (Elasri et al. 2001). This advocates that plants might be employing root-exuded molecules in the rhizosphere to obtain benefit of this bacterial information structure and control colonizing populations (Lugtenberg and Kamilova 2009; Lopez-Raez et al. 2012). Exudates from pea seedlings comprise compounds that impersonate components of QS molecules which advocate that plants are capable of selecting their microbial colleagues (Teplitski et al. 2000; Fatima et al. 2010). Perez-Montano et al. (2013) documented that *Oryza sativa* and *Phaseolus vulgaris* roots and seeds secrete molecules which exclusively meddle with the capability of plant-associated bacteria to develop biofilms, a crucial feature for bacteria-eukaryotic host communication. Plant host species have developed responses to AHLs. *Medicago truncatula* on contact to a broad concentration series of AHLs responded with a primary decline in different protein volume followed by increase of the same proteins afterward (Mathesius et al. 2003; Hartmann and Schikora 2012). A number of these proteins involved members of cytoskeleton structure/function, defense/stress response, isoflavone production, and metabolic enzyme families. This presents an interesting area of research as to how bacteria communicate among themselves and how plants have developed mechanisms to react to these signal compounds.

In the recent past, many articles and scientific literature have been published on the specific and general aspects of quorum sensing in plant pathogens and beneficial rhizobacteria (Singh et al. 2012; Hartmann and Schikora 2012; Hartmann et al. 2014; Kalia 2015; Schikora et al. 2016). In this chapter, we have reviewed extensive and updated literature to address the role of quorum sensing in plant

growth-promoting rhizobacteria (PGPR), possible interaction mechanisms, and signaling in the rhizosphere relative to plant-microbe interaction.

16.2 Diversity of Quorum-Sensing Signal Molecules and Its Detection

Various types of quorum-sensing network, its regulatory mechanism involved in production of signal molecules, and gene expression have been reviewed by various workers (Atkinson and Williams 2009; Papenfort and Bassler 2016) and are not the subject for discussion of this chapter. However, we have briefly summarized here important aspect. Among different Gram-negative bacteria, biosynthesis of N-acyl homoserine lactones (HSL) takes place in several deviations of the molecular structure. The range of HSL molecules varies from short (C4-, C6-, and C8-) carbohydrate side chains to long (C12-, C14-, or even longer) side chains and consists of unsubstituted in addition to OH- and oxo-C3-substituted compounds. Despite the fact that HSLs are the universal autoinducers in Gram-negative bacteria, arrangements like AI-2 (alternative autoinducer; furanosyl borate diester), AI3, and quinolones (PQS) and a range of extra minute compounds are known as signaling molecules (Effmert et al. 2012). Additionally, lipid compounds, like cis-11-methyl-2-dodecenoic acid (also called as diffusible signal factor or DSF) (Wang et al. 2004a) and 3-hydroxy-palmitate methyl ester (3OH-PAME) (Flavier et al. 1997), have been recognized as QS-mediating molecules. Moreover, cyclic compounds, such as 2-heptyl-3-hydroxy-4-quinolone (PQS) and diketopiperazines (DKZ), also have been recommended as QS signals of *Pseudomonads* (Holden et al. 1999; McKnight et al. 2000). In Gram-positive bacteria, a range of incomplete cyclic peptides, AI-2 and butyrolactone, control cellular functions and activities via perceiving the cell quantity. AI-2 was anticipated as a "universal" QS indicator in bacteria, but this task is still uncertain since it might just be a secreted product of a common metabolic network (Folcher et al. 2001; Winzer et al. 2002; Lyon and Novick 2004). Diverse types of quorum-sensing molecules and their corresponding producing bacteria are presented in Table 16.1.

Cell-to-cell communication between rhizosphere microbes probably takes place universally since several strains obtained from the rhizosphere have been documented to produce QS signals. For instance, it has become evident that a diversity of proteobacterial rhizosphere isolates secrete and/or react to N-acyl homoserine lactone (AHL) QS signals, together with strains associated to species or genera of *Pseudomonas chlororaphis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Burkholderia*, *Serratia*, *Erwinia*, and *Ralstonia*, in addition to rhizobial species (Ferluga et al. 2008). AHLs have also developed to work as interkingdom messenger molecules affecting plant gene interpretation, the initiation of systemic plant resistance, and influencing plant growth and development (Venturi and Fuqua 2013). In recent times, new categories of signals (e.g., pyrones and dialkylresorcinols) secreted by Gram-negative bacteria have been revealed which are predicted by *LuxR* proteins and found to be strongly connected to the AHL-responsive LuxR

Table 16.1 Signal molecules of common PGPR

PGPR	QS network	Major signal molecules	References
Acinetobacter sp.	AHL	N-Acyl-L-HSL; N-(3-oxoacyl)-L-HSL; N-(3-hydroxyacyl)-L-HSL	Atkinson and Williams (2009)
Pseudomonas fluorescens	-Do-	3-OH-C6-HSL;3-OH-C7-HSL; 3-OH-C8-HSL; 3-OH-C10-HSL, C 6-HSL, C8-HS	Khan et al. (2005)
P. fluorescens CHA0	Non-AHL QS signal compounds		Kay et al. (2005)
Pseudomonas sp.	-Do-	N-Acyl-L-HSL; N-(3-oxoacyl)-L-HSL; N-(3-hydroxyacyl)-L-HSL; 2-heptyl-3-hydroxy-4-quinolone (PQS)	Williams and Camara (2009) and Hartmann and Schikora (2012)
Pseudomonas aeruginosa	-Do-	N-(3-Oxododecanoyl)-homoserine lactone (OdDHL); N-butyrylhomoserine lactone (BHL); 2-heptyl-3-hydroxy-4-quinolone (PQS); 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS)	Lee and Zhang (2015)
Rhodopseudomonas sp.	-Do-	N-(p-Coumaroyl)-HSL; R = OH (pC-HSL)	Atkinson and Williams (2009)
Rhizobium sp.	-Do-	N-Acyl-L-HSL; N-(3-oxoacyl)-L-HSL; N-(3-hydroxyacyl)-L-HSL	Sanchez-Contreras et al. (2007)
Bradyrhizobium sp.	-Do-	N-(p-Coumaroyl)-HSL; R = OH (pC-HSL)	Sanchez-Contreras et al. (2007)
Sinorhizobium meliloti	-Do-	3-Oxo-C16	Mathesius et al. 2003 and Hartmann et al. (2014)
Mesorhizobium huakuii	-Do-	C8-HSL	Wang et al. (2004b) and Braeken et al. (2008)
Bacillus subtilis	LuxS	Peptides	Duanis-Assaf et al. (2016)
Pantoea agglomerans YS19	AHL	N-3-Oxooctanoyl-L-homoserine lactone	Jiang et al. (2015)
Stenotrophomonas maltophilia	Diffusible signal factor (DSF)	cis-11-Methyl-2-dodecenoic acid diffusible signal factor (DSF)	Alavi et al. (2013)
			Ryan et al. (2015)
Burkholderia sp.	Diffusible signal factor (DSF)/AHL	cis-11-Methyl-2-dodecenoic acid diffusible signal factor (DSF); N-acyl-L-HSL; N-(3-oxoacyl)-L-HSL; N-(3-hydroxyacyl)-L-HSL	Schmid et al. (2012), Chapalain et al. (2013), Suppiger et al. (2013), and Ryan et al. 2015
Ochrobactrum sp. Pv2Z2	AHL	3O-C7-HSL; 3OHC7-HSL	Imran et al. (2014)
Serratia plymuthica HRO-C48	AHL	3-Oxo-C6	Pang et al. (2009)
Gluconacetobacter diazotrophicus PAL5	AHL	C6-, C8-, C10-, C12-, C14-HSL; 3-oxo-C10-, C12-, C14-HSL	Nieto-Penalver et al. (2012)

family (Brameyer et al. 2015); it is at present unidentified whether these signals are formed by rhizobacteria. One more group of QS signals in Gram-negative bacteria is the DSF family (diffusible signal factor, which are cis-2-unsaturated fatty acids); more bacterial species are presently being identified which generate DSF, together with rhizosphere-inhabiting species such as *Burkholderia* spp. and *Stenotrophomonas maltophilia* (Ryan et al. 2015). Fascinatingly, bacterial DSF signal molecules have also been currently resolved to bring about innate immunity in plants, therefore performing as interkingdom signal molecules (Kakkar et al. 2015). Several Gram-positive bacterial inhabitants in the rhizosphere utilize peptides (also known as pheromones) as QS signaling compounds; probably these molecules participate in numerous regulatory functions both at the intra- and interspecies level (Bassler 2002; Monnet et al. 2016).

An accurate, exact, and responsive chemical examination of quorum-sensing autoinducer compounds was a necessary requirement for novel studies of quorum-sensing-associated regulation in bacteria. By employing these methods, a detailed tracking of these QS compounds in the habitat and inside eukaryotic cell, populated by HSL-producing bacteria, was made possible (Gotz et al. 2007; Hartmann and Schikora 2012). In case of quorum-sensing compounds pertaining to N-acyl homoserine lactone group, it has been proved lucky for the progress of study in this area that the first accessible chromatographic tools were soon aided by extremely sensitive and specific biosensors. These biosensors get benefit of the careful establishment of promoters of HSL-regulated genes by autoinducer molecules. Different existing operon fusion constructs of HSL-activated genes with the *lux-casette*, *gfp*, *rfp*, or *lacZ* have been evaluated by Fekete et al. (2010b). Additionally, the quorum-sensing-controlled violacein secretion by *Chromobacterium violaceum* can be utilized effectively to initiate HSL production or deterioration, respectively (McClean et al. 1997). The indicated constructs are also present on plasmids and can be transmitted to other bacteria. On the other hand, HSL-biosensor bacteria should contain their personal HSL-secreting genes deleted or inactivated to circumvent self-activation. The constructs generally have different precision for both short and long side chain HSLs, but there are also reporter plasmids that permit recognition of most HSLs with comparable sharpness (Thomson et al. 2000; Andersen et al. 2001). However, one has to be cautious in the utilization of these biosensors, as their report may be somewhat partial and has to be incremented with other resources of chemical or immunological metabolite analysis. The existence of HSLs in definite environments and their ecological importance have been encouraged by the use of green fluorescent protein (GFP) or red fluorescent protein/DsRed (RFP) stuck to HSL-regulated promoters. The potency of HSL down to 20 nmol l^{-1} can be identified by means of these bioreporter constructs. However, this recognition is relatively discriminatory, because, for example, in the case of the reporter strain *Pseudomonas putida* F117, the confined reporter plasmid pAS-C8 is 100 times extra susceptible to 3-oxo-C12-HSL than C12-HSL (Steidle et al. 2001). Using these constructs, the in situ secretion of HSL compounds can be, for example, discovered on the surface of roots, consequently ensuing in the regulation of "landscapes" of HSLs on occupied surfaces (Gantner et al. 2006).

In microcolonies or polymer matrix-surrounded biofilms, where the dispersion is limited, the local concentration of HSLs can reach high peak values. By using mathematical models for the computation of the autoregulated HSL secretion in bacteria and restricted dispersion (Muller et al. 2006), local concentrations in the mmol l^{-1} range can be calculated, accepting just a volume of a 5-μm cube with enclosed *Burkholderia cepacia*. This fact can have ecological importance for communication with eukaryotic hosts inhabited by HSL-producing bacterial microcolonies or biofilms that could also add to compensate the potential deterioration of HSL by quorum-quenching reactions. With reference to chemical analysis, GC-based methods of HSL quantification were established first. To amplify the sensitivity of the technique, for example, selective ion monitoring of the mass spectrometry (MS) detection or derivatization of the ß-oxo group to an oxime was applied (Charlton et al. 2000). As analyzed by Fekete et al. (2007), reversed-phase HPLC coupled with MS for selective detection has been useful in nearly all cases (Morin et al. 2003). Frommberger et al. (2004) established a micro-electrospray interface to MS after nano-LC separation of the HSLs. Electrokinetic chromatography (MEKC) also has been employed effectively for the recognition of HSLs and detection by MS. The most effective separation of HSLs is with UPLC analysis, as described in detail by Li et al. (2006). The classification of enantiomers of HSLs in biological matrices also is achievable by means of an optimized GC-MS approach (Malik et al. 2009). The maximum precision of molecular mass detection of HSLs has been completed by using the positive ion Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) with mass errors of the peaks less than 0.1 ppm, as described by Fekete et al. (2007).

Nevertheless, still after employing this highly resolving analytical instrument, it is suitable to use two independent analytical approaches (e.g., UPLC and FTICR-MS) to clearly recognize HSL molecules, particularly when the recognition is from very complex matrices, such as nutrient broth medium, frequently used in microbiology (Hartmann and Schikora 2012). One more autonomous technique for the examination of HSL molecules is based on immunochemistry. From several labs, monoclonal antibodies (MAB) have been produced against several HSL molecules (Kaufmann et al. 2006, 2008; Chen et al. 2010a, b). These MABs not only allow the research of the biological impact of scavenging HSL but also the investigation of reduced sample sizes and the localization of the allocation of HSL secreted by bacteria connected with eukaryotes (Park et al. 2007; Hartmann and Schikora 2012). For more details, readers are being suggested to read specific review article on the subject (Rumbaugh 2011).

16.3 Quorum Sensing in Plant Growth-Promoting Rhizobacteria

Quorum sensing provides a great competing benefit to bacteria enhancing their likelihood to stay alive, while they can explore more difficult habitats. QS in bacterial conversation is connected with the manufacturing and discharge of signal

molecules, termed autoinducers, into the surrounding medium. On recognition of the signal compounds at a given concentration, transcription of definite genes controlled by this system is stimulate or withdrawn in the bacteria. There are different microbial mechanisms regulated by QS which include DNA transferase by conjugation, bioluminescence, siderophore production, biofilm formation, and moving ability of some bacteria, also termed as "swarming" (Fray 2002; Barriuso et al. 2008b). Streptomycetes, with high G+C-content, have been shown to control spore development as well as antibiotic manufacturing by a quorum-sensing indicator called A-factor. The separation of AHLs from bacteroids of *R. leguminosarum* advocates that quorum sensing might play a role in the mature nodule (Daniels et al. 2002). It is hypothesize that quorum sensing influences population flow in connection with host plants. Both siderophores and HSLs have been recommended to participate as chemical signal molecules for interspecies conversation among bacteria (Guan and Kamino 2001). However, insufficient information is available related to interspecies conversation in the natural microbial habitat. Mathesius et al. (2003) documented better discharge of AHL mimics in exudates of *Medicago truncatula*. The chemical composition of such active quorum sensing mimicking secondary metabolites is presently unidentified and also needs additional explanation (Teplitski et al. 2000; Chen et al. 2002; Podile et al. 2014).

QS-regulated gene expression is based not only on signal compounds but also on bacterial population thickness (Williams 2007). The requirement for a minimum level of the primary PGPR inocula to promote plant growth considerably sustains the thought that quorum sensing by microorganisms participates in plant-rhizobacteria communications (Persello-Cartieaux et al. 2003). Bacteria can respond to QS-like molecules secreted by other rhizospheric bacteria (Steidle et al. 2001) and by plants (Teplitski et al. 2000) and even eradicate the QS signal compounds secreted by other bacterial species (Dong et al. 2002). Other than producing regulatory peptides, *Bacillus* secretes enzymes to degrade the AHL moieties produced by Gram-negative bacteria. Genes encrypting for AHL-degrading enzymes, *aiiA*, have been established in *B. thuringiensis* and different subspecies (Lee et al. 2002). The occurrence of such proteins permits *Bacillus* strains to split the lactone bond of AHLs via hydrolysis, signifying a method for autoinducer-degrading activity, permitting these bacteria to struggle with other Gram-negative bacteria. Bacterial functions in the rhizosphere can, therefore, be changed directly by plants or other microorganisms via QS molecules (Podile et al. 2014).

In addition to motility and QS, bacterial major outer membrane protein (MOMP) also performs a crucial task in initial host identification. The MOMP of *Azospirillum brasilense* demonstrated better adhesion factor to exudates of cereals than exudates of legumes and tomatoes and could work as a bond implicated in root adsorption and cell accumulation of the bacterium (Burdman et al. 2001). Bacterial lipopolysaccharides (LPS), particularly the O-antigen chain, can also cooperate in root habitation (Dekkers et al. 1998a, b). On the other hand, it is strain related since the O-antigenic side chain of *Pseudomonas fluorescens* WCS374 does not help in potato root attachment (De Weger et al. 1989), while the O-antigen chain of

P. fluorescens PCL1205 is implicated in tomato root colonization (Dekkers et al. 1998b). Several workers (Simons et al. 1996; Dekkers et al. 1998a; Compant et al. 2010) also reported that high bacterial growth rate and capability to produce vitamin B1 and secrete NADH dehydrogenases help in plant colonization.

Endophytes comprising a vital constituent of plant structure are frequently reported assisting in plant defense reactions by quorum-preventing methods. Fascinatingly however, endophytes are repeatedly observed to have quorum-sensing mechanisms that permit them to sustain their own inhabitation in host plants and counteract plant pathogens. For instance, strain PsJNT is described to set up endophytic relations with different plants and acknowledged to develop plant-rooting structure with improved vascular arrangements, enhance quantity of chlorophyll and phytohormones, and offer resistance to phytopathogens. Fascinatingly, *Burkholderia phytofirmans* strain PsJNT was reported to secrete quorum autoinducer 3-hydroxy-C8-homoserine lactone (Sessitsch et al. 2005). In addition, endophytic *Serratia plymuthica* with enormous biological control capability was found to hold high amount of homoserine lactone (HSL), namely, C4-HSL, C5-HSL, C6-HSL, C7-HSL, C8-HSL, 3-oxo derivatives (3-oxo-C6-HSL, 3-oxo-C7-HSL, 3-oxo-C8 HSL), and 3-hydroxy derivatives (3-hydroxy-C6-HSL, 3-hydroxy-C8-HSL). These AHL molecules were due to two quorum-sensing mechanisms in *S. plymuthica* (Liu et al. 2011). Additionally, the olive plant epiphyte (*Pantoea agglomerans*) and endophyte (*Erwinia toletana*) linked with olive knot infection were observed for the discharge of signals analogous to AHLs. This chemical communication changed the virulence of pathogen *Pseudomonas savastanoi* pv. *savastanoi* blamed for olive knot. This work is an illustration of tripartite connections among plant and connected microbes (Hosni et al. 2011).

The genome sequence of endophytic *Gluconacetobacter diazotrophicus* PAL5 based on *Saccharum officinarum* exposed the existence of quorum-sensing mechanisms and identification of eight AHLs, viz., C6, C8, C10, C12, and C14-HSL (Nieto-Penalver et al. 2012). A current description from Dourado et al. (2013) demonstrated the exploitation of quorum-sensing compounds for *Methylobacterium* (famous for displaying endophytic lifestyle) communications with plants. A series of genes were up- and downregulated in *Methylobacterium* and host plant at the same time facilitating colonization and symbiotic relations, presenting the reliance of plant-endophyte relations on quorum-sensing mechanisms. Rhizobacteria are extensively recognized to improve production of plants by nitrogen fixation and production of siderophores and phytohormone, decrease plant stress, induce systemic resistance, and have capability to attenuate phytopathogenic signals (Liu et al. 2012). Thus, maintaining quorum-sensing mechanisms and autoinducers may allow the endophytic isolates to talk with other connected endophytes in addition to the host plant, thus preserving symbiotic relationship and habitation inside the inner tissues of plants. Surely, there is a deficiency of information on such organization, which needs to be examined in deepness to search for the possible plant physiological modifications and resistance reactions such as release of ethylene, salicylic acid, and defense proteins during the initial stages of colonization.

16.4 Recent Reports on Quorum-Sensing-Associated Functions in Plant Growth-Promoting Bacteria

The rising demand for food and the apprehension related to food quality are the compelling activities advancing to new approaches in agriculture. An effective plant protection mechanism possesses a huge potential to make certain an adequate and high-quality food delivery. Biocontrol agents are well recognized and widely used; however their potential is not yet fully exploited. These days numerous products based on bacterial inoculum, primarily consisting of *Bacillus*, *Pseudomonas*, or *Serratia* spp., arrived at the market. The use of N_2-fixing *Rhizobia* (e.g., *Sinorhizobium meliloti*), with improved secretion of specific AHLs, might augment the useful effects of bacteria and increase the effect to plant species generally not connected with the specific strain (Zarkani et al. 2013; Hernandez-Reyes et al. 2014). Further, a better comprehension of the communication among bacterial quorum-sensing compounds and eukaryotic host cells can unlock novel strategies in agriculture. Throughout the infection procedure, QS molecules administer the bacterial capability to form biofilms and other density-regulated traits. Those compounds participate in key role in the communication among bacterial and plant cells. Several workers documented the role of quorum sensing in plant disease control and phytopathogen transmission. Some of the reports are summarized briefly.

Barriuso et al. (2008a) reported the role of N-acyl-homoserine lactone (AHL) quorum-sensing signaling compounds in plant growth promotion and the initiation of defense against salt stress. They utilized two Gram-negative, plant growth-promoting rhizobacteria, designated as M12 and M14, and were identified by 16S rDNA sequencing as *Burkholderia graminis* species. Both strains were found to produce a diversity of N-acyl-homoserine lactone (AHL) quorum-sensing signaling compounds. AHL generation was examined in vitro by thin-layer chromatography by applying AHL biosensors, and the characteristic of the AHLs produced was decided by liquid chromatography-tandem mass spectrometry. The in situ secretion of AHLs by M12 and M14 in the rhizosphere of *Arabidopsis thaliana* plants was distinguished by co-inoculation with green fluorescent protein-based biosensor strains and confocal laser scanning microscopy. To establish both plant growth promotion and defense against salt stress, these PGPRs were examined on wild-type tomato plants, in addition to their matching transgenics expressing *YenI* (short-chain AHL producers) and *LasI* (long-chain AHL producers). In wild-type tomato plants, it was found that only M12 improved the plant growth and this result vanished in both transgenic lines. On the opposing, M14 did not encourage development in wild-type tomatoes but did so in the *LasI* transgenic line. Resistance to salt stress was stimulated by M14 in wild-type tomato, but this outcome vanished in both transgenic lines. The strain M12, however, did not stimulate salt resistance in wild-type tomato but did so in *LasI* tomato plants. These outcomes disclose that AHL QS signaling compounds decide the capability of both PGPR strains M12 and M14 to enhance plant growth and to activate protection against salt stress.

Johnson and Walcott (2013) reported that *Acidovorax citrulli* convert from saprobic to pathogenic growth for seed-to-seedling distribution of bacterial fruit blotch

(BFB) of cucurbits; they speculate that quorum sensing was implicated in the regulation of this procedure. Using *aacI* (*luxI* homologue) and *aacR* (*luxR* homologue) mutants of AAC00-1, they examined the task of QS in watermelon seed colonization and seed-to-seedling distribution of BFB. *aacR* and *aacI* mutants of AAC00-1 inhabited germinating watermelon seed at wild-type levels; on the other hand, BFB seed-to-seedling distribution was influenced in a cell thickness-attached approach. There were no important distinctions in BFB seedling transmission among watermelon seed penetrated with approximately 1×10^6 CFU of AAC00-1, the *aacR* or *aacI* deletion mutants (95.2, 94.9, and 98.3% BFB occurrence, correspondingly). On the contrary, when seed inoculum was decreased in the order of $1x10^3$ CFU $seed^{-1}$, BFB seed-to-seedling transmission dropped to 34.3% for the *aacI* mutant, which was considerably low than the wild type (78.6%). Fascinatingly, BFB seed-to-seedling distribution for the *aacR* mutant was not significantly unusual to the wild-type strain. This information advocates that QS takes part in the regulation of genes implicated in seed-to seedling spreading of BFB.

Alavi et al. (2013) accounted the role of DSF quorum-sensing system in controlling the progressive impact of *Stenotrophomonas maltophilia* on plants. They reported that the quorum-sensing molecule DSF (diffusible signal factor) is accountable for the directive of phenotypes in pathogenic *Stenotrophomonas*; to date, no helpful results were documented to be managed by it. They examined the role of DSF in the plant growth-promoting model strain *S. maltophilia* R551-3 using functional and transcriptomic analyses. For this intention, these workers correlated the wild-type strain with a mutant deficient in the *rpfF* (regulation of pathogenicity factors) gene that is necessary for the synthesis of DSF. Oilseed rape seeds coated with the wild-type strain demonstrated a statistically significant enhancement in germination rate compared with those coated with the *rpfF* mutant. Likewise, the wild-type strain displayed improved plant growth promotion and a better effectiveness in colonizing oilseed rape compared to the mutant strain. Furthermore, only the wild type was competent of establishing organized cell masses both in vitro and in the rhizosphere, a quality decided by DSF. Gene transcription analyses revealed that many genes documented to participate in plant inhabitation (e.g., cell motility, chemotaxis, multidrug efflux pumps, biofilm formation) are controlled by the *rpf*/DSF system in *S. maltophilia.* Additionally, these workers discovered novel prospective traits of spermidine, mainly for both growth enhancement and stress protection. In general, these results elucidated an association among the regulation of DSF and the constructive communication outcome with the plant host.

Zuniga et al. (2013) evaluated that by using appropriate mutant strains of *Burkholderia phytofirmans* PsJN, data can be acquired showing the significance of N-acyl homoserine lactone-mediated quorum sensing in well-organized inhabitation of *Arabidopsis thaliana* plants and the organization of an advantageous communication. These workers also noticed that bacterial deterioration of the auxin indole-3-acetic acid (IAA) takes part in plant growth-promoting characters and is crucial for successful root colonization.

Perez-Montano et al. (2014) found that bacterial surface components, particularly exopolysaccharides, in association with bacterial quorum-sensing molecules

are vital for the formation of biofilms within the majority of species as examined until now. Biofilm formation permits soil bacteria to inhabit their neighboring territory and stay alive under frequent ecological stresses such as drought and nutrient limitation. This form of life is regularly important for continued existence in bacteria of the genera *Mesorhizobium*, *Sinorhizobium*, *Bradyrhizobium*, and *Rhizobium*. They also established that biofilm construction is essential for a most favorable root colonization and symbiosis among *S. fredii* SMH12 and *Glycine max* cv Osumi. In this bacterium, nod gene-activating flavonoids and the NodD1 protein are necessary for the evolution of the biofilm configuration from monolayer to microcolony. QS mechanisms are also essential for the complete growth of both types of biofilms. In fact, both the *nodD1* mutant and the lactonase strain (the lactonase enzyme stop AHL buildup) are imperfect in soybean root inhabitation. The destruction of the lactonase strain in its colonization capability results in the decline of the symbiotic parameters. Fascinatingly, NodD1 jointly with flavonoids induces certain quorum-sensing mechanisms involved in the growth of the symbiotic biofilm. Therefore, *S. fredii* SMH12 through distinctive key compound, the flavonoid, competently forms biofilm, colonizes the legume roots, and induces the production of Nod factors, necessary for fruitful symbiosis. Oslizlo et al. (2015) demonstrated that *Bacillus subtilis* isolated from tomato rhizosphere displayed variety of the ComQXPA quorum-sensing mechanisms. This QS mechanism controls the secretion of antipathogenic and biofilm-activating compounds, for example, surfactins, which are responsible for the biocontrol activity of this bacterium.

Paungfoo-Lonhienne et al. (2016) established the role of quorum sensing in colonization and biofilm formation by *Burkholderia* Q208. They accounted that *Burkholderia* strain Q208, a PGPR of Australian sugarcane, exhibits an extremely conserved quorum-sensing mechanism, nominated as BraI/R, which is programmed by a cluster of three genes (*braI*, *rsaL* and *braR*), the results of which create and react to *N*-dodecanoyl-3-oxo-homoserine lactone. In the biofilm *Burkholderia braI* is upregulated (twofold), while, strangely, *rsaL* and *braR* are downregulated (to 0.35- and 0.45-fold of reference levels, respectively). The absolute counts of raw reads of *rsaL* (16,000) and *braR* (15,500) are higher than the mean (700) read number over all expressed genes, signifying that even though these genes are downregulated, BraI/R quorum sensing by *Burkholderia* Q208 continues to be effective in the sugarcane rhizosphere.

16.5 Role of Quorum Sensing in Rhizosphere Signaling and Plant-Microbe Interactions

The rhizosphere is a highly complex microecological niche rich in nutrient released by plant root and provides suitable environment for growth and multiplication of an array of soil microbial populations. Primary and secondary metabolites released in the form of plant root exudates are believed to shape, signal, and interfere with rhizosphere microflora by attracting beneficial microflora and combating pathogenic microflora. In a review by Venturi and Keel (2016) described various issues related

to signaling in rhizosphere and divided the process in three groups: (i) signaling between microbes, (ii) from plants to microbes, and (iii) microorganisms to plants. Two major groups of small signaling molecules are recognized. First is the QS molecules released by bacteria and volatile organic compounds (VOCs) released by various bacteria and fungi. VOCs are assumed to play significant task in long-distance communication within microbial populations, microbe-microbe, along with plant-microbe cooperation within the rhizosphere (Bitas et al. 2013). VOCs are also known to also work as intra- and interspecies signals by influencing gene expression and microbial functions such as biofilm, virulence, and stress tolerance (Audrain et al. 2015). Various rhizobacteria isolated from rhizosphere are known to produce QS signal molecules, and respond to these molecules. For example species of *Burkholderia*, *Pseudomonas*, *Rhizobia*, and *Sinorhizobium* as depicted in Table 16.1, and impact of QS on plant-microbe interaction is presented in Fig. 16.1.

Phytocompounds secreted by plant roots promote microbial interaction and also influence plant-microbe interactions (Zhang et al. 2015). Plant-produced signals have been studied only in well-established association such as legume-rhizobia symbiosis and mycorrhizal associations as reviewed by other workers and are not topic of discussion here (Downie 2010; Oldroyd 2013). The role of QS in

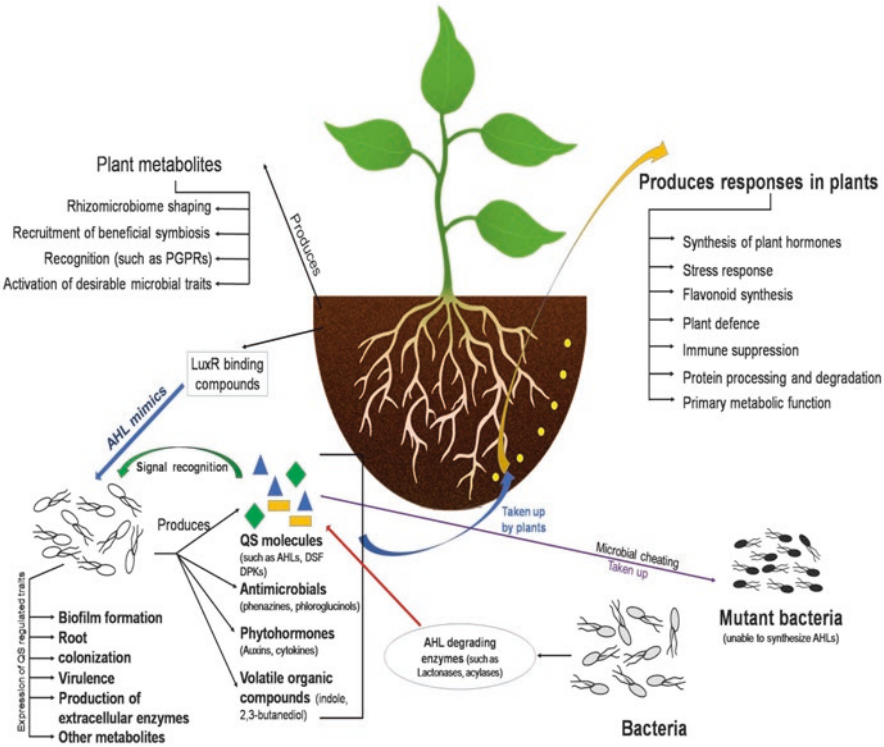


Fig. 16.1 Role of quorum sensing in microbe-plant interactions and rhizosphere signaling

plant-microbe association is now becoming more evident, since many rhizobacteria employ QS molecules to colonize plant surface or plant-associated environment through QS-mediated gene expression (Von Bodman et al. 2003; Newton and Fray 2004). On the other hand, plant-derived compounds are reported to interfere in bacterial quorum sensing. Recently a bacterial subfamily of LuxRs proteins produced by bacteria interacts with plant small molecules and not with QS. LuxRs is expected to respond plant signals indicating a more complex interkingdom signaling mechanism (Venturi and Fuqua 2013; Gonzalez and Venturi 2013). Various signals produced by PGPR are now characterized, and the best studied AHLs are found to influence plant physiology and plant-microbe interaction such as induction of plant defense against pathogens, pest, and abiotic stressor, which results in promotion of plant growth and development (Shoresh et al. 2010; Zamioudis and Pieterse 2012; Cameron et al. 2013; Pieterse et al. 2014).

Terrestrial plants related to different genera are recognized to generate AHL-mimic molecules for defense system in opposition to pathogen and communication with connected bacterial communities, both inside and outside the plant tissues (Perez-Montano et al. 2013). Quorum-mimicking AHLs are synthesized and secreted in close proximity by different plant species varying from seedlings to a mature plant (Teplitski et al. 2011).

Mathesius et al. (2003) have reported the modulatory role of signaling molecules, AHLs, on plant physiology based on differential proteome analysis and found that protein-related defense, stress, flavonoid metabolism, hormones, and many regulatory proteins were differentially expressed in plants treated with AHLs. von Rad and his colleagues have reported the upregulation of auxin and downregulation of cytokinin genes and influence the ratio of auxin and cytokinin in the treated model plant with C6-HSL (von Rad et al. 2008). Hartmann and Schikora (2012) and Schenk et al. (2012) proposed a double role of the AHL molecules in *Arabidopsis thaliana.* Short acyl chain AHLs, like C4 or C6, were revealed to increase the growth rate, primarily elongating the roots (von Rad et al. 2008; Bai et al. 2012; Liu et al. 2012; Schenk et al. 2012), in contrast to molecules with longer acyl chains (e.g., C12 or C14).

Recently, Hartmann et al. (2014) described the impact of AHLs on plant growth in plant species and found that it is more complex. However, in some studied cases, it may be very specific such as in mung bean and *Medicago truncatula* plants. Long-chain 3-oxo-C14-HSL produced by *Sinorhizobium meliloti* showed increase in root nodulation in *Medicago truncatula* (Veliz-Vallejos et al. 2014). It was interesting to note that the increased number of nodules was observed only after a treatment with 3-oxo-C14-HSL, the predominant AHL of *S. meliloti*, and treatment with other AHLs showed no effect. In mung bean plants, only the 3-oxo-C10-HSL, but not the unsubstituted C10-HSL or C12-HSL, was able to induce adventitious roots (Bai et al. 2012).

In a study conducted on barley treated with C6-, C8-, and C10-HSLs indicated modulatory role in the activity of glutathione *S-transferase* and *dehydroascorbate reductase*. On the other hand, in yam bean, no influence was measured (Gotz-Rosch et al. 2015). Yet another interesting example is the modification of plant cell walls

in AHL-primed plants. In this primed stage, plants upregulated the transcription of numerous genes pertaining to secondary metabolism (e.g., phenols). In consequence, upon a challenge with pathogens, those plants accumulate callose and phenolic compounds (Schenk and Schikora 2015). In a recent review article, Schikora et al. (2016) described the effect of quorum-sensing molecules of the N-acyl homoserine lactone group on plant physiology and significance in the development of stress tolerance mechanism in plants against stressors (Fig.16.1).

16.6 Conclusion and Future Direction

Research carried out in the last decades has shown that quorum sensing is a widespread global regulatory mechanism of gene expression in a density-dependent manner among several bacteria including both pathogenic and beneficial species. Plant-associated bacteria such as PGPR, both free living and symbiotic, have been investigated, which use QS to regulate specific traits. Some of these are important in the interaction with other bacteria or the host plant. These bacteria produce small molecules called autoinducer. Various types of complex QS network are present in bacteria, but the most commonly studied system in Gram-negative bacteria is found to possess AHL-based LuxR/LuxI homologous systems. The signal molecules contribute not only in signaling within bacterial population in the rhizosphere but also contribute in plant-microbe interactions.

Interestingly plants are able to react or hamper bacterial QS which clearly indicated its significance in plant-bacteria interactions. Many bacteria in rhizosphere produce AHL-degrading enzymes, thus exhibiting phenomenon of quorum quenching. On the other hand, plant metabolites can also inhibit QS thus showing QS-mimic activity. Recent reports indicated that bacteria produce compounds which act as receptor for plant signals. Researchers have provided evidences that the treatment of plant with AHLs results in plant response which induces resistance to pathogens and stressor. Studying the dynamics of AHL production and degradation and response of plant-associated microbial biome will certainly help to fully explore the role of QS in plant-microbe interaction.

Now it has been established that plant is able to control the recruitment of root microbiome and to select specific microbes of desired function. Therefore, there is a greater need to understand how plant root-associated bacteria such as free-living PGPR are recruited by plants. Further, the role of QS-mediated signaling and other signaling mechanisms in the rhizosphere contributing in the establishment and maintenance of dynamic root microbiome needs to be studied. It is expected that an enhanced understanding on all these aspects will open new avenues to modulate root microbiome through the use of appropriate consortium of beneficial microbes for improved crop productivity and soil health.

Acknowledgment We are grateful to the Chairman, Department of Agricultural Microbiology, AMU, Aligarh, India for providing support to complete this task. We are also thankful to Mr. Faizan Abul Qais, research scholar, Department of Agricultural Microbiology, AMU, Aligarh, for his cooperation in preparing Fig. 16.1 of this chapter.

References

Ahmad I, Aqil F, Ahmad F et al (2008) Quorum sensing in bacteria: potential in plant health protection. In: Ahmad I, Hayat S, Pichtel J (eds) Plant-bacteria interactions: strategies and techniques to promote plant growth. Wiley, Germany, pp 129–153

Ahmad I, Khan MSA, Husain FM et al (2011) Bacterial quorum sensing and its interference: methods and significance. In: Ahmad I, Ahmad F, Pichtel J (eds) Microbes and microbial technology: agricultural and environmental applications. Springer, New York, pp 127–161

Alavi P, Muller H, Cardinale M et al (2013) The DSF quorum sensing system controls the positive influence of *Stenotrophomonas maltophilia* on plants. PLoS One 8(7):e67103

Andersen JB, Heydorn A, Hentzer M (2001) gfp-based N-acyl homoserine lactone sensor systems for detection of bacterial communication. Appl Environ Microbiol 67:575–585

Atkinson S, Williams P (2009) Quorum sensing and social networking in the microbial world. J R Soc Interface 6:959–978

Audrain B, Farag MA, Ryu CM et al (2015) Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev 39:222–233

Bai X, Todd CD, Desikan R et al (2012) *N*-3-oxo-decanoyl-L homoserine lactone activates auxin-induced adventitious root formation via hydrogen peroxide- and nitric oxide-dependent cyclic GMP signaling in mung bean. Plant Physiol 158:725–736

Barriuso J, Solano BR, Fray RG et al (2008a) Transgenic tomato plants alter quorum sensing in plant growth-promoting rhizobacteria. Plant Biotechnol J 6:442–452

Barriuso J, Solano BR, Lucas JA et al (2008b) Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria. (PGPR). In: Ahmad I, Pichtel J, Hayat S (eds) Plant-bacteria interaction, strategies and techniques to promote plant growth. Wiley, Germany, pp 1–13

Bassler BL (2002) Small talk. Cell-to-cell communication in bacteria. Cell 109:421–424

Bitas V, Kim HS, Bennet JW et al (2013) Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol Plant-Microbe Interact 26:835–843

Braeken K, Daniels R, Ndayizeye M et al (2008) Quorum sensing in bacteria-plant interactions. In: Nautiyal C, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, Berlin, pp 265–289

Brameyer S, Bode HB, Heermann R (2015) Languages and dialects: bacterial communication beyond homoserine lactones. Trends Microbiol 23:521–523

Burdman S, Dulguerova G, Okon Y et al (2001) Purification of the major outer membrane protein of *Azospirillum brasilense*, its affinity to plant roots, and its involvement in cell aggregation. Mol Plant-Microbe Interact 14:555–558

Cameron DD, Neal AL, van Wees SC et al (2013) Mycorrhiza-induced resistance: more than the sum of its parts? Trends Plant Sci 18:539–545

Chapalain A, Vial L, Laprade N (2013) Identification of quorum sensing-controlled genes in *Burkholderia ambifaria*. Microbiol Open 2:226–242

Charlton TS, De Nys R, Netting A et al (2000) A novel and sensitive method for the quantification of N-acyl 3-oxohomoserine lactones using gas chromatography-mass spectrometry: application to a model bacterial biofilm. Environ Microbiol 2:530–541

Chen X, Schauder S, Potier N et al (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415:545–549

Chen X, Buddrus-Schiemann K, Rothballer M et al (2010a) Detection of quorum sensing molecules in *Burkholderia cepacia* culture supernatants with enzyme-linked immunosorbent assays. Anal Bioanal Chem 398:2669–2676

Chen X, Kremmer E, Gouzy MF et al (2010b) Development and characterization of rat monoclonal antibodies for N-acylated homoserine lactones. Anal Bioanal Chem 398:2655–2667

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants. Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Daniels R, De Vos DE, Desair J et al (2002) Quorum sensing in *Rhizobium etli* CNPAF512 affects growth and symbiotic nitrogen fixation. J Biol Chem 277:462–468

De Weger LA, Bakker PAHM, Schippers B et al (1989) *Pseudomonas* spp with mutational changes in the O-antigenic side chain of their lipopolysaccharides are affected in their ability to colonize potato roots. In: Lugtenberg BJJ (ed) Signal molecules in plant-microbe interactions. Springer, Berlin, pp 197–202

Dekkers LC, Phoelich CC, van der Fits L et al (1998a) A site specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens* WCS365. PNAS 95:7051–7056

Dekkers LC, van der Bij AJ, Mulders IHM et al (1998b) Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH, ubiquinone oxidoreductase (*nuo*) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol Plant-Microbe Interact 11:763–771

Dong H, Gusti AR, Zhang Q et al (2002) Identification of quorum-quenching *N*-acyl homoserine lactonases from *Bacillus* species. Appl Environ Microbiol 68:1754–1759

Dourado MN, Bogas AC, Pomini AM et al (2013) *Methylobacterium*-plant interaction genes regulated by plant exudate and quorum sensing molecules. Braz J Microbiol 44:1331–1339

Downie JA (2010) The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. FEMS Microbiol Rev 34:150–170

Duanis-Assaf D, Steinberg D, Chai Y et al (2016) The luxs based quorum sensing governs lactose induced biofilm formation by *Bacillus subtilis*. Front Microbiol 6:1517

Eberhard A (1972) Inhibition and activation of bacterial luciferase synthesis. J Bacteriol 109:1101–1108

Effmert U, Kalderas J, Warnke R et al (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703

Elasri M, Delorme S, Lemanceau P et al (2001) Acylhomoserine lactone production is more common among plant associated *Pseudomonas* spp than among soil borne *Pseudomonas* spp. Appl Environ Microbiol 67:1198–1209

Fatima Q, Zahin M, Khan MSA et al (2010) Modulation of quorum sensing controlled behaviour of bacteria by growing seedling, seed and seedling extracts of leguminous plants. Indian J Microbiol 50:238–242

Fekete A, Rothballer M, Frommberger M et al (2007) Identification of bacterial N-acyl homoserine lactones (AHLs) with a combination of ultra-performance liquid chromatography (UPLC), ultra-high-resolution mass spectrometry, and in-situ biosensors. Anal Bioanal Chem 387:455–467

Fekete A, Rothballer M, Hartmann A et al (2010) Identification of bacterial autoinducers. In: Kraemer R, Jung K (eds) Bacterial signaling. Wiley, Germany, pp 95–111

Ferluga S, Steindler L, Venturi V (2008) N-acyl homoserine lactone quorum sensing in Gram-negative rhizobacteria. In: Karlovsky P (ed) Secondary metabolites in soil ecology. Springer, Berlin, pp 69–90

Flavier AB, Clough SJ, Schell MA et al (1997) Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. Mol Microbiol 26:251–259

Folcher M, Gaillard H, Nguyen LT et al (2001) Pleiotropic functions of a *Streptomyces pristinaespiralis* autoregulator receptor in development, antibiotic biosynthesis, and expression of a superoxide dismutase. J Biol Chem 276:44297–44306

Fray RG (2002) Altering plant–microbe interaction through artificially manipulating bacterial quorum sensing. Ann Bot 89:245–253

Frommberger M, Schmitt-Kopplin P, Ping G et al (2004) A simple and robust set-up for on-column sample preconcentration-nano-liquid chromatography-electrospray ionization mass spectrometry for the analysis of N-homoserine lactones. Anal Bioanal Chem 378:1014–1020

Galloway WR, Hodgkinson JT, Bowden SD et al (2011) Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and Al-2 quorum sensing pathways. Chem Rev 111:28–67

Gantner S, Schmid M, Durr C et al (2006) In situ quantitation of the spatial scale of calling distances and population density-independent N-acylhomoserine lactone-mediated communication by rhizobacteria colonized on plant roots. FEMS Microbiol Ecol 56:188–194

Gonzalez JE, Keshavan ND (2006) Messing with bacterial quorum sensing. Microbiol Mol Biol Rev 70:859–875

Gonzalez JF, Venturi V (2013) A novel widespread interkingdom signaling circuit. Trends Plant Sci 18:167–174

Götz C, Fekete A, Gebefuegi I et al (2007) Uptake, degradation and chiral discrimination of N-acyl-D/L-homoserine lactones by barley (*Hordeum vulgare*) and yam bean (*Pachyrhizus erosus*) plants. Anal Bioanal Chem 389:1447–1457

Götz-Rösch C, Sieper T, Fekete A et al (2015) Influence of bacterial N-acyl-homoserine lactones on growth parameters, pigments, antioxidative capacities and the xenobiotic phase II detoxification enzymes in barley and yam bean. Front Plant Sci 6:205

Guan LL, Kamino K (2001) Bacterial response to siderophore and quorum sensing chemical signals in the seawater microbial community. BMC Microbiol 1:27

Hartmann A, Schikora A (2012) Quorum sensing of bacteria and trans-kingdom interactions of *N*-acyl homoserine lactones with eukaryotes. J Chem Ecol 38:704–713

Hartmann A, Rothballer M, Hense BA et al (2014) Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. Front Plant Sci 5:131

Hernández-Reyes C, Schenk ST, Neumann C et al (2014) N-acyl homoserine lactones-producing bacteria protect plants against plant and human pathogens. Microb Biotechnol 7:580–588

Holden MT, Ram Chhabra S, de Nys R et al (1999) Quorum-sensing cross-talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other Gram-negative bacteria. Mol Microbiol 33:1254–1266

Hosni T, Moretti C, Devescovi G et al (2011) Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease. ISME J 5:1857–1870

Imran A, Saadalla MJA, Khan SU et al (2014) *Ochrobactrum* sp. Pv2Z2 exhibits multiple traits of plant growth promotion, biodegradation and N-acyl-homoserine-lactone quorum sensing. Ann Microbiol 64:1797–1806

Jiang J, Wu S, Wang J et al (2015) AHL-type quorum sensing and its regulation on symplasmata formation in *Pantoea agglomerans* YS19. J Basic Microbiol 55:607–616

Jimenez PN, Koch G, Thompson JA et al (2012) The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. Microbiol Mol Biol Rev 76:46–65

Johnson KL, Walcott RR (2013) Quorum sensing contributes to seed-to-seedling transmission of *Acidovorax citrulli* on watermelon. J Phytopathol 161:562–573

Kakkar A, Nizampatnam NR, Kondreddy A (2015) *Xanthomonas campestris* cell–cell signaling molecule DSF (diffusible signal factor) elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan. J Exp Bot 66:6697–6714

Kalia VC (ed) (2015) Quorum sensing vs. quorum quenching: a battle with no end in sight. Springer, India

Kaplan HB, Greenberg EP (1985) Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. J Bacteriol 163:1210–1214

Kaufmann GF, Sartorio R, Lee SH et al (2006) Antibody interference with N-acyl homoserine lactone-mediated bacterial quorum sensing. J Am Chem Soc 128:2802–2803

Kaufmann GF, Park J, Mee JM et al (2008) The quorum quenching antibody RS2-1G9 protects macrophages from the cytotoxic effects of *Pseudomonas aeruginosa* quorum sensing signaling molecule N-3-oxo dodecanoylhomoserine lactone. Mol Immunol 45:2710–2714

Kay E, Dubuis C, Haas D (2005) Three small RNAs jointly ensure secondary metabolism and biocontrol in *Pseudomonas fluorescens* CHA0. Proc Natl Acad Sci U S A 102:17136–17141

Kendall MM, Sperandio V (2007) Quorum sensing by enteric pathogens. Curr Opin Gastroenterol 23:10–15

Khan SR, Mavrodi DV, Jog GJ et al (2005) Activation of the *phz* operon of *Pseudomonas fluorescens* 2-79 requires the LuxR homolog PhzR, *N*-(3-OHHexanoyl)-l-homoserine lactone produced by the LuxI homolog PhzI, and a cis-acting *phz* box. J Bacteriol 187:6517–6527

Lee J, Zhang L (2015) The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein Cell 6:26–41

Lee SJ, Park SY, Lee JJ et al (2002) Genes encoding the *N*-acyl homoserine lactone-degrading enzyme are widespread in many subspecies of *Bacillus thuringiensis*. Appl Environ Microbiol 68:3919–3924

Li X, Fekete A, Englmann M et al (2006) Development of a solid phase extraction-ultra pressure liquid chromatography method for the determination of N-acyl homoserine lactones from bacterial supernatants. J Chromatogr A 1134:186–193

Liu X, Jia J, Popat R et al (2011) Characterisation of two quorum sensing systems in the endophytic *Serratia plymuthica* strain G3: differential control of motility and biofilm formation according to life-style. BMC Microbiol 11:26

Liu F, Bian Z, Jia Z et al (2012) The GCR1 and GPA1 participate in promotion of *Arabidopsis* primary root elongation induced by N-acyl-homoserine lactones, the bacterial quorum sensing system. Mol Plant-Microbe Interact 25:677–683

López-Ráez JA, Bouwmeester H, Pozo MJ (2012) Communication in the rhizosphere, a target for pest management. In: Lichtfouse E (ed) Agroecology and strategies for climate change, sustainable agriculture reviews. Springer, Netherlands, pp 109–133

Lugtenberg BJ, Kamilova F (2009) Plant growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Lyon GJ, Novick C (2004) Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. Peptides 25:1389–1403

Malik AK, Fekete A, Gebefuegi I et al (2009) Single drop microextraction of homoserine lactones based quorum sensing signal molecules, and the separation of their enantiomers using gas chromatography mass spectrometry in the presence of biological matrices. Microchim Acta 166:101–107

Mathesius U, Mulders S, Gao M et al (2003) Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. PNAS 100:1444–1449

McClean KH, Winson MK, Fish L (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of the violacein production and inhibition for the detection of N-acyl homoserine lactonase. Microbiology 143:3703–3711

Mcknight SL, Iglewski BH, Pesci EC (2000) The Pseudomonas quinolone signal regulates rhl virulence factor production and biofilm formation in *Pseudomonas aeruginosa*. J Bacteriol 182:2702–2708

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Monnet V, Juillard V, Gardan R (2016) Peptide conversations in Gram-positive bacteria. Crit Rev Microbiol 42:339–351

Morin D, Grasland B, Vallee-Rehel K et al (2003) On-line high performance liquid chromatography-mass spectrometry detection and quantification of N-acyl homoserine lactone quorum sensing signal molecules, in the presence of biological matrices. J Chromatogr A 1002:79–92

Müller J, Kuttler C, Hense BA (2006) Cell-cell communication by quorum sensing and dimension-reduction. J Math Biol 53:672–702

Newton JA, Fray RG (2004) Integration of environmental and host-derived signals with quorum sensing during plant-microbe interactions. Cell Microbiol 6:213–224

Nieto-Penalver CG, Bertini EV, de Figueroa LIC (2012) Identification of N-acyl homoserine lactones produced by *Gluconacetobacter diazotrophicus* PAL5 cultured in complex and synthetic media. Arch Microbiol 194:615–622

Oldroyd GE (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

Oslizlo A, Stefanic P, Vatovec S et al (2015) Exploring ComQXPA quorum sensing diversity and biocontrol potential of *Bacillus* spp. isolates from tomato rhizoplane. Microb Biotechnol 8:527–540

Pang Y, Liu X, Ma Y et al (2009) Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of *Serratia plymuthica* are dependent on N-acyl homoserine lactones. Eur J Plant Pathol 124:261–268

Papenfort K, Bassler BL (2016) Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol 14:576–588

Park J, Jagasia R, Kaufmann GF et al (2007) Infection control by antibody disruption of bacterial quorum sensing signaling. Chem Biol 14:1119–1127

Paungfoo-Lonhienne C, Lonhienne TGA, Yeoh YK et al (2016) Crosstalk between sugarcane and a plant-growth promoting *Burkholderia* species. Sci Rep 6:37389

Pereira CS, Thompson JA, Xavier KB (2013) AI-2-mediated signalling in bacteria. FEMS Microbiol Rev 37:156–181

Perez-Montano F, Jimenez-Guerrero I, Sanchez-Matamoros C et al (2013) Rice and bean AHL-mimic quorum-sensing signals specifically interfere with the capacity to form biofilms by plant-associated bacteria. Res Microbiol 164:749–760

Pérez-Montaño F, Jiménez-Guerrero I, Del Cerro P et al (2014) The symbiotic biofilm of *Sinorhizobium fredii* SMH12, necessary for successful colonization and symbiosis of *Glycine max* cv Osumi, is regulated by quorum sensing systems and inducing flavonoids via NodD1. PLoS One 9(8):e105901

Persello-Cartieaux F, Nussaume L, Robaglia C (2003) Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell Environ 26:189–199

Pieterse CM, Zamioudis C, Berendsen RL et al (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375

Podile AR, Vukanti RVNR, Sravani A et al (2014) Root colonization and quorum sensing are the driving forces of plant growth promoting rhizobacteria (PGPR) for growth promotion. Proc Natl Acad Sci India Sect B Biol 80:407–413

Rumbaugh KP (ed) (2011) Quorum sensing: methods and protocols. Methods in molecular biology. Springer, New York

Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2:a012427

Ryan RP, An SQ, Allan JH et al (2015) The DSF family of cell–cell signals: an expanding class of bacterial virulence regulators. PLoS Pathog 11:e1004986

Sanchez-Contreras M, Bauer WD, Gao MS et al (2007) Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Philos Trans R Soc B 362:1149–1163

Schenk ST, Schikora A (2015) AHL-priming functions via oxylipin and salicylic acid. Front Plant Sci 5:784

Schenk ST, Stein E, Kogel KH et al (2012) *Arabidopsis* growth and defense are modulated by bacterial quorum sensing molecules. Plant Signal Behav 7:178–181

Schikora A, Schenk ST, Hartmann A (2016) Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the *N*-acyl homoserine lactone group. Plant Mol Biol 90:605–612

Schmid N, Pessi G, Deng Y et al (2012) The ahl- and bdsf-dependent quorum sensing systems control specific and overlapping sets of genes in *Burkholderia cenocepacia* H111. PLoS One 7(11):e49966

Sessitsch A, Coenye T, Sturz AV et al (2005) *Burkholderia phytofirmans* sp. *nov*., a novel plant-associated bacterium with plant-beneficial properties. Int J Syst Evol Microbiol 55:1187–1192

Shoresh M, Harman GE, Mastouri F et al (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43

Simons M, van der Bij AJ, de Weger LA (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. Mol Plant-Microbe Interact 9:600–607

Singh BN, Singh HB, Singh A et al (2012) *Lagerstroemia speciosa* fruit extract modulates quorum sensing-controlled virulence factor production and biofilm formation in *Pseudomonas aeruginosa*. Microbiology 158:529–538

Steidle A, Sigl K, Schuhegger R et al (2001) Visualization of Nacylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. Appl Environ Microbiol 67:5761–5770

Suppiger A, Schmid N, Aguilar C (2013) Two quorum sensing systems control biofilm formation and virulence in members of the *Burkholderia cepacia* complex. Virulence 4:400–409

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant-Microbe Interact 13:637–648

Teplitski M, Mathesius U, Rumbaugh KP (2011) Perception and degradation of N-acyl homoserine lactone quorum sensing signals by mammalian and plant cells. Chem Rev 111:100–116

Thomson NR, Crow MA, Mcgowan SJ et al (2000) Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. Mol Microbiol 36:539–556

Veliz-Vallejos DF, van Noorden GE, Yuan M et al (2014) A *Sinorhizobium meliloti*-specific N-acyl homoserine lactone quorum-sensing signal increases nodule numbers in *Medicago truncatula* independent of autoregulation. Front Plant Sci 5:551

Venturi V, Fuqua C (2013) Chemical signaling between plants and plant-pathogenic bacteria. Annu Rev Phytopathol 51:17–37

Venturi V, Keel C (2016) Signaling in the Rhizosphere. Trends Plant Sci 21:187–198

Verma SC, Miyashiro T (2013) Quorum sensing in the squid-vibrio symbiosis. Int J Mol Sci 14:16386–16401

von Bodman SB, Bauer WD, Coplin DL (2003) Quorum sensing in plant-pathogenic bacteria. Annu Rev Phytopathol 41:455–482

von Rad U, Klein I, Dobrev PI et al (2008) The response of *Arabidopsis thaliana* to N -hexanoyl DL-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229:73–85

Wang LH, He Y, Gao Y et al (2004a) A bacterial cell-cell communication signal with cross-kingdom structural analogues. Mol Microbiol 51:903–912

Wang H, Zhong Z, Cai T et al (2004b) Heterologous overexpression of quorum-sensing regulators to study cell-density-dependent phenotypes in a symbiotic plant bacterium *Mesorhizobium huakuii*. Arch Microbiol 182:520–525

Waters CM, Bassler BL (2005) Quorum sensing, cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Williams P (2007) Quorum sensing, communication and cross-kingdom signaling in the bacterial world. Microbiology 153:3923–3938

Williams P, Câmara M (2009) Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. Curr Opin Microbiol 12:182–191

Winzer K, Hardie KR, Williams P (2002) Bacterial cell-to-cell communication: sorry, can't talk now-gone for lunch! Curr Opin Microbiol 5:216–222

Wynendaele E, Bronselaer A, Nielandt J et al (2013) Quorumpeps database: chemical space, microbial origin and functionality of quorum sensing peptides. Nucleic Acids Res 41:D655–D659

Yamada Y, Nihira T (1998) Microbial hormones and microbial chemical ecology. In: Barton DHR, Nakanishi K (eds) Comprehensive natural products chemistry. Elsevier Sciences, Amsterdam, pp 377–413

Zamioudis C, Pieterse CM (2012) Modulation of host immunity by beneficial microbes. Mol Plant-Microbe Interact 25:139–150

Zarkani AA, Stein E, Rohrich CR et al (2013) Homoserine lactones influence the reaction of plants to rhizobia. Int J Mol Sci 14:17122–17146

Zhang Y, Ruyter-Spira C, Bouwmeester HJ (2015) Engineering the plant rhizosphere. Curr Opin Biotechnol 32:136–142

Zúñiga A, Poupin MJ, Donoso R (2013) Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of *Arabidopsis thaliana* by *Burkholderia phytofirmans* PsJN. Mol Plant-Microbe Interact 26:546–553

Microorganisms: Role for Crop Production and Its Interface with Soil Agroecosystem

17

Dhiman Mukherjee

Abstract

Throughout the world agriculture has need to twofold increase in food production by 2050 in order to meet the burgeoning population with decrease its necessity on factory made fertilizers and plant protection chemicals. This may be attained through exploring multiple options of utilizing beneficial microorganisms and its suitable interaction in agroecosystem of the concerned surroundings. Our agricultural system is a multifaceted system of exchanges between plants and microorganisms. Increasing demand for economically well-matched, surroundings sociable technique in farming that might be able to provide sufficient nutrients for the growing human inhabitants through upgrading of the worth and scale of farming yield with the help of eco-friendly microbes present in nature. In this aspect, microorganisms play a key role. Beneficial effects of microorganisms on herbal progress mainly include uptake of major soil nutrients mainly NPK, etc., advanced growth of young branches and roots, improvement of soil productivity, and lastly proper nitrogen fixations and acquisition of soil nitrogen. Some of the frequently used beneficial microbes in agriculture globally include *Bacillus*, *Azospirillum*, *Trichoderma*, *Rhizobia*, *Mycorrhizae*, *Pseudomonas*, *Streptomyces*, and many other species. Exploring modern techniques with molecular biology helps to exploit valuable microbes and its products that leads to enhancing farm productivity and improvement of soil quality on sustainable basis.

Keywords

Agroecosystem • Crop diversification • Microbes • Nutrient mobilization • Plant

D. Mukherjee (✉)
Bidhan Chandra KrishiViswavidayalaya, Directorate of Research,
Kalayani 741235, West Bengal, India
e-mail: dhiman_mukherjee@yahoo.co.in

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_17

17.1 Introduction

During the precedent century, mechanization of agriculture has aggravated a remarkable increase in efficiency, which gives a better quantity and quality of food accessible to the common people. Under the present circumstances, nowadays, it is vital to preserve that high efficiency, but it is becoming imperative to alter the surrounding as little as conceivable (Mukherjee 2015a). Clearly we must then head for a more eco-friendly and sound farming practices while maintaining soil agroecosystem and microbial veracity in the surroundings. Microbes, though they have a dreadful agent of disease, also play a beneficial role in agriculture. For example, they help to accumulate atmospheric N into an easily available form such as soluble nitrates, which act as environmentally friendly plant nutrients. Proper knowledge and exploration of such microorganisms for agricultural production system are an important aspect of current farming technology. The distinctiveness of microorganisms and its frequently changeable character and biosynthetic capabilities, under a certain environmental condition, have made them likely aspirants for solving mainly complicated tribulations in the biological sciences and other fields as well. Different measures in which microorganisms have been used in the past century to advance in agricultural sciences, food processing, food safety and quality, technology intervention, genetic engineering, ecological safety, more effective treatment of farming, and municipal wastes provide a most striking evidence of success. Microorganisms acts well only when they are accessible with appropriate and best possible environment for metabolizing their substrates mainly accessible water, oxygen (depending on whether the microorganisms are obligate aerobes or facultative anaerobes), pH and temperature of their environment. Meanwhile, availability of different types of culture (microbes) or inoculants in the market increased due to its novel technologies. Since microbes are helpful in eliminating evils with synthetic pesticides, they are currently extensively useful in agriculture and natural farming (Mukherjee 2013a). Active soil microbiological processes help to enhance the release of different nutrients from organic substances; this leads to better plant nutrition and uptake by crop. Information regarding microbe's soil interaction and factors on which they depend makes possible the exercise of many microbes in agriculture. Symbiotic atmospheric nitrogen accumulations need a comprehensive use in pulse-based production.

17.2 Microbe's Efficacy in Farming Practices

An agricultural practice begins with photosynthesis – the translation of solar energy into element form. It's an amazing process, but not a particularly efficient one. Observation by different concerned revealed that, quick mounting crop such as maize, sugarcane etc. can fix a highest 8–9% of sun energy. These processes exploit wavelengths that green vegetation does not (Fischer et al. 2014). Photosynthetic or phototropic bacteria are autonomous self-sufficient microorganisms which can utilize solar system of energy and transform the secretion from crop roots,

carbon-based substance and detrimental gases into plant useful substances like amino acids, sugars, nucleic acids, and various other metabolites. These bacteria mostly utilize directly by field crop etc. to augment yield efficiency and also some extent to other advantageous microorganisms. For example, VAM fungi help to improve exploitation of available phosphate in soil and directly help plant growth (Mukhrjee 2014). VAM fungi lying with other microbe's *viz. Rhizobium*, *Azotobactor* etc. and amplify the capability of flora to fix nitrogen. Economical valuable microorganisms utilize atmospheric nitrogen for better plant growth, putrefy natural waste and residues, detoxify pesticide, restrain plant disease, improve plant nutrient availability, and construct bioactive substances mainly active hormones, vitamins, and various enzymes that stimulate plant growth. However, harmful agricultural microorganisms are those that can encourage plant diseases, rouse soil-associated pathogens, immobilize macro- and micronutrients, and produce toxic and putrescent substances that badly influence plant expansion and healthiness.

17.2.1 Microorganism and Soil

Microorganism involvement in soil offers the substratum for crops, plants, and other living organisms. They represent a sympathetic habitat for microbes and are occupied by an extensive series of microbes, comprising algae, viruses, fungi, bacteria, and protozoa and many other (Mukherjee 2008). Microorganisms in the topsoil are important, improving soil productivity, cycling of nutrient compounds in the biosphere, and sources of manufacturing products such as growth hormones, enzymes, antibiotics, vitamins, etc. Few microbes help to change soil configurations or structure, which might be beneficial for crop production and productivity as well. But certain microbes in agroecosystem are the contributory agents of a variety of plant and animal sicknesses. The crop and living thing vestiges deposited within the earth contribute organic substances. Soil microorganisms break a series of carbon-based resources and utilize a piece of these breakdown foodstuffs to create or synthesize a sequence of compound that make up a humus substance composed of residual organic matter not readily decomposed by microorganisms. Humus exists in soil mainly in four forms, viz., polysaccharides, humic substances, other non-humic substances, and humin. This can help the physicochemical properties of soil in many ways by improving the texture and arrangement of the soil, contributes to its buffering ability, and boosts the productivity of soil, by improving water holding capacity. Soil structure plays a significant responsibility in persuading the nature and distribution of pore space and water availability. These alter the heterogeneity of microbial habitats. Thus, physical characteristics have direct and measurable effects on microbial activity inside the soil. These comprise constraints on the habitable pore space available for microorganisms, the opportunities for transport of microbes, and the effects on microbial makeup and metabolism. A well-developed soil structure is expected to encourage microbial activity. Fungal hyphae play a predominantly vigorous protagonist in aggregating and stabilizing soil structure

(Young and Crawford 2004). Guggenberger et al. (1999) persuaded fungal growth by addition of starch to soil and observed the expansion of macroaggregate structures following the enlargement of hyphae between microaggregates. Bossuyt et al. (2001) establish to facilitate inhibition of fungal activity that led to the reduced formation of macroaggregates.

Fungal hyphae of AMF (*arbuscular mycorrhizal* fungi) may have a diameter of 1 μm, allowing them to penetrate very small soil pores, without the physical movement of soil particles. AMF are likely to help the formation of aggregates. Rillig et al. (2002) found that aggregate formation could be predicted by the occurrence of glomalin, a protein only produced by AMF. The option of diversified cropping sequence could influence the development of topsoil aggregate stability and degree of fungal associations. These influence the cropping pattern of the particular zone (Mukherjee 2016a). Compounds released from decomposing organic material influence soil microbial activity. The extent to which these organic matters such as polysaccharides are altered by microbial activity can provide an indication of the type of microorganism involved. Thus, for example, a low glucosamine/muramic acid relation is linked with bacterial movement (Glaser et al. 2004), while fungal action can be recognized by the nature of PLFA outlines (Bardgett and McAlister 1999). Six et al. (2001) establish that modification of plant-derived carbon increases with falling aggregate size. This is significant because the humic compounds they form due to decomposition are recognized to be important in contributing to structural stability through attachment amid contiguous clay particles. Microbiological process play important role in soil structure, as we know that structure itself acts in the reverse direction toward supply a very strong control on the eminence of microbial action.

17.2.2 Microorganism for Soil Strength and Nutrient Mobilization

Soil agroecosystem is inhabited by various groups of microorganisms, which are its living component. This represents the major and most unlikely biotic group in soil, with an estimation of one million to one billion microbes in 1 g of agricultural topsoil. Microorganisms hold the ability to give an integrated determination of soil physical condition, an aspect that could not be attained with physical/chemical measures and/or analyses of diversity of higher organisms. The aboriginal rhizospheric microbial inhabitants of farming soils are greatly influenced by agricultural practices (e.g., soil nurturing, period, stubble preservation, blazing, etc.), crop genotype, as well as soil category (Berg and Smalla 2009; Reeve et al. 2010). Various reports revealed that crop exudates may cause changes to soil uniqueness such as texture, buffering capacity, and organic carbon availability, impacting the multiplicity and action of diverse microbes (Haichar et al. 2008). Bio-augmentation, the accumulation of microorganisms to farming soils, thus becomes a precious impact on soil microbiological processes (Mukherjee 2008). Microbes and microbial cycle play a dynamic effect in mobilization and transport of nitrogen, phosphorus, and sulfur and the decay of organic residue. They influence plant vital nutrient cycle and

carbon pool in a worldwide scale. Changes in microbe's congregation help to notice change in the soil's physicochemical activity, thus giving an early symbol of soil improvement or an early warning of soil degradation (Mukherjee 2013c). Biofertilizers play a critical role in crop nutrition accessibility and an input of various nutrient movements in plant-soil continuum.

17.3 Biofertilizers and Crops

This can be elaborated as microbes that assist crop vegetation uptake of plant nutrients by their exchanges in the rhizosphere when applied through seed or soil (Singh and Mukherjee 2009). This helps to enhance a certain microbiological methodology in soil system which augments the extent of accessibility of nutrients in a form easily assimilated by crop or vegetation (Mukherjee 2012). Different kinds of microorganisms and its association with plant-soil system are being subjugated in the manufacture of biofertilizers, and its role varies in diverse plants or crops (Tables 17.1 and 17.2).

17.4 Role of Microorganisms in Various Crop Production

Microbial inoculants include three major groups: (1) AMF, (2) plant growth-promoting rhizobacteria (PGPR), and (3) symbiotic nitrogen-fixing rhizobia. The valuable role of each category has been worked out separately in diverse crops by

Table 17.1 Different types of biofertilizer and its role in potential crops

	Groups	Examples
Nitrogen accumulating		
1.	Free-living	*Anabaena*, *Klebsiella*, *Clostridium*, *Beijerinckia*, *Azotobacter*, *Nostoc*
2.	Symbiotic	*Rhizobium*, *Frankia*, *Anabaena azollae*
3.	Associative symbiotic	*Azospirillum*
Phosphorus solubilizing		
1.	Bacteria	*Pseudomonas striata*, *Bacillus circulans*, *Bacillus megaterium*, *Bacillus subtilis*
2.	Fungi	*Aspergillus awamori*, *Penicillium* sp.
Phosphorus mobilizing		
1.	*Arbuscular mycorrhiza*	*Glomus* species, *Gigaspora* species, *Acaulospora* species, *Scutellospora* species, and *Sclerocystis* species
2.	*Ectomycorrhiza*	*Amanita* species, *Laccaria* species, *Pisolithus* species, *Boletus* species
Biofertilizers for micronutrients		
	Silicate and zinc solubilizers	*Bacillus* species
Plant growth-promoting rhizobacteria		
	Pseudomonas	*Pseudomonas fluorescens*

Table 17.2 Recommendation and method of biofertilizer application in different crops

Crop	Recommended biofertilizer	Application method
Field crops		
Pulses		
Black gram, chickpea, pea, groundnut, soybean, beans, lentil, lucern, berseem, green gram, cowpea, and pigeon pea	*Rhizobium*	Seed treatment
Cereals		
Wheat, oat, barley	*Azotobacter/Azospirillum*	Seed treatment
Rice	*Azospirillum*	Seed treatment
Oil seeds		
Mustard, seasum, linseeds, sunflower, castor	*Azotobacter*	Seed treatment
Millets		
Pearl millets, finger millets, kodo millet	*Azotobacter*	Seed treatment
Maize and sorghum	*Azospirillum*	Seed treatment
Forage crops and grasses		
Bermuda grass, Sudan grass, Napier grass, Para grass, star grass, etc.	*Azotobacter*	Seed treatment
Other misc. plantation crops		
Tobacco	*Azotobacter*	Seedling treatment
Tea, coffee	*Azotobacter*	Soil treatment
Rubber, coconuts	*Azotobacter*	Soil treatment
Agroforestry/fruit plants		
All fruit plants/agroforestry (herb, shrubs, annuals, and perennial) plants for fuel wood fodder, fruits, gum, spice, leaves, flowers, nuts, and seeds purpose	*Azotobacter*	Soil treatment
Leguminous plants/trees	*Rhizobium*	Soil treatment

different workers (Dobbelaere et al. 2001; Barea et al. 2002; Murray 2011; Verma et al. 2010, Mukherjee 2014b). PGPRs play a notable function in crop productivity and enhancement of soil fertility. This helps to reduce harmful ecological impacts resulting from sustained use of chemical fertilizers, herbicides, and plant protection chemicals. PGPR was first illustrated by Kloepper and Schroth (1978) to explain various microbes which inhabit the rhizosphere of plants, mounting in, on, or around plant tissues that inspire herbal development by several mechanisms. Moreover, various works are being taken to assess crop development effects by applying diverse microbial alteration or consortia, such as AMF-PGPR, symbiotic nitrogen-fixing rhizobia, etc. (Swarnalakshmi et al. 2013). However, knowing the appliances of crop expansion is important to decide what type of microorganism is better to use

with which plant in a given situation. Soil microbes were widely deliberate by various workers in crop production due to its high affinity in enhancing crop yield (Somers et al. 2004). The research involving the utilization of PGPRs was done mainly on herbaceous plants in open-field environments and on horticultural crops. Furthermore, their incorporation has freshly extended both in agroforestry and in phytoremediation of contaminated soils. Strains of *Azospirillum* (Dobbelaere et al. 2001), *Bacillus* (Kokalis-Burelle et al. 2006), and *Pseudomonas* (Meyer et al. 2010) had been used in an extensive variety of viable beneficial crops. The consumption of useful microorganisms in the substitute or the decrease of inorganic has been so far supported from a different corner (Dobbelaere et al. 2003).

Beneficial microbes such as PGPRs and diazotrophs (PGPFs) can lead to a good position in this main confront, as they fulfill important ecosystem functions for crop and soil ecosystem. How useful microbes and its interface with soil agroecosystem helps to augment plant rooting which is only partly recognized, as quite a few aspects have to be measured, mainly (1) the fabrication of metabolites associated with root growth and pathogen control (phytohormones, antimicrobials, antibiotics) and (2) the exertion to distinguish the precise/entire actions, owing toward the enhanced accessibility of nutrient. Although in more than one and a half decade, fungi and bacteria have been recurrently demonstrated to endorse herbal development and repress crop pathogens, this knowledge has yet to be extensively exploited in agricultural biotechnology (Berg 2009). Beneficial microorganisms play a critical role in the enhancement of crop yield particularly maize, rice, and wheat.

17.4.1 Maize

The global production of maize was 883 MT (2011). In 2050, it is estimated that the requirement of maize and its produce in the emerging nations will be a nearly twofold increase (Rosegrant et al. 2009). Rice exhibits an extensive compliance to different environments, which makes it the most widespread crop around the globe. It can grow in dearth circumstances or in shallow label of water (up to 50 cm of water) and in a wide diversity of latitudes and up to 3000 m altitude. For this cause, it is considered a strategic crop for foodstuff safety in the globe by the FAO. With regard to maize, it represents a major renewable supply for food, feed, and industrial raw material which makes it the most widely grown crop worldwide. Although in the past few years, wheat has undergone an impressive yield increase, annual yield increase began to slow down in 1995 and is currently stagnating in almost each nation (Reynolds et al. 2009). Crystal-clear connection has been observed with rising frequency of interfering climatic factors such as spring drought during stem elongation, heat stress around flowering time and during grain filling out since 1995 (Lobell et al. 2011). During that same instance, global population increased from 5.8 billion to 6.6 billion and is predictable to exceed ten billion by 2050 (Dixon et al. 2009). Utilization of plant growth-promoting substances or any other microbial inoculants would establish a biological alternate for healthy yield of these crops. There are many free-living nitrogen-fixing bacteria, such as *Burkholderia* sp.,

Azospirillum sp., *Azotobacter* sp., *Herbaspirillum seropedicae*, *Pseudomonas* sp., and *Bacillus* sp. (Riggs et al. 2001). An experiment revealed that a sturdy boost in whole crop and grain dry weight was obtained when maize plants were inoculated with *Burkholderia cepacia* (Riggs et al. 2001). Krey et al. (2013) observed the role of PGPR on phosphorus nutrition, and he found that field application of *P. fluorescens* DR54 on maize increased crop development and P pools in soil. From these observations, mainly through the P-deficient treatment, so use of *P. fluorescens* DR54 on P poor soils, and concluded that PGPR and phosphate nourishments should be use separately.

17.4.2 Rice

Paddy crop uses significantly high amounts of agrochemical input in the form of fertilizer and protection chemicals. This leads to soil pollution that is destructive to the environment and surroundings in terms of soil quality and physical condition, emergence of resistant biotype of crop pathogens, and abolition of microbes in soil, which take part in crop cultivation. Microbes help in enhancing soil productivity and rice yield (Febri et al. 2013).The mechanisms engaged by microorganisms in attractive rice progress and economic production include growth-regulating substance construction, phosphate solubilization, nitrogen fixation, and siderophore production. Four fungi (*Aspergillus niger*, *T. harzianum*, *T. virens*, and *Gliocladium virens*) were scrutinized for their outcome on sprouting and sapling weight of paddy. Observations showed that shoot height and fresh mass of paddy seedlings noteworthily increased (Mishra and Sinha 2000). The major restrictive nutrient for rice crops is nitrogen, and merely one-third of the applied nitrogen as chemical nourishment is directly used by this crop (Buresh et al. 2008). Thus, it becomes important to find an alternative to decrease and optimize the usage of N fertilizers applied to rice crops; plenty of information explain that diazotrophic bacteria are helpful in this regard (Araújo et al. 2013; Mukherjee 2013b). This also helps to produce IAA. Numerous PGPR reports consider that phytostimulation is mainly due to phytohormone release by the bacteria. Thus, the genus *Burkholderia* has been revealed to be the most widespread rice growth-endorsing bacteria able to produce plant hormones. However, few other options such as *Azospirillum*, *Bacillus*, *Paenibacillus*, *Brevundimonas*, *Serratia*, *Herbaspirillum*, and *Xanthomonas* improve crop growth by phytostimulation. Many of these synthesize IAA, gibberellin, and ethylene.

17.4.2.1 Paddy and Microbe's Interaction

Crops and microbial interactions occur in different ways. These may comprise among others the phyllosphere, endosphere, and rhizosphere as core spaces. The phyllosphere is connected with the aerial domain of the plant and the endosphere being linked with the internal cellular systems and its attendant convey systems. Plant exudes act as a signal (phenolic compounds) to attract microbes (Bhattacharyya and Jha 2012; Bais et al. 2004). Blilou et al. (2000) recognized paddy root exudates, categorized in two groups, amino acids (methionine, etc.) and carbohydrates

(mannose, galactose, glucose, and glucuronic acid). The profusion of paddy root exudates might draw microbes to colonize roots that penetrate to the root tissue. Reinhold-Hurek and Hurek (1997) examined the ability of *Azoarcus to colonize* paddy roots endophytically. This invades intercellularly and enters deeply into the vascular system mainly xylem vessels and help to spread into paddy shoot. Blilou et al. (2000) observed that appearance of a lipid transfer protein (LTP) gene is regulated in *Oryza sativa* roots in reaction to mycorrhizal fungus *Glomus mosseae*. Further, Rediers et al. (2003) examined *Pseudomonas stutzeri* A15 genes; its strain is capable to endorse rice growth. Mwashasha et al. (2016) work on certain microbes in enlightening the harvest potential of Basmati 317 rice. A study was conducted in the experimental farm of Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. A study was conducted with microbial concentrations and inoculants under randomized block design. The concentrations at three levels (10^9cfu spores^{-1} ml^{-1}, 10^7cfu spores^{-1} ml^{-1}, and 10^5cfu spores^{-1} ml^{-1}) comprised the main plots, while microbial inoculants (16 and two controls) were the subplots. Results enumerate that crops inoculated with elevated microbial concentration were enhanced in relation to the yield and yield-attributing parameters compared to those treated with low microbial concentrations. More number of tillers, high panicle length and more 100 grain weight of plants observed with species of *Brevundimonas*, *Bacillus*, *Enterobacter*, *Trichoderma*, and *Aspergillus*, were better than for the control plants. The above work confirms that PGPM inoculants improved plant growth and ultimately productivity. As far as rice disease is concerned, the three main rice pathogens *Xanthomonas oryzae*, *Rhizoctonia solani*, and *Magnaporthe oryzae* are responsible for bacterial leaf blight, sheath blight, and blast on rice plants, respectively. The majority of the studies in rice biocontrol are focused on treatment and prevention of these diseases (Han et al. 2005). *Bacillus* and *Pseudomonas* are the predominant PGPR genera used against those pathogens, owing to its antagonism against growth of several fungal and bacterial microorganisms. These PGPRs usually construct siderophores, antibiotics, quitinases, and proteases, which could be accountable for the antagonism against pathogens. The yield of disease control inside a close chamber or in greenhouse and field experiments was satisfactory, reducing the severity of diseases up to 90% subjected to the PGPR, pathogen, and paddy variety (Filippi et al. 2011).

17.4.3 Wheat

Wheat utilization of PGPR that enhances plant growth and productivity and helps to reduce the usage of inorganic agrochemicals has become one of the important aspects and strategies for supportable farming (Mukherjee 2015b). For example, inoculation of the wheat seed with ACC deaminase producer *P. fluorescens* strains allowed the diminishing of N, P, and K fertilizer rates (Shaharoona et al. 2008), and in general, crops presented higher grain yields, harvest index, and protein content with lower fertilizer doses, along with PGPR, than those conventionally applied (Rosas et al. 2009). This improved grain production of wheat crop, found with

consortia of PGPR and AMF, particularly if they show diverse and harmonizing abilities. Singh and Kapoor (1999) worked on various inoculations with the AMF *Glomus* sp. and two phosphate (PO_4^{3-})-solubilizing microorganisms (PSM), *Bacillus circulans* and *Cladosporium herbarum*, with or without rock phosphate in a natural P-deficient sandy soil on wheat crops. The significant enhancement of stubbles and economic yields due to inoculation with the consortia could be attributed to a high absorption of nutrients. The consortia of AMF and PGPR on wheat crops were investigated in a 2-year experiment in different agroclimate zones of India at seven different locations spreading from the Himalayan foothills to the Gangetic belt, and it was seen that dual inoculation of this cereal increased crop yield, grain yield, and soil value and the nutrient deduction by wheat. In addition, it was observed that yield retorts to inoculants were maximum at locations with previous low yields (Mäder et al. 2011).

17.4.4 Legumes

Soil microbes play a key function in legume production and enhancement of soil productivity with the help of biofertilizer, etc., as per Tables 17.2 and 17.3 legumes are used in rotation, they increase the nitrogen availability and reduce the exercise of synthetic fertilizers. A number of studies suggest that legume residues can supply nitrogen up to 36–266 kg/ha. The total supply of nitrogen mainly depends on environmental conditions, microorganism biomass, management practices used (e.g., tillage), and the legume species. Legumes form a symbiotic association with *Rhizobium*, root-nodule bacteria that fix atmospheric nitrogen to ammonium, and thus acquire nitrogen from the soil and the atmosphere. Pulses exploit soil microorganisms to draw nitrogen from the air. The fixed nitrogen is incorporated into legumes' biomass in the form of amino acids and proteins. Crop rotations that include actively fixing legumes can reduce nitrogen fertilizer needs because a number of fixed nitrogen is returned to the soil with incorporation of crop remains by various microbiological activities and by direct release of growth-promoting

Table 17.3 Magnitude of natural nitrogen fixed by *Rhizobium* in different crops

Host group	*Rhizobium* species	Crops	N fix kg/ha
Pea	*Rhizobium leguminosarum*	Green pea, lentil	60–130
Soybean	*R. japonicum*	Soybean	60–108
Lupini	*R. lupine orinthopus*	Lupinus	65–92
Alfalfa	*R. meliloti*, *Medicago*, *Trigonella*	Melilotus	110–160
Beans	*R. phaseoli*	Phaseoli	70–115
Clover	*R. trifolii*	Trifolium	120–132
Cowpea	*R. species*	Moong, pigeon pea, cowpea, groundnut	60–110
Cicer	*R. species*	Bengal gram	70–120

substances into the soil via root exudation and root death. Many farmers in Darjeeling-Sikkim Himalaya under organic farming use leguminous cover crops as green manures for the enhancement of soil quality and health (Mukherjee 2010). Incorporation of a pulse-based system into rotation could be helpful for succeeding plant yields. For example, regardless of fertilizer application, grain yields following legume rotations are often 20–30% higher than continuous grain rotations. In some cases, improved synchrony between nutrient accessibility and crop need could also be gained by using an amalgamation of legume residue and chemical fertilizer (Singh and Mukherjee 2009).

17.4.5 Sugarcane

Sugarcane is one of the most vital cash crops in India and throughout the world. This crop is mainly utilized for sugar and ethanol (as biofuel) manufacturing. Sugarcane consumed a high amount of fertilizer and is quite often affected by various fungal and bacterial diseases for which chemical treatments are not recommended. For good harvest of sugarcane crop, around 200–400 kg N/ha is required, which is expensive and perilous for the environment. In this direction utilization of PGPR can reduce the cost of nourishment and ecological risk and repress the disease as well (Samina 2011). PGPRs are incredibly well recognized mostly for their good supply of nitrogen to the plant root zone. A number of PGPR like *Enterobacter*, *Klebsiella*, *Azospirillum*, *Gluconacetobacter*, *Herbaspirillum*, *Pseudomonas*, etc. have been scrutinized from sugarcane (Samina 2011). Literature revealed that a few bacterium species help to reduce the use of fertilizer in sugarcane production and help to enhance its yield potentials. A novel work published in *Microbial Biotechnology* elaborates the root of sugarcanes inhabited by a new bacterium *Burkholderia australis*; this assists to augment crop growth via nitrogen fixation technique. Microbes and particularly bacteria play a vital function in sugarcane production, where they help mineralized the organic matter present in soil for easy release of major plant nutrients or help in atmospheric nitrogen fixation (Labandera-Gonzalez et al. 2001). Paungfoo-Lonhienne from the University of Queensland, Australia, and his associate workers found that bacteria help to break the waster product from sugarcane or livestock manure, to ensure enhanced normal nourishment for the coming generation of crop production. They hope to conduct field tests with a view to assist the expansion of commercial products that will be utilized for the better health and productivity of sugarcane crops and to reduce the utilization of commercial fertilizers (Chanyaratn et al. 2014).

17.4.6 Potato

Potato is one of the most important crop throughout the world and its important day by day increasing. Microbial inoculants particularly biofertilizers play a key role to enhance its productivity. This crop needs higher nutrients due to its sparse root

system. Hilly soil are acidic in nature and, P-fixing problem has been seen, thus it becomes urgent to take up location-friendly approaches by integrated use of biofertilizers, synthetic fertilizers, and organic manures in correct quantity for optimum potato yield in this region. In potato, primarily nonsymbiotic N fixer, plant growth-promoting bacteria, and PSB have been found helpful in rainfed environments, predominantly in hills. Under this agroecological zone, this crop grows during pre-*kharif* season and soils contain high organic matter. Mostly the soil of this region is acidic in nature, and a high quantity of phosphorus fixation is observed. High fixation of phosphorus leads to insoluble compound of Al and Fe ions. At Shimla, *Azotobacter* utilization has been found helpful and augmented the economic production of this crop in rainfed condition in the absence of N. This revealed that as nitrogen rate enhances, the influence of nonsymbiotic nitrogen fixer is reduced and it became nonsignificant at 180 kg N/ha. *Azotobacter* inoculation enhanced leaf nitrogen, NUE, as well tuber yield, mainly at a lesser nitrogen level. Inoculation of tuber with *Azotobacter* only improved the foliage nitrogen, perhaps owing to increased N availability from the soil to the crop at the early stage of growth when plant needs are high. During harvesting application of *Azotobacter* alone or with nitrogen considerably improved quantity of medium- and big-sized tubers. The sole application of *Azotobacter* improved the economic yields by 22q/ha above control and improved NUE considerably in company of lesser rate of nitrogen, i.e., at 25% and 50% of recommended nitrogen. Use of nitrogen along with inoculation of tuber with *Azotobacter* culture proved to be extra productive of total tuber numbers yield and recovery (Jatav et al. 2013). One more work in Shimla revealed that, during pre-*kharif* of 2007, application of phosphate and PSB, either sole application or mixture, significantly increased tuber yield. Further, other studies during the same period and in the same place concluded that seed inoculation with PSB in conjunction with 50, 75, and full dose of phosphate application resulted in not only higher yields but also better nutrient utilization as was evident by its constructive outcome on nutrient uptake and resulted in high revival of NPK in potatoes. This may be due to the useful result of PSB in the acidic soils by the discharge of native P there; this in turn makes sufficient labile phosphorus in soil solution around the root zone as directed by the higher NPK recoveries. The positive outcome of PSB endorsed the discharge of P from inorganic fractions of Al-P, Fe-P, and Ca-P and reduced P-fixing ability of the acidic soil (Jatav et al. 2013). Available crop growth-enhancing microbes particularly bacteria had immense potential in improving crop growth phase, economic produce, and macro- and micronutrient economy. Bacterial cultures of *Bacillus subtilis* and *Bacillus cereus* (growth-promoting bacteria) were evaluated in potato crop. Result revealed that, *Bacillus cereus* was better than *Bacillus subtilis* in all concentrations of nutrients. As far as economic produce is concerned, utilization of *Bacillus cereus* economized on NPK fertilizer dose by 25%. *Bacillus subtilis* and *Bacillus cereus* separately augmented the tuber yield of potato (Jatav et al. 2013). Inoculation with phosphobacteria (*Pseudomonas striata*) considerably improved the tuber yield at Shillong (Meghalaya) during pre-*kharif* of 1999 and 2000. However, its result on crop stand

was not noteworthy compared with those without culture. At Shillong, the use of PSB with different phosphatic fertilizers using two potato cultivars Kufri Megha and Kufri Jyoti improved yield of crop and tuber size. In a study at Shillong, during the pre-*kharif* of 1996–1998, the biofertilizers (*Azotobacter* and/or phosphorus inoculant culture of *Pseudomonas striata*) were evaluated in blend with N at 0, 50, 100, and 150 kg/ha on the economic production of potato cv. Kufri Jyoti. The tuber yield, tuber number, and tuber size were augmented with rising rates of N. Inoculation with *Azotobacter* and *P. striata* resulted in the highest tuber production and tuber number regardless of N rates, though differences in tuber production due to inoculation of different biofertilizers at 100 and 150 kg N/ha were not significant. B:C ratio improved with enhancing N rates and was highest with *Azotobacter* and *P. striata* inoculation (Jatav et al. 2013). Results from 3 years work in Shillong revealed that pooled employ of *Azotobacter* + phosphorus bacteria gave higher tuber production and gross outcome compared to their separate use of biofertilizer and control Rifampicin-nalidixic acid resistant mutants of a crop growth-promoting *Pseudomonas* sp., strain PsJN, were evaluated for their capacity to encourage in vitro expansion of potato. Few other root colonization strains such as MFE (a consistent growth advocate) were positively linked with crop expansion stimulation (Frommel et al. 1993).

17.5 Plant Growth-Promoting Microorganisms and Yield Potential

Agricultural industries rely heavily on the utilization of inorganic chemical doses, weedicide, and pesticides. Agriculture biotechnologies play a crucial position in increasing microbial inoculants to get better crop expansion and suppress plant disease, with the main aim of sinking reliance on commercial chemicals (Adesemoye et al. 2009). Many aspects need to be considered during the progress of such inoculants on a commercial scale (Berg 2009), including selection of appropriate plant growth-promoting (PGP) microorganisms based on target host plant, soil type, aboriginal microbial communities, ecological conditions, inoculant density, suitability of carriers, and compatibility with integrated crop management. The PGPRs are capable to encourage crop act by way of a wide assortment of mechanism (Saharan and Nehra 2011). Various works revealed that crop inoculation with PGPR helps to augment plant nutrient uptake and increase defense mechanism against pathogens (Maksimov et al. 2011). Furthermore, few crop growth-promoting rhizobia are capable to construct phytohormones, boost the population of other useful microbes, and control the detrimental ones in the rhizosphere (Saharan and Nehra 2011; Bhattacharyya and Jha 2012). Thus, plants competent to employ a larger mass of these microbes into their rhizospheres present greater endurance, growth, and reproduction (Gholami et al. 2009) and as an effect higher competitive ability.

17.6 Microbe Biotechnology and Sustainable Farming

Microbes are microscopic in nature; they belong to different groups such as viruses, protozoa, microalgae, fungi, and bacteria. These microorganisms survive in various media and diverse environments. Different natures of microorganisms and its habitats imitate a vast range of metabolic and biochemical character, which is due to the difference in hereditary configuration and natural selection in microbial populations. Genomes is a hereditary substance in the DNA of a particular creature (Yang et al. 2009). Obtaining the entire genome sequence of a microorganism gives a vital message regarding its ecology, but it is only the main step in the direction of understanding a microbe's biological capability and modifying them, if required, for farming purposes (<http://microbialbiotechnology.puchd.ac.in>). Microbial biotechnology is a noteworthy area that promotes food security and food safety and basic study in agriculture and allied sciences (Mosttafiz et al. 2012). Currently mounting awareness is to be paid toward the expansion of eco-friendly farming system, in which the high productivity of crops and animals is ensured using their usual adaptive potentials, with a least commotion of the milieu (Noble and Ruaysoongnern 2010). The major impact of an agricultural microbiologist on sustainable or eco-friendly farming practices would be to alternate various agrochemicals (mineral fertilizers, pesticides) with different sorts of microbial preparations (Weishampel and Bedford 2006). Different culture of various microbial species are use for improvement of crop and plant production (Andrews et al. 2010). Moreover, different methods for increasing the efficiency of nutrition and self-protective kinds of microbial mutualists require to be developed. For the dietetic types, an efficient colonization of crop plant in a host-specific mode is the most favorable, and the impacts of helpful symbionts are augmented in comparison to their host specificity. The use of various microbial symbiotic signals or their products for reorganizing crop growth or cynical functions may represent a good meadow for agricultural biotechnology. Avenues for future expansion of agri-farming microbiology may engross the edifice of novel multipartite endo- and ectosymbiotic communities based on comprehensive genetic and molecular (metagenomic) analyses. For harmonizing the host crop metabolism, an amalgamation of nitrogen and phosphorus providing symbionts would appear proficient, including the VAM fungi in association with endosymbiotic rhizobia (Shtark et al. 2010).

17.7 Application of Microbial Technology in Agroecosystem

Microbe-based symbiosis in crop plant ecosystem would be effective for the progress of sustainable agriculture in sort to ensure livelihood food security with least uproar of the surroundings. An efficient utilization of symbiotic microbial communities is promising using molecular approaches that depend on the stability of microbial pools which are circulating recurrently amid soil, plant, and animal which provided niches in usual and agricultural ecosystems (Kupriyanov et al. 2010). This helps to create extremely fruitful microbe-based sustainable agricultural systems.

17.7.1 Natural System

Different natures of microorganisms and different germs found in soil ecosystem are to be productive for enhancing agricultural or plant production and productivity too. Scientist try to develop biofertilizers and biopesticides to assist plant growth, curb problem of various pests mainly crop pest, weeds, pests, and plant pathogen diseases. Microbes that grow in the soil actually assist vegetation to absorb higher nutrients and to some extent also help in nutrient mobilization too (Mukherjee 2014a). Various crops and plants and these harmonious microorganisms are associated with "nutrient recycling," as per our above discussion. The microbes assist the crop to obtain valuable energy derivatives. Plant or crop gives their ravage by-products for the microbes to use for food or as source of energy. Various technical persons and workers utilized these pleasant microbes to develop biofertilizers. Primary nutrients mainly N and P are vital and critical for crop growth. Primary nutrients live obviously in the milieu but crop have a partial capacity to take out them. Phosphorus plays a critical role in crop stress tolerance, maturity, quality, and directly or indirectly nitrogen fixation. *Penicillium bilaii* is one of the most significant fungi, which helps to release phosphate from the soil agro-system. *Rhizobium* is a bacteria, which is linked with plant's roots in the cell, called nodules. Nodules act as biological factories, which facilitate to utilize N from the atmosphere and change it into an organic form that the crop can utilize.

17.7.2 Biopesticides

Microbes found in the earth are mostly eco-friendly to crop and soil agroecosystem. These pathogens or microorganisms may damage the crop plant to some extent. Few important biological kits, which use these problem enhancing microorganisms to manage pests and weeds naturally. These mostly act as biopesticides and are helpful in improving crop productivity, by reducing population of harmful pests.

17.7.3 Bioherbicides

Weeds are trouble for farmers. These are undesirable and difficult to control. They compete with crops in various ways, such as for space, light, water, and most importantly nutrients. They also act as host to various insect and disease pests, block irrigation and canal systems, etc. The use of mycoherbicide or bioherbicide plays a noteworthy function in controlling weeds without ecological hazards posed by synthetic agrochemicals and herbicides. Microorganisms have omnipresent genes that can attack the argument genes of the weeds, there by carnage it. Bioherbicides can stay alive in the surroundings as long as for the next mounting season where there will be more weeds to infect. It is a bit low cost compared to synthetic pesticides and thus could essentially reduce agricultural operating cost if managed appropriately.

17.7.4 Bioinsecticides

Modern biotechnological tools can also assist in mounting to artificial insecticides to fight against various pests. Microbes in the earth that will hit fungi, viruses, or bacteria are used as formulation products to scrap next to various diseases, etc. Various formulations used for seed, etc. carry these helpful organisms to protect the crop during the significant seedling stage. Bioinsecticides do not continue long in the milieu and have shorter shelf lives; they are efficient in minute amount; safe to mankind and vertebrates compared to artificial pesticides; extremely precise, often affecting only a single group of insect; and slow in action. They have an extremely precise mode of action, and the duration of its application is relatively critical.

17.7.5 Fungal Bioinsecticides

Fungi are the source of diseases in some 150 diverse insects, and this sickness producing traits of fungi is being use as bioinsecticides for various disease control (Reinhart and Callaway 2006). Various fermentation techniques are used for a large assembly of fungi. Effective sporulents are collected and packaged for further utilization in insect-ridden fields. Inoculated spores, when applied ultimately, cause death. Utilization of fungi sources is optional by some scientists as having the greatest source for enduring insect control (Mishra and Sinha 2006). This is because these bioinsecticides attack in a diverse form of action, and it is very difficult for the insect to develop resistance behaviors against these products.

17.7.6 Virus-Based Bioinsecticides

A report revealed that baculoviruses affect insect pests like corn borers, potato beetles, flea beetles, and aphids. Specific strains are effective against a certain range of bacteria and insect population. Bertha armyworms are affected by a specific strain and attack canola, flax, and vegetable crops. Conventional pesticides are ineffective against this worm.

17.8 Microorganisms and Its Association with Crop Sequencing

Crop sequence that profoundly modifies the soil ambiance and microorganism habitat under various sequences plays a key role for eco-friendly farming concept. The series of crop in rotation influences not only the utilization of valuable nutrients from a soil but also the return of crop residues, the progress and allocation of biopores, and the dynamics of microbial community (Ball et al. 2005). This helps to develop soil arrangement. These include better quantitative linkages amid soil

arrangement and crop expansion and nutrient mobilization through plant remain incorporation. Crop rotations in farming system intermediate between conventional and organic are known as "integrated" or "low input," for example, the Linking Environment and Farming (LEAF) initiative (www.leafuk.org). Use of crop rotation, which involves the sequential production of different plant species on the same land, has been in existence for thousands of years. Crop rotations comprise of grass, clover legumes etc. within the series of crops, given their importance to many cropping systems. The capability of legumes to fix nitrogen (N) from air helped maintain the performance of an alternate crop pattern in most agricultural cropping systems well into the twentieth century. However, in the concluding half of the twentieth century, the improved accessibility of N from the industrial source and pesticides to manage pests, diseases, and weeds reduced the requirement for comprehensive rotations. European and North American farmers mechanized their operations and used artificial inputs to optimize the crop yields (Robson et al. 2002). Widespread acceptance of short cereal or pulse/oilseed rotations or monocultures has allowed the farming community to specialize, increase crop productivity and quality, and fetch good marketing price. The reliance on alternate crop in various crop sequences in European and North American conventional farming has decreased steadily since the early 1950s, and continuous monocultures and little rotations are now widespread in temperate parts of the developed world (Karlen and Sharpley 1994). Crop rotations directly modify soil arrangement and ultimately microbial activity in soil bionetwork. Any increase in soil quality in organic rotations involves an improvement in soil structure. For example, organic agriculture generally improves counting of pore space and earthworm abundance and increases aggregate size and development. This results in favorable soil structure, i.e., a structure that contains an assortment of distinct aggregates from 2 to 50 mm equivalent spherical diameter (Ball and Douglas 2003). Structural development can change with the phase of the rotation. There is a vibrant interrelationship amid soil configuration and soil organic matter decomposition and stabilization and microbial activity. Soil microbes take part in a key role in the configuration of structure. The importance of different forms of organic carbon input for the expansion of soil arrangement has also been the subject of many studies. Plants provide the largest input of carbon pool to most soils (Rees et al. 2005). These different natures of carbon incorporation are likely to have distinct influences on the arrangement of soil aggregate. The physical incorporation of organic substance is likely to have an important influence on structural formation. Straw amalgamation can get better soil structure by promoting aggregation and influencing soil microbial system and help to improve crop productivity under different cropping sequences (Mukherjee 2014c). Pathogens and nonmobile pests that have short life spans in the soil and a precise or narrow host range (e.g., nematodes) are mainly susceptible to the addition of nonhost crops in the rotation. However, crop rotation does not control all soilborne pathogens adequately. Some soils encompass the ability to suppress these types of pathogens, and plants cultivated in them exhibit less disease, even within the same crop sequence and under similar environmental conditions.

17.9 Microorganism and Crop-Weed Competition

Crop and weed competition is accountable for significant yield losses in agricultural ecosystems, which might vary depending on the species analyzed and the existing ecological conditions (Mukherjee and Singh 2005). Weeds have a very high viable ability due to its definite biological characteristics especially C4 plant, with distinct root canopy which allows for better and deeper exploitation of space, nutrients, and various available water from soil ecosystem (Mukherjee 2016b). Plants are capable to endorse change in the soil microbial community through the exudation of diverse combinations of organic compounds by the roots depending on environmental conditions (Massenssini et al. 2013). Crops cultivated under best conditions or in low phosphorus or nitrogen availability tend to display a noteworthy difference in the root exudate composition, which in turn causes change in the inhabitants' solidity of microorganism groups in the soil. Soil microbes take part in a primary and effective role in determining the aggressive ability of various weeds and crops (Massenssini 2014). Weeds have a similar performance to that of persistent crops found in different natural ecosystems (Reinhart and Callaway 2006). Few studies revealed that weeds are capable to connect with *arbuscular mycorrhizal* (AM) fungi and that the effects of this association may vary from positive to negative, depending on the environmental conditions (Massenssini 2014). Furthermore, the occurrence of a competing plant may alter weed root association by AMF. Fialho (2014) found that *Bidens pilosa* and *Eleusine indica* showed an elevated mycorrhizal association when grown in contest with maize plant. This leads to increase in AMF colonization to the competitive strategy of these weeds. In other works, mycorrhizal association of the weeds *Ageratum conyzoides*, *Ipomoea ramosissima*, and *B. pilosa* varied depending on the uniqueness of the competitor species (Massenssini 2014). Weeds might encompass different competitive strategies and may have constructive relations through different microbial groups. The configuration of the soil microbial population is responsive to competition between plants. In common, antagonism promotes change in the makeup of the earth microbial community, making it diverse from that found when plants are grown in monoculture.

17.10 Plant Growth and Soil Microbe Interaction

Microbes in the earth may play a fundamental function in determining the constitution of plant communities. Microbes alter the physicochemical property of the environment; are straightly involved in the transformations of nitrogen, phosphorus, and sulfur; and form mutualistic associations with plants; these activities result in greater plant growth (Sylvia et al. 2005). Usually soil microbes are high near root zone of plant, where more amount of organic substances exuded from root are available. Availability of root exudate quality influences the availability of microbes in the earth ecosystem (Wolfe and Klironomos 2005). These microbes will work together with the crop and have either positive, neutral, or negative intrusion on crop expansion and help to determine the nature of crop communities. Symbiotic

relations amid fungi and crops are present in a wide range of soil and land ecosystems and occupy a hefty section of plant taxa (Brundrett 2009). It is believed that at least 85% of plant group are capable to set up symbiotic relations with fungi, of which 70% are associated with individuals of the phylum *Glomeromycota*, forming the AM (Wang and Qiu 2006). Thus, mycorrhizal associations are very much precious for terrestrial ecosystems owing to its wide geographical distribution and the huge total number of plant kingdom involved. It was believed for the past few decades that the family Cyperaceae was unable to correlate with mycorrhizal fungi (Brundrett 2009), but recent evidence has shown otherwise (Bohlen 2006). Various species of Cyperaceae are capable to relate with AM fungi and dark septate endophytes (DSE), but the intensity of root colonization could differ based on the environment in which the samples were collected, the season of the year, or the phenological phase of the crop (Wang and Qiu 2006). Thus, the inclusion of soil microbes in research effective for crop-microbe relations can be decisive for the association of a species in a given environment (Van Grunsven 2009). Plant community and its distribution pattern were decided by composition of various mycorrhizal associations (Shah et al. 2008) or it may help to create other genus (Chen et al. 2004). Experimental evidence suggests that mycorrhizal associations can determine the coexistence of different plants (Van der Heijden et al. 2003). A study revealed that few bacteria help to fix nitrogen from air and form symbiotic relation with crops (Franche et al. 2009; Mukherjee 2016c). Nitrogen-fixing bacteria are capable to form symbiotic relation with plant roots, leading to the structures called nodules, and have a higher specificity with the host (Bhattacharjee et al. 2008). Bacteria associated with these nodules provide more than 90% of nitrogen supply to the plant or crop (Franche et al. 2009). Nitrogen fixing microorganism help the plant to improve its growth and yield under poor nitrogen supply soil surroundings, which are often associated to the resource full outcome that pulse or legume species have on other plant species (Mukherjee 2015c). Phosphate-solubilizing microorganisms (PSB) present in soil naturally or introduced artificially by various cultures, etc. help to solubilize adsorbed phosphorus by soil mineral complex through cation exchange mechanisms and have great capacity to encourage crop growth. Few works revealed that inoculation of this group of microbes into the root zone of many plant species led to increased uptake of phosphorus by the plants, besides higher growth (Kumar and Narula 1999). However, these microbes provide benefit indirectly to plant, since soluble phosphorus is found in the soil solution and is not directly moved to the crop or plant (Rodrýìguez and Fraga 1999). In this context crops capable to employ larger densities of these microbes can gain a competitive benefit over others, mainly in soils where availability of phosphorus is scarce.

17.10.1 Mechanisms of Disease Suppression

Worldwide, plant protection and good crop strength are always a challenge by rising, reemerging, and widespread crop pathogens. The use of various agrochemicals in terms of weedicide, fungicide, etc. has led to ecological apprehensions and

pathogen resistance, forcing invariable expansion of new agents. Rhizospheric microorganisms that repress crop pathogens might be used as biocontrol agents and can be considered as an alternative to chemical pesticides. A number of sites of action for crop pathogen repression include direct inhibition of pathogen expansion through making antibiotics, hydrogen cyanides (HCN), toxins, and hydrolytic enzymes (chitinases, proteases, lipases) that degrade virulence factors or pathogen cell wall components (Whipps 2001; Compant et al. 2005). Antibiotics are a regular part of the defensive arsenals of microorganisms or bacteria, such as *Pseudomonas* species (e.g., *Pseudomonas fluorescens* strains) (Haas and Defago 2005) and *Bacillus* species (e.g., *Bacillus subtilis*) (Kim et al. 2003), in addition to fungal species such as *Trichoderma*, *Gliocladium*, *Ampelomyces*, and *Chaetomium* (Kaewchai et al. 2009), and as a result, these organisms encompass immense potential for soil conditioning. Multifunctional organisms such as *Trichoderma harzianum* Rifai 1295-22 emerge to augment crop growth by solubilizing phosphate and micronutrient requisite by plants, such as iron and manganese, and also by suppressing plant pathogens. HCN production suppresses microbial development and may slow down pathogens such as root knot, bacterial canker, and black rot in tomato and tobacco (Lanteigne et al. 2012). However, HCN might be harmful to crop by inhibiting energy metabolism and reducing root growth. A range of bacterial genera produce HCN, mainly *Alcaligenes*, *Aeromonas*, *Bacillus*, *Rhizobium*, and *Pseudomonas* spp. Pathogen suppression can also occur competitively through indirect inhibition. Selected fungi and bacteria create siderophores as iron-chelating agents particularly in iron deficiency, including *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Serratia*, and *Azospirillum* (Lily et al. 2015). Other actions associated with disease repression include commencement of the plant's individual defense system, known as induced systemic resistance (ISR). This ISR is triggered by various volatile compounds released by PGP bacteria and fungi, ensuing an improved appearance of defense-connected genes in the host (Hossain et al. 2007; Naznin et al. 2014). Soil-borne microorganisms interact with plant roots and soil constituents at the root-soil interface, where root exudates and rotting plant substances provide sources of carbon compounds for the heterotrophic biota (Bisseling et al. 2009). Once a seed starts to germinate, a relatively large quantity of carbon and nitrogen compounds, i.e., sugars, organic acids, amino acids, and vitamins, are excreted into the adjacent environment. This attracts a huge mass of microbes inducing forceful competition between the diverse species (Okon and Labandera-Gonzales 1994). Microorganisms affect activities of soilborne pathogens, mainly through competition, antibiosis, lysis, and hyperparasitism. Competition takes place for space and nutrients at the root surface. Antagonistic microorganisms can frequently give a series of diverse antimicrobial secondary metabolites and/or extracellular lytic enzymes. Direct postitive effect on crops are exerted by rhizosphere microbes through a phytostimulation and a biofertilization of crops these processes involve production of phytohormones, nonsymbiotic nitrogen fixation, and the raise of accessibility of phosphate and other nutrients in the soil (Burdman et al. 2000). Various crops are involved in a multifaceted system of connections with microbes; some of those are useful, others are harmful, but the previous are by far the largest

and still widely unexplored and complex part (Mukherjee 2016d). Benefits to crops from host-PGPR relations have been shown to include plant health and growth, repress disease-causing microorganisms, and speed up nutrient accessibility and absorption (Mantelin and Touraine 2004; Yang et al. 2009). These helpful things on vegetation can be achieved by the direct interaction between PGPR and their host plant and are also indirectly due to their antagonistic activity next to crop pathogens.

Soilborne microorganisms interact with plant roots and soil constituents at the root-soil interface, where root exudates and decaying plant material provide sources of carbon compounds for the heterotrophic biota (Bisseling et al. 2009). The number of bacteria in the rhizosphere (the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms) and rhizoplane (the external surface of roots together with closely adhering soil particles and debris) is higher than in the soil devoid of plants; this happens because soils devoid of plants are poor in many attractive substances secreted from the roots. As soon as a seed starts to germinate, large amounts of carbon and nitrogen compounds, i.e., sugars, organic acid, amino acids, and vitamins, are excreted into the surrounding environment. This attracts a large population of microorganisms inducing vigorous competition between the different species (Okon and Labandera-Gonzales 1994). Moreover, rhizosphere microbiomass typically differs between plant species (Bisseling et al. 2009). Beneficial microorganisms are known to be biocontrol agents and/or growth promoters. There are several modes of action by which they can be beneficial to plant health, which can be related to an indirect or a direct positive effect. Microorganisms have indirect positive effects on plants, affecting adversely the population density, dynamics, and metabolic activities of soilborne pathogens, mainly through competition, antibiosis, lysis, and hyperparasitism. Competition takes place for space and nutrients at the root surface; competitive colonization of the rhizosphere and successful establishment in the root zone are prerequisites for effective biocontrol. Antagonistic microorganisms can often produce a range of different antimicrobial secondary metabolites and/or extracellular lytic enzymes. Hyperparasitism is well documented for *Trichoderma*; it involves secretion of chitinases and cellulases, contact with the pathogen, coiling of hyphae around the hyphae of the pathogen, enzymatic digestion of its cell wall, and penetration. Direct positive effects on plants are exerted by rhizosphere microorganisms through a phytostimulation and a biofertilization of plants; these processes involve production of phytohormones, nonsymbiotic nitrogen fixation, and increase of availability of phosphate and other nutrients in the soil (Burdman et al. 2000). Plants are involved in a complex network of interactions with microorganisms, some of those are beneficial, others are detrimental, but the former are by far the largest and still widely unexplored and complex part. Benefits to plants of host-PGPR interactions have been shown to include plant health and growth, suppress disease-causing microbes, and accelerate nutrient availability and assimilation (Mantelin and Touraine 2004; Yang et al. 2009; Mukherjee 2016d). These effects on plants can be achieved by the direct interaction between PGPR and their host plant and areal, so indirectly due to their antagonistic activity against plant pathogens. Direct

stimulation includes several mechanisms such as production of 1-aminocyclopropane-1-carboxylate (ACC)-deaminase to reduce ethylene levels in the rhizosphere of developing plants; making crop growth regulators like auxins, cytokines, gibberellins, and certain volatiles; biological nitrogen accumulation; solubilization of mineral like phosphorus and other nutrients; etc (Desbrosses et al. 2009). Indirect stimulation is associated with biocontrol, by means of an antagonistic action next to phytopathogenic microbes inducing plant systemic resistance responses, interfering in the bacterial quorum sensing (QS) systems, etc. A number of works demonstrate that PGPR can use more than one of these mechanisms for accomplishing crop growth augmentation (Bashan and Holguin 1997; Ahmad et al. 2008).

17.11 Conclusions

Various microorganisms and their diverse metabolic behaviors are of noteworthy value in terms of their sustainable existence on our planet, including reusing of elements and other compounds, on which primary productivity depends on. Microbes are indispensable in degradation of ecological wastes and restitution of tarnished ecosystems. Conservation of diversity of microbes in a microbial system is essential in the maintenance of species diversity of higher organisms and management of strategies such as plant disease management and nutrient management. Scheming the soil microflora to augment the predominance of beneficial and effective microorganisms can assist to improve and maintain the soil physicochemical aspect. The proper and customary trappings of natural amendments are often an important aspect of any strategy to exercise such control.

References

Adesemoye A, Torbert H, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Andrews M, Hodge S, Raven JA (2010) Positive plant microbial interactions. Ann Appl Biol 157:317–320

Araújo AES, Baldani VLD, Galisa PS, Pereira JA, Baldani JI (2013) Response of traditional upland rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the northeast region of Brazil. Appl Soil Ecol 64:49–55

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Ball BC, Douglas JT (2003) A simple procedure for assessing soil structural, rooting and surface conditions. Soil Use Manag 19:50–56

Ball BC, Bingham I, Rees RM, Watson CA, Litterick A (2005) The role of crop rotations in determining soil structure and crop growth conditions. Can J Soil Sci 85:557–577

Bardgett RD, McAlister E (1999) The measurement of soil fungal: bacterial biomass ratios as an indicator of ecosystem self regulation in temperate meadow grasslands. Biol Fertile Soils 29:282–290

Barea JM, Azcón R, Azcón A (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81:343–351
Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). Can J Mirobiol 43:103–121
Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18
Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. Microbiol Ecol 68:1–13
Bhattacharjee R, Singh A, Mukhopadhyay S (2008) Use of nitrogen-fixing bacteria as biofertiliser for non- legumes: prospects and challenges. Appl Microbiol Biotechnol 80(2):199–209
Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350
Bisseling T, Dangl JL, Schulze-Lefert P (2009) Next-generation communication. Science 324:691–692
Blilou I, Ocampo JA, García-Garrido JM (2000) Induction of Ltp (Lipid Transfer Protein) and Pal (Phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus Glomus mosseae. J Exp Bot 51:1969–1977
Bohlen PJ (2006) Biological invasions: linking the aboveground and below ground consequences. Appl Soil Ecol 32(1):1–5
Bossuyt H, Denef K, Six J, Frey SD, Merckx R, Paustian K (2001) Influence of microbial populations and residue quality on aggregate stability. Appl Soil Ecol 16:195–208
Brundrett M (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320(1):37–77
Burdman S, Jurkevitch E, Okon Y (2000) Recent advance in the use of plant growth promoting rhizobacteria (PGPR) in agriculture. In: SubbaRao NS, Dommergues YR (eds) Microbial interaction, In Agriculture Forestry, vol II. Science Publishers, Enfield, pp 229–250
Buresh RJ, Reddy KR, van Kessel C (2008) Nitrogen transformations in submerged soils. In: Schepers JS, Raun WR (eds) Nitrogen in agricultural systems. Agronomy monograph, vol 49. ASA, CSSA, and SSSA, Madison, pp 401–436
Chanyarat PL, Thierry GA, Lonhienne YK, Yeoh R, Webb I, Prakash L, Cheong XC, Phaik-Eem L, Mark A, Ragan S, Hugenholtz P (2014) A new species of Burkholderia isolated from sugarcane roots promotes plant growth. Microb Biotechnol 39(4):175–187
Chen X, Tang J, Fang Z, Shuijin H (2004) Effects of weed communities with various species numbers on soil features in a subtropical orchard ecosystem. Agric Ecosyst Environ 102(3):377–388
Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms. Appl Environ Microbiol 71(9):4951–4959
Desbrosses G, Contesto C, Varoquaux F, Galland M, Touraine B (2009) PGPR–Arabidopsis interactions is a useful system to study signalling pathways involved in plant developmental control. Plant Signal Behav 4:321–323
Dixon J, Brau HJ, Kosina P, Crouch J (2009) Wheat facts and futures. CIMMYT, Mexico, pp 56–74
Dobbelaere S, Vanderleyden J, Okon Y (2001) Plant growth promoting effects of diazotrophs in the rhizosphere. CRC Crit Rev Plant Sci 22:107–149
Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149
Febri D, Nasser NK, Tiben EM, Abuelhassan NN, Isahak A, Zain CRC, Yusoff WMW (2013) Microbial involvement in growth of Paddy. Curr Res J Biol Sci 5(6):285–290
Fialcho CMT (2014) Interação entre micro-organismos do solo, plantas daninhase as culturas do milho e da soja. 2013. 75 f. Tese (Doutoradoem Fitotecnia) – Universidade Federal de Viçosa, Viçosa, pp 65–78

Fischer T, Byerlee D, Edmeades G (2014) Crop yields and global food security. Australian Centre for International Agricultural Research, Canberra, Monograph no. 158

Filippi MCC, da Silva GB, Silva-Lobo VL, Côrtes MVCB, Moraes AJG, Prabhu AS (2011) Leaf blast (*Magnaporthe oryzae*) suppression and growth promotion by rhizobacteria on aerobic rice in Brazil. Biol Control 58:160–166

Franche C, Lindstrom K, Elmerich C (2009) Nitrogen fixing bacteria associated with leguminous and non leguminous plants. Plant Soil 321(1):35–39

Frommel MI, Nowak J, Lazarovits G (1993) Treatment of potato tubers with a growth promoting *Pseudomonas* sp.: plant growth responses and bacterium distribution in the rhizosphere. Plant Soil 150(1):51–60

Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int J Biol Life Sci 5(1):35–40

Glaser B, Turrion MB, Alef K (2004) Amino sugars and muramic acid–biomarkers for soil microbial community structure analysis. Soil Biol Biochem 36:399–407

Guggenberger G, Elliott ET, Frey SD, Six J, Paustian K (1999) Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. Soil Biol Biochem 31:407–419

Haas D, Defago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Haichar F, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W (2008) Plant host habitat and root exudates shape soil bacterial community structure. ISME J:1221–1230

Han J, Sun L, Dong X, Cai Z, Xiaolu S, Yang H (2005) Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst Appl Microbiol 28:66–76

Hossain M, Sultana F, Kubota M, Koyama H, Hyakumachi M (2007) The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in Arabidopsis Thaliana by activation of multiple defense signals. Plant Cell Physiol 48(12):1724–1736

Jatav MK, Dua VK Kumar M, Kumar S, Sharma RP, Bairwa RC (2013) Bio fertilizers for sustaining potato productivity under North-western and North-eastern Hills (Internet), pp 67–83

Kaewchai S, Soytong K, Hyde KD (2009) Mycofungicides and fungal biofertilizers. Fungal Divers 38:25–50

Karlen DL, Sharpley AN (1994) Management strategies for sustainable soil fertility. In: Hatfield JL, Karlen DL (eds) Sustainable agricultural systems. CRC Press, Boca Raton, pp 47–108

Kim H, Park J, Choi SW, Choi KH, Lee G, Ban S, Lee C, Kim CS (2003) Isolation and characterization of Bacillus strains for biological control. J Microbiol 41(3):196–201

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the IVth international conference on plant pathogenic bacteria. Station de Pathologie Vegetaleet. Phyto-Bacteriologie 2:879–882

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006) Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl Soil Ecol 31(1–2):91–100

Krey T, Vassilev N, Baum C, Eichler-Löbermann B (2013) Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions. Eur J Soil Biol 55:124–130

Kumar V, Narula N (1999) Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. Biol Ferti Soils 28(3):301–305

Kupriyanov AA, Semenov AM, Van Bruggen AHC (2010) Transition of entheropathogenic and saprotrophic bacteria in the niche cycle: animals–excrement–soil–plants–animals. Biol Bull 3:263–267

Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Responses of agronomically important crops to inoculation with *Azospirillum*. Aust J Plant Physiol 28:871–879

Lanteigne C, Gadkar V, Wallon T, Novinscak A, Filion M (2012) Production of DAPG and HCN by pseudomonas sp. LBUM300 contributes to the biological control of bacterial canker of tomato. Phytopathology 102(10):967–973

Lily P, LuzE de Bashan, Bashan Y (2015) Assessment of affinity and specificity of Azospirillum for plants. Plant Soil 399:389–414
Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science 333:616–620
Mäder P, Kaiser F, Adholeya A, Singh R, Uppal HS, Anil K (2011) Inoculation of root microorganisms for sustainable wheat-rice and wheat-black gram rotations in India. Soil Biol Biochem 43:609–619
Maksimov I, Abizgildina R, Pusenkova L (2011) Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (review). Appl Biochem Microbiol 47(4):333–345
Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55:27–34
Massenssini AM (2014) Contribuição da microbiota do solo para o sucessocompetitivo de plantas. 2014. 95 f. Tese (DoutoradoemMicrobiologiaAgrícola) – Universidade Federal de Viçosa, Viçosa, pp 64–87(http://posmicrobiologiaagricola.ufv.br/equipe/mauricio-dutra-costa/)
Massenssini AM, Bouduki VHA, Melo CAD, Totola MR, Ferreira FA, Costa MD (2013) Soil microorganism and their role in the interaction between weeds and crops. PlantaDaninha Viscosa-MG 32(4):873–884
Meyer JB, Lutz MP, Frapolli M, Péchy-Tarr M, Rochat L, Keel C, Défago G, Maurhofer M (2010) Interplay between wheat cultivars, biocontrol pseudomonas, and soil. Appl Environ Microbiol 76(6):196–204
Mishra DS, Sinha AP (2000) Plant growth promoting activity of some fungal and bacterial agents on rice seed germination and seedling growth. Trop Agric 77:188–191
Mishra S, Sinha SP (2006) Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. Afr J Biotechnol 7(17):3326–3331
Mosttafiz S, Rahman M, Rahman M (2012) Biotechnology: role of microbes in sustainable agriculture and environmental health. Internet J Microbiol 10(1):1–6
Mukherjee D (2008) Effect of different biofertilizer and organic source of nutrient along with chemical fertilizer on wheat under mid hill situation. Indian Agric 52(1&2):49–52
Mukherjee D (2010) Productivity, profitability and apparent nutrient balance under different crop sequence in mid hill condition. Indian J Agric Sci 80(5):420–422
Mukherjee D (2012) Influence of combined application of bio and inorganic fertilizers on growth and yield of soyabean (Glycin max (L) Merill). Indian Agric 56(3 & 4):107–112
Mukherjee D (2013a) Organic agriculture. In: Rodriguez H, Ramanjaneyulu R, Sarkar NC, Maity R (eds) Advances in agro-technology: a text book, Compilation of international research work. Puspa Publishing House, Kolkata, pp 43–81
Mukherjee D (2013b) Nutrient use efficiency for maximization of crop productivity. In: Hemantaranjan A (ed) Advances in plant physiology, An International Treatise Series, vol 14. Scientific Publishers, Jodhpur, pp 173–209
Mukherjee D (2013c) Studies on resource management for sustainable ecosystem in Eastern Himalaya. Asian J Agric Food Sci 1(5):222–235
Mukherjee D (2014a) Influence of integrated nutrient management on productivity, nutrient uptake and economics of maize (*Zea mays*) –yellow sarson (*Brassica rapa*) cropping system under rainfed mid hill condition. Indian J Agron 59(2):221–228
Mukherjee D (2014b) Effect of forest microhabitat on growth of high altitude plants in Darjeeling Himalaya. J Interacademicia 18(1):20–30
Mukherjee D (2014c) Nutrient and its management: Prospect and challenges under the changing environment scenario. In: Hemantaranjan A (ed) Advances in plant physiology, vol 15. Scientific Publishers, Jodhpur, pp 413–442
Mukherjee D (2015a) Food security: a world wide challenge. Res Rev: J Agric Allied Sci (RRJAAS) 4(1):3–5
Mukherjee D (2015b) Influence of various tillage option along with nutrient management practices in maize-wheat cropping system under mid hill situation of West Bengal. Ann Plant Sci 4(3):1008–1015

Mukherjee D (2015c) Integrated nutrient management practices for enhancing blackgram (Vigan mungo L. Hepper) production under mid hill situation in North Eastern Himalaya. J Food Legumes 28(1):83–85
Mukherjee D (2016a) Evaluation of different crop sequence productivity potential, economics and nutrient balance under new alluvial situation of NEPZ. Int J Horticult Agric 1(1):5
Mukherjee D (2016b) Influence of transplanting time, plant geometry and nutrient management on growth and economics of *Centella asiatica*: valuable NTFPs. Int J For Usufructs Manag (IJFUM) 17(2):37–45
Mukherjee D (2016c) Effect of various sources of nutrients on growth and productivity of Indian mustard (*Brassica juncea*) under terraced cultivation. J Agric Eng Food Technol 3(3):167–171
Mukherjee D (2016d) Conservation farming: an approach of sustainable forest ecosystem. MFP News Lett 26(2):5–10
Mukherjee D, Singh RP (2005) Relative performance of new generation herbicides on weed density, yield and N,P uptake behavior in transplanted rice (*Oryza sativa* L.) Indian J Agric Sci 75(12):820–822
Murray JD (2011) Invasion by invitation: rhizobial infection in legumes. Mol Plant Microb Interact 24:631–639
Mwashasha RM, Hunja M, Kahangi EM (2016) The effect of inoculating plant growth promoting microorganisms on rice production. Int J Agric Res 9(3):34–44
Naznin H, Kiyohara D, Kimura M, Miyazawa M, Shimizu H, Hyakumachi M (2014) Systemic resistance induced by volatile organic compounds emitted by plant-growth promoting fungi in *Arabidopsis thaliana*. PLoS One 9(1):64–78
Noble AD, Ruaysoongnern S (2010) The nature of sustainable agriculture. In: Dixon R, Tilston E (eds) Soil microbiology and sustainable crop production. Springer Science and Business Media B.V, Berlin, pp 1–25
Okon Y, Labandera-Gonzales CA (1994) Agronomic application of Azospirillum: an evaluation of 20 years worldwide field inoculation. Soil Biol Biochem 26:1591–1601
Rediers H, Bonnecarrere V, Rainey PB, Hamonts K, Vanderleyden J, De Mo R (2003) Development and application of a DapB-based in vivo expression technology system to study colonization of rice by the endophytic nitrogen fixing bacterium *Pseudomonas stutzeri* A15. Appl Environ Microbiol 69:6864–6874
Rees RM, Bingham IJ, Baddeley JA, Watson CA (2005) The role of plants and land management in sequestering soil carbon in temperate arable and grassland ecosystems. Geoderma 128:130–154
Reeve J, Schadt C, Carpenter-Boggs L, Kang S, Zhou J, Reganold JP (2010) Effects of soil type and farm management on soil ecological functional genes and microbial activities. ISME J 4:1099–1107
Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. New Phytol 170(3):445–457
Reinhold-Hurek B, Hurek T (1997) *Azoarcus* spp. and their interactions with grass roots. Plant Soil 194:57–64
Reynold M, Foulkes MJ, Gustavo A, Slafer GA, Berry P, Parry MAJ (2009) Raising yield potential in wheat. J Exp Bot 60:1899–1918
Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW (2001) Enhanced maize productivity by inoculation with diazotrophic bacteria. Aust J Plant Physiol 28:829–836
Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil 238:325–333
Robson MC, Fowler SM, Lampkin NH, Leifert C, Leitch M, Robinson D, Watson CA, Litterick AM (2002) The agronomic and economic potential of break crops for ley/arable rotations in temperate organic agriculture. Adv Agron 77:369–427
Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17(1):319–339
Rosas MF, Chiou B, Medeiros ES, Wood DF, Williams TG, Mattoso LHC (2009) Effect of fiber treatments on tensile and thermal properties of starch/ethylene vinyl alcohol copolymers/coir biocomposites. Bioresour Technol 100(21):5196–5202

Rosegrant MR, Ringler C, Sulser TB, Ewing M, Palazzo A, Zhu T (2009) Agriculture and food security under global change, Prospects for 2025/2050. International Food Policy Research Institute, Washington, DC, pp 145–178

Saharan B, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21:1–30

Samina M (2011) Plant growth promoting bacteria associated with sugarcane. In: Book: bacteria in agrobiology: crop ecosystem, pp 165–187

Shah MA, Reshi Z, Rashid I (2008) Mycorrhizal source and neighbour identity differently influence *Anthemis cotula* L. invasion in the Kashmir Himalaya, India. Appl Soil Ecol 40(2):330–337

Shaharoona B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of pseudomonas for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.) Appl Microbiol Biotechnol 79:147–155

Shtark OY, Borisov AY, Zhukov VA, Provorov NA, Tikhonovich IA (2010) Intimate associations of beneficial soil microbes with host plants. In: Dixon R, Tilston E (eds) Soil microbiology and sustainable crop production. Springer Science and Business Media B.V, Berlin, pp 119–196

Singh S, Kapoor KK (1999) Inoculation with phosphate solubilizing microorganisms and a vesicular Arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in a sandy soil. Biol Fertil Soils 28:139–144

Singh RK, Mukherjee D (2009) Influence of biofertilisers, fertility levels and weed management on chickpea (*Cicer arietinum* L.) under late sown condition. Ann Agric Res New Ser 30(3&4):116–120

Six J, Guggenberger G, Paustian K, Haumaier L, Elliott ET, Zech W (2001) Sources and composition of soil organic matter fractions between and within soil aggregates. Eur J Soil Sci 52:607–618

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Swarnalakshmi K, Prasanna R, Kumar A, Pattnaik S, Chakravarty K, Shivay YS (2013) Evaluating the influence of novel cyanobacterial biofilmed biofertilizers on soil fertility and plant nutrition in wheat. Eur J Soil Biol 55:107–116

Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (2005) Principles and applications of soil microbiology, 2nd edn. Prentice Hall, Upper Saddle River, p 640

Van der Heijden MGA, Wiemken A, Sanders IR (2003) Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytol 157(3):569–578

Van Grunsven RHA (2009) Release from soil pathogens plays an important role in the success of invasive Carpobrotus in the Mediterranean. S Afr J Bot 75(1):172–175

Verma JP, Yadav J, Tiwari KN, Lavakush K, Singh V (2010) Impact of plant growth promoting rhizobacteria on crop production. Int J Agric Res 5:954–983

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16(5):299–363

Weishampel P, Bedford B (2006) Wetland dicots and monocots differ in colonization by Arbuscular mycorrhizal fungi and dark septate endophytes. Mycorrhiza 16(7):495–502

Whipps J (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Wolfe BE, Klironomis JN (2005) Breaking new ground: soil communities and exotic plant invasion. Bio Sci 55(6):477–487

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Young IM, Crawford JW (2004) Interactions and self-organization in the soil-microbe complex. Science 304:1634–1637

Microbes: Bioresource in Agriculture and Environmental Sustainability

18

Prachi Bhargava, Ankit K. Singh, and Reeta Goel

Abstract

The concept of sustainable agriculture is a response to the decline in the quality of the natural resource base associated with modern agriculture. The relationship between agriculture, the global environment, and social systems suggests that agricultural development results from the complex interaction of a multitude of factors. Dependence on chemicals for further agricultural needs will result in future loss in soil physical condition, feasibility of water pollution, and calculated burden on the fiscal system. Inaugurating an ecological friendly parallel mechanism on earth is of vital importance. The exploitation of beneficial microbes as a biofertilizer has become paramount importance in agriculture sector for their potential role in food safety and sustainable crop production. This chapter focuses on the use of microbes as bioresource in agriculture which is the backbone of economies of most of the developing nations and specifically on the use of PGP microbes. The knowledge gained from the literature appraised herein will help us to understand the physiological bases of biofertilizers toward sustainable agriculture in reducing problems associated with the use of chemical fertilizers.

Keywords

Sustainable agriculture • Plant growth-promoting regulators • Biofertilizers • Biofungicide • Bioremediation

P. Bhargava • A.K. Singh
Institute of Biosciences and Technology, Shri Ramswaroop Memorial University, Lucknow, Uttar Pradesh, India
e-mail: prachicbsh@rediffmail.com; prachi.bio@srmu.ac.in

R. Goel (✉)
Department of Microbiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India
e-mail: rg55@rediffmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_18

18.1 Introduction

The concept of agricultural sustainability although controversial is useful because it captures a set of concerns about agriculture which is conceived as the result of the coevolution of socioeconomic and natural systems (Altieri 1995). Agriculture is the backbone of mostly all developing economies, contributing to the whole economy of such nations and determining the general standard of life to a greater extent of 50% and so of the population. Soil productivity is a significant factor for success of agricultural production rather than soil fertility (Day and Bassuk 1994). Status of nutrients existing in the soil and its physical ability determines the fecundity of soil (Stine and Weil 2002; Onduru et al. 2006). The intensive agricultural technologies, which ensured a "green revolution" in the middle of the twentieth century, had an unpredictably high biological expenditure, contributing to worldwide pollution, bad climate change, and loss of biodiversity (Vance 1998). Very few soil microflora have been extensively used as plant growth-promoting agents including *Rhizobia*, *Azospirillum*, mycorrhizal fungi, and bioagents. Soil microbial gathering is multiplex and dynamic and varies in composition among dissimilar levels and compartments, which represents a real challenge in soil ecology. These microbes are known for their ability to fix atmospheric nitrogen in association with plants forming nodules in roots. They belong to the family *Rhizobiaceae*, are symbiotic, and can fix nitrogen 50–100 kg/ha in association with pods only (Azevedo et al. 2000). Irrigation problems, fragmented land holdings, seed problems, and lack of market understanding are some of the challenges faced in order to follow the concept of sustainable agriculture in India.

18.2 Microbial Biotechnology and Its Application in Agriculture

Microbes are extremely diverse and comprise of bacteria, archaea, and almost all the protozoans. They also include algae, fungi, and few animals like rotifers. Microbes are cosmopolitan in biosphere, present in soil and hot springs be it 7 miles in deep sea or 40 miles high in the atmosphere. This fact is based on "applied coevolutionary research" (Arnold et al. 2010), representing the environment molecular chemical process for communal adaptation and equivalent evolution of plant and microbial partners. Now progressive attention has been compensated to the development of sustainable agriculture in which the high productivities of plants and animals are ensured by means of their earthy adaptational potentials, with a negligible perturbation of the environment (Noble and Ruaysoongnern 2010). The future of progress of agricultural microbiology envisages very few important environmental and hereditary challenges obligatory by the broad application of symbiotic microbes. This study stands upon the pillar of "applied coevolutionary research" (Arnold et al. 2010), suggesting the ecological and chemical mechanisms for common adaptation and equivalent evolution of plant and microbial partner.

The wide application of microorganisms in sustainable agriculture is due to the genetic reliance of plants on the beneficial functions given by symbiotic cohabitants (Noble and Ruaysoongnern 2010). The best studied genetic models for nutritional symbioses are two-partite plant-microbe family, such as the N_2-fixing legume-*Rhizobia* nodular symbioses (Franche et al. 2009). Taxonomically, the diversity of *Rhizobia* (over 20 distinct lineages of α- and β-proteobacteria), which diverged from a universal relative a lot before the legume hosts originated (Balach et al. 2007), assume that few *Rhizobia* may ignite the nodular symbiosis avoiding the Nod factor-dependent pathway (Masson-Boivin et al. 2009). *Rhizobium*, *Azospirillum*, and *Azotobacter* being the common nitrogen fixers along with cyanobacteria and phosphate-solubilizing bacteria like *B. magaterium* and *Pseudomonas striata*, and phosphate-mobilizing mycorrhiza are widely accepted as biofertilizers. The consolidation of biofertilizers (N-fixers) plays key function in rising soil fertility, yield attributing characters, and thereby final yield. In addition, their utilization in soil minimizes the extent use of chemical fertilizers (Mishra et al. 2013).

Thus, there is a basic need to improve the efficiency and fulfill the deficient amounts of external inputs by putting to work the finest combinations of valuable bacteria in sustainable agrifield production systems (Hayat et al. 2010). Although organic farming has been demonstrated to supply high organic matter inputs to soil with reduced reliance on synthetic compost and pesticides, the high reliance on tillage for organic production can reduce soil and water conservation through erosion and compaction (Peigné et al. 2007).

18.2.1 Biofertilizers

The term biofertilizer represents everything from organic fertilizer to plant residues that contain living microorganisms, colonize the rhizosphere of the plant, and increase the availability of primary nutrients and growth stimulus to target crop (Bhattacharjee and Dey 2014). Agriculture is the socialization of plants, animals, and many more life forms for food, fibers, biofuels, and additional payload for the well-beingness of human existence (Roychowdhury et al. 2014). Increasing the use of chemical fertilizers in agriculture makes country self-dependent in food production, but it deteriorates environment and causes negative impacts on living beings. The surplus use of chemical fertilizers in agriculture is costly and also has various adverse effects on soils as depletion of water holding capacity, soil fertility, and disparity in soil nutrients (Youssef and Eissa 2014). To contract with low cost-effective and eco-friendly fertilizers which work without disturbing nature, certain species of microorganisms are widely used which have unique properties to provide natural products and serve as a good substitute of organic fertilizers (Deepali and Gangwar 2010). Biofertilizer can also make plants resistant to adverse environmental stresses. Control of root-knot disease of soybean caused by *Meloidogyne javanica* may be explored through the use of BAU (biofungicide) and BINA (fertilizer) for eco-friendly management avoiding chemical nematicides. The suitable application and use of biofertilizers will not merely have an impact

on sustainable agriculture's economic growth, but it will also supply boost to a sustainable ecosystem and holistic well-being (Bhattacharjee and Dey 2014). Biological soil distinctiveness such as microbial biomass, ecosystem structures, activities, and functions may offer considerable information on ecological and anthropogenic influences on agricultural soils (Hartmann et al. 2006).

Biofertilizers are essential components of incorporated nutrient management. These potential biological fertilizers would play a key role in efficiency and sustainability of soil and also conserve the environment as eco-friendly and cost-effective inputs for the farmers. Organisms that are commonly used as biofertilizer components are nitrogen fixers (N-fixer), potassium solubilizer and phosphorus solubilizer, or with the combination of molds or fungi. India is one of the foremost countries in biofertilizer manufacture and utilization. In order to promote the eco-friendly practices in agriculture by biofertilizers, five biofertilizers, namely, *Rhizobium*, *Azotobacter*, phosphate-solubilizing bacteria, *Azospirillum*, and *mycorrhiza*, have been included in the FCO (Pindi 2012). Beneficial microorganisms in biofertilizers speed up and advance plant growth and protect plants from pests and diseases (El-Yazeid et al. 2007). Living microorganisms have specific functions to reinforce plant growth and so are being used in the preparation of biofertilizer. Microorganism converts composite nutrients into uncomplicated nutrients for the availability of the plants (Sahu et al. 2012). If the microbial inoculants are not applied properly, the benefits from the biofertilizer might not be obtained. Throughout widely accepted application, one should always remember that most of the microbial biofertilizers are heterotrophic, i.e., they cannot prepare their own food and depend upon the organic carbon of soil for their energy requirement and growth. Additionally, biofertilizers can proceed as a renewable addition to chemical fertilizers and organic manures. They have the ability to create natural resistance in plants against pests and soilborne diseases, because antibodies are formed and beneficial microorganisms contribute in the soil to increase fertility. The microorganisms intended for the biofertilizer are bacteria of *Pseudomonas*, *Bacillus*, and *Lactobacillus*, photosynthetic bacteria, nitrogen-fixing bacteria, fungi of *Trichoderma*, and yeast. *Rhizobium* is the most studied and important genera of nitrogen-fixing bacteria (Odame 1997).

The chemical fertilizers and pesticides have remarkable harmful long-term residual effect not only on the soil health and crop productivity but also on the level of contamination of the groundwater level. Eventually, they are integrated into the food chain in the ecosystem that causes human health hazards. Unlike chemical pesticides and fertilizers, biopesticides and biofertilizers contain viable population of the selected microbes which may be colonized in the soil ecosystem (Datta 2012). The use of biofertilizer, against chemical fertilizers, offers cost-effective and ecological remuneration by way of soil health and efficiency to farmers. Thus, biofertilizers can be expected to reduce the use of chemical fertilizers and pesticides (Sahu et al. 2012).

18.2.2 Biopesticides

In the past 50 years of history, the pesticides have played a crucial role in increasing the agricultural productivity all over the world. But the extensive uses of chemical pesticides have adverse effects on human health. Indiscriminate use of chemical pesticides contributed in failure of soil productivity along with addition of salts to the soil. In recent years, crop protection based on biological management of crop pests with microbial pathogens like virus, bacteria, fungi, and nematodes has been accepted as a valuable tool in pest management (Anand et al. 2009). Biopesticides are prepared from naturally occurring substances that manage pests by nontoxic mechanisms and in eco-friendly manner. They may be extracted from animals (e.g., nematodes), plants (*Chrysanthemum*, *Azadirachta*), and microbes (e.g., *Bacillus thuringiensis*, *Nuclear polyhedrosis virus*, *Trichoderma*) and include living organisms (natural enemies) and their products (microbial and phytochemicals products) or by-products (semichemicals) (Mazid et al. 2011). The appropriate use of eco-affable microbial biopesticide can be engaged in recreation of sustainable organic crop production by providing a stabile pest management program. Thus, microbial biopesticides are those microorganisms that promote plant growth by controlling phytopathogenic agents through an extensive diversity of mechanisms such as production of antibiotics, siderophores, HCN, production of hydrolytic enzymes, and obtained and induced systemic resistance (Chandler et al. 2008). Biopesticides play very important role in reducing the environmental issues such as harmful residues left in food, feed, and fodder which cause environmental pollutions as well as reduce the use of chemical pesticides which cause hazardous effects on soil fertility, and most of the countries have amended their policies to ensure minimal use of chemical pesticides and encourage the use of biopesticides. Although the potential use of biopesticides and biofertilizers for promoting sustainable agriculture has been recognized for years, their demands have increased now in view of the organic farming to produce safe and healthy food.

Biopesticides can be broadly categorized into three major classes:

Table 18.1 shows the different biopesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides.

Biopesticides or natural pesticides based on pathogenic microbes being precise to a target pest offer an ecologically sound and effective solution to pest problems.

Table 18.1 Important microorganisms used as biopesticides

S. No.	Types	Included substances	Examples
1	Microbe-based biopesticides	Bacterium, fungus, virus, protozoan, or algae	*Bacillus thuringiensis* or *Bt*
2	Chemical-based biopesticides	Plant extracts, fatty acids, or pheromones	Redolence plant extract
3	Plant-incorporated protectants(PIPs)	Genetically engineered/cry gene	Production of cry protein

The majority of widely known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis*, or *Bt*, which can kill certain insects in cabbage, potato, and additional crops. *Bt* generates a protein that is unsafe to particular insect pests. The most commonly used biopesticides, i.e., living organisms, are pathogenic for the pest of interest. These comprise biofungicides (*Trichoderma*), bioherbicides (*Phytophthora*), and bioinsecticides (*Bacillus thuringiensis*) (Mazid et al. 2011). Conventional pesticides, by contrast, are generally man-made resources that directly destroy or inactivate the pest (Datta 2012). The pesticide-induced diseases are autism, asthma and learning disabilities, reproductive misfunction and birth defects, diabetes, Parkinson's and Alzheimer's diseases, and several types of cancer (Owens et al. 2010). The use of eco-friendly biopesticides is an effective tool to overcome these problems.

18.2.3 Bioherbicides

Bioherbicides have shown huge potential as a renewable and eco-friendly source of increasing crop yield. They are prepared by using live formulations of valuable microorganisms. When it is added to seed, root, or soil, it mobilizes the availability and utility of the microorganisms and thus improves the soil health. Bioherbicides offer a sustainable, minimum cost, and environmentally friendly approach to balance conventional process that helps meet to necessitate for new weed executive strategies (Boydston et al. 2008). The term bioherbicide is used for herbicides based on several living organisms (e.g., fungi, bacteria, viruses, protozoa). When growing seeds are in touch with the herbicide, the increase of emerging roots and/or shoots is repressed, but preemergent herbicides might not be valuable without good contact with germinating weed seeds (Altland et al. 2003).

Many plants which are used as bioherbicides are also the important producers of essential oils in the world (Table 18.2). The conventional biological approach presents a natural foe that spreads throughout the region where the target weed is present (Frantzen et al. 2001). The classical approach is subjected to stern regulations because of the introduction of potentially destructive pathogens to agricultural invention. The bioherbicide access relies on natural enemies imbibed within the native range of the weed to raise significant harm to the weed and cut down the negative impact on crop yield (Frantzen et al. 2001). Onen et al. (2002) quoted that the essential oils extracted from leaves and flowers of five diverse plant species (*Mentha spicata* subsp. *spicata*, *Artemisia vulgaris*, *Salvia officinalis*, *Ocimum basilicum*, *Thymbra spicata* subsp. *spicata*) were enormously phytotoxic to seed germination and seedling growth of eight weed species from diverse families (*Cardaria draba*, *Agrostemma githago*, amaranth, *Echinochloa crus-galli*, *Chenopodium album*, *Reseda lutea*, *Rumex crispus*, *Trifolium pratense*) (Cai and Gu 2016).

Table 18.2 Plants used as bioherbicides

S. No.	Plants	Scientific name	Family	Industrially/pharmaceutically important compound
1	Eucalyptus	*Eucalyptus* spp.	Myrtaceae	Pharmaceutical, antiseptic, repellent, flavoring, fragrance, and industrial uses
2	Lawson cypress	*Chamaecyparis Lawsoniana*	Cupressaceae	Broom, hedge, resin, wood
3	Rosemary	*Rosmarinus Officinalis*	Lamiaceae	Herbal oil (indigestion, stress relief, pain relief, immune system boost, and more)
4	White cedar	*Thuja Occidentalis*	Cupressaceae	The neurotoxic composite *thujone*
5	Amaranth	*Amaranthus Retroflexus*	Amaranthaceae	Cosmetics, shampoos pharmaceuticals, rubber chemicals
6	Purslane	*Portulaca Oleracea*	Portulacaceae	Abnormal uterine bleeding, asthma, diabetes, oral lichen planus
7	Knapweed	*Acroptilon Repens*	Asteraceae	Stimulant, styptic

18.3 Need of Beneficial Microbes

The microbial world is the largest unknown reservoir of multifariousness on earth. They comprise of the prevalent living mass on the planet earth (Russo et al. 2012). To advance the sustainability of existing agricultural system and to provide a higher quality to our agricultural merchandise, beneficial microbes are used (Adhya et al. 2015). The responsible use of native microorganisms to get monetary, social, and environmental asset is inherently striking and determines a magnificent evolution of research from traditional technologies to modern techniques to provide a capable system to protect environment and new methods of environmental monitoring (Cai et al. 2013). Figure 18.1 depicts the different roles played by the microorganisms in maintaining the sustainability of the ecosystem.

18.3.1 In Remediation

At a specified position, many factors influence the pace of biodegradation process, namely, soil moisture, soil pH, and availability of oxygen, accessibility of nutrients, pollutant concentration, and occurrence of suitable microbe. At higher rates, many hydrocarbons are easily degradable during aerobic metabolism, and only few hydrocarbons are biodegradable through anaerobic metabolism at comparatively lower rates. Native microbes are inhabited by aquatic plus oil-bearing deep subsurface environments. Oxygen is the key aspect that plays a crucial part in the biodegradative process.

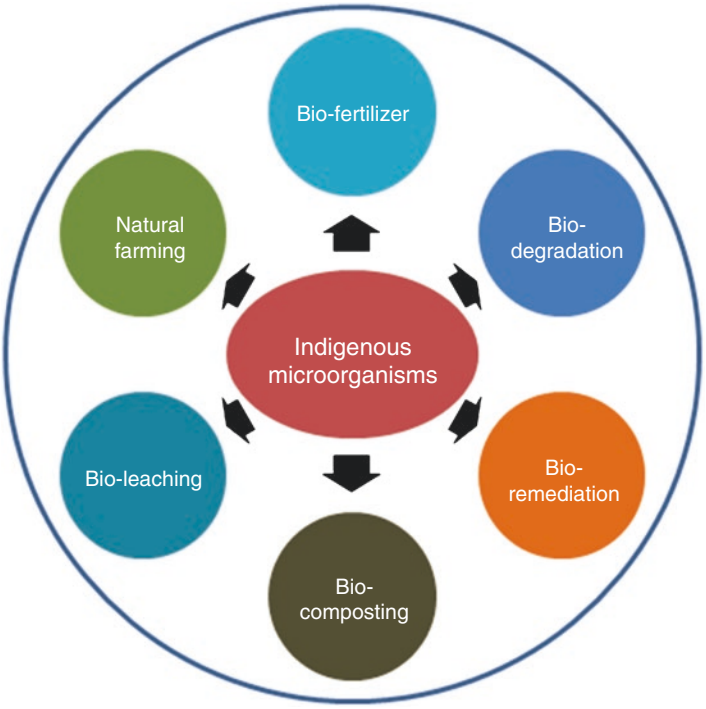


Fig. 18.1 Applications of microbes in a sustainable ecosystem

18.3.2 As a Biofertilizer

Indigenous microorganisms (IMOs) are a set of innate microbial consortium that inhabits the soil and the surfaces of the whole living things inside and out which have the capability in biodegradation, N_2 fixation, phosphate solubilizers, improving soil fertility, and plant growth promoters. Indigenous microorganisms don't contain a single culture of beneficial microorganisms but a combination of dissimilar advantageous microbes; it is a village of good bacteria that are living mutually in harmony with the rest of the environment. They create the optimum and favorable environment to improve and maintain soil flora and soil fauna in addition to the other microbes which in turn hold up the significant life of higher foliage and animals including humans.

18.3.3 Bioleaching

Bioleaching refers to the exchange of solid metal values into their water-soluble forms by the exercise of microorganisms. For example, in the case of copper, copper sulfide is microbially oxidized to $CuSO_4$, metal values are present in the aqueous phase, and the left over solids are discarded. The mass of naturally occurring

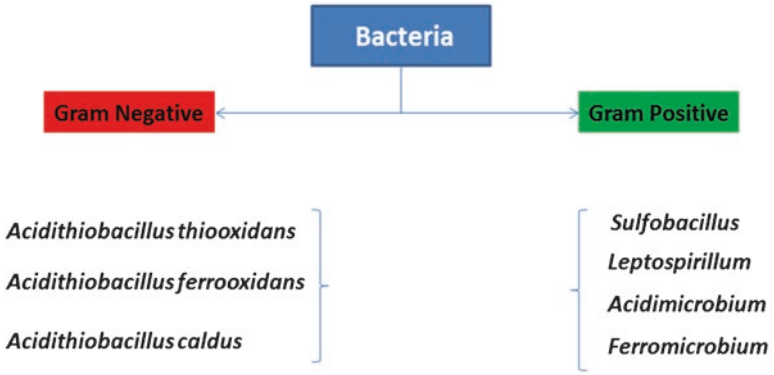


Fig. 18.2 A list of some identified potential microorganisms concerned in bioleaching

bacteria and fungi executes frequent physiologically important reactions that enable them to grow and reproduce. Mineralytic effects of bacteria and fungi on minerals are mainly based on three ideologies, acidolysis, complexolysis, and redoxolysis (Mishra et al. 2005). The entrapment of metal cations from insoluble ores through complexation and biological oxidation operation is referred to as bioleaching (Rohwerder et al. 2003). Bacterial leaching which is similar to any additional process concerning living beings is influenced by environmental, biological, and physicochemical factors that involved in the yield of metal extraction (Torma 1977; Lundgren and Silver 1980). Currently, the standard leaching bacteria correspond to the genus *Acidithiobacillus* (formerly *Thiobacillus*). Few microbes used for bioleaching are mentioned in Fig. 18.2 (Kelly and Wood 2000).

18.3.4 Biodegradation

Soil contamination which is on rise due to industrial and urban wastes generated by the human activities is of huge environmental concern (Ghosh and Singh 2005). One of the chief environmental hazards is the slow/least rate of deprivation or non-biodegradability of the organic resources under natural state, e.g., plastics (Sangale et al. 2012). Various forms of plastics such as nylon, polystyrene, polyethylene terephthalate, polycarbonate, polyurethane, polyethylene, polypropylene, polytetrafluoroethylene, and polyvinyl chloride are being constantly used in our day-to-day life (Smith 1964). The biodegradation through microorganisms such as bacteria, fungi, and algae is a usual process of degrading materials (Rutkowska et al. 2002). In this procedure, a range of dissimilar types of microbes are needed, in which one must lead to disruption of the polymer into their minor constituents, one utilizes the monomers and expel simple waste compounds as by-products, and one uses the expelled waste. Xenobiotic compounds are also present in the surroundings, and they are highly thermodynamically stable. The main concern with xenobiotic compounds is the toxicity risk they cause to public health. Various lethal effects on

humans such as severe carcinogenic, teratogenic, and mutagenic effects are exhibited through xenobiotic compounds. Microorganisms play a chief role in degradation of xenobiotics. They convert toxic contaminants into less hazardous or nonhazardous substances. Examples of anaerobic and aerobic xenobiotic degradative microbes are *Pseudomonas*, *Gordonia*, *Bacillus*, *Rhodococcus*, *Moraxella*, *Micrococcus*, *Escherichia*, *Pandoraea*, and *Sphingobium*, and anaerobic xenobiotic degradative bacteria are *Methanosaeta desulfotomaculum*, *Pelotomaculum*, *Syntrophobacter*, *Methanospirillum desulfovibrio*, and *Syntrophus* (Varsha et al. 2011; Chowdhury et al. 2008).

18.3.5 Bio-composting

India produces an average of 270 million tons of liquid effluent which is discharged to rivers or sea every year (Zeyer et al. 2004). During the production of sugar products such as pressmud, bagasse and sugarcane residues are produced. Methane embraces one of the six greenhouse gases accountable for the global warming that must be condensed, in order to undertake climate change. About 30% of the global anthropogenic emissions of methane to the atmosphere is released from landfills. The notion of recycling waste nutrients and organic material back to agricultural land is possible and desirable (Tweib et al. 2011).

18.3.6 Natural Farming

Lactic acid bacteria are omnipresent microorganisms that can be beneficial in crops and livestock production. LAB (lactic acid bacteria) have been documented as harmless for human consumption (Ikeda et al. 2013) and used in food maintenance since years ago by countless world cultures. LAB are used with IMOs (indigenous microorganisms) in usual farming in making compost used for soil preparation prior to planting. LAB are extensive in nature and are valuable probiotics for our digestive systems. They are among the majority of important groups of microbes used in fermentation of food, enhancing to the texture and taste of fermented stuffs and inhibiting food spoilage caused by other microorganisms (Ikeda et al. 2013).

18.4 Other Potential Roles Played by Microorganisms

The soil rhizosphere is a huge reservoir of microbial diversity. Microbes perform plentiful metabolic functions vital for their own maintenance and can assist the biosphere directly or indirectly through nutrient recycling, environmental detoxification, soil health improvement, wastewater treatment, etc. (Sengupta and Gunri 2015). The cooperative genome of rhizosphere microbial population of invasive plant roots is bigger in comparison to that of plants and is referred to as microbiome (Bulgarelli et al. 2013).

18.4.1 Plant Growth Producer and Regulators (PGPRs)

PGPRs assist in solubilization of mineral phosphates and other nutrients, improve resistance to stress, stabilize soil aggregates, and advance soil constitution and organic matter content. PGPRs keep hold of more soil organic N and additional nutrients in the plant-soil system; thus, they help in dropping the need for N and P fertilizer and enhance the rate to let go of the nutrients. Some of the associative and free-living rhizosphere bacteria apply valuable effects and improve growth of several crop plants; therefore, they are called plant growth-promoting *Rhizobacteria* (PGPR) (Kloepper et al. 1980; Bashan and Holguin 1998). The most possible candidates for PGPR are *Herbaspirillum* spp. and *Acetobacter diazotrophicus* for sugarcane (Baldani et al. 1997); *Azoarcus* spp. for kallar grass; and species of *Arthrobacter*, *Alcaligenes*, *Acinetobacter*, *Serratia*, *Streptomyces*, *Azospirillum*, *Bacillus*, *Agrobacterium*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Burkholderia*, *Cellulomonas*, *Frankia*, *Rhizobium*, *Bradyrhizobium*, *Pantoea*, *Pseudomonas*, and *Thiobacillus* for different legumes and nonlegumes.

18.4.2 Phosphorous (P) Solubilization

Phosphate-solubilizing microorganisms include several bacteria and growth medium containing tricalcium, iron, and aluminum phosphate, hydroxy apatite, bone meal, rock phosphate, and some insoluble phosphate compounds (Bhattacharjee and Dey 2014). There are considerable populations of phosphate-solubilizing bacteria in soil and in plant *rhizospheres.* These include both aerobic and anaerobic strains, with a dominance of aerobic strains in submerged soils (Roychowdhury et al. 2014). Bacteria are more effective in phosphorus solubilization than in fungi (Alam et al. 2002). The use of phosphate-solubilizing microorganisms boost crop yields up to 70%. Combined inoculation of arbuscular mycorrhiza and phosphate-solubilizing bacteria enhanced uptake of equally native P from soil and P coming from the phosphatic rock. There are significant populations of phosphate-solubilizing bacteria in soil and in plant *rhizospheres*. The most resourceful PSM belong to the genera *Bacillus* and *Pseudomonas* among bacteria and *Aspergillus* and *Penicillium* among fungi. There are seven genera of these fungi that produce arbuscular mycorrhizal symbiosis with plants. They are *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, *Entrophospora*, *Archaeospora*, and *Paragonimus.*

18.4.3 Microbes in Bioremediation

The frequent urbanization and industrialization over the precedent many decades have resulted in contamination of all the components of the atmosphere, that is, air, water, and soil including our food. As is clear from the word itself, bioremediation involved two components: first, “the bio,” i.e., the live component, and, second, “remediation,” i.e., the management of the pollutant. The word denotes the

occurrence of some pollutant in the matrix which is to be remediated. The petroleum-based products are major basis of energy for industries and daily life. So, accidental spills and leakages from oil tankers and ships occur frequently during the transportation. Its contamination negatively influences the soil microorganisms and plants, as well as polluted groundwater that can be used for consumption or farming. Recently, bioremediation is the most rising technology for treatment of petroleum-tainted sites. It is commercially useful and leads to deprivation or full mineralization of contaminants. Bioremediation technology is primarily based on biodegradation.

18.4.4 As Source of Bioenergy

In the current scenario, biofuels are receiving much attention worldwide because of early depletion of fossil fuels and their negative effect on global climate alteration through greenhouse gas emissions (Lang et al. 2001; Lee et al. 2010). The exercise of fossil fuels, particularly oil and gas, has accelerated in current years, and this triggers a global energy crisis. Renewable bioenergy is assumed as one of the ways to improve the current global warming calamity (Du et al. 2007). Currently, microalgae and *Cyanobacteria* intentionally supposed as the most promising candidates for the creation of different sources for biofuels (Sheehan et al. 1998). It is also predicted that microalgae and *Cyanobacteria* are capable to generate about ten times extra biodiesel per unit area of land than a distinctive terrestrial oleaginous crop (Chisti 2007; Rosenberg et al. 2008). Electrical energy was created from living cultures of *Escherichia coli* and *Saccharomyces* by using platinum electrodes. Moreover, they have simple growth necessities such as carbon dioxide, sunlight, and additional inorganic nutrients. Microalgae and *Cyanobacteria* can produce and accumulate huge quantities of neutral lipids (20–50% dry weight of biomass) and grow at elevated rates. Microalgae and *Cyanobacteria* sequester CO2 from flue gases are released from fossil fuel-fired power plants and other sources, thus dropping emissions of a major greenhouse gas (1 kg of dry microalgal biomass utilizes about 1.83 kg of CO2) (Brennan and Owende 2010; Mutanda et al. 2011; Rawat et al. 2013).

18.4.5 Microbes as Biotic Elicitors

Plants are known as the major basis of medicinally important compounds. Numerous plant products are used as pharmaceuticals, pigments, herbicides, etc. There are two types of elicitors: general elicitors and race-specific elicitors. While general elicitors are able to activate resistance together in host and nonhost plants, race-specific elicitors persuade defense responses important to disease confrontation only in specific host cultivars. Elicitor treatment is one of the effective approaches for improving secondary metabolite production in in vitro plant cell culture (Sivanandhan et al. 2011).

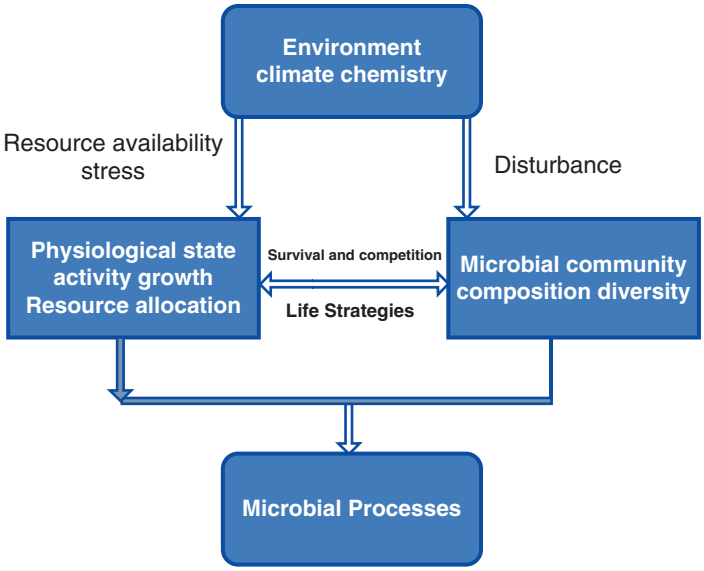


Fig. 18.3 Flowchart presenting the link among environmental drivers, microbial physiology, community composition, and ecosystem processes

18.4.6 Microbial Responses in Stress Agriculture

Climate change is one of the key issues that frequently affects the root activity, photosynthesis, functioning, and general morphology of the plant specimens plus their interactions. Alteration in the type of weather not only affects the prospective crop yield, but it may also alter the activities of pests and pathogens (Bhattacharyya et al. 2016). The microbial world is the major unexplored pool of biodiversity on earth. Bacteria, fungi, algae, *protozoa*, *actinomycetes*, and the infectious agents such as viruses are the things within the enormous resources of activities of microbial diversity (Andreote et al. 2014) (Fig. 18.3).

18.5 Conclusion

Go Green is what we all have learned from our past endeavors. This review has taught us to go back to our basics. Everything that we want to live is provided by nature. The necessity of the hour is to explore the vast pool of microorganisms and use them with the help of the latest scientific technologies to warfare the problems of agriculture and environment. The microbes are beneficial to mankind; it is on us whether we use them in a constructive or destructive way. They help to maintain the sustainability of the atmosphere and make us help to make this earth a healthy place to live in.

Acknowledgment PB thanks DST-SERB: SB/YS/LS-213/2013 for the financial support. The authors acknowledge Vivek Kumar for designing the figures and proofreadings of the above manuscript.

References

Adhya TK, Kumar N, Reddy G, Podile AR, Bee H, Samantaray B (2015) Microbial mobilization of soil phosphorus and sustainable P management in agricultural soils. Curr Sci 108(7):1280–1287

Alam S, Khalil S, Ayub N, Rashid M (2002) In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. Int J Agric Biol 4(4):454–458

Altieri MA (1995) Agroecology: the science of sustainable agriculture. Intermediate Technology Publications Ltd (ITP), London

Altland JE, Gilliam CH, Wehtje G (2003) Weed control in field nurseries. HortTechnology 13(1):9–14

Anand R, Prasad B, Tiwary BN (2009) Relative susceptibility of Spodoptera litura pupae to selected entomopathogenic fungi. BioControl 54(1):85

Andreote FD, Gumiere T, Durrer A (2014) Exploring interactions of plant microbiomes. Sci Agric 71(6):528–539

Arnold AE, Lamit LJ, Gehring CA, Bidartondo MI, Callahan H (2010) Interwoven branches of the plant and fungal trees of life. New Phytol 185(4):874–878

Azevedo JL, Maccheroni W Jr, Pereira JO, de Araújo WL (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron J Biotechnol 3(1):15–16

Balach D, Raja P, Kumar K, Sundaram SP (2007) Non-rhizobial nodulation in legumes. Biotechnol Mol Biol Rev 2(2):49–57

Baldani J, Caruso L, Baldani VL, Goi SR, Döbereiner J (1997) Recent advances in BNF with non-legume plants. Soil Biol Biochem 29(5):911–922

Bashan Y, Holguin G (1998) Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil Biol Biochem 30(8):1225–1228

Bhattacharjee R, Dey U (2014) Biofertilizer, a way towards organic agriculture: a review. Afr J Microbiol Res 8(24):2332–2343

Bhattacharyya PN, Goswami MP, Bhattacharyya LH (2016) Perspective of beneficial microbes in agriculture under changing climatic scenario: a review. J Phytology 8:26–41

Boydston RA, Collins HP, Vaughn SF (2008) Response of weeds and ornamental plants to potting soil amended with dried distillers grains. Hortscience 43(1):191–195

Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev 14(2):557–577

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EV, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838

Cai X, Gu M (2016) Bioherbicides in organic horticulture. Horticulturae 2(2):3

Cai M, Yao J, Yang H, Wang R, Masakorala K (2013) Aerobic biodegradation process of petroleum and pathway of main compounds in water flooding well of Dagang oil field. Bioresour Technol 144:100–106

Chandler D, Davidson G, Grant WP, Greaves J, Tatchell GM (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Technol 19(5):275–283

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306

Chowdhury S, Mishra M, Adarsh VK, Mukherjee A, Thakur AR, Chaudhuri SR (2008) Novel metal accumulator and protease secretor microbes from East Calcutta wetland. Am J Biochem Biotechnol 3:255–264

Datta S (2012) Biopesticides and fertilizers: novel substitutes of their chemical alternates. J Environ Res Dev 6(3):773–778
Day SD, Bassuk NLA (1994) Review of the effects of soil compaction and amelioration treatments on landscape trees. J Arboric 20(1):9–17
Deepali GK, Gangwar K (2010) Biofertilizers: an ecofriendly way to replace chemical fertilizers
Du Z, Li H, Gu T (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25(5):464–482
El-Yazeid AA, Abou-Aly HA, Mady MA, Moussa SA (2007) Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. Res J Agric Biol Sci 3(4):274–286
Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321(1–2):35–59
Frantzen J, Paul ND, Müller-Schärer H (2001) The system management approach of biological weed control: some theoretical considerations and aspects of application. BioControl 46(2):139–155
Ghosh M, Singh SA (2005) Review on phytoremediation of heavy metals and utilization of it's by products. Asian J Energy Environ 6(4):18
Hartmann M, Fliessbach A, Oberholzer HR, Widmer F (2006) Ranking the magnitude of crop and farming system effects on soil microbial biomass and genetic structure of bacterial communities. FEMS Microbiol Ecol 57(3):378–388
Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60(4):579–598
Ikeda DM, Weinert E Jr, Chang KC, McGinn JM, Miller SA, Keliihoomalu C, DuPonte MW (2013) Natural farming: lactic acid bacteria. Sustain Agric 8:3–4
Kelly DP, Wood AP (2000) Reclassification of some species of Thiobacillus to the newly designated genera Acidithiobacillus gen. nov., Halothiobacillus gen. nov. and Thermithiobacillus gen. nov. Int J Syst Evol Microbiol 50(2):511–516
Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886
Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB (2001) Preparation and characterization of bio-diesels from various bio-oils. Bioresour Technol 80(1):53–62
Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM (2010) Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 101(1):S75–S77
Lundgren DG, Silver M (1980) Ore leaching by bacteria. Ann Rev Microbiol 34(1):263–283
Masson-Boivin C, Giraud E, Perret X, Batut J (2009) Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends Microbiol 17(10):458–466
Mazid M, Khan TA, Mohammad F (2011) Role of secondary metabolites in defense mechanisms of plants. Biology and. Medicine 3(2):232–249
Mishra D, Kim DJ, Ahn JG, Rhee YH (2005) Bioleaching: a microbial process of metal recovery; a review. Met Mater Int 11(3):249–256
Mishra DJ, Rajvir S, Mishra UK, Kumar SS (2013) Role of bio-fertilizer in organic agriculture: a review. Res J Recent Sci 2:39–41
Mutanda T, Ramesh D, Karthikeyan S, Kumari S, Anandraj A, Bux F (2011) Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Bioresour Technol 102(1):57–70
Noble AD, Ruaysoongnern S (2010) The nature of sustainable agriculture. In: Soil microbiology and sustainable crop production. Springer, New York, pp 1–25
Odame H (1997) Biofertilizer in Kenya: research, production and extension dilemmas. Biotechnol Dev Monitor 30:20–23
Onduru DD, De Jager A, Wouters B, Muchera FN, Gachimbi L, Gachini GN (2006) Improving soil fertility and farm productivity under intensive crop-dairy smallholdings: experiences from farmer field schools in the highlands of Kiambu district, central Kenya. Middle-East J Sci Res 1(1):31–49

Onen H, Ozer Z, Telci I (2002) Bioherbicidal effects of some plant essential oils on different weed species. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Sonderheft 18:597–606
Owens K, Feldman J, Kepner J (2010) Wide range of diseases linked to pesticides. Pesticides and You 30(2):13–21
Peigné J, Ball BC, Roger-Estrade J, David C (2007) Is conservation tillage suitable for organic farming? A review. Soil Use Manag 23(2):129–144
Pindi PK (2012) Diversity of fungi at various depths of marine water. Res Biotechnol 3(4)
Rawat I, Kumar RR, Mutanda T, Bux F (2013) Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl Energy 103:444–467
Rohwerder T, Gehrke T, Kinzler K, Sand W (2003) Bioleaching review part a. Appl Microbiol Biotechnol 63(3):239–248
Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ (2008) A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr Opin Biotechnol 19(5):430–436
Roychowdhury DE, PauL MA, Banerjee SKA (2014) Review on the effects of biofertilizers and biopesticides on rice and tea cultivation and productivity. Int J Eng Sci Technol 2(8):96–106
Russo A, Carrozza GP, Vettori L, Felici C, Cinelli F, Toffanin A (2012) Plant beneficial microbes and their application in plant biotechnology. In: Innovations in biotechnology. Intech
Rutkowska M, Krasowska K, Heimowska A, Steinka I (2002) PT 02/04/262 Transl. Serial no. 14810-Effect of modification of poly (E-caprolactone) on its biodegradation in natural environments. Int Polym Sci Technol 29(11):77–84
Sahu D, Priyadarshani I, Rath B (2012) Cyanobacteria-as potential biofertilizer. CIB Tech J Microbiol ISSN: 2319-3867
Sangale MK, Shahnawaz M, Ade ABA (2012) Review on biodegradation of polythene: the microbial approach. J Bioremed Biodegr 3(10):1–9
Sengupta A, Gunri SK (2015) Microbial intervention in agriculture: an overview. Afr J Microbiol Res 9(18):1215–1226
Sheehan J, Dunahay T, Benemann J, Roessler PA (1998) Look back at the US Department of Energy's aquatic species program: biodiesel from algae. National Renewable Energy Laboratory; 328
Sivanandhan G, Mariashibu TS, Arun M, Rajesh M, Kasthurirengan S, Selvaraj N, Ganapathi A (2011) The effect of polyamines on the efficiency of multiplication and rooting of Withania Somnifera (L.) Dunal and content of some withanolides in obtained plants. Acta Physiol Plant 33(6):2279
Smith WM (1964) Manufacture of plastics, vol. 1. Technology and Engineering, Reinhold Pub. Corp, USA
Stine MA, Weil RR (2002) The relationship between soil quality and crop productivity across three tillage systems in south central Honduras. Am J Altern Agric 17(1):2–8
Torma AE (1977) The role of Thiobacillus ferrooxidans in hydrometallurgical processes. In: Advances in biochemical engineering, vol 6. Springer, Berlin/Heidelberg, pp 1–37
Tweib SA, Rahman RA, Khalil MS (2011) Composting of solid waste from wet market of Bandar Baru Bangi Malaysia. Aust J Basic Appl Sci 5(5):975–983
Vance CP (1998) Legume symbiotic nitrogen fixation: agronomic aspects. In: The Rhizobiaceae. Springer, Dordrecht, pp 509–530
Varsha YM, Naga Deepthi CH, Chenna S (2011) An emphasis on xenobiotic degradation in environmental clean up. J Bioremed Biodegr 11:1–0
Youssef MM, Eissa MF (2014) Biofertilizers and their role in management of plant parasitic nematodes. A review. E3 J Biotechnol Pharm Res 5(1):1–6
Zeyer J, Ranganathan LS, Chandra TS (2004) Pressmud as biofertilizer for improving soil fertility and pulse crop productivity. ISCB–Indo–Swis collaboration in Biotech. A report Portfolia first phase (1999–2004)

Arbuscular Mycorrhizal Symbiosis: A Promising Approach for Imparting Abiotic Stress Tolerance in Crop Plants

19

Purnima Bhandari and Neera Garg

Abstract

Arbuscular mycorrhizal (AM) fungi form symbiotic association with a majority of plant species and act as a bridge between soil and plants, improving both plant health and soil fertility. In the recent decade, several studies have highlighted the potential of using such beneficial microbes in bioremediation practices where AM fungi not only improve overall soil structure and fertility but also help in the adaptation of plants in regions facing abiotic constraints including drought stress, salinity stress and heavy metal stress. AM fungi also establish effective symbiosis with legumes which are the key nitrogen fixers in the agricultural land, thereby improving legume-rhizobial symbiosis and nitrogen fixation process even in severely disturbed environments. Based on recent available literature, this chapter summarizes (1) the probable underlying mechanism(s) at biochemical and molecular level adopted by AM fungi in imparting stress resistance in plants against salinity, drought stress and HM stress and (2) major prospects to be taken in the future in the current direction.

Keywords

Arbuscular mycorrhizal (AM) fungi • Drought • Salinity • Heavy metal (HM) toxicity • Oxidative stress • Antioxidants

P. Bhandari • N. Garg (✉)
Department of Botany, Panjab University, Chandigarh 160014, India
e-mail: gargneera@gmail.com; garg_neera@yahoo.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_19

19.1 Introduction

Plants represent a very dynamic system, reflecting a great capacity for adaptation in constantly fluctuating surroundings. This ability is particularly advantageous in the areas that are prone to intensive agriculture or biotic or abiotic vagaries. Among abiotic stresses, drought stress, soil salinity and accumulation of heavy metals (HM) in the soil are one of the major grounds of diminished plant performance and restrained crop yield worldwide. These stresses, particularly the abiotic ones, are expected to be intensified by the use of global crop production chains (López-Ráez 2016). In response, plants modify their root morphology, ultrastructure (Fusconi and Berta 2012) which may modulate their physiology and biochemistry to limit stress-induced damages and/or facilitate the repair of damaged systems (Patakas 2012; Latef et al. 2016). Various research efforts are being aimed (1) at developing strategies that could make agriculture more resilient and (2) mitigating the stress effects on crop yield which include selection of stress-adapted crop varieties and introduction of improved soil management and irrigation techniques (Campbell 2012; Chitarra et al. 2016). In addition, among the sustainable efforts, the role of root-associated microbes especially arbuscular mycorrhizal (AM) fungi in imparting stress tolerance has been exploited by many researchers in the recent years (Evelin et al. 2009; Garg and Chandel 2010; Hajiboland et al. 2010; Garg and Singla 2012; Ruiz-Lozano et al. 2012a; Kapoor et al. 2013; Garg and Pandey 2015; Garg and Bhandari 2016a, b).

AM fungi, belonging to the phylum Glomeromycota (Schüßler et al. 2001; Schüßler 2014), are the obligate biotrops that have been documented to form symbioses with the roots of more than 80% of terrestrial plant species (except in the plants belonging to families Amaranthaceae, Brassicaceae, Proteaceae, Commelinaceae, Polygonaceae, Cyperaceae, Juncaceae and Chenopodiaceae). They are ubiquitous soilborne fungi, whose origin and divergence dates back to over 450 million years (Redecker et al. 2000; Schüßler and Walker 2011; Gutjahr and Parniske 2013; Barea et al. 2014). Their association with host plant enhances mineral nutrition in exchange for the uptake of carbon (C) compounds derived from the photosynthetic process (Bucher et al. 2014) which results in positive host growth responses even under stressful conditions (Balestrini et al. 2015; Balestrini 2016). In general, it has been estimated that approximately 20% C of net primary productivity is allocated to AM fungus (Valentine et al. 2013; Fellbaum et al. 2014; Bücking and Kafle 2015) which is used to maintain and extend its hyphal network in the soil and in turn provide a majority of the plant nutrients (Leake et al. 2004; Ahanger et al. 2014).

Plant root, formation of fungal intraradical structures within roots (i.e. arbuscules and vesicles) and an extraradical mycelium (ERM) in the soil constitute important components of mycorrhizal symbiosis. The development of a functional mycorrhizal symbiosis requires a fine-tune coordination between the two partners (i.e. AM fungi and host plant) involving a series of recognition events leading to the morphological and physiological integration of these symbionts (Gianinazzi et al. 1995; Bucher et al. 2014). Primarily under nutrient-deficient conditions, this

communication leads to the establishment of association that starts in the rhizosphere with the production and exudation of signalling molecules – *strigolactones* (SL) by the host plants – which are recognized by AM fungi, thereby stimulating their hyphal growth (Matusova et al. 2005; Gomez-Roldan et al. 2007; López-Ráez et al. 2012; Ahanger et al. 2014) and giving rise to the so-called pre-symbiotic stage. In response, plants perceive diffusible fungal signals called 'Myc factors' at the plant plasma membrane due to lysine-motif (LysM) receptor kinases (Antolın-Llovera et al. 2012; Broghammer et al. 2012; Oldroyd 2013) that actively prepare the intracellular environment and induce symbiosis-specific responses in the host root, even in the absence of any physical contact (Parniske 2008; Genre and Bonfante 2010) ultimately leading to the formation of a highly branched, swollen and flattened characteristic fungal structure called *appressorium* on the root epidermal cells (Smith and Read 2008; Genre 2012). This event marks the initiation of the symbiotic phase of the interaction. Consequently, fungal hyphae penetrate into host roots, which are characterized by localized production of wall-degrading hydrolytic enzymes by the fungus and by the exertion of hydrostatic pressure by the hyphal tip (Kapoor et al. 2013). Colonization then progresses further to produce a characteristic tree-like structures – 'arbuscule' – which develop within the root cortical cells where the exchange of C from the host and nutrient from fungus occurs (Parniske 2008; Gutjahr and Parniske 2013; Barea et al. 2014). In addition, vesicles are also formed by some fungal species that serve as a storage structure. Following root colonization, AM fungi form extensive mycelial networks outside the root, i.e. ERM that helps in the acquisition of mineral nutrients from the strata, particularly those nutrients whose ionic forms have poor mobility or are present in low concentration in the soil solution such as phosphate and ammonia (Barea et al. 2014, 2005). As a consequence, they are being used as bio-fertilizers for enhancing plant growth and biomass production under stressful conditions, although at the moment at a lesser extent than conventional methods (Duhamel and Vandenkoornhuyse 2013; López-Ráez 2016). However, the exact mechanism(s) via which mycorrhizal fungi impart stress tolerance is still unknown. Thus, the present chapter appraises the probable underlying mechanism(s) of AM fungi that imparts tolerance to plants against various abiotic conditions (drought, salinity and HM toxicity).

19.2 AM Fungi and Drought Stress

Water stress may occur either due to excess of water (i.e. *flooding*) or water-deficit (i.e. *drought stress*) conditions. In the latter condition, the absence of adequate water table which is required for normal plant growth, development and reproducibility results in *drought/water stress* (Hasanuzzaman et al. 2013; Latef et al. 2016). It causes the dehydration of cells and osmotic imbalance (Mahajan and Tuteja 2005; Karthikeyan et al. 2016). In order to cope up with water-deficient conditions, plants have evolved a number of strategies all aimed for the optimization of water use such as morphological adaptations, stomatal closure to prevent leaf water loss, regulation of hydraulic conductivity, osmotic adjustment, reduction of growth and

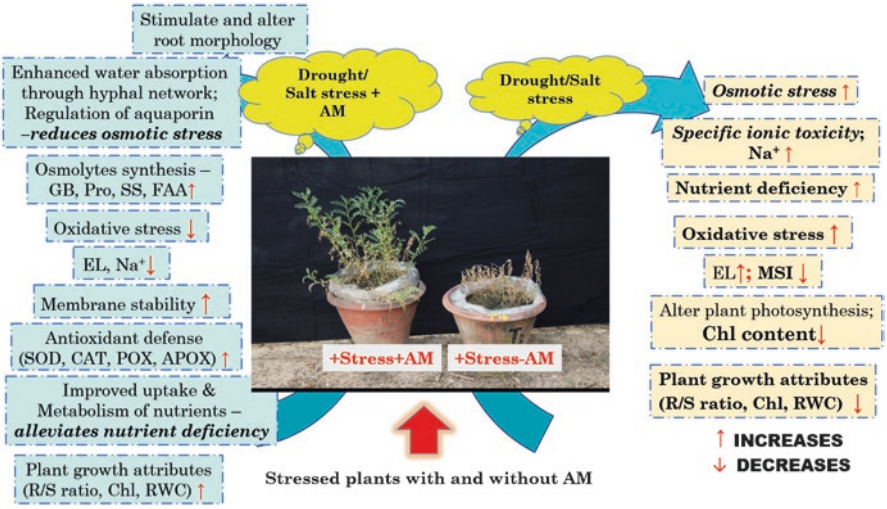


Fig. 19.1 Schematic representation of probable mechanism(s) through which arbuscular mycorrhiza (AM) fungi mediate improved tolerance in plants under drought and salinity

photosynthesis rate (López-Ráez 2016). In addition, to protect themselves and counteract the devastating effect of the stress, plants have developed mechanisms which involve induction of stress-responsive genes and proteins.

In addition, a growing evidence indicates that association with AM fungi can improve overall plant growth and reproducibility by improving root length, leaf area, plant biomass, plant tissue hydration and nutrient uptake under water-deficit condition (Al-Karaki et al. 2004; Nasim 2010; Kapoor et al. 2013), thus imparting drought resistance (Fig. 19.1). Several authors have attributed AM-mediated improvement in plant growth to the formation of extensive hyphal network and secretion of glomalin that not only enhances water uptake and nutrient acquisition but also enriches soil structural development, and stabilizes aggregate and soil structure, thus improving soil build-up (Augé 2001; Rillig 2004; Miransari 2010; Pagano 2014; Latef et al. 2016). The major mechanisms adopted by AM fungi in alleviating drought stress include direct uptake and translocation of water and mineral nutrients, improved osmotic adjustment, gas exchange attributes, transpiration thus water-use efficiency (Lee et al. 2012) and better protection against drought-induced ROS damage. Authors have ascribed these beneficial effects of AM fungi to the development of ERM that explore the distant areas, thus providing access to water-filled pores non-accessible by the roots (Smith et al. 2010). AM hyphal network associated with mycorrhizal plants has been estimated between 1 and 100 m/g of soil, thus increasing enormously the capacity for soil exploration (López-Ráez 2016), thereby resulting in acquisition of water that indirectly augments plant relative water content and photosynthetic rate. In one of the studies, Ruth et al. (2011) estimated that about 20% of root water uptake taken by roots of mycorrhizal barley plants is caused by the presence of fungal mycelium (Barea et al. 2014). Moreover,

AM-induced modification of root architecture and hydraulic conductance (*Lpr*) could also be ascribed as an important mean via which mycorrhiza imparts drought resistance. Several authors have ascribed the role of aquaporins (AQP) in regulating root hydraulic conductance (Uehlein et al. 2007; Ruiz-Lozano and Aroca 2010; Ruiz-Lozano et al. 2012b; Azcón et al. 2013; Li et al. 2013) and water movements. AQP are the small integral membrane proteins that constitute channels to facilitate the passive movement of water and small neutral molecules down a water potential gradient (Kaldenhoff and Fischer 2006; Maurel et al. 2015; López-Ráez 2016). In case of lettuce plants exposed to drought stress conditions, inoculation with *G. intraradices* or with the plant growth-promoting bacteria *Pseudomonas mendocina* modulated the expression of *PIP2* gene (Alguacil et al. 2009). On the contrary, Azcón et al. (2013) verified and reported that AM fungi enhanced the expression pattern under similar experimental conditions. Recently, Li et al. (2013) recorded an increased expression of two AQP genes (*GintAQPF1* and *GintAQPF2*) in both root cortical cells holding arbuscules and extraradical mycelia in +AM-inoculated *Zea mays* plants under stressful conditions.

Moreover, by extending their hyphae, mycorrhizal fungi have been stated to enhance the acquisition of mineral nutrients including Ca, Fe, K, Mg, P and Zn (Wu and Zou 2010; Bagheri et al. 2012; Gholamhoseini et al. 2013), thus alleviating drought-induced deficiency of these vital elements. While working on sunflower plant exposed to drought stress, Gholamhoseini et al. (2013) stated that inoculation with *G. mosseae* improved availability of P, thus minimizing the impact of stress on seed oil percentage and oil yield. Authors further confirmed that by improving root hydraulic conductivity, AM fungi improved the uptake and translocation of N, P and K that simultaneously augmented the levels of protein in drought-exposed plants (Gholamhoseini et al. 2013). AM-mediated availability of P in the host plant has been reported to alter positively various physiological processes including guard cell osmotic parameters and stomatal movements (Augé 2001).

AM fungi have also been suggested to mediate water movement via their effect on osmotic adjustments in the plant (Koltai and Kapulnik 2009) which could be attained by actively accumulating organic compounds including proline (Pro), soluble sugars (SS), glycine betaine (GB), etc. thus, positively regulating plant water content, cell turgor and related cellular processes (Hoekstra et al. 2001). Several studies have documented that by accruing compatible solutes (i.e. osmolytes) such as sugars, AM fungi lower the osmotic potential in drought-stressed mycorrhizal plants, thus conferring stress resistance (Abbaspour et al. 2012; Baslam and Goicoechea 2012; Yooyongwech et al. 2013). On the contrary, numerous studies have documented that inoculation with mycorrhizal fungi led to decrement in the levels of SS in several drought-exposed plants (Manoharan et al. 2010; Zhang et al. 2010). Similarly, AM-inoculated plants have been documented to accumulate higher levels of amino acids particularly Pro which subsequently enhanced drought tolerance in host plants (Ruíz-Sánchez et al. 2010; Kapoor et al. 2013; Rapparini and Penuelas 2014). In one of the studies, AM symbiosis enhanced Pro level by 29, 38 and 43% in drought-exposed *Zea mays* plants when inoculated with *Glomus mosseae* at three different concentrations (Abdelmoneim et al. 2014). However, on the

contrary, numerous studies have documented lower accumulation of Pro in mycorrhizal drought-stressed plants in comparison to non-mycorrhizal counterparts (Ruíz-Sánchez et al. 2010; Abbaspour et al. 2012; Doubková et al. 2013; Zou et al. 2013; Rapparini and Peñuelas 2014; Latef et al. 2016) which could be correlated with the ability of +AM plants to counteract stress. In another study, Zou et al. (2013) reported that when inoculated with *Funneliformis mosseae*, *Poncirus trifoliata* plants recorded lower tissue accumulation of this imino acid that related with improved plant growth and productivity under drought-stressed conditions. Authors postulated this lower accrual of Pro with inhibition of glutamate synthetic pathway and simultaneous enhanced Pro degradation pathway. However, Augé (2001) argued that despite of alteration in the levels of key solutes, the maintenance of root turgor upon mycorrhization during drought stress was related to changed apoplastic/symplastic water partitioning (Augé and Stodola 1990; Karthikeyan et al. 2016).

Studies have further depicted that AM-mediated alleviation of drought stress could also be allied with enhancement observed in the activities of antioxidants such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) in plants (Wu and Zou 2010; Ruíz-Sánchez et al. 2010; Baslam and Goicoechea 2012) which not only lower the build-up of toxic metabolites including superoxide radical ($O_2^{\bullet-}$), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) but also maintain membrane stability and integrity and other vital plant physiological processes under stressed conditions. For instance, while working on rice, Ruíz-Sánchez et al. (2010) recorded AM-mediated enhancement in photosynthetic performance in stressed conditions which they allied with the accrual of antioxidant compounds including glutathione (GSH) that not only lowered the build-up of cellular H_2O_2 but also maintained membrane stability. In addition to basic antioxidants, flavonoids have been reported to play a crucial role as ROS scavengers, thus imparting drought resistance to plants (Abbaspour et al. 2012). Volatile organic compounds such as isoprenoids (Rapparini et al. 2008; Rapparini and Peñuelas 2014), apocarotenoids (Walter and Strack 2011) and strigolactones (Lopez-Ráez et al. 2008) act as a supplementary protective systems against abiotic stresses including drought (Peñuelas and Munné-Bosch 2005; Vickers et al. 2009; as reviewed by Latef et al. 2016).

Plant hormones are considered indispensable for plant growth and development. In addition to the above-mentioned roles, several studies have reported enhanced levels of abscisic acid (ABA) upon mycorrhization that regulate the activity of plant stomata and enhance water efficiency under water-deficit conditions (Ludwig-Müller 2000; Aroca et al. 2008; Ruiz-Lozano et al. 2009; Calvo-Polanco et al. 2013; Miransari 2016). Moreover, for an effective mycorrhizal symbiosis, ABA has been found to be necessary for development and maintenance of arbuscules (Herrera-Medina et al. 2007; Martín-Rodríguez et al. 2011; Pozo et al. 2015). Thus, improved levels of ABA in mycorrhizal-stressed plants promote stress tolerance and at the same time enhance and maintain AM symbiosis (López-Ráez 2016). Once symbiosis is established within the host roots, AM fungi will regulate ABA levels when plants are exposed to drought conditions (Aroca et al. 2008; Calvo-Polanco et al. 2013). In their study, Aroca et al. (2008) recorded AM-induced induction of gene

LsNCED2 in drought-exposed *Lactuca sativa* plants, thus regulating plant ABA levels. In addition, mycorrhizal associations have been stated to positively regulate expression levels of certain genes such as P5CS (gene involved in synthesis of Pro) and genes encoding for late embryogenesis abundant (LEA; involved in ion association, membrane integrity, protein stabilization and folding and antioxidant defence response) proteins (Porcel et al. 2004, 2005), thereby directly contributing to enhanced drought tolerance (Ruiz-Lozano et al. 2006; Ahanger et al. 2014).

19.3 AM Fungi and Salinity Stress

Soil salinization is another major abiotic constraint that impedes plant growth and development by (a) inducing *osmotic stress* that leads to alteration in net assimilation capacity, leaf expansion rate, photosynthesis and its attributes (Raziuddin et al. 2011); (b) *specific ion toxicity* that disturbs the acquisition of mineral elements and disturbs ionic homeostasis; and (c) *oxidative stress* that elevates the build-up of reactive oxygen species (ROS), thereby mediating damage to lipids, proteins and nucleic acids (Hasanuzzaman et al. 2013; Hashem et al. 2014; Ahmad et al. 2016; Garg and Bhandari 2016a, b). A growing body of evidence exists that highlights the role of AM fungi as stress alleviator under saline conditions (Evelin et al. 2009; Garg and Manchanda 2009b; Ruiz-Lozano et al. 2012a; Porcel et al. 2012; Aroca et al. 2013; Kapoor et al. 2013; Hashem et al. 2014; Garg and Bhandari 2016a, b; Garg and Pandey 2015, 2016; Garg and Singla 2015, 2016). However, mycorrhizal symbiosis has been frequently documented to enhance the resilience of host plants to salinity stress, with even greater consistency than to drought stress (Koltai and Kapulnik 2009; Nasim 2010). Despite the beneficial effects of AM fungi recorded under saline stress, a large number of studies indicate that high concentrations of soluble salts in the rooting medium negatively affect symbiotic establishment in a number of plant species (Juniper and Abbott 2006; Giri et al. 2007; Sheng et al. 2008; Aroca et al. 2013; Estrada et al. 2013a; Porcel et al. 2015; López-Ráez 2016) due to the negative effect of salt on both fungus and on host plant. For instance, authors demonstrated that high concentrations of salt inhibited germination of spores/fungal propagules and hyphal growth and development, thus negatively influencing fungal colonization (Juniper and Abbott 2006; Jahromi et al. 2008). However, host plant is considered more sensitive than AM fungus because even in disturbed soils, AM fungal propagules never disappear completely, and whenever fungal colonization occurs, even to a small extent, it induces beneficial effects on the host stressed plant (Barea et al. 2014). Moreover, AM fungal species display differential colonizing ability, thus varying in their ability to alleviate salt stress among the plant genotypes. For instance, in one of the studies, our lab demonstrated greater effectiveness of *R. irregularis* in alleviating salt stress than *F. mosseae* in pigeon pea genotypes (Garg and Pandey 2015). Moreover, the genotype with higher per cent colonization and responsiveness showed higher stress tolerance than the ones with lower ability to form effective AM symbiosis.

Improved plant vigour and productivity as observed under salt-stressed conditions in AM-inoculated plants could not be a result of a single mechanism but a number of different mechanisms operating simultaneously under saline conditions which include improved nutrient uptake especially P, AM-mediated effects on water absorption and translocation, enhanced defence response (enzymatic as well as non-enzymatic antioxidants), synthesis of osmolytes, compartmentalization or reduction in toxic ion accumulation and increased photosynthetic system (Fig. 19.1).

In order to alleviate salt-induced *physiological drought conditions* (a condition where the plant is unable to utilize/uptake water from the rhizosphere due to higher concentrations of soluble salts), mycorrhizal inoculations enhance root hydraulic conductivity (Smith et al. 2010; Kapoor et al. 2013) by altering the morphology of root in a structural, spatial, quantitative and temporal manner (Kapoor et al. 2008) which not only results in production of greater root system (Khalil et al. 2011; Hajiboland 2013) and better root system architecture (RSA) in mycorrhizal plants but also allows exploration of a large soil volume, thus enhancing plant salt tolerance (Chatzistathis et al. 2013; Wu et al. 2013). Several reports have highlighted the potential of mycorrhizal fungi in maintaining better water status and sustaining higher RWC over the non-AM plants under osmotic stress conditions (Aroca et al. 2006, 2007; Jahromi et al. 2008; Porcel et al. 2012; Kapoor et al. 2013; Barea et al. 2014). Recently, Akhzari et al. (2016) reported significant higher leaf RWC and survival capacity values in AM-inoculated plants of *Melilotus officinalis* in comparison to non-AM counterparts under varying regimes of salt stress.

Besides, AM-mediated improvement in water status could also be attributed to fine regulation of root water uptake that depends on *Lpr* values, which depend eventually on the functioning of AQP (Aroca et al. 2012). Several authors have documented that AM fungi modify expression pattern of AQP of the host plants by increasing (Aroca et al. 2007), decreasing (Jahromi et al. 2008) or with no effect (Aroca et al. 2007) on the expression of AQP. Such results specify that each AQP has its specific function under each environmental stress situation (Aroca et al. 2007) and that each plant will respond differently to each colonizing fungus (Calvo-Polanco et al. 2013). In 2007, Aroca and colleagues reported that out of four aquaporin genes, three *PIP* genes displayed higher expressions in *Phaseolus vulgaris* plants inoculated with *G. intraradices* under specific conditions of drought, cold and salinity. Moreover, downregulation of plant AQP as observed under mycorrhizal plants under water-deficit condition allows conservation of water in plant tissues and substantiates the beneficial effect of AM fungi in maintaining higher water status of host plants under adverse conditions (Porcel et al. 2006; Aroca et al. 2007; Hajiboland 2013). In addition, AM fungi have been stated to affect events such as phosphorylation and dephosphorylation that enable to switch a channel on or off (Johansson et al. 1998; Calvo-Polanco et al. 2013), thus helping in regulating water transport.

AM inoculation can also modulate plant water status by accumulating osmolytes such as free amino acids (FAA), Pro, GB, SS and organic acids (Garg and Manchanda 2009b; Garg and Chandel 2011a; Garg and Baher 2013; Evelin and Kapoor 2014) which not only lower down osmotic potential but also permit cells to maintain

turgor-related processes (Ruiz-Lozano et al. 2012a). In comparison to non-AM counterparts, Abdel Latef and Chaoxing (2014) reported enhanced build-up of soluble proteins and FAA in pepper plants inoculated with *G. mosseae* when subjected to saline conditions. AM-mediated improvement observed in levels of Pro and GB has been correlated with the maintenance and protection of thylakoid membranes against the ROS damage by several authors (Yang et al. 2008; Talaat and Shawky 2014; Latef et al. 2016). In one of the studies, Garg and Baher (2013) accredited higher accumulation of Pro in mycorrhizal-inoculated chickpea plants to the substantial enhancement observed in Pro anabolic enzymes – P5CS and GDH activities, with a concomitant decline in Pro catabolic enzyme – ProDH activity under varying degree of salt stress, with higher accumulation recorded in tolerant genotype than the sensitive one. In contrast, lower accrual of Pro in mycorrhizal plants when compared with non-AM plants under various levels of salt stress have been reported (Jahromi et al. 2008; Sheng et al. 2011; Evelin et al. 2013; Abdel Latef and Chaoxing 2014) which could be considered as a reflection of an enhanced salt resistance in mycorrhizal plants (Hajiboland 2013). At the molecular level, Jahromi et al. (2008) studied the expression of gene encoding δ1-pyrroline-5-carboxylate synthetase (*LsP5CS*) in *Lactuca sativa* plants under varied salt treatments (0–100 mM NaCl) and demonstrated a higher expression of *LsP5CS* in non-AM plants than AM plants at 50 mM NaCl, although at 100 mM, the levels were similar, indicating that AM-inoculated plants suffered lower amount of stress when compared with non-AM plants which may be credited to the primary salt avoidance mechanisms operating in the former than the latter (Al-Karaki 2006; Evelin et al. 2012). In another study, Sheng et al. (2011) ascribed AM-mediated enhanced accumulation of GB with osmotic adjustment and subsequent efficient photosynthesis process operating in salinity-stressed maize plants. In addition, authors further recorded higher build-up of organic acids in mycorrhizal-stressed plants. Apart from playing as a role of osmoprotectant, organic acids have been stated to counteract excess of cations, thus maintaining pH homeostasis (Hatzig et al. 2010). Moreover, as argued by Kapoor et al. (2013), excess of them (especially malic acid) could enhance sugar synthesis through facilitation of CO_2 delivery to Calvin cycle. Similarly, accumulation of SS has also been considered as another defence strategy employed by mycorrhizal plants under salinity (Sheng et al. 2011; Garg and Chandel 2011b; Talaat and Shawky 2014; Abdel Latef and Chaoxing 2014). According to Abd-El Baki et al. (2000), sugars have been described to prevent structural changes in soluble protein and maintain osmotic equilibrium in plant cells, thus protecting membrane integrity. High accumulation of SS in mycorrhizal-treated plants might be explained by the sink effect of fungus demanding sugars from the shoot tissues (Augé 2001), increased rates of photosynthesis and of C compounds to the root system, hydrolysis of starch and higher concentration of organic acid in AM plants (Sheng et al. 2011; Kapoor et al. 2013). In the recent past, many studies have documented that by altering the activities of biosynthetic enzymes, fungal colonization induces higher accumulation of trehalose, an important osmoprotectant, thus complementing legume-*Rhizobium* symbiosis under different abiotic stresses (Ocón et al. 2007; Garg and Chandel 2011a; Garg and Pandey 2016; Garg and Singla 2016).

Besides alleviating osmotic stress, colonization of host plants with glomeromycotan fungi has been reported to prevent Na^+ translocation to shoot tissues and simultaneously enhances absorption of K^+ under saline conditions (Giri et al. 2007; Zuccarini and Okurowska 2008; Evelin et al. 2012; Hajiboland 2013; Garg and Pandey 2015; Garg and Bhandari 2016b), thereby maintaining ionic balance and preventing disruption of cellular processes such as protein synthesis (Ruiz-Lozano et al. 2012a). Researchers have related such effect of fungus with its capacity to retain toxic ions in intraradical fungal hyphae or to its compartmentalization strategy in the root cell vacuoles that prevent them from being transported into the shoots (Cantrell and Linderman 2001; Al-Karaki 2006; Ruiz-Lozano et al. 2012a; Hajiboland 2013) and ensured higher K^+/Na^+, Ca^{2+}/Na^+ and NO_3^-/Cl^- ratios in the tissues, thereby directing towards the smooth metabolic functioning of the processes in the plant (Kapoor et al. 2013). Moreover, AM-facilitated maintenance of higher ionic ratios in the host plant is achieved by regulating the expression and activity of transporters that are involved in the uptake of nutrients such as K^+, Na^+ and of H^+ pumps that generate the driving force for the transport of ions (Parida and Das 2005; Kapoor et al. 2013). In addition to transporters, influx of ion can occur via cyclic nucleotide-gated ion channels (CNGC) that have been proposed as the potent candidate genes for the studies related with amelioration of salt stress in mycorrhizal plants (Talke et al. 2003; Porcel et al. 2012). Recently, Estrada et al. (2013c) demonstrated differential expression levels for Na^+, K^+ transporters of *Z. mays* putatively involved in maintaining Na^+/K^+ homeostasis in roots during AM colonization.

In addition to K^+, Ca^{2+}, ERM of AM fungi, displays the ability to proliferate and exploit the rhizospheric area, thus stimulating the uptake of other mineral components including N, P, Mg, Cu, Fe and Zn, thereby alleviating salt-induced *mineral deficiency* (Garg and Manchanda 2009b; Estrada et al. 2013a, b; Hajiboland 2013; Garg and Pandey 2015). However, among them, P is considered as the most important element which is absorbed at a higher rate by the host plant via the activity of fungus that produces different enzymes including phosphatases (Miransari 2016), thus enhancing the availability of P under NaCl-stressed condition which ultimately contributes towards the maintenance of integrity of vacuolar membranes and facilitates the compartmentalization of Na^+ within vacuoles. Consequently, various researchers have hypothesized/considered AM-mediated improvement in P nutrition to be the main mechanism responsible for imparting enhanced tolerance in AM-inoculated plants under saline conditions (Copeman et al. 1996; Al-Karaki et al. 2001; Zarea et al. 2013; Garg and Pandey 2015). Similarly, improved P nutrition has often been allied with the enhancement observed in growth rate, antioxidant defence production as well as with nodulation and N_2 fixation efficiency in mycorrhizal legume plants exposed to saline environment (Garg and Manchanda 2008; Garg and Bhandari 2016a, b; Garg and Singla 2015, 2016; Kapoor et al. 2013). In one of the studies, inoculation with *F. mosseae* improved root nodulation and led to higher fixation of atmospheric N_2 in pigeon pea genotypes, thus enabling plants to overcome the adverse effect of salinity (Garg and Manchanda 2008). Similarly, inoculation with AM fungi not only improved the pink colour of leghaemoglobin (LHb) and pigment content but also led to higher nitrogenase (N_2ase) activity, hence

higher efficiency of N_2 fixation in mycorrhizal plants (Garg and Manchanda 2008; Hameed et al. 2014; Garg and Pandey 2016;). Recently, Abd-Alla et al. (2014b) ascribed the enhancement observed in N_2 fixation in faba bean to mycorrhizal colonization that accelerated the mobilization of P, Fe, K and other minerals which are involved in synthesis of N_2ase and LHb contents (Abd-Alla et al. 2014a). Thus, it could be hypothesized that both mycorrhizal symbiosis and *Rhizobial* symbioses often act synergistically and augment the tolerance of inoculated plants to salinity (Rabie and Almadini 2005). Moreover, by facilitating the uptake of nutrients including K^+, Ca^{2+} and Mg^{2+}, mycorrhiza alleviate salt-induced-specific effects on chlorophyll (Chl) degradation and leaf senescence (Kaya et al. 2009; Evelin et al. 2012; Hajiboland 2013; Talaat and Shawky 2014), thereby improving Chl concentration. Similarly, Hajiboland et al. (2010) validated in their study that colonization with the *R. irregularis* improved net assimilation rates by elevating stomatal conductance and by protecting photochemical processes of PS II against salt stress. Besides, few studies have reported that mycorrhizal fungi enhance Si uptake in plants (Kothari et al. 1990; Clark and Zeto 1996; Garg and Bhandari 2016b), thus imparting stress resistance to host plants (Nogueira et al. 2002). Recently, while working on *Cicer arietinum* genotypes exposed to salinity stress, Garg and Bhandari (2016b) documented enhanced uptake of this beneficial element under stressed conditions which not only imparted stress resistance to plants but also improved plant productivity.

In addition, AM-mediated-enhanced salt tolerance could also be ascribed to its ability to eliminate the build-up of stress metabolites, thus reducing membrane lipid peroxidation by enhancing the levels of both enzymatic and non-enzymatic defence compounds in salt-exposed plants (Garg and Manchanda 2009a; Kapoor et al. 2013; Hameed et al. 2014; Garg and Singla 2015; Garg and Bhandari 2016a). Recently, in one of the studies, Garg and Bhandari (2016a) recorded higher plant biomass in +AM plants under salt stress which they ascribed to improved efficacy of antioxidant defence machinery and efficient recycling of ascorbate (ASA) and glutathione (GSH) that maintained redox balance in mycorrhizal chickpea plants. Similar results were obtained by Garg and Singla (2015) under the same experimental conditions. Subsequently, studies have reported lower rates of membrane peroxidation, thus lowering the rate of electrolyte leakage (EL) in mycorrhizal plants compared to non-mycorrhizal counterparts under salt stress (Garg and Manchanda 2009b; Kaya et al. 2009; Evelin et al. 2012). Moreover, AM-facilitated reduction in peroxidation of membrane lipids and, hence, EL could also be ascribed to the maintenance of higher Ca^{2+}/Na^+ ratio as recorded in different studies (Kapoor et al. 2013; Garg and Bhandari 2016a; Garg and Pandey 2015).

19.4 AM Fungi and Heavy Metal Stress

Apart from drought stress and salinity stress, accumulation of toxic metals such as cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) (which are not biologically functional in nature) in the soil or within plants is considered as another abiotic stress that negatively affects environmental health and threatens ecosystem

sustainable food production/sustainability. Such HMs are produced by various natural activities as well as by different anthropogenic activities such as metal smelting, use and drainage of chemical agro-products (e.g. fertilizers and pesticides), mining tail dumping and burning of fossil fuels (Wang and Chen 2006; Barea et al. 2014; Singh et al. 2016). According to Gohre and Paszkowski (2006), these metals are grouped into one category of elements having specific weight >5 g cm^{-3}. Some of these metals are essential plant micronutrients such as Cu, Fe, Mn, Ni and Zn and are required for beneficial plant growth and development, while others have no known biological function such as Cd, Pd and Hg (Kapoor et al. 2013). These elements can either be absorbed in soil particles or leached into groundwater (Nasim 2010). At elevated levels, HMs have been documented to cause several morphological, physiological, biochemical and structural changes in plants including inhibition of seed germination, decrease in root elongation, growth inhibition, disturbance in cellular homeostasis, suppression of photosynthesis rate, leaf chlorosis and premature leaf senescence (Benavides et al. 2005; Goncalves et al. 2007; Drzewiecka et al. 2012). In addition, toxic metals have been reported to get translocated to different plant parts where they interfere with active sites of many enzymes including phosphatase, ATPase and enzymatic antioxidants (Verma and Dubey 2003; Drzewiecka et al. 2012; Latef et al. 2016), thereby destroying protein structures and replacing crucial elements resulting in deficiency symptoms (Khanday et al. 2016). Like other stresses, metal toxicity has been advocated to augment build-up of ROS, thus altering membrane integrity and stability. Studies have further demonstrated that HMs, if taken in excessive amount, negatively disturb legume-rhizobial symbiosis and affect the survival and ability of rhizobia to form N_2-fixing nodules (Garg and Bhandari 2012; Garg and Kaur 2012).

Several studies have validated that AM fungi play a vital role in improving growth and productivity of host plants in metal-contaminated soils (Garg and Aggarwal 2011; Garg and Singla 2012; Garg and Kaur 2013a, 2013b; Garg and Bhandari 2014; Nadeem et al. 2014; Garg and Chandel 2015). However, HMs have been described to greatly influence mycorrhizal colonization. Studies have documented that the presence of HM in excess concentrations not only reduces spore germination, mycelia growth, degree of colonization and sporulation of these fungi but also causes a significant impact on their ecology and diversity (Klauberg-Filho et al. 2005; Folli-Pereira et al. 2013). Despite this fact, even in highly contaminated soils, AM fungal colonization occurs thus inducing beneficial effects on the stressed host plant (Gamalero et al. 2009; Barea et al. 2014). In addition, more than 30 species of AM fungi have been identified in contaminated soils worldwide and some at high frequencies, such as *Paraglomus occultum*, *G. clarum*, *G. intraradices* and *Scutellospora pellucida* (Folli-Pereira et al. 2013). Studies have further depicted that spores isolated from polluted soils are more tolerant to and germinated better in HM-polluted soil in comparison to spores isolated from non-polluted soils (Leyval et al. 1995; Gaur and Adholeya 2004). According to Shalabyl (2003), such naturally occurring resistance is likely due to phenotypic plasticity rather than genetic changes in the spores as tolerance gets lost after one generation in the absence of HM (Koltai and Kapulnik 2009).

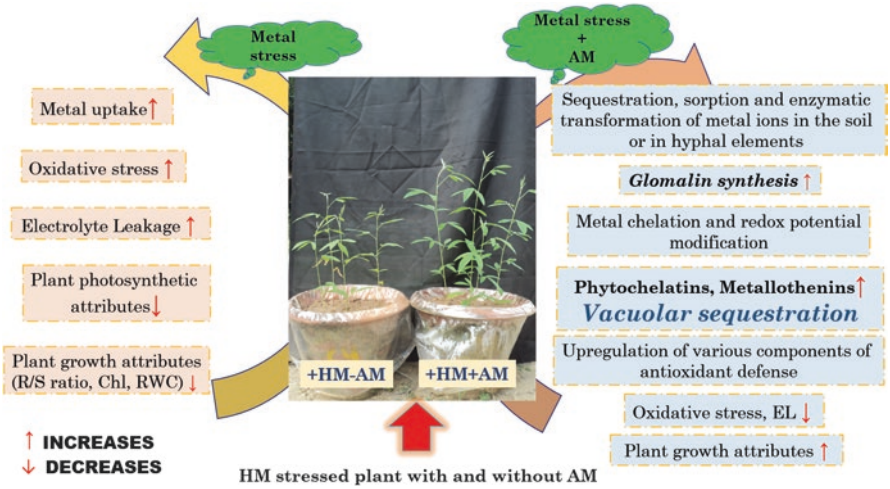


Fig. 19.2 Schematic representation of probable mechanism(s) through which arbuscular mycorrhiza (AM) fungi mediate improved tolerance in plants under HM stress

Numerous studies have highlighted the potential of glomeromycotan fungi in buffering metal toxicity and improving growth and productivity in metal-contaminated soils (Hildebrandt et al. 2007; Upadhyaya et al. 2010; Garg and Aggarwal 2011; Garg and Kaur 2013a, b; Muleta and Woyessa 2012; Garg and Bhandari 2012, 2014). However, the role of mycorrhiza in imparting metal resistance in host plant depends on several factors including the type of AM fungal species, plant genotype and nature as well as on the type of metal present in the soil (Sudová et al. 2008). For instance, in one of their studies, Garg and Aggarwal (2012) demonstrated higher colonizing ability of AM fungi with tolerant pigeon pea genotype than the sensitive one that conferred higher degree of protection in the former than the latter. Several possible mechanism(s) have been proposed via which AM fungi alleviate HM stress (Fig. 19.2).

The first mechanism includes restriction of HM ions by the compounds secreted by the mycorrhizal fungus which includes production of glomalin, extracellular chelation, cell wall binding and HM accumulation in ERM (Colpaert et al. 2011). As discussed previously, glomalin is a glycoprotein, first believed to be a hydrophobin, later identified as likely a 60-kDa heat shock protein homolog (Gadkar and Rillig 2006; Barea et al. 2014) and produced by AM fungi which has been documented to irreversibly bind with metals comprising Cu, Cd and Zn (Gonzalez-Chavez et al. 2004; Cornejo et al. 2008), thereby lowering their availability and leading to stabilization of metals in root rhizosphere (i.e. phyto-stabilization). Metals can also be precipitated onto polyphosphate granules, resulting in compartmentalization into plastids or other membrane-rich organelles (Turnau et al. 1993; Kaldorf et al. 1999; Koltai and Kapulnik 2009). Besides, AM fungi have been reported to accumulate toxic metal ions on their spore walls and hyphae as well as

in the vacuoles, thus retaining low cytoplasmic concentrations (Gonzalez-Guerrero et al. 2007). In one of the studies, Ferrol et al. (2009) validated that extraradical spores and intraradical vesicles of AM fungi, isolated from a Cu-enriched medium, contained a high build-up of toxic ion of Cu. In addition, several researchers have opined vesicles of the intraradical mycelium to function as storage structures for HM (Orlowska et al. 2008; Amir et al. 2014). Besides, fungal cell wall is composed of chitin which contains free amino, negatively charged hydroxyls and carboxyls groups that display very efficient binding ability with toxic metal ions (Barea et al. 2014), thus arresting them in soil matrix itself. In one of the studies, Gonzalez-Guerrero et al. (2008) reported higher concentrations of Cu, Zn and Cd elements that were partly localized in the fungal cell wall of *R. irregularis* (Amir et al. 2014). Moreover, many filamentous fungi have been commercially used as bio-sorbents due to their tendency to sorb trace elements (Morley and Gadd 1995; Nasim 2010). Consequently, AM fungi have been validated to lower the translocation of metals from roots to aerial organs (Garg and Bhandari 2012, 2014; Garg and Kaur 2012; Muleta and Woyessa 2012). In one of the studies, Garg and Kaur (2013b) demonstrated that in comparison with non-AM pigeon pea plants, colonization with *F. mosseae* led to significant immobilization of metals – Cd and Zn in roots – thus leading to lower translocation of toxic ions in above-ground plant organs in mycorrhizal-stressed plants. Similarly, colonization with *R. intraradices* restricted large amount of Pb in roots of woody legume *Robinia pseudoacacia*, thus preventing future damage to above-ground parts (Yang et al. 2016). However in some cases, mycorrhizal plants such as in red kidney bean (Rabie 2005) have been reported to exhibit improved (HM) uptake and root-to-shoot transport (i.e. phytoextraction; Göhre and Paszkowski 2006; Koltai and Kapulnik 2009).

Once transcended through the fungal wall, other defence-related mechanisms start operating that include compartmentalization of HM in vacuoles, modification of HM influx transporter processes and an enhancement in the efflux of toxic ions via the cell membrane (Meharg 2003; Ouziad et al. 2005). Fungal vacuoles seemed to play a crucial role in regulating cytosolic metal ion levels with simultaneous detoxification of potentially toxic ions. Moreover, excess of metal ions could be translocated to the vacuolar region through the activity of specific metal transporters like vacuolar Zn transporter (GintZnT1) or ABC transporter (GintABC1) (González-Guerrero et al. 2005, 2010). On the other hand, adsorption of metal ions onto plant or fungal cell walls (Joner et al. 2000) could also be considered as an additional mechanism which is facilitated by the chelation of metals by compounds including siderophores and metallothioneins (MT) that are secreted by mycorrhizal fungi or by plant-derived compounds comprising phytochelatins (PC) or phytates (Joner and Leyval 1997; Koltai and Kapulnik 2009; Garg and Bhandari 2014). MT are the cysteine-rich polypeptides that can chelate metals and sustain cellular metal homeostasis or sequestration through binding metals with thiol (-SH) group of their cysteine residues (Anjum et al. 2015; Latef et al. 2016). In one of the studies, MT – GmarMT1 and GintMT1 – have been reported to provide enhanced tolerance against metals such as Cd and Cu in *Gigaspora margarita* and *Glomus intraradices*,

respectively (Lanfranco et al. 2002; Gonzalez-Guerrero et al. 2007). Further, Lanfranco et al. (2002) validated that exposure to Cu upregulated the gene expression entirely in the symbiotic mycelium (Latef et al. 2016). Lately, four fungal genes have been documented to be involved in the sustenance of cellular homeostasis against HM stress which includes (1) *GrosMT1* from *Gigaspora rosea* (Stommel et al. 2001), (2) *GinZnT1* from *G. intraradices* (González-Guerrero et al. 2005), (3) *GmarMT1* from *Gigaspora margarita* (BEG 34, Gonzalez-Guerrero et al. 2007) and (4) *GintABC1* from *G. intraradices* (Gonzalez-Guerrero et al. 2007). Both *GrosMT1* and GinZnT1 have been described to assist in vacuolar Zn compartmentalization; *GmarMT1* regulates the fungal redox potential and confers tolerance against oxidative stress, while *GintABC1* codes for a polypeptide of 434 amino acids and actively participates in detoxification of Cu and Zn (as reviewed by Latef et al. 2016). In one of the studies, *GintGRX1* – a first characterized glomeromycotan glutaredoxin – has been reported to work efficiently against oxidative stress (Benabdellah et al. 2009). In case of legumes, several experiments were conducted by Garg and Aggarwal (2011) and Garg and Chandel (2011b) who evaluated the role of *F. mosseae* in the alleviation of Cd and Pb toxicities in pigeon pea genotypes and documented that mycorrhizal fungi enhanced PCs and GSH levels in stressed plants that eventually resulted in sequestration of metal ions, thus helping legume species to thrive well in multi-metal-contaminated soils. Recently, while working on legume plant – *Sophora viciifolia* exposed to Pb stress – Yang et al. (2016) reported that inoculation with *R. irregularis* increased the expression of phytochelatin synthase gene (*PCS1*), thus helping in immobilization of Pb in the roots of mycorrhizal-stressed plants.

Besides, numerous studies have documented that by up-regulating the levels of antioxidant enzymes such as SOD, CAT and POX, AM fungi augment the ROS-scavenging capacity of plants under metal stress conditions, thus imparting stress tolerance. For instance, Garg and Aggarwal (2012) demonstrated higher levels of SOD, CAT, POX and GR, as well as higher GSH/GSSG ratios in mycorrhizal pigeon pea plants in comparison with non-AM plants exposed to Cd and Pb stress that correlated with lower build-up of stress metabolites in the former than the latter. Similarly, in their study, Garg and Kaur (2013a) concluded that mycorrhization with propagules of *F. mosseae* attenuated the phytotoxic effects of Cd and Zn in nodules of pigeon pea plants in a genotype-dependent manner by reducing metal uptake and ROS levels and by augmenting defence responses that subsequently improved the N_2-fixing ability of nodules under stressed conditions. Indirectly, by enhancing water uptake, nutrient acquisition especially P via their hyphae, AM fungi mediate promotion in the plant growth under metal-contaminated environment (Abdel Latef 2011, 2013; Garg and Singla 2012; Garg and Bhandari 2013; Garg and Chandel 2015). Similarly, under As stress, inoculation with *F. mosseae* improved plant RWC and Chl level in AM-colonized pea plants when compared with non-AM counterparts (Garg and Singla 2012). Authors related such effects with the AM-mediated augmentation in SS and GB content (Garg and Singla 2012) as well as with elevated FAA and Pro contents (Abdel Latef 2011) under metal stress.

19.5 Conclusion and Future Prospects

The use of plant microbes as bio-fertilizer and bio-protector offers a potential tool for sustainable agriculture especially under stressed conditions. The current review summarizes the potential of AM fungi in regulating plant growth and productivity under unfavourable conditions. However, even though plant-AM association is older than 450 million years, in-depth studies are required at molecular, physiological and biochemical levels in order to have a better insight about the underlying mechanisms involved in AM-induced-enhanced stress tolerance against varying abiotic stresses. Another important factor to consider includes a sound knowledge of community structure of the AM fungi of a particular environment and evaluation of the functional diversity of these symbionts so as to exploit their full potential under different remediation practices. Deciphering how these beneficial microorganisms would act and interact with roots of the host plant genotype as well as with other soilborne microbes in the mycorrhizosphere will contribute towards the designing of strategies for sustainable agriculture.

Acknowledgement The authors are grateful to the Department of Biotechnology (DBT) and Department of Science and technology (DST-PURSE scheme), Government of India, for providing financial assistance for undertaking the research in the above context.

References

Abbaspour H, Saeid-Sar S, Afshari H, Abdel-Wahhab MA (2012) Tolerance of mycorrhiza infected Pistachio (*Pistacia vera* L.) seedlings to drought stress under glasshouse conditions. J Plant Physiol 169:704–709

Abd-Alla MH, El-Enany AW, Nafady NA, Khalaf DM, Morsy FM (2014a) Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiol Res 169(1):49–58

Abd-Alla MH, Issa AA, Ohyama T (2014b) Impact of harsh environmental conditions on nodule formation and dinitrogen fixation of legumes. In: Ohyama T (ed) Agricultural and biological sciences, Advances in biology and ecology of nitrogen fixation. InTechOpen, Rijeka, pp 131–193

Abd-El Baki GK, Siefritz F, Man PM, Weiner H, Kaldenhoff R, Kaiser WM (2000) Nitrate reductase in *Zea mays* L under salinity. Plant Cell Environ 23:515–521

Abdel Latef AA (2011) Influence of arbuscular mycorrhizal fungi and copper on growth, accumulation of osmolyte, mineral nutrition and antioxidant enzyme activity of pepper (*Capsicum annuum* L.) Mycorrhiza 21:495–503

Abdel Latef AA (2013) Growth and some physiological activities of pepper (*Capsicum annuum* L.) in response to cadmium stress and mycorrhizal symbiosis. J Agric Sci Technol 15:1437–1448

Abdel Latef AA, Chaoxing H (2014) Does the inoculation with *Glomus mosseae* improve salt tolerance in pepper plants? J Plant Growth Regul 33:644–653

Abdelmoneim TS, Moussa T, Almaghrabi OA, Alzahrani HS, Abdelbagi I (2014) Increasing plant tolerance to drought stress by inoculation with arbuscular mycorrhizal fungi. Life Sci J 11:10–17

Ahanger MA, Hashem A, Abd-Allah EF, Ahmad P (2014) Arbuscular mycorrhiza in crop improvement under environmental stress. In: Ahmad P, Rasool S (eds) Emerging technologies and management of crop stress tolerance. Elsevier, Amsterdam, pp 69–95

Ahmad P, Abdul Latef AA, Hashem A, Abd-Allah EF, Gucel S, LSP T (2016) Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. Front Plant Sci 7:347

Akhzari D, Pessarakli M, Ebrahimi M (2016) Effects of arbuscular mycorrhizal fungi on seedling growth and physiological traits of *Melilotus officinalis* L. grown under salinity stress conditions. Commun Soil Sci Plant Anal 47(7):2261. doi:10.1080/00103624.2016.1146897

Alguacil MM, Kohle RJ, Caravaca F, Roldána A (2009) Differential effects of *Pseudomonas mendocina* and *Glomus intraradices* on lettuce plants physiological response and aquaporin *PIP2* gene expression under elevated atmospheric CO_2 and drought. Microb Ecol 58:942–95l

Al-Karaki GN (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci Hortic 109:1–7

Al-Karaki GN, Hammad R, Rusan M (2001) Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. Mycorrhiza 11:43–47

Al-Karaki G, McMichael B, Zak J (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Amir H, Jourand P, Cavaloc Y, Ducousso M (2014) Role of mycorrhizal fungi in the alleviation of heavy metal toxicity in plants. In: Zakaria M, Solaiman ZM, Abbott LK, Varma A (eds) Mycorrhizal fungi: use in sustainable agriculture and land restoration, soil biology, vol 41. Springer-Verlag, Berlin, pp 241–258

Anjum NA, Hasanuzzaman M, Hossain MA, Thangavel P, Roychoudhury A, Gill SS, Rodrigo MA, Adam V, Fujita M, Kizek R, Duarte AC (2015) Jacks of metal(loid) chelation trade in plants – an overview. Front Plant Sci 6:192

Antolın-Llovera M, Ried MK, Binder A, Parniske M (2012) Receptor kinase signaling pathways in plant-microbe interactions. Annu Rev Phytopathol 50:451–473

Aroca R, Ferrante A, Vernieri P, Chrispeels MJ (2006) Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. Ann Bot 98:1301–1310

Aroca R, Porcel R, Ruiz-Lozano JM (2007) How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? New Phytol 173:808–816

Aroca R, Vernieri P, Ruiz-Lozano JM (2008) Mycorrhizal and nonmycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. J Exp Bot 59:2029–2041

Aroca R, Porcel R, Ruiz-Lozano JM (2012) Regulation of root water uptake under abiotic stress conditions. J Exp Bot 63:43–57

Aroca R, Ruiz-Lozano JM, Zamarreño ÁM, Paz JA, García-Mina JM, Pozo MJ, López-Ráez JA (2013) Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. J Plant Physiol 170:47–55

Augé RM (2001) Water relations, drought and vesicular mycorrhizal fungi symbiosis. Mycorrhiza 11:3–42

Augé RM, Stodola AJW (1990) Apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted Rosa plants. New Phytol 115:285–295

Azcón R, Medina A, Aroca R, Ruiz-Lozano JM (2013) Abiotic stress remediation by the arbuscular mycorrhizal symbiosis and rhizosphere bacteria/yeast interactions. In: Frans J. de Bruijn (ed) Molecular microbial ecology of the rhizosphere (vol. 2, 1st edn). Wiley, Hoboken, pp 991–1002

Bagheri V, Shamshiri MH, Shirani H, Roosta H (2012) Nutrient uptake and distribution in mycorrhizal pistachio seedlings under drought stress. J Agric Sci Technol 14(Suppl):1591–1604

Balestrini R (2016) Biological potential of arbuscular mycorrhizal fungi. In: Arora NK, Mehnaz S, Balestrini R (eds) Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 127–135

Balestrini R, Lumini E, Borriello R, Bianciotto V (2015) Plant–soil biota interactions. In: Paul EA (ed) Soil microbiology, ecology and biochemistry, 4th edn. Academic/Elsevier, London/San Diego/Oxford

Barea JM, Azcón R, Azcón-Aguilar C (2005) Interactions between mycorrhizal fungi and bacteria to improve plant nutrient cycling and soil structure. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer-Verlag, Berlin, pp 195–212

Barea JM, Pozo MJ, López-Ráez JA, Aroca R, Ruíz-Lozano JM, Ferrol N, Azcón R, Azcón-Aguilar C (2014) Arbuscular mycorrhizas and their significance in promoting soil-plant system sustainability against environmental stresses. In: Rodelas MB, González-López J (eds) Beneficial plant-microbial interactions ecology and applications. CRC Press/Taylor & Francis, London, pp 353–387

Baslam M, Goicoechea N (2012) Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza 22:347–359

Benabdellah K, Merlos MA, Azcón-Aguilar C, Ferrol N (2009) GintGRX1, the first characterized glomeromycotan glutaredoxin, is a multifunctional enzyme that responds to oxidative stress. Fungal Genet Biol 46:94–103

Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34

Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, Maolanon N, Vinther M, Lorentzen A, Madsen EB, Jensen KJ, Roepstorff P, Thirup S, Ronson CW, Thygesen MB, Stougaard J (2012) Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. Proc Natl Acad Sci U S A 109(34):13859–13864

Bucher M, Hause B, Krajinski F, Küster H (2014) Through the doors of perception to function in arbuscular mycorrhizal symbioses. New Phytol 204:833–840

Bücking H, Kafle A (2015) Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. Agronomy 5:587–612

Calvo-Polanco M, Sánchez-Romera B, Aroca R (2013) Arbuscular mycorrhizal fungi and the tolerance of plants to drought and salinity. In: Aroca R (ed) Symbiotic endophytes, soil biology 37. Springer-Verlag, Berlin/Heidelberg, pp 271–288

Campbell B (2012) The global imperative. Drought affects us all. In perspectives: legislating change. What should governments do to enhance sustainable agriculture and mitigate droughts? Nat Outlook Agric Drought 501:s12–s14

Cantrell IC, Linderman RG (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. Plant Soil 233:269–281

Chatzistathis T, Orfanoudakis M, Alifragis D, Therios I (2013) Colonization of Greek olive cultivars' root system by arbuscular mycorrhiza fungus: root morphology, growth and mineral nutrition of olive plants. Sci Agric 70(3):185–194

Chitarra W, Pagliarani C, Maserti B, Lumini E, Siciliano I, Cascone P, Schubert A, Gambino G, Balestrini R, Guerrieri E (2016) Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiol 171(2):1009–1023

Clark RB, Zeto SK (1996) Mineral acquisition by mycorrhizal maize grown on acid and alkaline soil. Soil Biol Biochem 28(I-II):1495–1503

Colpaert JV, Wevers JHL, Krznaric E, Adriaensen K (2011) How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Ann For Sci 68:17–24

Copeman RH, Martin CA, Stutz JC (1996) Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. Hortic Sci 31:341–344

Cornejo P, Meier S, Borie G, Rillig MC, Borie F (2008) Glomalin-related protein in a Mediterranean ecosystem affected by copper smelter and its contribution to cu and Zn sequestration. Sci Total Environ 406:154–160

Doubková P, Vlasáková E, Sudová R (2013) Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on *Knautia arvensis* plants in serpentine soil. Plant Soil 370:149–161

Drzewiecka K, Mleczek M, Waśkiewicz A, Goliński P (2012) Oxidative stress and phytoremediation. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer Science+Business Media, LLC, Berlin, pp 425–449

Duhamel M, Vandenkoornhuyse P (2013) Sustainable agriculture: possible trajectories from mutualistic symbiosis and plant neodomestication. Trends Plant Sci 18:597–600

Estrada B, Aroca R, Barea JM, Ruíz-Lozano JM (2013a) Native arbuscular mycorrhizal fungi isolated from a saline habitat improved maize antioxidant systems and plant tolerance to salinity. Plant Sci 201–202:42–51

Estrada B, Aroca R, Maathuis FJM, Barea JM, Ruiz-Lozano JM (2013b) Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant Cell Environ 36(10):1771–1782

Estrada B, Barea JM, Aroca R, Ruiz-Lozano JM (2013c) A native *Glomus intraradices* strain from a Mediterranean saline area exhibits salt tolerance and enhanced symbiotic efficiency with maize plants under salt stress conditions. Plant Soil 366:333–349

Evelin H, Kapoor R (2014) Arbuscular mycorrhizal symbiosis modulates antioxidant response in salt-stressed *Trigonella foenum-graecum* plants. Mycorrhiza 24(3):197–208

Evelin H, Giri B, Kapoor R (2012) Contribution of Glomus intraradices inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed Trigonella foenum-graecum. Mycorrhiza 22(3):203–217

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Evelin H, Giri B, Kapoor R (2013) Ultrastructural evidence for AMF mediated salt stress mitigation in *Trigonella foenum-graecum*. Mycorrhiza 23:71–86

Fellbaum CR, Mensah JA, Cloos AJ, Strahan GE, Pfeffer PE, Kiers ET, Bucking H (2014) Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. New Phytol 203:646–656

Ferrol N, Gonzalez-Guerrero M, Valderas A, Benabdallah K, Azcon-Aguilar C (2009) Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. Phytochem Rev 8:551–559

Folli-Pereira MS, Meira-Haddad LSA, Houghton CMNSVC, Kasuya MCM (2013) Plant-Microorganism Interactions: Effects on the Tolerance of Plants to Biotic and Abiotic Stresses. In: Hakeem KR, Ahmad P, Ozturk M (eds) Crop Improvement, Springer Science+Business Media, Berlin, pp 209–238

Fusconi A, Berta G (2012) Environmental stress and role of arbuscular mycorrhizal symbiosis. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants. Springer, New York, pp 197–214

Gadkar V, Rillig MC (2006) The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. FEMS Microbiol Lett 263:93–101

Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514

Garg N, Aggarwal N (2011) Effects of interactions between cadmium and lead on growth, nitrogen fixation, phytochelatin and glutathione production in mycorrhizal *Cajanus cajan* (L.) Millsp. J Plant Growth Regul 30:286–300

Garg N, Aggarwal N (2012) Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of *Cajanus cajan* (L.) Millsp. Genotypes grown in cadmium and lead contaminated soils. Plant Growth Regul 66:9–26

Garg N, Baher N (2013) Role of arbuscular mycorrhizal symbiosis in proline biosynthesis and metabolism of *Cicer arietinum* L. (chickpea) genotypes under salt stress. J Plant Growth Regul 32:767–778

Garg N, Bhandari P (2012) Influence of cadmium stress and arbuscular mycorrhizal fungi on nodule senescence in *Cajanus cajan* (L.) Millsp. Int J Phytoremediation 14:62–74

Garg N, Bhandari P (2014) Cadmium toxicity in crop plants and its alleviation by arbuscular mycorrhizal (AM) fungi: an overview. Plant Biosyst 148:609–621

Garg N, Bhandari P (2016a) Interactive effects of silicon and arbuscular mycorrhiza in modulating ascorbate-glutathione cycle and antioxidant scavenging capacity in differentially salt-tolerant *Cicer arietinum* L. genotypes subjected to long-term salinity. Protoplasma. doi:10.1007/s00709-015-0892-4

Garg N, Bhandari P (2016b) Silicon nutrition and mycorrhizal inoculations improve growth, nutrient status, K^+/Na^+ ratio and yield of *Cicer arietinum* L. genotypes under salinity stress. Plant Growth Regul 78:371–387

Garg N, Chandel S (2010) Arbuscular mycorrhizal networks: process and functions. Rev Agron Sustain Dev 30:581–599

Garg N, Chandel S (2011a) The effects of salinity on nitrogen fixation and trehalose metabolism in mycorrhizal *Cajanus cajan* (L.) Millsp. plants. J Plant Growth Regul 30:490–503

Garg N, Chandel S (2011b) Effect of mycorrhizal inoculation on growth, nitrogen fixation, and nutrient uptake in *Cicer arietinum* (L.) under salt stress. Turk J Agric For 35:205–214

Garg N, Chandel S (2015) Role of arbuscular mycorrhiza in arresting reactive oxygen species (ROS) and strengthening antioxidant defense in *Cajanus cajan* (L.) Millsp. nodules under salinity (NaCl) and cadmium (Cd) stress. Plant Growth Regul 75(2):521–534

Garg N, Kaur H (2012) Influence of zinc on cadmium-induced toxicity in nodules of pigeonpea (*Cajanus cajan* L. Millsp.) inoculated with arbuscular mycorrhizal (AM) fungi. Acta Physiol Plant 34(4):1363–1380

Garg N, Kaur H (2013a) Response of antioxidant enzymes, phytochelatins and glutathione production towards Cd and Zn stresses in *Cajanus cajan* (L.) Millsp. genotypes colonized by arbuscular mycorrhizal fungi. J Agron Crop Sci 199(2):118–133

Garg N, Kaur H (2013b) Impact of cadmium-zinc interactions on metal uptake, translocation and yield in pigeonpea genotypes colonized by arbuscular mycorrhizal fungi. J Plant Nutr 36(1):67–90

Garg N, Manchanda G (2008) Effect of Arbuscular mycorrhizal inoculation of salt-induced nodule senescence in *Cajanus cajan* (pigeon pea). J Plant Growth Regul 27:115–124

Garg N, Manchanda G (2009a) ROS generation in plants: boon or bane? Plant Biosyst 143(1):81–96

Garg N, Manchanda G (2009b) Role of arbuscular mycorrhizae in the alleviation of ionic, osmotic and oxidative stresses induced by salinity in *Cajanus cajan* (L.) Millsp. (Pigeonpea). J Agron Crop Sci 195:110–123

Garg N, Pandey R (2015) Effectiveness of native and exotic arbuscular mycorrhizal fungi on nutrient uptake and ion homeostasis in salt-stressed *Cajanus cajan* L. (Millsp.) genotypes. Mycorrhiza 25(3):165–180

Garg N, Pandey R (2016) High effectiveness of exotic arbuscular mycorrhizal fungi is reflected in improved rhizobial symbiosis and trehalose turnover in *Cajanus cajan* genotypes grown under salinity stress. Fungal Ecol 21:57–67

Garg N, Singla P (2012) The role of *Glomus mosseae* on key physiological and biochemical parameters of pea plants grown in arsenic contaminated soil. Sci Hortic 143:92–101

Garg N, Singla P (2015) Naringenin-and *Funneliformis mosseae*-mediated alterations in redox state synchronize antioxidant network to alleviate oxidative stress in *Cicer arietinum* L. genotypes under salt stress. J Plant Growth Regul 34(3):595–610

Garg N, Singla P (2016) Stimulation of nitrogen fixation and trehalose biosynthesis by naringenin (Nar) and arbuscular mycorrhiza (AM) in chickpea under salinity stress. Plant Growth Regul. doi:10.1007/s10725-016-0146-2

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86:528–534

Genre A (2012) Signalling and the re-structuring of plant cell architecture in am symbiosis. In: Perotto S, Baluška F (eds) Signaling and communication in plant symbiosis Vol 11 of the series – signaling and communication in plants. Springer-Verlag, Berlin, pp 51–71

Genre A, Bonfante P (2010) The making of symbiotic cells in arbuscular mycorrhizal roots. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 57–71

Gholamhoseini M, Ghalavand A, Dolatabadian A, Jamshidi E, Khodaei-Joghan A (2013) Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. Agric Water Manag 117:106–114

Gianinazzi S, Gianinazzi-Pearson V, Franken P, Dumas-Gaudot E, van Tuinen D, Samra A, Martin-Laurent F, Dassi B (1995) Molecules and genes involved in mycorrhizal functioning. In: Stoechi V, Bonfante P, Nuti M (eds) Biotechnologies of ectomycorrhizae. Springer, New York, pp 67–76

Giri B, Kapoor R, Mukerji KG (2007) Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. Microb Ecol 54:753–760

Gohre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhiza symbiosis to heavy metal phytoremediation. Planta 223:1115–1122

Gomez-Roldan V, Roux C, Girard D, Bécard G, Puech V (2007) Strigolactones: promising plant signals. Plant Signal Behav 2:163–164

Goncalves JF, Becker AG, Cargnelutti D, Tabaldi LA, Pereira LB, Battisti V, Spanevello RM, Morsch VM, Nicoloso FT, Schetinger MRC (2007) Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings. Braz J Plant Physiol 19:223–232

González-Chávez MC, Carrillo-González R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323

González-Guerrero M, Azcón-Aguilar C, Mooney M, Valderas A, MacDiarmid CW, Eide DJ, Ferrol N (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Oenet Biol 42:130–140

Gonzalez-Guerrero M, Cano C, Azcon-Aguilar C, Ferrol N (2007) GintMT1 encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17:327–335

Gonzalez-Guerrero M, Melville LH, Ferrol N, Lott JNA, Azcon-Aguilar C, Peterson RL (2008) Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. Can J Microbiol 54:103–110

González-Guerrero M, Benabdellah K, Valderas A, Azcón-Aguilar C, Ferrol N (2010) GintABC1 encodes a putative ABC transporter of the MRP subfamily induced by Cu, Cd, and oxidative stress in *Glomus intraradices*. Mycorrhiza 20:137–146

Gutjahr C, Parniske M (2013) Cell and developmental biology of the arbuscular mycorrhiza symbiosis. Annu Rev Cell Dev Biol 29:593–617

Hajiboland R (2013) Role of arbuscular mycorrhiza in amelioration of salinity. In: Ahmad P, Azooz M, Prasad M (eds) Salt stress in plants. Springer, New York, pp 301–354

Hajiboland R, Aliasgharzadeh N, Laiegh SF, Poschenrieder C (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. Plant Soil 331:313–327

Hameed A, Dilfuza E, Abd-Allah EF, Hashem A, Kumar A, Ahmad P (2014) Salinity stress and arbuscular mycorrhizal symbiosis in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, vol 1. Springer Science+Business Media, New York, pp 139–159

Hasanuzzaman M, Gill SS, Fujita M (2013) Physiological role of nitric oxide in plants grown under adverse environmental conditions. In: Tuteja N, Gill SS (eds) Plant acclimation to environmental stress. Springer Science+Business Media, New York, pp 269–322

Hashem A, Abd-Allah EF, Alqarawi AA, El-Didamony G, Alwhibi M, Egamberdieva D, Ahmad P (2014) Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. Pak J Bot 46:2003–2013

Hatzig S, Kumar A, Neubert A, Schubert S (2010) PEP-carboxylase activity: a comparison of its role in a C4 and a C3 species under salt stress. J Agron Crop Sci 196:185–192

Herrera-Medina MJ, Steinkellner S, Vierheilig H, Bote JAO, Garrido JMG (2007) Abscisic acid determines arbuscule development and functionality in the tomato arbuscular mycorrhiza. New Phytol 175:554–564

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6:431–438

Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM (2008) Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. Microb Ecol 55:45–53

Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, Kjellbom P (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. Plant Cell 10:451–459

Joner EJ, Leyval C (1997) Uptake of 109Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol 135:353–360

Joner EJ, Briones R, Leyval C (2000) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226:227–234

Juniper S, Abbott LK (2006) Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. Mycorrhiza 16:371–379

Kaldenhoff R, Fischer M (2006) Aquaporins in plants. Acta Physiol 187:169–176

Kaldorf M, Kuhn AJ, Schroder WH, Hildebrandt U, Bothe H (1999) Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. J Plant Physiol 154:718–728

Kapoor R, Sharma D, Bhatnagar AK (2008) Arbuscular mycorrhizae in micropropagation systems and their potential applications. Sci Hortic 116:227–239

Kapoor R, Evelin H, Mathur P, Giri B (2013) Arbuscular mycorrhiza: approaches for abiotic stress tolerance in crop plants for sustainable agriculture. In: Tuteja N, Gill SS (eds) Plant acclimation to environmental stress. Springer Science+Business Media, LLC, Berlin, pp 359–401

Karthikeyan B, Abitha B, Henry AJ, Sa T, Joe MM (2016) Interaction of rhizobacteria with arbuscular mycorrhizal fungi (AMF) and their role in stress abetment in agriculture. In: Pagano MC (ed) Recent advances on mycorrhizal fungi, fungal biology. Springer International Publishing, Basel, pp 117–142

Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL, Cullu MA (2009) The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. Sci Hortic 121:1–6

Khalil HA, Eissa AM, El-Shazly SM, Aboul-Nasr AM (2011) Improved growth of salinity-stressed citrus after inoculation with mycorrhizal fungi. Sci Hortic 130:624–632

Khanday M, Bhat RA, Haq S, Dervash MA, Bhatti AA, Nissa M, Mir MR (2016) Arbuscular mycorrhizal fungi boon for plant nutrition and soil health. In: Hakeem KR, Akhtar J, Sabir M (eds) Soil science: agricultural and environmental Prospectives. Springer International Publishing, Basel, pp 317–332

Klauberg-Filho O, Siqueira JO, Moreira FMS, Soares CRFS, Silva S (2005) Ecologia, funçãoe potencial de aplicação de FMAs em condições de excesso de metais pesados. Tópicos em ciência do solo. Soc Bras Cienc Solo 4:85–144 (in Portuguese)

Koltai H, Kapulnik Y (2009) Effect of arbuscular mycorrhizal symbiosis on enhancement of tolerance to abiotic stresses. In: White JF, Torres MS (eds) Defensive mutualism in microbial symbiosis. CRC Press/Taylor & Francis, Boca Raton, pp 217–234

Kothari SK, Marschner H, Romheld V (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. New Phytol 116:637–645

Lanfranco L, Bolchi A, Ros EC, Ottonello S, Bonfante P (2002) Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol 130:58–67

Latef AAHA, Hashem A, Rasool S, Abd-Allah EF, Alqarawi AA, Egamberdieva D, Jan S, Anjum NA, Ahmad P (2016) Arbuscular mycorrhizal Symbiosis and abiotic stress in plants: a review. J Plant Biol 59:407

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot 82:1016–1045

Lee BR, Muneer S, Avice JC, Jin Jung W, Kim TH (2012) Mycorrhizal colonisation and P-supplement effects on N uptake and N assimilation in perennial ryegrass under well-watered and drought-stressed conditions. Mycorrhiza 22:525–534

Leyval C, Singh BR, Joner EJ (1995) Occurrence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. Water Air Soil Pollut 84:203–216

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B (2013) First cloning and characterization of two functional aquaporin genes from an Arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 197:617–630

López-Ráez JA (2016) How drought and salinity affect arbuscular mycorrhizal symbiosis and strigolactone biosynthesis? Planta 243(6):1375–1385

Lopez-Ráez JA, Charnikhova T, Gomez-Roldan V, Matusova R, Kohlen W, De Vos R, Verstappen F, Puech-Pages V, Bécard G, Mulder P, Bouwmeester H (2008) Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytol 178:863–874

López-Ráez JA, Bouwmeester H, Pozo MJ (2012) Communication in the rhizosphere, a target for pest management. Lichtfouse E Sustainable agriculture reviews, 8; Agroecology and strategies for climate change, sustainable agriculture reviews, Springer, Dordrecht, 109–133

Ludwig-Müller J (2000) Hormonal balance in plants during colonization by mycorrhizal fungi. In: Kapulnik Y, Douds D (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 263–285

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Manoharan PT, Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Sharma MP, Muthuchelian K (2010) Influence of AM fungi on the growth and physiological status of *Erythrina variegata* Linn. grown under different water stress conditions. Eur J Soil Biol 46:151–156

Martín-Rodríguez JA, Leon-Morcillo R, Vierheilig H, Ocampo JA, Ludwig-Muller J, García-Garrido JM (2011) Ethylene-dependent/ethylene-independent ABA regulation of tomato plants colonized by arbuscular mycorrhiza fungi. New Phytol 190:193–205

Matusova R, Rani K, Verstappen AWF, Franssen FWA, Beale MH, Bouwmeester HJ (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiol 139:920–934

Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in plants. Physiol Rev 95:1321–1358

Meharg AA (2003) The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. Mycol Res 107:1253–1265

Miransari M (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Review. Plant Biol 12:563–569

Miransari M (2016) Stress and mycorrhizal plant. In: Pagano MC (ed) Recent advances on mycorrhizal fungi, fungal biology. Springer International Publishing, Basel, pp 63–79

Morley GF, Gadd GM (1995) Sorption of toxic metals by fungi and clay minerals. Mycol Res 99:1429–1438

Muleta D, Woyessa D (2012) Importance of arbuscular mycorrhizal fungi in legume production under heavy metal-contaminated soils. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, London, pp 219–241

Nadeem SM, Ahmad M, Zahir ZA, Javid A, Ashraf M (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Research review paper. Biotechnol Adv 32:429–448

Nasim G (2010) The role of arbuscular mycorrhizae in inducing resistance to drought and salinity stress in crops. In: Ashraf M, Ozturk M, Ahmad MSA (eds) Plant adaptation and phytoremediation. Springer, Dordrecht, pp 119–141

Nogueira MA, Cardoso E, Hampp R (2002) Manganese toxicity and callose deposition in leaves are attenuated in mycorrhizal soybean. Plant Soil 246:1–10

Ocón A, Hampp R, Requena N (2007) Trehalose turnover during abiotic stress in arbuscular mycorrhizal fungi. New Phytol 174:879–891

Oldroyd GED (2013) Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

Orlowska E, Mesjasz-Przybylowicz J, Przybylowicz W, Turnau K (2008) Nuclear macroprobe studies of elemental distribution in mycorrhizal and non-mycorrhizal roots of Ni-hyperaccumulator Berkheya coddii. X-Ray Spectrom 37:129–132

Ouziad F, Hildlebrandt U, Schmelzer E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. J Plant Physiol 162:634–649

Pagano MC (2014) Drought stress and mycorrhizal plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses. Springer Science+Business Media, New York, pp 97–110

Parida SK, Das AB (2005) Salt tolerance and salinity effects on plants. Ecotoxicol Environ Saf 60:324–349

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Patakas A (2012) Abiotic stress-induced morphological and anatomical changes in plants. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer Science+Business Media, LLC, Berlin, pp 21–39

Peñuelas J, Munné-Bosch S (2005) Isoprenoids: an evolutionary pool for photoprotection. Trends Plant Sci 10:166–169

Porcel R, Azcón R, Ruiz-Lozano JM (2004) Evaluation of the role of genes encoding for D1-pyrroline-5-carboxylate synthetase (P5CS) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. Physiol Mol Plant Pathol 65:211–221

Porcel R, Azcón R, Ruiz-Lozano JM (2005) Evaluation of the role of genes encoding for dehydrin proteins (LEA D-11) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. J Exp Bot 56:1933–1942

Porcel R, Aroca R, Azcón R, Ruiz-Lozano JM (2006) PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. Plant Mol Biol 2006:389–404

Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi – a review. Agron Sustain Dev 32:181–200

Porcel R, Redondo-Gómez S, Mateos-Naranjo E, Aroca R, Garcia R, Ruiz-Lozano JM (2015) Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. J Plant Physiol 185:75–83

Pozo MJ, López-Ráez JA, Azcón C, García-Garrido JM (2015) Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytol 205:1431–1436

Rabie GH (2005) Contribution of arbuscular mycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy metal-polluted soil. Afr J Biotechnol 4(4):332–345

Rabie GH, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. Afr J Biotechnol 4:210–222

Rapparini F, Peñuelas J (2014) Mycorrhizal fungi to alleviate drought stress on plant growth. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, vol 1. Springer, New York, pp 21–42

Rapparini F, Llusià J, Peñuelas J (2008) Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of *Artemisia annua*. Plant Biol 10:108–122

Raziuddin F, Hassan G, Akmal M, Shah SS, Mohammed F, Shafi M, Bakht J, Zhou W (2011) Effects of cadmium and salinity on growth and photosynthesis parameters of *Brassica* species. Pak J Bot 43:333–340

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921
Rillig MC (2004) Arbuscular mycorrhizae, glomalin and soil quality. Can J Soil Sci 84:355–363
Ruiz-Lozano JM, Aroca R (2010) Host response to osmotic stresses: stomatal behaviour and water use efficiency of arbuscular mycorrhizal plants. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function, 2nd edn. Springer Science +Business Media B.V, Dordrecht, pp 239–256
Ruiz-Lozano JM, Porcel R, Aroca R (2006) Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes. New Phytol 171:693–698
Ruiz-Lozano JM, Alguacil MM, Bárzana G, Vernieri P, Aroca R (2009) Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. Plant Mol Biol 70:565–579
Ruiz-Lozano JM, Porcel R, Azcón C, Aroca R (2012a) Regulation by Arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. J Exp Bot 63(11):4033–4044
Ruiz-Lozano JM, Porcel R, Bárzana G, Azcón R, Aroca R (2012b) Contribution of arbuscular mycorrhizal symbiosis to plant drought tolerance: state of the art. Plant responses to drought stress. Springer, Heidelberg, pp 335–362
Ruíz-Sánchez M, Aroca R, Muñoz Y, Armada E, Polón R, Ruiz-Lozano JM (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J Plant Physiol 167:862–869
Ruth B, Khalvati M, Schmidhalter U (2011) Quantification of mycorrhizal water uptake via high-resolution on-line water content sensors. Plant Soil 342:459–468
Schüβler A (2014) Glomeromycota: species list. [WWW document] URL http: // schuessler.user-web.mwn.de/amphylo. Accessed 1 Aug 2016
Schüßler A, Walker C (2011) Evolution of the 'plant-symbiotic' fungal phylum, Glomeromycota. In: Pöggeler S, Wöstemeyer J (eds) Evolution of fungi and fungal like organisms. Springer-Verlag, Berlin/Heidelberg, pp 163–185
Schüβler A, Schwarzott D, Walker C (2001) A new fungal phylum, the glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421
Shalabyl AM (2003) Responses of arbuscular mycorrhizal fungal spores isolated from heavy metal-polluted and unpolluted soil to Zn, Cd, Pb and their interactions in vitro. Pak J Biol Sci 6:1416–1422
Sheng M, Tang M, Chen H, Yang B, Zhang F, Huang Y (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza 18:287–296
Sheng M, Tang M, Zhang F, Huang Y (2011) Influence of arbuscular mycorrhiza on organic solutes in maize leaves under salt stress. Mycorrhiza 21:423–430
Singh BR, Singh A, Mishra S, Naqvi AH, Singh HB (2016) Remediation of heavy metal- contaminated agricultural soils using microbes. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 115–132
Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, San Diego
Smith SE, Facelli E, Pope S, Smith FA (2010) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326:3–20
Stommel M, Mann P, Franken P (2001) EST-library construction using spore RNA of the arbuscular mycorrhizal fungus *Gigaspora rosea*. Mycorrrhiza 10:281–285
Sudová R, Doubkova P, Vosatka M (2008) Mycorrhizal association of *Agrostis capillaris* and *Glomus intraradices* under heavy metal stress: combination of plant clones and fungal isolates from contaminated and uncontaminated substrates. Appl Soil Ecol 40:19–29
Talaat NB, Shawky BT (2014) Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. Environ Exp Bot 98:20–31
Talke IN, Blaudez D, Maathuis FJM, Sanders D (2003) CNGCs: prime targets of plant cyclic nucleotide signalling? Trends Plant Sci 8:286–293

Turnau K, Kottke I, Oberwinkler F (1993) Element localization in mycorrhizal roots of *Pteridium aquilinum* L. Kuhn collected from experimental plots treated with cadmium dust. New Phytol 123:313–324

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R (2007) Arbuscular mycorrhizal symbiosis and plant aquaporin expression. Phytochemistry 68:122–129

Upadhyaya H, Panda SK, Bhattacharjee MK, Dutta S (2010) Role of arbuscular mycorrhizal in heavy metal tolerance in plants: prospects for phytoremediation. J Phytol 2:16–27

Valentine AJ, Mortimer PE, Kleinert A, Kang Y, Benedito VA (2013) Carbon metabolism and costs of arbuscular mycorrhizal associations to host roots. In: Aroca R (ed) Symbiotic endophytes, soil biology, vol 37. Springer-Verlag, Berlin, pp 233–252

Verma S, Dubey R (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci 164:645–655

Vickers CE, Gershenzon J, Lerdau MT, Loreto F (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nature Chem Ecol 5:283–291

Walter MH, Strack D (2011) Carotenoids and their cleavage products: biosynthesis and functions. Nat Prod Rep 28:663–692

Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnol Adv 24:427–451

Wu QS, Zou YN (2010) Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. Sci Hortic 125:289–293

Wu QS, Zou YN, He XH (2013) Mycorrhizal symbiosis enhances tolerance to NaCl stress through selective absorption but not selective transport of K^+ over Na^+ in trifoliate orange. Sci Hortic 160:366–374

Yang X, Liang Z, Wen X, Lu C (2008) Genetic engineering of the biosynthesis of glycine betaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. Plant Mol Biol 66:73–86

Yang Y, Liang Y, Han X, Chiu T-Y, Ghosh A, Chen H, Tang M (2016) The roles of arbuscular mycorrhizal fungi (AMF) in phytoremediation and tree-herb interactions in Pb contaminated soil. Sci Rep 6:20469. doi:10.1038/srep20469

Yooyongwech S, Phaukinsang N, Cha-Um S, Supaibulwatana K (2013) Arbuscular mycorrhiza improved growth performance in *Macadamia tetraphylla* L. grown under water deficit stress involves soluble sugar and proline accumulation. Plant Growth Regul 69:285–293

Zarea MJ, Goltapeh EM, Karimi N, Varma A (2013) Sustainable agriculture in saline-arid and semiarid by use potential of AM fungi on mitigates NaCl effects. In: Goltapeh EM, Danesh YR, Varma A (eds) Fungi as bioremediators, soil biology 32. Springer-Verlag, Berlin, pp 347–369

Zhang HH, Tang M, Chen H, Zheng C, Niu Z (2010) Effect of inoculation with AM fungi on lead uptake, translocation and stress alleviation of *Zea mays* L., seedlings planting in soil with increasing lead concentrations. Eur J Soil Biol 46:306–311

Zou YN, Wu QS, Huang YM, Ni QD, He XH (2013) Mycorrhizal-mediated lower proline accumulation in *Poncirus trifoliata* under water deficit derives from the integration of inhibition of proline synthesis with increase of proline degradation. PLoS One 8:1–8

Zuccarini P, Okurowska P (2008) Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. J Plant Nutr 31(3):497–513

An Insight into Genetically Modified Crop-Mycorrhizal Symbiosis

20

D. Mohandass and T. Muthukumar

Abstract

Genetically modified crops (GMCs) are currently widely used in agricultural biotechnology where plants are engineered to express characters that defend them against different abiotic and biotic stresses. Many studies have revealed that GMCs have sequential benefits for the environment, human well-being, and farmers' economic growth, especially in densely populated countries. Several studies revealed that GMCs can significantly affect the soil microorganisms even their symbiosis with plants. Of these, arbuscular mycorrhizal fungi (AMF) are a good example for the widespread symbiotic relationship as they are associated with maximum crop species and provide several benefits in various agroecosystems. The AMF association can show an imperative functional character in the acquisition of nutrients by the crop plants. In this case, the associated response of transgenic crops and soil microorganisms in relation with AMF may be positive, negative, and neutral. Moreover, GMCs may influence AMF either directly and indirectly through modifications in root exudation or through discrepancies in the variety and action of soil microorganisms. Although *Bacillus thuringiensis* (Bt) corn is extensively cultivated, a limited number of studies have investigated the interaction of altered lines of Bt corn with symbiotic AMF. These studies pointed out that AMF colonization of genetically modified Bt corn lines differs with quantity and kind of engineered traits. Many research studies reported that GMCs do not affect AMF and failed to find any variations between non-Bt and Bt crops. In contrast, some studies reported a substantial decrease in AMF colonization levels. Therefore, we gathered the information available on the influence of GMCs on AMF in this chapter and consider that it will explore interesting insights on mycorrhizal symbioses in the modern agroecosystems.

D. Mohandass • T. Muthukumar (✉)
Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu 641046, India
e-mail: tmkum@yahoo.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_20

Keywords
Arbuscular mycorrhizal fungi (AMF) • Agroecosystems • *Bacillus thuringiensis* (Bt) • Rhizosphere • Soil fungi

Terms, Units, and Acronyms

AM	Arbuscular mycorrhizal
AMF	Arbuscular mycorrhizal fungi
Bt	*Bacillus thuringiensis*
GMC	Genetically modified crop
GMCs	Genetically modified crops

20.1 Introduction

For two decades, genetically modified crops (GMCs) have been successfully introduced and cultivated in about 28 countries worldwide, and the produces are currently available in the respective local markets (Prakash et al. 2011; James 2012; Zhang et al. 2016). Over 90% of the 179.7 million hectares under commercial GMC cultivation is in the USA, Brazil, Argentina, India, and Canada (James 2013; Fig. 20.1). Among the GMCs with different traits, herbicide-resistant cultivars occupy the major area under GMC cultivation followed by Bt crops and the combination of herbicide-resistant and Bt crops (Beckie and Hall 2014; Fig. 20.2). This suggests that cultivation of GMCs may not be harmful to the surrounding ecosystem such as the plant-beneficial soil microorganisms. Nevertheless, several researchers suspect that rapid and widespread adoption of GMC cultivation may influence nontarget microorganisms like bacteria (Saxena and Stotzky 2001a; Wu et al. 2004; Castaldini et al. 2005), fungi (Ferreira et al. 2003; Turrini et al. 2004a, b; Oliveira et al. 2008), protozoa (Donegan et al. 1996; Griffiths et al. 2008), nematodes (Khan et al. 2010; Hoss et al. 2011), and soil invertebrates (Hönemann and Nentwig 2009; Liu et al. 2009) in the soil environment. These organisms play a crucial role in upholding plant health and fertility of the soils through the decomposition of organic matter and nutrient mineralization (Smith and Read 2008; Willis et al. 2013). Of these, arbuscular mycorrhizal fungi (AMF) are pervasive soil microorganisms that play a pivotal role in the sustainable soil environment as they form a mutualistic symbiosis with around 70–90% of the terrestrial plant taxa (Wang and Qiu 2006; Parniske 2008). AMF render several benefits to their associated host plants including the uptake and transport of nutrients from the nutrient-stressed soils to the plant roots, in exchange for carbohydrates. In addition, AMF also play a crucial role in imparting tolerance against various abiotic and biotic stresses in their associated host plants resulting in increased growth and productivity (Steinkellner et al. 2012; Hajiboland 2013).

Scientists and farmers presume that transgenic crops might negatively influence AMF abundance and diversity in cultivated soils. Changes in the diversity or activities of soil microorganisms are known to affect AMF in the soil. Therefore, any undesirable influence of GMC cultivation on soil microbial communities would

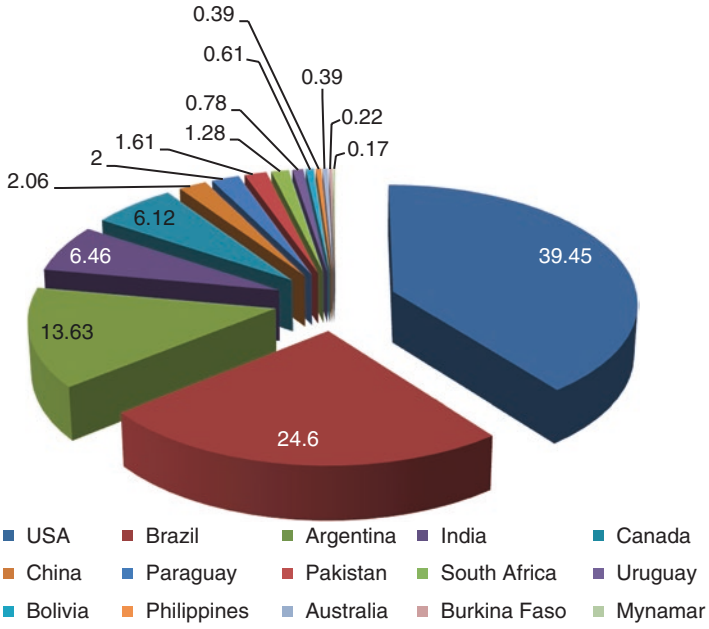


Fig. 20.1 Percentage area of the top 15 countries (total 179.7 million hectares) under commercial genetically modified crop cultivation as of 2015 (Data from GeneWatch 2016)

subsequently induce several negative consequences on AMF community structure, functions, and diversity. However, few studies have examined the actual impact of GMCs on AMF. Studies examining the influence of GMC on arbuscular mycorrhizal (AM) symbiosis have shown both positive and negative effects of GMCs on AM formation and function (Liu 2010; Zeng et al. 2015). For example, GMCs could negatively influence AM formation by reducing the colonization rate of the fungi by reducing the growth rate of the fungal hyphae in the soil (Cheeke et al. 2012a; Zeng et al. 2015). The root exudates of Bt corn considerably reduce the presymbiotic hyphal formation during the colonization process (Turrini et al. 2004a). Therefore, the percentage root length colonized by AMF in Bt corn is often lower than those of the wild-type (Castaldini et al. 2005; Tan et al. 2011). Contrarily, several studies have also revealed the lack of any significant influence of GMCs on AM fungal colonization, spore abundance, or diversity (Liu 2010; Hannula et al. 2014).

Thus, it would be interesting to evaluate the impact of GMCs on AM formation and function in agroecosystems. Most of the studies assessing the impact of Bt corn on AMF were primarily conducted during a single cropping season or year, in spite of the fact that Bt proteins could sustain their effect for 180–234 days in the soil (Saxena and Stotzky 2001b). However, a study conducted by Zeng et al. (2014) on the seasonal effects of GMCs on AMF revealed no significant influence of season on AMF colonization (Zeng et al. 2014). Nevertheless, considerable variations in soil nutrients and AMF colonization have been reported due to GMC cultivation (Liu 2010; Hannula et al. 2014). Therefore, we assessed the effects of GMCs on the

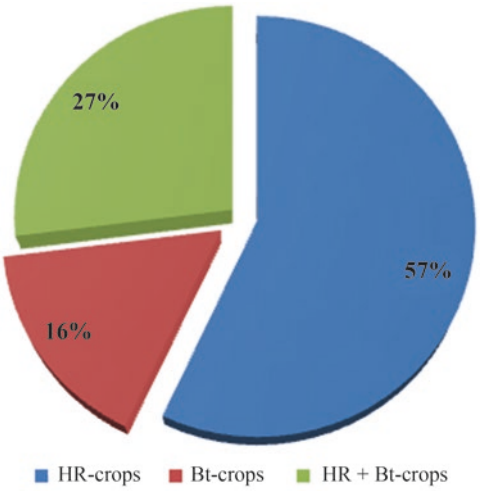


Fig. 20.2 Percentage global area under genetically modified crop cultivation with specific traits (*HR* herbicide resistant, *Bt* pest resistant) in 2013 (Data from James 2013)

functioning of AMF among the agricultural practices which would bring important insights to understand the advantages and disadvantages of the GMC cultivation to the human health and environment. Here, we analyzed the impact of GMCs on AMF colonization, structure, functions, spore diversity, and soil dynamics. We also discuss whether GMCs will be beneficial to nontarget organisms like AMF and their related environments.

20.2 GMCs and Nutrient Enhancement

GMCs are genetically modified and do not occur naturally. Initially, many experts and farmers failed to appreciate both the cultivation and consumption of GMCs (Wohlfender-Bühler et al. 2016). This arose from the assumption that GMCs might be nutritionally inferior due to their short growth period and the presence of transgenes. In contrast, researchers believed that genetic modification may boost nutrients and vitamins in genetically modified (GM) food crops like rice, cassava, millet, potato, and other crops that are rich in nutrients (Anderson 2001; Nottingham 2002; Motavalli et al. 2004). In addition, it could be beneficial for the small farmers by reducing the incidence and severity of pest and diseases in crops (Klümper and Qaim 2014). Thus, GMCs are widely preferred in developing countries. But, there tends to be a basic variation in the creation of GMCs and GM foods that are cultivated and used in developing and developed countries. In developed countries, cultivation of GMCs is directed towards high productivity, whereas in developing countries, the goal of developing GMCs is to enhance the nutritional contents of the foods to ensure adequate nutrient consumption by its people.

The World Health Organization indicated that malnutrition is an important cause of all childhood deaths in developing countries. Threefold juveniles in developing countries are malnourished and fivefolds have stunted growth, and thus there is

strong evidence to show that these children are deficient in iron, vitamins, zinc, and iodine (Anderson 2001). Thus, scientists strongly believe that GMCs are the only probable solution to improve children's vitamin and nutrition intake in developing countries. Therefore, transgenic crops could improve the nutrient content of the principal foods that lack some macronutrients such as amino acids; micronutrients, such as iron; and vitamins like vitamin A (Anderson 2001).

Certain crops like rice and cassava are rich in calories but are deficient in vitamin A, iron, and protein (Montagnac et al. 2009; Ali et al. 2016). Therefore, by introducing transgenes into these crops, we can enhance nutrients like vitamin A, iron, and protein for these commonly cultivated crops (Zhu et al. 2007). The GM rice named "golden rice" containing beta-carotene, which is converted into vitamin A (Ye et al. 2000), is yet to be introduced for commercial cultivation. Moreover, the practice of farming GMCs could diminish the rate of chronic disease by increasing nutritional values of the foods consumed. Interestingly, GMCs let farmers use fewer biocides, as the crops are pathogen resistant. It benefits the environment, reduces production costs, and is less labor intensive, and crop yields are generally higher. Therefore, it is suggested that GMCs are beneficial to human health and agriculture, commercially viable, and environment-friendly (Wohlfender-Bühler et al. 2016). Nevertheless, a holistic method is needed to investigate the effect of GMCs on both environmental and human health impacts.

20.3 Influence of GMCs on Soil Microorganisms

Analyzing the impact of GMCs on soil microorganisms could identify important ecological risks associated with GM cultivation that would be fundamental for the accomplishment of biogeochemical cycles and soil fertility (Giovannetti et al. 2005). Transgenic crops can exudate as much as 20% of assimilates into plant rhizosphere soil as root exudates during their growth cycle (Whipps 2001). Several studies demonstrate the impact of GMCs on soil microorganisms (Giovannetti et al. 2005). Of these, the majority of the studies revealed that GMCs influence soil microorganisms either negatively or positively through direct and indirect effects (Motavalli et al. 2004; Liu 2010). Cultivation of GMCs may also result in the transfer of the transgenes to their wild relatives, weeds, and may also induce trait modifications in nontarget plant species (Motavalli et al. 2004; Zeng et al. 2014, 2015). The direct influence of GMCs includes transgenic proteins that induce various effects against insects or pathogenic fungi and bacterial communities in the soil. Generally, the cauliflower mosaic virus (CaMV) 35S promoter used in the development of transgenic plants often increases the environmental risks accompanying GMCs (Ho et al. 1999). The CaMV 35S viral promoter stimulates the production of transgenic proteins in all plant tissues. These transgenic proteins, when released from the plants, accumulate and do not degrade easily in the soil. Thus, these transgenic proteins remain stable for a prolonged period based on their potential bioavailability and persistence affecting the soil microorganisms directly (Saxena et al. 1999; Zwahlen et al. 2003; Giovannetti et al. 2005). Other direct effects include

alterations in soil microbial mobility in response to changes in the quality and quantity of root exudates, changes in microbial activity, and alterations in microbial populations resulting from GMC management practices like the application of chemical and biofertilizers and pesticide and tillage applications (Motavalli et al. 2004).

Researchers strongly believe the possibility of gene flow from GMCs to closely related crop varieties and wild relatives through cross-pollination. Since most crops tend to outbreed, it is reasonable to believe that gene flow might also occur with transgenic crops (Mercer and Wainwright 2008; Prakash et al. 2011). However, researchers are not fully aware of any actual gene transfer from the GMCs to their wild relatives. In terms of nontarget species, some transgenic characteristics like pesticidal toxins expressed by Bt genes may affect nontarget species as well as crop pests. Though field studies revealed a certain level of variations in soil microbial communities associated with non-Bt and Bt crops (Cheeke et al. 2011, 2012a), most of these variations were not overtly negative (Yang et al. 2002; Xue et al. 2005). Nevertheless, certain studies have also adequately demonstrated that GMCs could negatively influence soil microorganisms and nontarget species. Therefore, proper monitoring and management of agricultural practices for GMCs are necessary while releasing them for cultivation.

The indirect effects of GMCs on microbial communities in the soils are often complex and are highly influenced by the nature and composition of root exudates and plant metabolic activity. Previous studies on the indirect effect of transgenic crops on microbe-mediated processes in the soil suggest changes in the quantity and composition of crop residues from transgenic crops. For example, the low N content of the Bt corn residues affects its decomposition and mineralization resulting in its persistence in the soil for a long time after the crop harvest (Motavalli et al. 2004). Therefore, transgenic Bt corn residues containing transgenic proteins may reduce the microbe-mediated nutrient processes in the soil (Motavalli et al. 2004). This is further confirmed by studies where frequent cultivation of transgenic crops has been shown to accelerate the accumulation and persistence of Bt proteins in the soil (Saxena et al. 2010; Bakhsh et al. 2015). Therefore, soil microorganisms are influenced by Bt toxins that originate either from the transformation process or plant tissue culture procedures, instead of inserted genes. All these findings do suggest that GMCs significantly altered structure and functions of soil microorganisms.

20.4 Impact of GMCs on Soil Fungi

Most studies that determined the effect of transgenic proteins on soil fungi failed to find any strong negative impact of these proteins on saprotrophic fungi (Table 20.1) (Saxena and Stotzky 2001a; Koskella and Stotzky 2002; Ferreira et al. 2003; Icoz et al. 2008; Oliveira et al. 2008). For example, there were no significant differences in culturable soil fungal populations when soil was amended with purified Cry1Ab or Cry1Ac protein (Donegan et al. 1995). In the same study, a transient increase in culturable fungal populations was also reported in soils amended with Bt cotton.

Table 20.1 Overview of studies reporting the influence of genetically modified (GM) crops on non-mycorrhizal and arbuscular mycorrhizal fungi (AMF) by the influence of different modified traits was examined from survey of different literatures

Impacts of GMCs	GM plant names	References
Neutral	Alfalfa (*Medicago sativa*), American elm (*Ulmus americana*), aspen (*Populus tremula* x *P. tremuloides*), birch (*Betula pendula*), brinjal (*Solanum melongena*), canola (*Brassica napus*), cotton (*Gossypium hirsutum*), flax (*Linum usitatissimum*), potato (*Solanum tuberosum*), soybean (*Glycine max*), tobacco (*Nicotiana sylvestris*), tomato (*Solanum lycopersicum*)	Donegan et al. (1999), Newhouse et al. (2007), Kaldorf et al. (2002), Seppänen et al. (2007), Turrini et al. (2004b), Flores et al. (2005), Blackwood and Buyer (2004), Tan et al. (2010), Kuramae et al. (2013), Verbruggen et al. (2012), Cheeke et al. (2013), Naef and Defago (2006), Lawhorn et al. (2009), Saxena and Stotzky (2001), Hart et al. (2009), Powell et al. (2009), de Vaufleury et al. (2007), Zeng et al. (2015), Knox et al. (2008), de Souza Vieira et al. (2011), Li et al. (2011), Wrobel-Kwiatkowska et al. (2012), Donegan et al. (1996), Götz et al. (2006), Weinert et al. (2009), Gschwendtner et al. (2010), Hannula et al. (2010), Wu et al. (2009), Ren (2006), Lee et al. (2011), Chun et al. (2012), Powell et al. (2007), Weaver et al. (2007), Liang et al. (2015), Vierheilig et al. (1993), and Girlanda et al. (2008)
Negative	Canola (*Brassica napus*), corn (*Zea mays*), rice (*Oryza sativa*)	Dunfield and Germida (2001), Xue et al. (2005), Icoz et al. (2008), Oliveira et al. (2008), Turrini et al. (2004a), Cheeke et al. (2011), Xue et al. (2011), Fließbach et al. (2012), Tan et al. (2011), Cheeke et al. (2013), Turrini et al. (2004a), Castaldini et al. (2005), Cheeke et al. (2012), Seres et al. (2014), Cowgill et al. (2002), Hannula et al. (2012), Hannula et al. (2013), Xue et al. (2005), Donegan et al. (1995), Liu et al. (2008), Wu et al. (2004), Lu et al. (2010), Yang et al. (2002), Tahiri-Alaoui et al. (1994), Vierheilig et al. 1995), Medina et al. (2003), and Tilston et al. (2013)
Positive	Barrel medic (*Medicago truncatula*), papaya (*Carica papaya*)	Boisson-Dernier et al. (2001), Cortet et al. (2006), Kremer and Means (2009), Wei et al. (2006), O'Callaghan et al. (2008), Henault et al. (2006)

Similarly, a lack of difference in culturable fungal populations was also reported in soil microcosms cultivated with non-Bt and Bt corns (Saxena and Stotzky 2001b; Flores et al. 2005). Nevertheless, the influence of Bt corn biomass buried in litterbags on soil fungal communities involved in the litter decomposition as confirmed by T-RELP was attributed to environmental factors rather than the presence of Cry3 Bb protein (Table 20.1; Xue et al. 2011). The slow decomposition rate of Bt corn, canola, potato, rice, and tobacco residues in the soil was attributed to the higher lignin content in these residues (Saxena and Stotzky 2001b; Stotzky 2004; Flores

et al. 2005; Poerschmann et al. 2005). Contrarily, no significant variations in lignin content between Bt and non-Bt cultivars (Jung and Sheaffer 2004; Mungai et al. 2005; Lang et al. 2006) and subsequently a slightly higher decomposition rates have been observed for Bt than non-Bt crop residues (Lehman et al. 2008; Tarkalson et al. 2008; Wu et al. 2009).

Cultivation of genetically engineered Bt potato failed to exhibit any negative effect on soilborne pathogens like *Fusarium* sp., and *Pythium* sp., in the plant rhizosphere under field conditions (Donegan et al. 1996). A mass spectrometric study showed that some of the Bt and non-Bt corn lines differed more in their volatile organic compound composition than the presence of Cry protein (Naef et al. 2006). This finding is an example of the pleiotropic effect occurring in GMCs that can alter the nature of the transgenic plant tissue, potentially affecting the degrading time of the residues and fungal community structure (Naef et al. 2006).

Moreover, the influence of transgenic plants on AM formation may vary with GMC varieties. For example, the Bt corn event Bt 176 exhibited reduced AM colonization levels and arbuscule development when compared to another Bt corn line event Bt 11 and a non-Bt parental isoline during primary stages of plant development. In addition, both the Bt cultivars had less arbuscule formation than the parental corn lines (Castaldini et al. 2005). Besides these, other studies pertaining to Bt and non-Bt corn failed to reveal any differences in the intensity or frequency of AM root colonization (de Vaufleury et al. 2007). In summary, GMCs significantly affect all groups of fungi including saprophytic, pathogenic, and mycorrhizal fungi; however, the response of all the fungal groups varies with respect to their transgenic Bt lines.

20.5 Mycorrhizal Associations in GMCs

Mycorrhizal fungi are one of the important soil organisms that can be affected by farming of transgenic crops. These fungi play important roles in the soil ecosystem as nutrient recyclers and plant symbionts (Cheeke et al. 2012b). The AMF association is an ideal example to understand the fundamentals and mechanisms involved in GMC-microbe symbiosis. Any influence of GMCs on AMF might have a more immediate consequence on GMCs farming because these fungi associate with a wide range of crop species (Wang and Qiu 2006). AMF are mostly beneficial to plant development, crop productivity, and ecosystem processes (Smith and Read 2008). The mycorrhizal benefit to the plants is normally mutualistic with the exchange of nutrients and carbon between the associating partners. In GMCs, the gene insertion may induce modifications in plant physiology, although the transgenic protein may concentrate either in the rhizosphere or in the plant tissues affecting mycorrhization and mycorrhizal benefits. A study examining the effect of GMCs on mycorrhizal formation showed that the root exudates of Bt corn (event Bt 176) significantly reduced the development of presymbiotic hyphae by *Funneliformis mosseae* (=*Glomus mosseae*), and 36% of the appressoria failed to produce viable infection pegs to penetrate plant roots (Cheeke et al. 2012a). Thus Bt isoline failed

to acquire the same amount of colonization as their non-Bt isoline conspecifics. Although this study suggested that the host recognition mechanism of the fungus was not disrupted by the presence of GMCs, some unknown plant/fungal factors might have limited the colonization of AMF in this Bt isoline (Cheeke et al. 2012b). Several greenhouse studies demonstrate that the mycorrhizal colonization and spore abundance in Bt corn were lower than their parental lines indicating that AMF is likely to colonize and benefit the GMCs under optimum conditions (Cheeke et al. 2011). Moreover, the study also suggested that the amount of fertilizers and the quantity of AMF inoculum added had a greater influence on the AMF colonization levels both in Bt and non-Bt isolines (Cheeke et al. 2011). Therefore, the interaction between GMCs and AMF should be closely monitored, particularly in low-input farming systems where reliance on a healthy soil microbial community for maintaining plant fitness and nutrition is of great relevance.

20.6 Incidence and Diversity of AMF in GMCs

Agriculture biotechnology introduced GMCs into the modern cropping systems which might have resulted in unintentional adverse consequences on the surrounding ecosystem, such as plant-beneficial AMF diversity (Liang et al. 2015). AMF have been considered as an important nontarget organism for studying the effect of GMCs on the environment (Turrini et al. 2015). For instance, several GMCs either had a higher or lower amount of secondary metabolites or alterations in crop chemistry that were not directly linked to the specific genes introduced (Smith and Read 2008; Pu et al. 2012; Hannula et al. 2014). The overall structure and functions of belowground microbial communities may be affected due to the GMCs (Liang et al. 2015). Therefore, GMCs may cause changes in AMF occurrence and diversity that could influence the variety of AMF associated with the target and nontarget plant species. Several studies have also shown that GMCs can change the diversity of the whole rhizosphere-associated fungal and bacterial communities, but sometimes there may be little or no variations among soil microbial communities (Pu et al. 2012; Meyer et al. 2013; Liang et al. 2014). Moreover, many study results showed that GMCs can change the development of AMF like a delay in colonization, decrease in presymbiotic hyphal growth, and effects on the regular appressoria development that reflects negative impacts of GMCs on AMF (Vierheilig et al. 1995; Turrini et al. 2004a). In contrast to some studies revealing a significant impact of Bt corn on AMF community structure (Castaldini et al. 2005; Tan et al. 2011; Cheeke et al. 2012a), others suggest that GMCs do not affect AMF community structure (Turrini et al. 2004a; Powell et al. 2007; Cheeke et al. 2011; Verbruggen et al. 2012; Hannula et al. 2012; Meyer et al. 2013). The percentage of AMF root colonization increased from juvenile to mature GM wheat plants, and therefore the *Pm3b* mildew resistance transgene had no strong impact on AM root colonization (Meyer et al. 2013). The Shannon diversity index for AMF diversity revealed negligent differences between GMCs and non- GMCs (Liang et al. 2015). In contrast, a decline in AMF colonization levels in different Bt corn lines has also been reported

(Cheeke et al. 2012a). In summary, GMCs do not have a much positive influence on AMF colonization and diversity as shown by several experimental studies. However, further studies are mandatory to evaluate the structure and growth of AMF colonization and diversity on different GMCs that use long-term harvesting under natural environment.

20.7 Possible Mechanisms of Interaction Between AMF and GMCs

Certain key factors through which GMCs might influence the AMF include altered gene expression levels in plant roots, changes in the quality and quantity of the root exudates, unintentional modifications of chemical concentrations of host plant, and the persistence of genetically modified proteins in the soil (Liu 2010). Few studies involving Bt corn and Dm-AMP1 aubergine plants have demonstrated the exudation of transgenic proteins from the roots through root exudates (Saxena et al. 1999; Turrini et al. 2004b). It is well established that the toxins or antimicrobial substances present in the plant root exudates may affect nontarget soil microorganisms and their communities (Siciliano and Germida 1999; Griffiths et al. 2007). Turrini et al. (2004a) also showed that the exudates originating from Bt 176 corn roots negatively affected the development of the presymbiotic hyphae, compared to Bt 11 and non-Bt plants. But, no such negative effect on the host recognition system of AMF or steps leading to the establishment of mycorrhizal symbiosis by *F. mosseae* was evident for the antimicrobial protein Dm-AMP1 exudated from aubergine (*Solanum melongena* L.) cv. Violetta roots (Table 20.1) (Turrini et al. 2004a, b). Turrini et al. (2004b) hypothesized that AMF like *F. mosseae* lack suitable binding sites for Dm-AMP1 on the hyphae as this protein induces membrane destabilization only after binding to membrane patches containing sphingolipids (Thevissen et al. 2000, 2003). In addition, the typical growth enhancement of GMCs due to the establishment of AMF symbiosis suggests that the symbiosis established was functional. As the development of AMF mycelium is sensitive to the nutritional changes in the soil environment, this AMF parameter has often been used as an indicator to assess the impact of different biological or chemical substances on AMF (Turrini et al. 2004a). Moreover, the capability of AMF mycelium to tolerate plant defense compounds indicates the ability of AMF to tolerate plant secondary metabolites like phenolics, phytoalexins, glucanases, and chitinases, which exhibit a transient increase in root cells during initial stages of the symbiosis establishment (Dumas-Gaudot et al. 1994; Volpin et al. 1994). In a field experiment, the linear relation between leaf chlorophyll content of corn plants and AMF colonization at harvest indirectly suggests that the carbon fixation or allocation might be an overriding factor controlling AMF colonization in GMCs (Cheeke et al. 2013). This view is supported by the presumption that changes in soil nutrient availability might influence mycorrhizal colonization levels both in Bt and non-Bt plants. Readily available nutrients in the soil may reduce AM colonization in plant roots as the carbon cost of supporting the fungal symbionts exceeds the benefits received (Johnson et al. 2003;

Cheeke et al. 2011). Thus the nature of the relationship may tilt from mutualism to parasitism (Johnson et al. 2003). Future investigation on the variations in AMF community composition in relation to changes in soil nutrient availability in non-Bt and Bt corn would help to explore and understand the nature of the interactions between these plant-fungus associates (Cheeke et al. 2013).

20.8 Variations in the Mycorrhizal Dependency of GMCs and Non GMCs

Colonization of plant roots by AMF is commonly influenced by several factors like soil, host plant, pathogens, drought, and local environmental conditions (Cheeke et al. 2012b; Zeng et al. 2015). Among these, soil factors are prominent in influencing AMF as colonization levels tend to vary with soil types. Many studies have reported differences in the levels of AMF colonization between Bt and non-Bt plants. Of these, lower level or reduced AMF colonization was reported in genetically modified corn and tobacco (Turrini et al. 2004a; Castaldini et al. 2005; Cheeke et al. 2011, 2012a). In contrast, no differences were observed in the extent of AMF colonization between non-Bt and Bt plants (Kaldorf et al. 2002; Xue et al. 2005; Flores et al. 2005; Naef et al. 2006; de Vaufleury et al. 2007). Moreover, incorporation of transgenic Bt proteins in the substrates negatively influences root colonization by AMF in many cultivated non-Bt plants (Yang et al. 2002). These variations in Bt-induced effects on root colonization by AMF were also affected by local environmental conditions, cultural practices, and GMC remnants in the soil (Xue et al. 2011). Moreover, the persistence and biological activity of Bt toxins in the soil are critical parameters for examining the risk potentiality and environmental prospects of GMC remnants in the agricultural soils (Icoz and Stotzky 2008).

Variations in the root colonization levels by AMF in GMCs were influenced by high P fertilization in the crop varieties of GM corn and wheat (Hetrick et al. 1995; Kaeppler et al. 2000; Sawers et al. 2008). Nevertheless, the mycorrhizal responsiveness may vary with crop varieties (Kaeppler et al. 2000; Schultz et al. 2001; Gao et al. 2007; Seifert et al. 2009). Significant variation in mycorrhizal colonization was induced by various Bt proteins, particularly Cry1Ab protein (Turrini et al. 2004a; Castaldini et al. 2005; Ren 2006; de Vaufleury et al. 2007; Cheeke et al. 2012a; Seres et al. 2014). The varied expression levels of modified genes on GMC roots are often exemplified by the modifications in the root exudates, persistence of transgenic proteins in agricultural soils, and modifications in the chemical composition of GMCs which may all influence AMF. Hence, both intraradical and extraradical structures of AMF in GMCs are exposed to both antimicrobial and pesticidal toxins. Additionally, exposure to toxins like Bt could be experienced throughout the life cycle of AMF and even longer when these toxins are exudated throughout the entire growth period of the GMCs (Rui et al. 2005). Consequently, GMC plants may impact AMF development over their whole life cycle by exudating antimicrobial compounds that may cause variation in the mycorrhizal colonization between non-Bt and Bt lines (Liu 2010).

20.9 Assessing Effects of GMCs and AMF on Soil Dynamics

Agricultural practices that involve the utilization of chemical fertilizers, biocides, tillage, and monocultures are harmful to AMF (Gosling et al. 2006). However, studies on effects of GMCs and the activity of AMF on soil dynamics might be important functional attributes in most plants. Carpenter (2011) reported in a study that GMCs could influence the soil functions like the movement of nutrients, cycling of N, and decomposition of wastes. Available evidence does indicate that the interaction between GMCs and AMF on soil dynamics could be either beneficial or harmful (Liu 2010; Wróbel-Kwiatkowska et al. 2012). This could arise from the fact that GMCs might drive nontarget organisms like the AMF; however, their role and functions could vary based on their level of transformation. The nontarget effect of GMCs on microorganisms involved in microbe-mediated activities in the soil appears due to the introduction of Cry proteins into the soil through the cultivation of GMCs (Masoero et al. 1999; Escher et al. 2000; Saxena and Stotzky 2001a; Dinel et al. 2003; Manachini et al. 2004; Rui et al. 2005; Griffiths et al. 2005). A 3-year field study confirmed the release of Cry protein into the soil by Bt corn in the form of root exudates continuously throughout the plant growth period. However, the levels of soil Cry proteins were not correlated with the specific stage of plant growth (Nguyen Thu 2004; Baumgarte and Tebbe 2005). Thus, frequent cultivation of GMCs leads to the continuous release and accumulation of Cry proteins in the rhizosphere. In addition, the binding of Cry proteins to soil components can result in their accumulation and persistence in soils that are repeatedly cultivated with GMCs on a large scale (Tabashnik 1994; Saxena and Stotzky 2001a; Muchaonyerwa et al. 2004). Besides, Lilley et al. (2006) reviewed the information available on the influence of insecticidal Cry proteins originating from GMCs on soil ecosystems, including soil microorganisms, microbe-mediated activities, and soil-inhabiting invertebrates and the fate and persistence of Cry proteins in the soil. There is always the possible risk of Cry protein accumulation with increasing cultivation and use of GMCs. But, experimental studies suggest that Cry proteins do not remain for a long time and degrade rapidly in the soil (Icoz and Stotzky 2008). Hopkins and Gregorich (2003) also showed that the Bt endotoxin in corn residues is highly unstable and rapidly decomposes in field soils, but a small portion of it may be secured from degradation in relatively recalcitrant form.

Although information dealing with Bt transgenic crops in relation to soil ecosystems have been published (Shelton et al. 2002; Saxena and Stotzky 2003), certain studies have highlighted nontarget effects of GMCs on AMF (Saxena and Stotzky 2003; O'Callaghan et al. 2005). The different effects of GMCs on soil microorganisms are mainly at the rhizosphere level, where root exudates directly affect the composition of microbial soil communities (Wieland et al. 2001; Chelius and Triplett 2001; Mansouri et al. 2002; Gomes et al. 2003; Lynch et al. 2004).

20.10 Do GMCs Influence AMF Diversity?

Certain studies indicate that the intensity of local environmental conditions affects AMF abundance, spore diversity, and community composition resulting in structural and functional variations (Glinka and Hawkes 2014). However, AMF community is critical for plant nutrition availability (Li et al. 1997), disease tolerance (Selosse et al. 2004), and plant productivity through ecological functioning (vander Heijden et al. 1998). The diversity and abundance of AMF spores associated with GMCs and non-GMCs depends on local environmental conditions. However, field-grown Bt and non-Bt corns did not show significant variations in AMF spore densities or root colonization (Kabir et al. 1998; Oehl et al. 2005; Isobe et al. 2008). In contrast, low AMF spore diversities were found to be associated with GMCs in the field experiments compared to those in natural systems (Helgason et al. 1998; Oehl et al. 2005). Nevertheless, low AMF diversity is also common in crops cultivated under continuous monocropping (Helgason et al. 1998; Oehl et al. 2005).

Previous studies demonstrate that the expression of the Cry1Ac protein in GM cotton plants does not affect the endophytic fungal communities as indicated by multivariate analysis. This statistical approach combines different measurements from the sample and recognizes relations and interactions between factors and hence is a powerful tool to understand external influences on biodiversity (Andreote et al. 2009). For this reason, many studies have employed this important statistical technique to calculate the impacts of GM plants on their associated microbial communities (Andreote et al. 2008). Several factors like GM parent, growth phase, field site, season, and cultivar were used to study the effects of GMCs on AMF communities (Cheeke et al. 2013). Among these, factors like field site, season, plant growth phase, and year were found to affect the AMF communities in a significant manner, while GM traits have either a transient or no effect (Table 20.1; Kabir et al. 1998; Castaldini et al. 2005; Grigera et al. 2007; Schaarschmidt et al. 2007). Similarly, Cotton et al. (2015) detected that the communities of AMF can change dramatically between years of cropping. Contrarily, AMF species diversity assessed by Shannon diversity index was almost similar for transgenic soybean cultivar (ZD91) containing the *Arabidopsis* cystathionine γ-synthase (*AtD-CGS*) gene and the wild-type line (ZD) within the similar growth phase during two consecutive years (Liang et al. 2015). The colonization of AMF and spore diversity was lower in Bt rice cultivars than those of the parental lines (Yang et al. 2002; Ren 2006). In summary, the results on the effect of GMCs on AMF diversity are rather inconsistent as some studies reveal a neutral response, while others showed negative responses.

20.11 Effects of GMC Plant Residues on AMF

The GMC remnants present in the soil containing Bt proteins can be identified using enzyme-coupled immunosorbent assay (Zeng et al. 2015). The strong variation found in the concentrations of the Cry1Ab protein in roots of dissimilar Bt corn line indicates that the manifestation of this protein varies among transgenic

cultivars developed using different transformation events (Zeng et al. 2015). The protein residues released from Bt plant roots may remain active and persist for a prolonged time in the soil (Crecchio and Stotzky 1998; Saxena and Stotzky 2001a; Stotzky 2004). Nevertheless, the low concentrations of Cry1Ab protein in the plant rhizosphere and bulk soil than in the plant roots of some Bt plants suggest the lack of accumulation of Bt proteins (Zeng et al. 2015). Further, the expression level of the Bt proteins in the plant roots of GMCs can also influence the AMF colonization (de Vaufleury 2007). Hence, the variations in the concentrations of the Cry1Ab protein in the plant roots of Bt and non-Bt crop cultivars could affect plant-AMF symbioses differently.

In addition, higher levels of AMF colonization were observed in the two Bt than the non-Bt corn lines suggesting that continuous cultivation of Bt corn lines does not adversely affect AMF colonization (Zeng et al. 2015). It also appears that the Bt protein remnants in the plant rhizospheres act as an organic matter amendment and not as a toxin (Saxena and Stotzky 2001b). However, an experimental study showed that GMC residues significantly reduced the proportion of colonization by indigenous AMF as assessed after 4 months of plant growth (Zeng et al. 2015). This could be related to the slower decomposition rate of the GMC residues than that of non-GMC residues (Saxena and Stotzky 2000; Dinel et al. 2003; Stotzky 2004; Flores et al. 2005; Castaldini et al. 2005; Raubuch et al. 2007; Cheeke et al. 2012a; Zeng et al. 2015). Moreover, there is also a probability that GMC toxic residues might negatively affect the organisms that biologically decompose organic matter in the soil, some of which are also known to stimulate mycorrhization. This effect can be elaborated through the discharge of toxic exudates by the plant roots of GMCs which subsequently get sorbed to clay particles or organic matter and maintain biocidal activity (Saxena et al. 1999, 2002; Saxena and Stotzky 2000; Lehman et al. 2008).

At harvest, large quantities of plant residue enter into the soil and are subsequently decomposed (Saxena et al. 1999; Saxena and Stotzky 2000). These GMC residues may bind to soils of humic acids and clays and retain their pursuit for a substantial period. Such persistence of the GMC residues may cause an adverse effect on AMF and other soil microorganisms (Saxena and Stotzky 2001b). The lower degradation rate of GMC residues was attributed to the increased lignin contents in the remnant compound of GMC. However, the recent evidence put forward the differences in phytoconstituents and microbial communities between GMCs and their near isogenic lines and may be reliable for the differences in mineralization amounts of the GMC residues (Poerschmann et al. 2005; Naef et al. 2006; Raubuch et al. 2007). Moreover, the association between plants and soil microbial community may induce changes in the phytoconstituents of plants and thus affect mineralization of their residues (Roessner et al. 2000; Cellini et al. 2004; Liu et al. 2005; Watanabe et al. 2007; Raubuch et al. 2007).

20.12 Impact of GMCs Cultural Practices on AMF

Sustainable agricultural practices provide various advantages like ecological benefits, economical viability, and social security for the production of human food (Gips 1987). It aims to recycle the detritus in the farming systems with few peripheral inputs and to conserve the high biodiversity of the agroecosystems. Further, the sustainable farming also helps in biological weed control and improves exploitation of soil-plant-microbe interactions for plant nutrition and defends against pests and pathogens (Edwards et al. 1990). Traditional agricultural practices benefit several soil microorganisms including AMF that provide minerals to plants and are directly involved in crop production (Plenchette et al. 2005). There are numerous agronomic practices that directly influence the AMF prevalence and activity. A few crop modifications comprise processes like breeding, application of pesticides or growth regulators, coating the seeds with fungicides, and modifications in soils which include the application of fertilizers, farming to destroy weeds, biocide application, tillage, and fallow (Plenchette et al. 2005). The breeding programs are often conducted in soils where mineral nutrients are not limiting plant growth, thus indicating that plant breeding conditions may not support optimum AMF colonization or activity among GMCs (Azcon and Ocampo 1981; Plenchette et al. 2005). In addition, the application of inorganic fertilizers to improve soil fertility decreases mycorrhizal development. But this influence depends on the nutrient requirement of the crop that is selected. Therefore, crops like GMCs which have high growth rates, high nutrient demand, and high mycorrhizal dependency may affect the structure and function of AMF if the nutrient demand of these crops is satisfied through fertilizers. However, James (2013) repatriated that the utilization of GMCs by farmers worldwide has reduced the pesticide usage by half thereby saving their chemical costs substantially, and this also reduced the exposure of farmers to insecticides. This clearly indicates that GMCs may benefit sustainable agriculture management by reducing the usage of synthetic chemicals in cultivation. Additionally, growth regulators produced by bioinoculants like PGPRs have the ability to increase the root proliferation of crops, and in turn, this facilitates the nutrient uptake of plants. This PGPR-mediated nutrient uptake by crops can be further enhanced by AMF resulting in increased crop growth and yield (Vafadar et al. 2014).

Studies on fertilizer consumption by GMC indicate that fertilizer usage would double for every 6% increase in GMC cultivable area. Nevertheless, the development of GM rice plants that are capable of acquiring and metabolizing N could substantially diminish the need for fertilizers for increasing crop yields (Shrawat et al. 2008). These nutrient efficient GMCs would reduce crop dependence on AMF and subsequently could alter AMF communities and their actions in the soil.

Another important agronomic activity that adversely affects the distribution of AMF propagules and mycorrhizal colonization is soil tillage (Smith 1978; Yocum et al. 1985). As this routine is common for both GMCs and non-GMCs, tillage would ultimately affect AMF. In summary, traditional agricultural practices may not be appropriate for GMCs which in turn inflict changes in AMF association and their community structure. Moreover, in-depth studies are necessary to evaluate and

develop farming practices that would suit coexistence and functioning of AMF GMCs in modern agroecosystems.

20.13 Future Prospects

Presently, the majority of the GMCs include simple manipulations like inserting genes for herbicide resistance or pest insect toxin. However, within two decades, a development of GMCs containing genes for desirable traits like high-temperature tolerance, apomixes, nitrogen fixation, denitrification inhibitor production, conversion from annual to perennial habit, and increased photosynthetic efficiency can be expected (Godfray et al. 2010). Nevertheless, understanding the consequence of GMCs on the activity of AMF colonization is an urgent practical implication to protect the environment, crops, and human health. The overview of the various study results showed that the risk evaluation of GMCs is consistent to affect AMF neutrally or negatively among the Bt crop plants. However, few studies demonstrate that GMCs positively influenced the AMF. Moreover, there is an absence of information in respect to the long-term assessment of AMF colonization, structures, spore diversity, and abundance among GMCs. Generally, AMF are more sensitive, the leftover residues of GMCs in farming practices may possibly affect colonization and diversity of AMF in subsequent crops that may be non-GMCs. Thus, GMC residues may possibly have a long-term effect in reducing the population and diversity of AMF. As GMCs have high Cry protein concentration, risk factor assessment should focus and identify the alterations in the genetics and physiology of the cultivated plants. Consequently, much emphasis should be laid on the different aspects of the risk assessment of GMCs especially in the areas of fungal ecology and community evaluation in both terrestrial and wetland ecosystems. Moreover, the effect of GMCs on AMF symbiosis under different observation methods and environmental situations should be performed to explore the actual benefits of the association. Available evidence does signify that the interaction between GMCs and AMF under realistic field conditions might be more complex than that can be predicted. However, the exploration of the GMC consequence on AMF in the line of threats and benefits would enable us to understand and develop strategies for the sustainable management of agronomy in the future. Further investigations should incorporate more molecular tools for the identification and quantification of AMF as it would be useful in estimating the consequences of each plant genetic insertion event on the AMF under different environmental and experimental conditions. Furthermore, it could also stretch a vibrant picture of the long-standing effects of GMCs on AMF formation and function with regard to plant growth in different soil conditions.

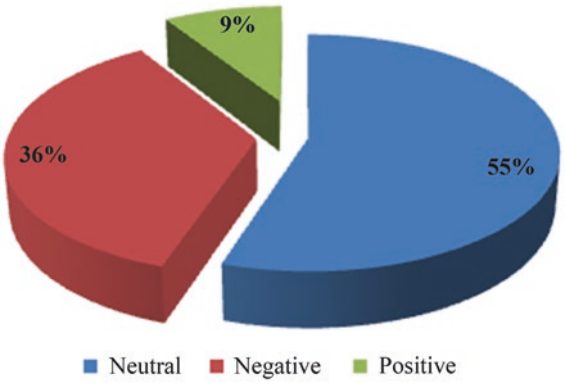


Fig. 20.3 Effect of genetically modified crops (GMCs) on arbuscular mycorrhizal fungi (AMF) as determined by the literature survey for the last two decades. The important consequences of GMCs on AMF are classified as neutral, negative, and positive

20.14 Conclusions and Future Considerations

GMCs are an important advancement in fast-growing agricultural biotechnology that is practiced worldwide. Earlier research has proved the benefits of GMC consequences on the environs and human health for the last two decades. Many experimental studies have also suggested that GMCs had few negative consequences on soil microorganisms and microbial community especially the AMF. In all these studies, several types of GMCs were investigated to test their consequences on AMF. However, Bt technology has been widely used to benefit cultivators to increase plant resistance to several diseases and to provide high yields.

A survey of the literature reporting the consequences of GMCs on AMF colonization, abundance, and community indicates that over half of the study results have reported a neutral effect (55%), followed by the negative (36%) and positive (9%) effects (Table 20.1; Fig. 20.3). Since last two decades, research outcomes have reported different responses to AM fungi; however, a number of research outcomes suggest beneficial or neutral effects on AMF colonization and fungal communities, although a negative effect in certain GMCs suggests that the improvement of transgenic lines in these plants should be avoided in the future for sustainable crop management. Further, studies during different seasons and in different sites through long-term experiments are necessary to validate the long-term influence of GMCs on AMF. Till date, this sort of information for GMCs is seriously lacking. Moreover, experiments are needed to systematically examine the interfaces between Bt toxins and soil biochemical and soil microorganism properties. The above issues should be taken into consideration for acceleration of current research progress and to make a clear understanding to validate the assumptions of GMCs on AMF and other soil microbial communities.

References

Ali A, Wani TA, Wani IA, Masoodi FA (2016) Comparative study of the physico-chemical properties of rice and corn starches grown in Indian temperate climate. J Saudi Soc Agri Sci 15:75–82

Anderson C (2001) The GM food potential. Strategic Analysis Paper. Published by Future Directions International Pty. Ltd. Australia

Andreote FD, Mendes R, Dini-Andreote F, Rossetto PB, Labate CA, Pizzirani-Kleiner AA, van Elsas JD, Azevedo JL, Araújo WL (2008) Transgenic tobacco revealing altered bacterial diversity in the rhizosphere during early plant development. Antonie Van Leeuwenhoek 93:415–424

Andreote FD, Carneiro RT, Salles JF, Marcon J, Labate CA, Azevedo JL, Araújo WL (2009) Culture-independent assessment of Rhizobiales-related Alphaproteobacteria and the diversity of Methylobacterium in the rhizosphere and rhizoplane of transgenic eucalyptus. Microbial Ecol 57:82–93

Azcon R, Ocampo JA (1981) Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytol 87:677–685

Bakhsh A, Khabbazi SD, Baloch FS, Demirel U, Caliskan ME, Hatipoglu R, Ozcan S, Ozkan H (2015) Insect-resistant transgenic crops: retrospect and challenges. Turk J Agri For 39:531–548

Baumgarte S, Tebbe C (2005) Field studies on the environmental fate of the Cry1Ab Bt toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol Ecol 14:2539–2551

Beckie HJ, Hall LM (2014) Genetically-modified herbicide resistant (GMHR) crops a two-edged sword? An Americas perspective on development and effect on weed management. Crop Protect 66:40–45

Blackwood CB, Buyer JS (2004) Soil microbial communities associated with Bt and non-Bt corn in three soils. J Environ Qual 33:832–836

Boisson-Dernier A, Chabaud M, Garcia F, Becard G, Rosenberg C, Barker DG (2001) *Agrobacterium rhizogenes*-transformed roots of *Medicago truncatula* for the study of nitrogen-fixing and endomycorrhizal symbiotic associations. Mol Plant-Microbe Interact 14:695–700

Carpenter JE (2011) Impacts of GM crops on biodiversity. GM Crops 2:1–17

Castaldini M, Turrini A, Sbrana C, Benedetti A, Marchionni M, Mocali S, Fabiani A, Landi S, Santomassimo F, Pietrangeli B, Nuti MP, Miclaus N, Giovannetti M (2005) Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Appl Environ Microbiol 71:6719–6729

Cellini F, Chesson A, Colquhoun I, Constable A, Davies HV, Engel KH, Gatehouseg AMR, Karenlamph S, Koki EJ, Leguayi J-J, Lehesrantah S, Noteborni HPJM, Pedersenk J, Smith M (2004) Unintended effects and their detection in genetically modified crops. Food Chem Toxicol 42:1089–1125

Cheeke TE, Pace BA, Rosenstiel TN, Cruzan MB (2011) The influence of fertilizer level and spore density on arbuscular mycorrhizal colonization of transgenic *Bt* 11 maize (*Zea mays*) in experimental microcosms. FEMS Microbiol Ecol 75:304–312

Cheeke TE, Coleman DC, Wall DH (2012a) Microbial ecology in sustainable agroecosystems. CRC Press. Taylor & Francis Group, UK

Cheeke TE, Rosenstiel TN, Cruzan MB (2012b) Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of Bt maize. Am J Bot 99:700–707

Cheeke TE, Cruzan MB, Rosenstiel TN (2013) Field evaluation of arbuscular mycorrhizal fungal colonization in *Bacillus thuringiensis* toxin-expressing (Bt) and non-Bt maize. Appl Environ Microbiol 79:4078–4086

Chelius MK, Triplett EW (2001) The diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microb Ecol 41:252–263

Chun Y, Kim H-J, Park K, Jeong S-C, Lee B, Back K, Kim H, Kim C-G (2012) Two-year field study shows little evidence that PPO transgenic rice affects the structure of soil microbial communities. Biol Fertil Soils 48(4):453–461

Cortet J, Andersen MN, Caul S, Griffiths B, Joffre R, Lacroix B, Sausse C, Thompson J, Krogh PH (2006) Decomposition processes under Bt (*Bacillus thuringiensis*) maize: results of a multi-site experiment. Soil Biol Biochem 38:195–199

Cotton TEA, Fitter AH, Miller RM, Dumbrell AJ, Helgason T (2015) Fungi in the future: inter-annual variation and effects of atmospheric change on arbuscular mycorrhizal fungal communities. New Phytol 205:1598–1607

Cowgill SE, Bardgett RD, Kiezebrink DT, Atkinson HJ (2002) The effect of transgenic nematode resistance on non-target organisms in the potato rhizosphere. J Appl Ecol 39:915–923

Crecchio C, Stotzky G (1998) Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. Soil Biol Biochem 30:463–470

de Souza Vieira PD, de Souza Motta CM, Lima D, Torres JB, Quecine MC, Azevedo JL, de Oliveira NT (2011) Endophytic fungi associated with transgenic and non-transgenic cotton. Mycology 2:91–97

de Vaufleury A, Kramarz PE, Binet P, Cortet J, Caul S, Andersen MN, Plumey E, Coeurdassier M, Krogh PH (2007) Exposure and effects assessments of Bt-maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. Pedobiologia 51:185–194

Dinel H, Schnitzer M, Saharinen M, Meloche F, Pare T, Dumontet S, Lemee L, Ambles A (2003) Extractable soil lipids and microbial activity as affected by Bt and non-Bt maize grown on a silty clay loam soil. J Environ Sci Health 38:211–219

Donegan KK, Palm CJ, Fieland VJ, Porteous LA, Ganio LM, Schaller DL (1995) Changes in levels, species, and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. Appl Soil Ecol 2:111–124

Donegan KK, Schaller DL, Stone JK, Ganio LM, Reed G, Hamm PB, Seidler RJ (1996) Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var *tenebrionis* endotoxin. Transgenic Res 5:25–35

Donegan KK, Seidler RJ, Doyle JD, Porteous LA, Digiovanni G, Widmer F, Watrud LS (1999) A field study with genetically engineered alfalfa inoculated with recombinant *Sinorhizobium meliloti*: effects on the soil ecosystem. J Appl Ecol 36:920–936

Dumas-Gaudot E, Asselin A, Gianinazzi-Pearson V, Gollotte A, Gianinazzi S (1994) Chitinase isoforms in roots of various pea genotypes infected with arbuscular mycorrhizal fungi. Plant Sci 88:27–37

Dunfield KE, Germida JJ (2001) Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. FEMS Microbiol Ecol 38:1–9

Edwards CA, Madden RLP, Miller RH, House G (1990) Sustainable agricultural systems. Soil and Water Conservation Society, Iowa

Escher N, Kach B, Nentwig W (2000) Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda). Basic Appl Entomol 1:161–169

Ferreira L, Molina J, Brasil C, Andrade G (2003) Evaluation of *Bacillus thuringiensis* bioinsecticidal protein effects on soil microorganisms. Plant Soil 256:161–168

Fließbach A, Messmer M, Nietlispach B, Infante V, Mader P (2012) Effects of conventionally bred and *Bacillus thuringiensis* (Bt) maize varieties on soil microbial biomass and activity. Biol Fertil Soils 48:315–324

Flores S, Saxena D, Stotzky G (2005) Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biol Biochem 37:1073–1082

Gao X, Kuyper TW, Zou C, Zhang F, Hoffland E (2007) Mycorrhizal responsiveness of aerobic rice genotypes is negatively correlated with their zinc uptake when nonmycorrhizal. Plant Soil 290:283–291

GeneWatch (2016) GM crops: current situation–Worldwide commercial growing. http://www.genewatch.org/sub-532326. Accessed on 10 Oct, 2016

Giovannetti M, Sbrana C, Turrini A (2005) The impact of genetically modified crops on soil microbial communities. Riv Biol 98:393–417

Gips (1987) Breaking the pesticide habit: alternative to twelve hazardous pesticides. International Alliance for Sustainable Agriculture, Minneapolis

Girlanda M, Bianciotto V, Cappellazzo GA, Casieri L, Bergero R, Martino E, Luppi AM, Perotto S (2008) Interactions between engineered tomato plants expressing antifungal enzymes and nontarget fungi in the rhizosphere and phyllosphere. FEMS Microbiol Lett 288:9–18

Glinka C, Hawkes CV (2014) Environmental controls on fungal community composition and abundance over 3 years in native and degraded shrub lands. Microbial Ecol 68:807–817

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Gomes NCM, Fagbola O, Costa R, Rumjanek NG, Buchner A, MendoncaHagler L, Smalla K (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Appl Environ Microbiol 69:3758–3766

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agri Ecosyst Environ 113:17–35

Götz M, Nirenberg H, Krause S, Wolters H, Draeger S, Buchner A, Lottmann J, Berg G, Smalla K (2006) Fungal endophytes in potato roots studied by traditional isolation and cultivation-independent DNA-based methods. FEMS Microbiol Ecol 58:404–413

Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Andersen MN, Cortet J, Messean A, Sausse C, Lacroix B, Krogh PH (2005) A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* CryIAb toxin. Plant Soil 275:135–146

Griffiths BS, Heckmann LH, Caul S, Thompson J, Scrimgeour C, Krogh PH (2007) Varietal effects of eight paired lines of transgenic Bt maize and near-isogenic non-Bt maize on soil microbial and nematode community structure. Plant Biotechnol 5:60–68

Griffiths BS, Caul S, Thompson J, Hackett CA, Cortet J, Pernin C, Krogh PH (2008) Soil microbial and faunal responses to herbicide tolerant maize and herbicide in two soils. Plant Soil 308:93–103

Grigera MS, Drijber RA, Wienhold BJ (2007) Redistribution of crop residues during row cultivation creates a biologically enhanced environment for soil microorganisms. Soil Till Res 94:550–554

Gschwendtner S, Esperschütz J, Buegger F, Reichmann M, Müller M, Munch JC, Schloter M (2010) Effects of genetically modified starch metabolism in potato plants on photosynthate fluxes into the rhizosphere and on microbial degraders of root exudates. FEMS Microbiol Ecol 76:564–575

Hajiboland R (2013) Role of arbuscular mycorrhiza in amelioration of salinity. In: Ahmad P, Azooz MA, MNV P (eds) Salt stress in plants: signalling, omics and adaptations. Springer Science+Business Media, New York, pp 301–354

Hannula SE, de Boer W, van Veen JA (2010) In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. Soil Biol Biochem 42:2211–2223

Hannula SE, de Boer W, van Veen JA (2012) A 3-year study reveals that plant growth stage, season and field site affect soil fungal communities while cultivar and GM-trait have minor effects. PLoS One 7:e33819. doi:10.1371/journal.pone.0033819

Hannula SE, de Boer W, Baldrian P, Van Veen JA (2013) Effects of genetically modified amylopectin-accumulating potato in decomposer processes and fungal diversity in litter and soil. Soil Biol Biochem 58:88–98

Hannula SE, de Boer W, van Veen JA (2014) Do genetic modifications in crops affect soil fungi? A review. Biol Fertil Soils 50:433–446

Hart MM, Powell JR, Gulden RH, Dunfield KE, Pauls KP, Swanton CJ, Klironomos JN, Antunes PM, Koch AM, Trevors JT (2009) Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn. Pedobiologia 52:253–262

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? Nature 394:431

Henault C, English LC, Halpin C, Andreux F, Hopkins DW (2006) Microbial community structure in soils with decomposing residues from plants with genetic modifications to lignin biosynthesis. FEMS Microbiol Lett 263:68–75

Hetrick BAD, Wilson GWT, Gill BS, Cox TS (1995) Chromosome location of mycorrhizal responsive genes in wheat. Can J Bot 73:891–897

Ho MW, Ryan A, Cummins J (1999) Cauliflower mosaic viral promoter – a recipe for disaster? Microb Ecol Health Dis 11:194–197

Hönemann L, Nentwig W (2009) Are survival and reproduction of *Enchytraeus albidus* (Annelida: Enchytraeidae) at risk by feeding on Bt-maize litter? Euro J Soil Biol 45:351–355

Hopkins DW, Gregorich EG (2003) Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. Eur J Soil Sci 54:793–800

Hoss S, Nguyen HT, Menzel R, Pagel-Wieder S, MiethlingGraf R, Tebbe CC, Jehle JA, Traunspurger W (2011) Assessing the risk posed to free-living soil nematodes by a genetically modified maize expressing the insecticidal Cry3Bb1 protein. Sci Total Environ 409:2674–2684

Icoz I, Stotzky G (2008) Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Res 17:609–620

Icoz I, Saxena D, Andow D, Zwahlen C, Stotzky G (2008) Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from *Bacillus thuringiensis*. J Environ Qual 37:647–662

Isobe K, Sugimura H, Maeshima T, Ishii R (2008) Distribution of arbuscular mycorrhizal fungi in upland field soil of Japan: 2. Spore density of arbuscular mycorrhizal fungi and infection ratio in soybean and maize fields. Plant Produc Sci 11:171–177

James C (2012) Global Status of Commercialized Biotech/GM Crops: 2012. ISAAA Brief No. 44. ISAAA, Ithaca, NY

James C (2013) Global status of commercialized biotech/GM crops: 2013. ISAAA Brief No. 46. ISAAA, Ithaca, NY

Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM, Allen EB (2003) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84:1895–1908

Jung HG, Sheaffer CC (2004) Lignin concentration of whole plants and stems of *Bt* corn hybrids. J Animal Sci 82:250–250

Kabir Z, OHalloran IP, Fyles JW, Hamel C (1998) Dynamics of mycorrhizal symbiosis of corn (*Zea mays* L.): effects of host physiology, tillage practice and fertilization on spatial distribution of extra-radical mycorrhizal hyphae in the field. Agric Ecosyst Environ 68:151–163

Kaeppler SM, Parke JL, Mueller SM, Senior L, Stuber C, Tracy WF (2000) Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. Crop Sci 40:358–364

Kaldorf M, Fladung M, Muhs HJ, Buscot F (2002) Mycorrhizal colonization of transgenic aspen in a field trial. Planta 214:653–660

Khan MQ, Abbasi MW, Zaki MJ, Khan SA (2010) Evaluation of *Bacillus thuringiensis* isolates against root-knot nematodes following seed application in okra and mungbean. Pak J Bot 42:2903–2910

Klümper W, Qaim M (2014) A meta-analysis of the impacts of genetically modified crops. PLoS One 9:e111629. doi:10.1371/journal.pone.0111629

Knox OGG, Nehl DB, Mor T, Roberts GN, Gupta VVSR (2008) Genetically modified cotton has no effect on arbuscular mycorrhizal colonisation of roots. Field Crops Res 109:57–60

Koskella J, Stotzky G (2002) Larvicidal toxins from *Bacillus thuringiensis* subspp. *kurstaki, morrisoni* (strain tenebrionis), and israelensis have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae in vitro. Can J Microbiol 48:262–267

Kremer RJ, Means NE (2009) Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur J Agron 31:153–161

Kuramae EE, Verbruggen E, Hillekens R, de Hollander M, Roling WFM, van der Heijden MGA, Kowalchuk GA (2013) Tracking fungal community responses to maize plants by DNA- and RNA-based pyrosequencing. PloS ONE 8:e69973. doi:10.1371/journal.pone. 0069973

Lang A, Abdel-Kader K, Arndt M, Bauchhenss J, Beck R, Benker U, Hermann A, Mautz D, Zellner M, Pommer G (2006) Monitoring the environmental impact of *Bt* maize: a research project of the Bavarian State Ministry for Health, Environment and Consumer Protection, and the Bavarian State Research Center for Agriculture. Mitt Biol Bundes Land Forst Berlin-Dahlem 403:136–139

Lawhorn CN, Neher DA, Dively GP (2009) Impact of coleopteran targeting toxin (Cry3Bb1) of Bt corn on microbially mediated decomposition. Appl Soil Ecol 41:364–368

Lee S-H, Kim C-G, Kang H (2011) Temporal dynamics of bacterial and fungal communities in a genetically modified (GM) rice ecosystem. Microb Ecol 61:646–659

Lehman RM, Osborne SL, Rosentrater KA (2008) No differences in decomposition rates observed between *Bacillus thuringiensis* and non-*Bacillus thuringiensis* corn residue incubated in the field. Agron J 100:163–168

Li SL, Zhao SJ, Zhao LZ, Li SL, Zhao SJ, Zhao LZ (1997) Variation in the responses of Alfalfa clone and cultivars of eggplant and cucumber and control of diseases. Acta Phytophylact Sin 24:117–120

Li X, Liu B, Cui J, Liu D, Ding S, Gilna B, Luo J, Fang Z, Cao W, Han Z (2011) No evidence of persistent effects of continuously planted transgenic insect-resistant cotton on soil microorganisms. Plant Soil 339:247–257

Liang J, Sun S, Ji J, Wu H, Meng F, Zhang M, Zheng X, Wu C, Zhang Z (2014) Comparison of the rhizosphere bacterial communities of Zigongdongdou soybean and a high-methionine transgenic line of this cultivar. PLoS One 9:e103343. doi:10.1371/journal.pone.0103343

Liang J, Meng F, Sun S, Wu C, Wu H, Zhang M, Zhang H, Zheng X, Song X, Zhang Z (2015) Community structure of arbuscular mycorrhizal fungi in rhizospheric soil of a transgenic high-methionine soybean and a near isogenic variety. PLoS One 10:e0145001. doi: 10.1371/journal.pone.0145001

Lilley AK, Bailey MJ, Cartwright C, Turner SL, Hirsch PR (2006) Life in earth: the impact of GM plants on soil ecology? Tren Biotech 24:9–14

Liu WK (2010) Do genetically modified plants impact arbuscular mycorrhizal fungi? Ecotoxicology 19:229–238

Liu B, Zeng Q, Yan FM, Xu HG, Xu CR (2005) Effects of transgenic plants on soil microorganisms. Plant Soil 271:1–13

Liu W, Hao Lu H, Wu W, Kun Wei Q, Xu Chen Y, Thies JE (2008) Transgenic Bt rice does not affect enzyme activities and microbial composition in the rhizosphere during crop development. Soil Biol Biochem 40:475–486

Liu B, Wang L, Zeng Q, Meng J, Hu W, Li X, Zhou K, Xue K, Liu D, Zheng Y (2009) Assessing effects of transgenic Cry1Ac cotton on the earthworm *Eisenia fetida*. Soil Biol Biochem 41:1841–1846

Lu H, Wu W, Chen Y, Wang H, Devare M, Thies JE (2010) Soil microbial community responses to Bt transgenic rice residue decomposition in a paddy field. J Soils Sed 10:1598–1605

Lynch JM, Benedetti A, Insam H, Nuti MP, Smalla K, Torsvik V, Nannipieri P (2004) Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol Fertil Soils 40:363–385

Manachini B, Landi S, Fiore MC, Festa M, Arpaia S (2004) First investigations on the effects of Bt-transgenic *Brassica napus* L. on the trophic structure of the nematofauna. IOBC/WPRS Bull 27:103–108

Mansouri H, Petit A, Oger P, Dessaux Y (2002) Engineered rhizosphere: the trophic bias generated by opine-producing plants is independent of the opine type, the soil origin, and the plant species. Appl Environ Microbiol 68:2562–2566

Masoero F, Moschini M, Rossi F, Prandini A, Pietri A (1999) Nutritive value, mycotoxin contamination and in vitro rumen fermentation of normal and genetically modified corn (Cry1A9b) grown in northern Italy. Maydica 44:205–209

Medina MJH, Gagnon H, Piche Y, Ocampo JA, Garrido JMG, Vierheilig H (2003) Root colonization by arbuscular mycorrhizal fungi is affected by the salicylic acid content of the plant. Plant Sci 164:993–998

Mercer KL, Wainwright JD (2008) Gene flow from transgenic maize to landraces in Mexico: an analysis. Agri Ecosyst Environ 123:109–115

Meyer JB, Song-Wilson Y, Foetzki A, Luginbühl C, Winzeler M, Kneubühler Y, Matasci C, Mascher-Frutschi F, Kalinina O, Boller T, Keel C, Maurhöfer M (2013) Does wheat genetically modified for disease resistance affect root-colonizing pseudomonads and arbuscular mycorrhizal fungi? PLoS One 8:e53825. doi:10.1371/journal.pone.0053825

Montagnac JA, Davis CR, Tanumihardjo SA (2009) Nutritional value of vassava for use as a staple food and recent advances for improvement. Compr Rev Food Sci Food Saf 8:181–194

Motavalli PP, Kremer RJ, Fang M, Means NE (2004) Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. J Environ Qual 33:816–824

Muchaonyerwa P, Waladde S, Nyamugafata P, Mpepereki S, Ristori GG (2004) Persistence and impact on microorganisms of *Bacillus thuringiensis* proteins in some Zimbabwean soils. Plant Soil 266:41–46

Mungai NW, Motavalli PP, Nelson KA, Kremer RJ (2005) Differences in yields, residue composition and N mineralization dynamics of *Bt*- and non-*Bt*-maize. Nutri Cycl Agroecosys 73:101–109

Naef A, Defago G (2006) Population structure of plant-pathogenic *Fusarium* species in overwintered stalk residues from Bt-transformed and non-transformed maize crops. Eur J Plant Pathol 116:129–143

Naef A, Zesiger T, Defago G (2006) Impact of transgenic *Bt* maize residues on the mycotoxigenic plant pathogen *Fusarium graminearum* and the biocontrol agent *Trichoderma atroviride*. J Environ Qual 35:1001–1009

Newhouse AE, Schrodt F, Liang HY, Maynard CA, Powell WA (2007) Transgenic American elm shows reduced Dutch elm disease symptoms and normal mycorrhizal colonization. Plant Cell Rep 26:977–987

Nguyen Thu H (2004) Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt Mais: Expression, Nachweis und Wirkung von rekombinantem Cry1Ab in heterologen Expressions systemen, Thesis, Georg-August-Universität, Göttingen, Germany

Nottingham S (2002) Genescapes: the ecology of genetic engineering. Zed Books, London

O'Callaghan M, Glare TR, Burgess EPJ, Malone LA (2005) Effects of plants genetically modified for insect resistance on nontarget organisms. Annu Rev Entomol 50:271–292

Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytol 165:273–283

Oliveira AP, Pampulha ME, Bennett JP (2008) A two-year field study with transgenic *Bacillus thuringiensis* maize: effects on soil microorganisms. Sci Tot Environ 405:351–357

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005) Managing arbuscular mycorrhizal fungi in cropping systems. Can J Plant Sci 85:31–40

Poerschmann J, Gathmann A, Augustin J, Langer U, Górecki T (2005) Molecular composition of leaves and stems of genetically modified *Bt* and near-isogenic non-*Bt* maize—characterization of lignin patterns. J Environ Qual 34:1508–1518

Powell JR, Gulden RH, Hart MM, Campbell RG, Levy-Booth DJ, Dunfield KE, Pauls KP, Swanton CJ, Trevors JT, Klironomos JN (2007) Mycorrhizal and rhizobial colonization of genetically modified and conventional soybeans. Appl Environ Microbiol 73:4365–4367

Powell JR, Levy-Booth DJ, Robert HG, Wendy LA, Rachel GC, Kari ED, Allan SH, Miranda MH, Sylvain L, Robert EN, Pauls KP, Peter HS, Clarence JS, Jack TT, John NK (2009) Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition. J Appl Ecol 46:388–396

Prakash D, Verma S, Bhatia R, Tiwary BN (2011) Risks and precautions of genetically modified organisms. ISRN Ecol 369573:13. doi:10.5402/2011/369573

Pu C, Liang J, Gao J, Wu C, Zhang M, Zhang Z, Cui Z, Cao H (2012) Effects of high producing methionine soybean transferred cystathionine γ-synthase gene on community structure of bacteria in soil. J Nanjing Agric Univ 35:8–14

Raubuch M, Roose K, Warnstorff K, Wichern F, Joergensen RG (2007) Respiration pattern and microbial use of field-grown transgenic *Bt*-maize residues. Soil Biol Biochem 39:2380–2389

Ren X (2006) Effect of Bt transgenic rice (KMD) on soil bacterial community and rhizosphere AM fungi. Dissertation, Zhejiang University. Hang Zhou, China

Roessner U, Wagner C, Kopka J, Trethewey RN, Willmitzer L (2000) Simultaneous analysis of metabolites in potato tuber by gas chromatography–mass spectrometry. Plant J 23:131–142

Rui YK, Yi GX, Zhao J, Wang BM, Li ZH, Zhai ZX, He ZP, Li QX (2005) Changes of *Bt* toxin in the rhizosphere of transgenic *Bt* cotton and its influence on soil functional bacteria. World J Microbiol Biotech 21:1279–1284

Sawers RJ, Gutjahr C, Paszkowski U (2008) Cereal mycorrhiza: an ancient symbiosis in modern agriculture. Trends Plant Sci 13:93–97

Saxena D, Stotzky G (2000) Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic *Bt* corn in vitro and in situ. FEMS Microbiol Ecol 33:35–39

Saxena D, Stotzky G (2001a) Bt toxin uptake from soil by plants. Nat Biotechnol 19:199

Saxena D, Stotzky G (2001b) Bt corn has higher lignin content than non-Bt corn. Amer J Bot 88:1704–1706

Saxena D, Stotzky G (2003) Fate and effects in soil of insecticidal toxins from *Bacillus thuringiensis* in transgenic plants. In: Collection of biosafety reviews. pp 7–83. International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Saxena D, Flores S, Stotzky G (1999) Insecticidal toxin in root exudates from *Bt* corn. Nature 402:480

Saxena D, Flores S, Stotzky G (2002) *Bt* toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biol Biochem 34:133–137

Saxena RK, Saxena KB, Varshney RK (2010) Application of SSR markers for molecular characterization of hybrid parents and purity assessment of ICPH 2438 hybrid of pigeon pea *Cajanus cajan* (L.) Millsp. Mol Breed 26:371–380

Schaarschmidt S, Gonzalez M-C, Roitsch T, Strack D, Sonnewald U, Hause B (2007) Regulation of arbuscular mycorrhization by carbon. The symbiotic interaction cannot be improved by increased carbon availability accomplished by root-specifically enhanced invertase activity. Plant Physiol 143:1827–1840

Schultz PA, Miller RM, Jastrow JD, Rivetta CV, Bever JD (2001) Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. Am J Bot 88:1650–1656

Seifert EK, Bever JD, Maron JL (2009) Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. Ecology 90:1055–1062

Selosse MA, Baudoin E, Vandenkoornhuyse P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. Comptes Rendus Biol 327:639–648

Seppänen S-K, Pasonen H-L, Vauramo S, Vahala J, Toikka M, Kilpeläinen I, Setälä H, Teeri TH, Timonen S, Pappinen A (2007) Decomposition of the leaf litter and mycorrhiza forming ability of silver birch with a genetically modified lignin biosynthesis pathway. Appl Soil Ecol 36:100–106

Seres A, Nagy KP, Saly P, Darvas B, Bakonyi G (2014) Arbuscular mycorrhizal fungi colonisation of *Cry3* toxin-producing *Bt* maize and near isogenic maize. Plant Soil Environ 60:569–573

Shelton AM, Zhao JZ, Roush RT (2002) Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annu Rev Entomol 47:845–881

Shrawat AK, Carroll RT, De Paw M, Taylor GJ, Good AG (2008) Genetic engineering of improved nitrogen use efficiency in rice by tissue-specific expression of alanine aminotransferase. Plant Biotech J 6:722–732

Siciliano SD, Germida JJ (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiol Ecol 29:263–272

Smith TF (1978) A note on the effect of soil tillage on the frequency and vertical distribution of spores of vesicular-arbuscular endophytes. Aust J Soil Res 16:359–361

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, Cambridge, UK

Steinkellner S, Hage-Ahmed K, Garcia-Garrido JM, Illana A, Ocampo JA, Vierheilig H (2012) A comparison of wild-type, old and modern tomato cultivars in the interaction with the arbuscular mycorrhizal fungus *Glomus mosseae* and the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Mycorrhiza 22:189–194

Stotzky G (2004) Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. Plant Soil 266:77–89

Tabashnik BE (1994) Evolution of resistance to *Bacillus thuringiensis*. Annu Rev Entomol 39:47–79

Tahiri-Alaoui A, Grison R, Gianinazzi-Pearson VT, Gianinazzi AS (1994) The impact of the constitutive expression of chitinases in roots of transgenic tobacco on arbuscular mycorrhizal fungi. In: Abstract 406 of the 7th international symposium on molecular plant–microbe interactions, Edinburgh, 26 June–1 July, 1994

Tan FX, Wang JW, Feng YJ, Chi GL, Kong HL, Qiu HF, Wei SL (2010) *Bt* corn plants and their straw have no apparent impact on soil microbial communities. Plant Soil 329:349–364

Tan F, Wang J, Chen Z, Feng Y, Chi G, Rehman SU (2011) Assessment of the arbuscular mycorrhizal fungal community in roots and rhizosphere soils of Bt corn and their non-Bt isolines. Soil Biol Biochem 43:2473–2479

Tarkalson DD, Kachman SD, Knops KMN, Thies JE, Wortmann CS (2008) Decomposition of *Bt* and non-*Bt* corn hybrid residues in the field. Nutri Cycl Agroecosys 80:211–222

Thevissen K, Osborn R, Acland D, Broekaert WF (2000) Specific binding site for an antifungal plant defensin from Dahlia (*Dahlia merckii*) are required for antifungal activity. Mol Plant Microb Interact 13:54–61

Thevissen K, Francois IEJA, Takemoto JY, Ferket KKA, Meert EMK, Cammue BPA (2003) DmAMP1, an antifungal plant defensin from dahlia (*Dahlia merckii*), interacts with sphingolipids from *Saccharomyces cerevisiae*. FEMS Microb Lett 226:169–173

Tilston EL, Halpin C, Hopkins DW (2013) Simultaneous down regulation of enzymes in the phenylpropanoid pathway of plants has aggregated effects on rhizosphere microbial communities. Biol Fertil Soils 50:455–463

Turrini A, Sbrana C, Nuti MP, Pietrangeli BM, Giovannetti M (2004a) Development of a model system to assess the impact of genetically modified plants and aubergine plants on arbuscular mycorrhizal fungi. Plant Soil 266:69–75

Turrini A, Sbrana C, Pitto L, Castiglione MR, Giorgetti L, Briganti R, Bracci T, Evangelista M, Nuti MP, Giovannetti M (2004b) The antifungal Dm-AMP1 protein from *Dahlia merckii* Lehm. expressed in *Solanum melongena* is released in root exudates and differentially affects pathogenic fungi and mycorrhizal symbiosis. New Phytol 163:393–403

Turrini A, Sbrana C, Giovannetti M (2015) Belowground environmental effects of transgenic crops: a soil microbial perspective. Res Microbiol 166:121–131

Vafadar F, Amooaghaie R, Otroshy M (2014) Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. J Plant Interact 9:128–136

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Verbruggen E, Kuramae EE, Hillekens R, de Hollander M, Kiers ET, Röling WF, Kowalchuk GA, van der Heijden MG (2012) Testing potential effects of maize expressing the *Bacillus thuringiensis* Cry1Ab endotoxin (Bt maize) on mycorrhizal fungal communities via DNA- and RNA-based pyrosequencing and molecular fingerprinting. Appl Environ Microbiol 78:7384–7392

Vierheilig H, Alt M, Neuhaus JM, Boller T, Wiemken A (1993) Colonization of transgenic Nicotiana sylvestris plants, expressing different forms of *Nicotiana tabacum* chitinase, by

the root pathogen *Rhizoctonia solani* and by the mycorrhizal symbiont *Glomus mosseae*. Mol Plant-Microbe Interact 6:261–264

Vierheilig H, Alt M, Lange J, Gut-Rella M, Wiemken A, Boller T (1995) Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*. Appl Environ Microbiol 61:3031–3034

Volpin H, Elkind Y, Okon Y, Kapulnik Y (1994) A vesicular arbuscular mycorrhizal fungus (*Glomus intraradix*) induces a defense response in alfalfa roots. Plant Physiol 104:683–689

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Watanabe T, Broadley MR, Jansen S, White PJ, Takada J, Satake K, Takamatsu T, Tuah SJ, Osaki M (2007) Evolutionary control of leaf element composition in plants. New Phytol 174:516–523

Weaver MA, Krutz LJ, Zablotowicz RM, Reddy KN (2007) Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Manag Sci 63:388–393

Wei XD, Zou HL, Chu LM, Liao B, Ye CM, Lan CY (2006) Field released transgenic papaya affects microbial communities and enzyme activities in soil. Plant Soil 285:347–358

Weinert N, Meincke R, Gottwald C, Heuer H, Gomes NCM, Schloter M, Berg G, Smalla K (2009) Rhizosphere communities of genetically modified zeaxanthin-accumulating potato plants and their parent cultivar differ less than those of different potato cultivars. Appl Environ Microbiol 75:3859–3865

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Wieland G, Neumann R, Backhaus H (2001) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Appl Environ Microbiol 67:5849–5854

Willis A, Rodrigues BF, Harris PJC (2013) The ecology of arbuscular mycorrhizal fungi. Crit Rev Plant Sci 32:1–20

Wohlfender-Bühler D, Feusthuber E, Wäger R, Mann S, Aubry SJ (2016) Genetically modified crops in Switzerland: implications for agrosystem sustainability evidenced by multi-criteria model. Agron Sustain Dev 36:1–16

Wróbel-Kwiatkowska M, Turnau K, Góralska K, Anielska T, Szopa J (2012) Effects of genetic modifications to flax (*Linum usitatissimum*) on arbuscular mycorrhiza and plant performance. Mycorrhiza 22:493–499

Wu WX, Ye QF, Min H, Duan XJ, Jin WM (2004) Bt transgenic rice straw affects the culturable microbiota and dehydrogenase and phosphatase activities in a flooded paddy soil. Soil Biol Biochem 36:289–295

Wu WX, Liu W, Lu HH, Chen YX, Devare M, Thies J (2009) Use of C-13 labeling to assess carbon partitioning in transgenic and nontransgenic (parental) rice and their rhizosphere soil microbial communities. FEMS Microbiol Ecol 67:93–102

Xue K, Luo HF, Qi HY, Zhang HX (2005) Changes in soil microbial community structure associated with two types of genetically engineered plants analyzing by PLFA. J Environ Sci-China 17:130–134

Xue K, Serohijos RC, Devare M, Thies JE (2011) Decomposition rates and residue-colonizing microbial communities of *Bacillus thuringiensis* insecticidal protein Cry3Bb-expressing (Bt) and non-Bt corn hybrids in the field. Appl Environ Microbiol 77:839–846

Yang YF, Yuan HX, Liu YL, Xu XP, Li BJ (2002) Research on root microorganism community of "RCH" transgenic rice. Chin J Agric Econ-China 10:29–31

Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305

Yocum DH, Larsen HJ, Boosalis MG (1985) The effects of tillage treatments and a fallow season on VA mycorrhizae in winter wheat. In: Molina R (ed) Proc. 6th North Am Conf on Mycorrhizae, Oregon State University, Corvallis

Zeng H, Tan F, Zhang Y, Feng Y, Shu Y, Wang JW (2014) Effects of cultivation and return of *Bacillus thuringiensis* (Bt) maize on the diversity of the arbuscular mycorrhizal community in soils and roots of subsequently cultivated conventional maize. Soil Biol Biochem 75:254–263

Zeng H, Tan F, Shu Y, Zhang Y, Feng Y, Wang J (2015) The Cry1Ab protein has minor effects on the arbuscular mycorrhizal fungal communities after five seasons of continuous Bt maize cultivation. PLoS One 10:e0146041. doi:10.1371/journal.pone.0146041

Zhang C, Wohlhueter R, Zhang H (2016) Genetically modified foods: a critical review of their promise and problems. Food Sci Human Wellness 5:116–123

Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci 12:548–555

Zwahlen C, Hilbeck A, Gugerli P, Nentwig W (2003) Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. Mol Ecol 12:765–775

An Expedition to the Mechanism of Plant–Microbe Interaction by Utilization of Different Molecular Biology Tools

21

Bitupon Borah, Babita Joshi, Debojit Kumar Sarmah, and Brijmohan Singh Bhau

Abstract

The global demand for food, animal feed, and plant-based products is increasing with the blast of population growth putting unprecedented pressure to the agriculture as the natural resources become diminished and the conventional system of cultivation is not sufficient to cope up with this. In addition to this, recent public concerns to the catastrophic effect of chemical fertilizer and pesticides to the livestocks and the environment led to the urgency of adopting sustainable agricultural practices. In sustainable agriculture, the plant–microbe interaction plays an imperative position which mainly confers the mechanism and utilization of beneficial microbes and their products for crop improvement, providing abiotic stress tolerance and control of plant diseases. The interaction between plants and microbes is a very complex and dynamic biological process which has evolved due to thousand years of coevolution between them. The plant–microbe interactions can provide the new imminent in various aspects of the mechanisms of how the microbes respond to perturbation, how chemical exudates released from plant roots, and how do they affect plant health and development. In the last two decades, molecular biology is being a powerful and precise tool becoming more commonly adopted and reliable for understanding of the plant–microbe

B. Borah • B. Joshi • B.S. Bhau (✉)
Plant Genomic Laboratory, Medicinal Aromatic & Economic Plants (MAEP) Group, Biological Sciences & Technology Division (BSTD), CSIR-North East Institute of Science and Technology, Jorhat, Assam 785006, India

Academy of Scientific and Innovative Research (AcSIR), CSIR-North East Institute of Science and Technology, Jorhat 785006, Assam, India
e-mail: bsbhau@gmail.com; bsbhau@neist.res.in

D.K. Sarmah
Plant Genomic Laboratory, Medicinal Aromatic & Economic Plants (MAEP) Group, Biological Sciences & Technology Division (BSTD), CSIR-North East Institute of Science and Technology, Jorhat, Assam 785006, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_21

interaction. For example, the introduction of next-generation sequencing giving multitude of nucleotide data in a very short duration also assists metagenomics which allows studying complete microbiota including non-culturable microbes. This book chapter is intended to chronicle the development of different molecular biology tools in studying the biosynthetic pathway secondary metabolites produced by microbes, diversity of microorganisms, and functional identification of induced genes in a plant–microbe interaction.

Keywords
Rhizobacteria • PGPR • Next-generation sequencing • Metagenomics • Non-culturable • Microbiota

21.1 Introduction

In a natural ecosystem there is always a competition for food and area that leads to the rise of different enemies. To combat successfully with these natural enemies, every living organism has adapted to their native environment and developed some defense mechanisms. Since most of the plants are immobile, they have to manage in the existing environment and utilize to its utmost (Chai et al. 2005); moreover plants require different micro- and macronutrient for maintaining a healthy life, but some of the nutrients are not readily available in the prescribed format (Fageria et al. 2011). So, the plants have to utilize a unique strategy to overcome this hurdle by attracting microorganisms which have the ability to convert the complex organic compounds into absorbable format. This type of phenomenon is termed as symbiosis, and it becomes an integral part of their survival (Ronald and Shirasu 2012). Various types of microbes are present in close proximity of the plants by involving as pathogens, saprophytes, epiphytes, endophytes, and mutualists. Pathogenic and mutualistic microbes are the major players that influence plant health (Goh et al. 2013). So the plants and microbes must coexist peacefully for the battle of survival, and plants itself determine whether the endeavor microbes are associates or antagonist. From several years, the plants and microbes acquired a number of mechanisms which can modulate outcomes of their interactions (Jones and Dangl 2006; Oldroyd 2013). Moreover, different abiotic ecological aspects such as temperature and light also effect the plant–microbe interactions (Hua 2013). Thus, plant–microbe interactions are a web of very complex and dynamic interconnecting biological process which can be classified as symbiotic, parasitic, and neutralistic (Newton et al. 2010). In case of positive interactions, different beneficial microbes such as endo- and ecto-mycorrhizal fungi, nitrogen-fixing bacteria, and plant growth-promoting rhizobacteria (PGPR) are involved as symbiotic and associative interactions which are yet to be explored. On the other hand, the negative interactions incorporate with the involvement of different types of parasitic plants, pathogenic bacteria, fungi, oomycetes, nematodes, and invertebrate herbivores (Halder and Sengupta 2015).

The rhizosphere is the junction of different interactions forming complex environments which are very crucial for plant health, and this is due to the root exudates released in the rhizosphere that attract different organisms (Berendsen et al. 2012). Due to the inherently complex nature of plant–microbe interactions, more powerful techniques are needed for unraveling the overall result of microbial activities in the rhizosphere and help researcher for better understanding of the prospective of crop plant. However, advances in methods based on nucleic acid analysis of the plant-associated microbiota especially by advancements in genomic (DNA and RNA) sequencing have dramatically accelerated research and are attested (Simon and Daniel 2011). And to understand the underlying mechanism of the linkages between microbial diversity and ecosystem functioning, effective high-throughput technologies are critical for advancing this mechanistic understanding (Fitter et al. 2005; Levin 2006). No doubt that the NGS technologies have revolutionized our understanding with the advantage of low cost and less laborious, but, however, function of host microbiome remains unclear. So there is a need of integrated information of microbial communities via different meta-omic technologies (Kothari et al. 2006). This advancement in sequencing technologies has given the metagenomic analysis to explore the entire spectrum and significant insight of plant–microbe interaction for complete understanding and hence discovery of novel genes.

21.2 Metagenomics

Since the interaction between plant and microbes present in the surrounding environment is very complex and dynamic, it arises some of the basic questions like what are the organisms acting there, how they interact, how do they affect plant health and development, how do they respond and adapt to environmental changes. Understanding these questions will lead to a better understanding of the association between microorganisms and plants. But while addressing these questions, traditional culture-based approach is not enough because only a tiny portion of the entire biomicrobiota is possible till now and the rest of the large portion is unculturable, and moreover, achieving culture conditions for isolating a single member from a consortium would be a daunting task because of (1) dependence on other organisms for significant practices, (2) unable to develop in vitro or (3) turn out as extinct in fossil records (Tringe and Rubin 2005). So there is a need of an effective technique to identify and to discover the knowledge treasure behind these unculturable microbes, and here it comes as non-culture-based approach, called "metagenomics," which has been rapidly adopted since the 1980s (Handelsman 2004). It is a technique which can directly access the genetic content of the whole group of organism by combining the genomic technology and bioinformatic tools. Metagenomics circumvents the requirement of isolation or cultivation of microbes and refers to the direct access to the total gene pool from their environment, without the need for prior culturing under laboratory conditions (Handelsman 2004). This study can lead to the discovery of new gene product and can reveal the complete metabolic pathways from the largely untapped microbial resource (Cowan et al.

2005). The main purpose of this approach is to develop a consensus of what microbes are present and their role in the particular environment (Jarvie and Harkins 2007).

21.2.1 Sample Enrichment

Metagenomic study involves the construction of a soil-based library that requires sufficient amounts of high-quality DNA, which is representative of the soil microbial community (Bertrand et al. 2005). But before that pre-enrichment of the sample can provide an attractive means of enhancing the screening hit rate and solving the problem of getting small portion of target genes from the total nucleic acid fraction (Cowan et al. 2005). There are different types of enrichment method depending upon the need. Generally, it can be divided into three broad categories: natural, artificial culture enrichment, and nucleic acid enrichment. Natural sample enrichment is the collection of metagenomic data from an enriched environment niches particularly those where the chances of expressing the target gene of interest are more. Example of natural enrichment is metagenomics that has been used to unlock novel cellulases from various natural environments (Alvarez et al. 2003). Culture enrichment is another method, which selectively favors the growth of only the target microorganisms. The selection can be based on nutritional, physical, or chemical criteria. Examples are the enrichment of carboxymethylcellulose in the culture for more chances of getting genes responsible for cellulose degrading enzymes (Rees et al. 2004; Eyers et al. 2004) in a study of induced xenobiotic to isolate xenobiotic degrading microbes. So this type of approach can be very useful for isolating genes from microbes which are very low in numbers in a particular environment or allows poorly cultivatable species to adapt culture conditions prior to isolation (Bollmann et al. 2007). Nucleic acid enrichment technique involves stable isotope probing (SIP), phage display, differential expression analysis (DEA) (Cowan et al. 2005), suppressive subtractive hybridization (SSH) (Sagerstrom et al. 1997), and affinity capture (Demidov et al. 2000).

21.2.2 DNA Extraction and Purification

After proper enrichment procedure, one of the crucial steps in metagenomic study is the process of DNA extraction. The major hurdle in metagenomic DNA extraction is the myriad nature or properties of the cells since the sample is a metagenome and required appropriate lysis technique. As a consequence, the harsh lysis methods can cause degradation of the DNA from some organisms (Tringe and Rubin 2005). So the isolation procedure of DNA should not physically disrupt the genetic material. Another important point of concern is that when association of a host and target community is taken place, the fractionation or selective lysis methods might fulfill the extraction of minimal host DNA (Burke et al. 2009; Thomas et al. 2010). A variety of methods are used in metagenomic studies for the isolation of nucleic acids directly from the soil sample (Handelsman et al. 1998) and can be broadly

divided into physical and chemical lysis method. Physical lysis methods include bead beating (Yeates et al. 1998), sonication (Purohit and Singh 2009), liquid nitrogen (Johnston and Aust 1994), and freeze thawing (Lee et al. 1996). Chemical lysis method uses chemicals like sodium dodecyl sulfate (SDS) (Gray and Herwig 1996) in combination with enzymes such as proteinase K and lysozyme, chelating agents such as EDTA and Chelex 100 (Robe et al. 2003), guanidine thiocyanate (Kauffmann et al. 2004), and various Tris and sodium phosphate buffers (Krsek and Wellington 1999). Such methods are relatively gentle, recovering higher-molecular-weight DNA, but are not as efficient as mechanical methods at lysing cells representative of all microbial genomes within a given environmental sample, but mechanical lysis method may produce fragment of metagenomic DNA though there is a great advantage of getting large amount of extracted DNA (Liesack and Stackebrandt 1992). A combination of both the methods, freeze thawing and lysozyme are also employed for some samples (Tsai and Olson 1991). Sometimes the lytic method results in poor yields and makes it difficult for the downstream processing since most of the microbes present in the soil are in the form of spores which are metabolically dormant and show high resistance to lytic agents (Steele and Streit 2006). In some cases where DNA yield is less e.g., groundwater, preamplification methods for the DNA are required. Multiple displacement amplification (MDA) is a commonly used technique that uses random hexamers and phage phi29 polymerase to amplify DNA in order to get high yield (Lasken 2009) and therefore extensively used in single-cell genomics and to some extent in metagenomics (Ishoey et al. 2008; Kozdroj 2010). Different types of extraction procedures are needed to be applied more precisely and have to be compared for the extraction of DNA. Generally almost all extracted soil DNA is contaminated with proteins, humic acids, polysaccharides, lipids, minerals, as well as eukaryotic DNA (Cullen and Hirsch 1998; Kozdroj 2010). So in most of the cases, an additional purification process is required for metagenomic DNA. Different chemicals such as potassium acetate, PEG, ethanol or isopropanol are used singly or in combination for the purification of DNA. Similar separation methods such as sephadex gel filtration, ion exchange chromatography column, and agarose or PVPP/PVP gel electrophoresis are extensively used for the binding and precipitation of proteins present in the crude extract of the DNA (Cullen and Hirsch 1998). Robe et al. (2003) stated the use of cesium chloride gradient centrifugation purification of high-quality DNA of about 100 kb size. Since, the above method is time consuming; therefore, other fast alternatives (yet with lower yield) have been applied nowadays. By using "ready-to-use" DNA extraction and purification kits, we can process different types of soil samples and get a relatively pure DNA in a short time.

21.2.3 Construction of Metagenomic Library

Isolation and purification of genetic material are followed by the construction of a metagenomic library. Library construction involves the cloning of environmental DNA fragments into a vector of choice and is transfected into an appropriate host

(Lorenz and Schleper 2002). On the basis of the insert capacity of vectors, libraries can be classified into two groups, viz., small inserts are constructed using plasmid vectors such as pBluescript SK+ (Stratagene, San Diego, California), lambda Zap II, and pCR-XL-TOPO which can carry DNA fragments up to 20 kb. Such libraries can be used for function-based screening, as small insert clones are better suited to *in vitro* expression than large DNA fragments (Healy et al. 1995), and for small insert libraries, robust lysis techniques can be employed as opposed to large insert (Riesenfeld et al. 2004). Large inserts are constructed in vectors like fosmids (25–40 kb), cosmids (25–35), yeast artificial chromosome YAC (40-50Kb), and bacterial artificial chromosome BAC (100–300 kb) (Li and Qin 2005; Shizuya et al. 1992). Large inserts are generally used for the study of genes and metabolic pathways; it is desirable to use clones with DNA inserts of high molecular weight, increasing the chances of finding positive hits during the screening of the library. Green and Keller (2006) reported that if the inserted clones are smaller in size, more numbers of clones are required for the better exposure of the metagenome. The host is another important point during library construction and choice of suitable host is of great importance (Gabor et al. 2004). A range of bacterial hosts, including *E. coli*, *Bacillus*, or *Streptomyces* strains have been used for library construction and screening. In most studies, *E. coli* has been the first choice and successfully been used as host for functional screens because its genome is well defined and easily transformable (Steele et al. 2009). However, the use of *E. coli* strains as a host has certain limitations as less than 0.01% of positive clones are typically identified during a single round of screening (Cowan et al. 2005) and as the host allows expression of only 40% of the genes present in a given sample which may be due to unrecognized signaling sequences of transcription of target genes (Craig et al. 2010; Parachin and Gorwa-Grauslund 2011). These types of limitations can be overcome by the introduction of new host from the genera *Streptomyces*, *Pseudomonas*, and *Bacillus* (Lorenz and Eck 2005; Aakvik et al. 2009). *Streptomyces* has been used to construct broad host-range vector, i.e., VECA, using *E. coli* as an initial host. Streptomycete genomes are characterized by high (>70%) G + C content (Binnie et al. 1997) and are therefore a potentially valuable host system for the expression of high G+C content genes since *E. coli* is unable to express 80% of promoters of *Actinobacteria*, due to the large difference in G+C content (Strohl 1992). Some *Archaea* genera, such as *Methanococcus*, *Pyrococcus*, *Sulfolobus*, and *Thermococcus*, have been widely used for the scheming of the stable host-vector system (Angelov and Liebl 2010).

21.2.4 Sequence Analysis

Metagenomics can be divided into two basic categories: a sequence-based and function-based technique. But both techniques start with isolation of environmental DNAs. Sequence-based metagenomics involves sequencing and analysis of DNA from environmental samples and with the advancement of new sequencing methodology and also with the advent of new improved bioinformatic tools, makes it more

useful in gene assembly, identification of new genes, exploring complete metabolic pathways, and analyzing the degree of diversity and the number of different bacterial species existing in a particular sample. But there are some limitations in sequence-based technique like the lack of functionality and biochemical parameters of the encoded enzymes. Another problem associated with sequence-based technique is its limitation with only known genes or it can reveal only partial genes, compelling researcher to do more subsequent labor-intensive study like expression analysis and detailed biochemical analysis.

In contrast, function-based metagenomics generally employs to screen for a particular function like identification of new antibiotics and antibiotic resistance gene, vitamin production, and pollutant degradation by isolating DNA from microbial communities since many proteins and enzymes with useful functions exist within microbes. The advantage of this function-based metagenomics is that the system can allocate the detection of novel enzymes in which their functions are unpredictable from the single DNA sequence. Thus it enables researchers to access the tremendous genetic diversity of unknown gene sequence in a microbial community, the structure of the desired protein or the microbe of origin. For sequence-/homology-based screening, significant advantage has been proposed by function-driven screening strategies (Suenaga 2012; Tuffin et al. 2009). Since there is no need of prior knowledge of the gene sequence for the target activity of interest, therefore it is estimated that functional screening increases the "novelty" hit rate and in addition increases the potential of recognizing new classes of genes for their known and novel functions (Sharma and Vakhlu 2014). Though the function-driven approach can completely identify active enzyme clones, still somehow it is lagging behind due to its slow, more labor-intensive and costly procedure and moreover it cannot tell much about what species the genetic material came from.

21.3 Next-Generation Sequencing

In the last couple of years, next-generation sequencing (NGS) technologies have remarkably accelerated the biological research by facilitating the production of large volumes of sequence data with lower price rate in comparison to traditional sequencing methods (Knief 2014). Sequencing technique was first introduced by Sanger in 1977 and involved the conventional method of DNA sequencing which is widely known as Sanger sequencing technology or first-generation technology. This technology is based on the amplification of a single-stranded DNA template (denaturing by application of heat) and chain termination with the use of dideoxynucleotides (ddNTPs) which prevents the integration or addition of further nucleotides. The chemically altered fluorescently labeled ddNTPs can inhibit phosphodiester bond formation and can identify the presence of nucleotide in the original DNA template (Sanger et al. 1977). Sanger sequencing technology can have an average read length of 800 bps. This technology was the most commonly used sequencing method till the 1990s and has led to complete many projects like International Human Genome Sequencing (Lander et al. 2001; Venter et al. 2001), Rice Genome

Project (Eckardt 2000), etc. However, every technology has its own limitations so as the Sanger sequencing. Its main disadvantages are that very less amount of DNA can be processed, high cost, low throughput, and quite difficult operation and have very limited use in deeper and more complex genomic investigation (Fullwood et al. 2009). So to overcome with this dilemma, newer sequencing technologies have been developed that can read the sequence of multiple DNA in parallel which is popularly known as "NEXT GENERATION SEQUENCING or High Throughput Sequencing technology or Second Generation Sequencing." This technology offers drastically faster and cost-effective gene sequence with a throughput on the gigabase (Gb) scale and can characterize the whole genomes and outlines the difference between them and is vastly superior to Sanger sequencing (Mamanova et al. 2010).

This technology also provides different possible applications such as whole-genome resequencing for variation analysis, quantitative detection of epigenomic dynamics, sequencing of RNA (RNA-seq) for transcriptome, and Chip-seq analysis for DNA–protein interactions (Lister et al. 2009). In addition to this interactome analysis for networks formed by protein–protein interactions (Arabidopsis Interactome Mapping Consortium 2011), hormone analysis for phytohormone-mediated cellular signaling (Kojima et al. 2009) and metabolome analysis for metabolic systems (Saito and Matsuda 2010) have been also implicated.

21.3.1 The Next-Generation Technologies Commercially Available Includes

1. Roche 454 (Margulies et al. 2005)
2. Illumina/Solexa (Bennett 2004; Bennett et al. 2005)
3. Ion torrent: proton/PGM sequencing (Jonathan and Rothberg 2011)
4. SOLiD (Sequencing by Oligonucleotide ligation Detection) (Shendure et al. 2005)

Roche 454 is based on pyrosequencing (sequencing by synthesis) technology and is the first commercially available platform as NGS sequencer. The second complete genome of an individual was sequenced with this platform (Wheeler et al. 2008). This technology makes the use of optical signals (here the pyrophosphate) released during nucleotide incorporation (Lui et al. 2012). The sequencer consists of a picotiter plate which has millions of wells, where the single-stranded DNA template along with the nucleotide reagents is released as a result of which hybridization takes place and generates an observable light which in turn is captured by an adapter and results in release of a sequence. At the beginning, the read length of this sequencer was low at around 100–150 bp. But later a new 454 GS FLX Titanium system has been launched which can generate a read length of ~700 bp–14 Gb data with accuracy of 99.9% within time period of 24 h (Huse et al. 2007).

Illumina/Solexa Genome Analyzer was the second sequencing platform to reach market which uses sequencing by synthesis approach (Bennett 2004) and recognized as the most adaptable and easiest sequencing technology based on the

reversible terminated chemistry concept (Canard and Sarfati 1994). The sequencer consists of a flow cell in which the primers and the DNA molecule are attached and each fluorescently labeled reversible terminator is added simultaneously into oligo-primed cluster fragments in flow-cell channels along with DNA polymerase. After generation of extended cluster sequence strands through bridge amplification, the template is then ready for sequencing. This technology is effective for sequencing of homopolymeric stretches with shorter sequence reads than pyrosequencing (Bentley 2006) which does not resolve short sequence repeats due to the use of reversible dye terminator nucleotide; base-substitution errors have been noted in this technology (Hutchison 2007).

Ion torrent/PGM (Personal Genome Machine) sequencing technology does not make use of optical signals; instead it is based on a well-characterized biochemical process like the detection of the protons released during the incorporation of nucleotide (Jonathan and Rothberg 2011). Emulsion PCR with 3 μ diameters of ion sphere particles can link and clonally amplified the DNA fragments with specific adapter. As sequencing proceed, incorporation of base protons (hydrogen ions) is released, and a signal is detected proportional to the number of base incorporated. The release of H+ (hydrogen ions) results in change in pH within the sensor wells and the change in pH is used to determine how many bases have been added in the sequence, and further data is analyzed.

SOLiD (Sequencing by Oligonucleotide Ligation and Detection) platform uses a unique sequencing by ligation approach. The ligation chemistry is based on the polony sequencing technique.

All the above technologies have been widely used in various field of biological sciences such as forensics (Weber-Lehmann et al. 2014), disease diagnosis (McCarthy et al. 2013), agri-genomics (Goddard and Hayes 2009; Van Borm et al. 2015), ancient DNA analysis (Poinar et al. 2006.), genetic diversity analysis (Fu and Peterson 2011), microbial diversity (Nikolaki and Tsiamis 2013), and plant–microbe interactions (Schenk et al. 2001; Knief 2014). This book chapter is mainly focused on outlying the application of next-generation technology on studying plant–microbe interactions.

The molecular mechanisms underlying these interactions are often regulated by hormones and have been revealed by gene expression profiling (Memelink 2009; Zhao et al. 2010). The study of microbial gene expressions using the NGS technology is known as metatranscriptomics, which is the most widely and commonly used approach in microbial research. The applications of these sequencing technologies are capable of explaining the complexity exhibited during plant–microbe interaction and lead to the opening of further more interesting topics involved in plant–microbe interaction.

Mosquera et al. (2009) carried out transcriptome analysis on the interaction between rice and rice blast fungal pathogen *Magnaporthe oryzae* and had led to the identification of biotrophy-associated secreted effector proteins which may prepare the plant cells for hyphal invasion. Another transcriptional profiling study between arbuscular mycorrhiza in Petunia hybrid has revealed the role of phosphate (Pi) in repressing essential symbiotic genes in the host (Breuillin et al. 2010). This

technology was successful to validate a hypothesis that some of the beneficial fungi (*Trichoderma* sp., *Piriformospora indica*) have little effect on host (barley) gene expression in the absence of pathogen (*Pseudomonas syringae*) (Brotman et al. 2012). Unno and Shinano (2013) did the transcriptomic analyses and have found that the microbiome present at the rhizosphere of *Lotus japonicus* leads to its better growth because these rhizospheric microbiome consists of some genes (such as alkaline phosphatase, citrate synthase) which are able to utilize the phytic acids and make it available for the plants in the soil which promotes its growth and development. These suggest that the presence of essential microbes is crucial for the better growth of the plant. Another transcriptome study has revealed that the epiphytic rhizosphere microbiome of soybean (*Glycine max*) was found to have an active role related to N, Fe, P, and K metabolism enabling proper growth of soybean (Mendes et al. 2014). Chhabra et al. (2013) identified genes and operons responsible for mineral phosphate solubilization in the barley rhizosphere using pyrosequencing 454 NGS technology. Certain phyllospheric bacteria present in association with tamarisk (*Tamarix nilotica*) contain few gene-encoding proteins that are involved in anoxygenic photosynthesis (bchY, pufM, and pufL) (Atamna et al. 2012). Metatranscriptomic and metabolomic studies of the plant microbiome are powerful tools that can provide complete picture of the active role of microbes toward its host at a given condition. Several transcripts were expressed differently at different stages of the host in the metatranscriptomic analysis of the rhizospheric microbiome of the *Arabidopsis* plants. Chaparro et al. (2014) reported that disease suppression genes (streptomycin synthesis) were significantly induced at bolting and flowering stages. However, in the mechanism of plant–microbe interaction, it is not always the microbes which are responsible for the association. Plants also exhibit developmental changes which are beneficial or harmful for the microbes. For example, in roots the developmental changes and expression of the genes have been well studied for the nodule formation in legume–rhizobium interactions (Stancey et al. 2006).

All the NGS technologies offer the useful analytical tools for the better understanding of plant–microbe interactions. Using such technology will be proven beneficial for all the plants by identifying the disease-causing pathogen and preventing it by breeding pathogen resistance varieties. Recently, a new technology has come up with better outcomes and results which is also known as "third-generation sequencing technology" which gives better read and proper picture for the molecular genomics research program. This includes the following technologies:

1. Helicos – Helicos Genetic Analysis System (Milos 2008)
2. PacBio RS- SMRT (Eid et al. 2009)
3. Oxford Nanopore Technologies (Clarke et al. 2009)

All the above sequencing technology emphasizes with the sequencing of a single molecule along with the real-time analysis.

21.4 Future Perspective

As the technologies progress, excellence can be seen in the metagenomic studies with the advent of next-generation sequencing which is not only fast but also reliable and low cost. However, it is needed for the lowering of the cost of metagenomic sequencing to meet the next-generation requisite. Thus, a higher coverage is required to obtain data of similar quality, and low-cost technique such as Illumina or SOLiD will help in sequencing up to the level of 40- to 50-fold. Though it can give broad spectrum of knowledge in understanding plant–microbe interaction, limitations are inconvenient in shorter reads and higher sequencing error rates. But future developments of the sequencing technology will really be interesting to witness the untold story of phyllosphere and rhizosphere research in even more detail. Another major constraint of metagenomic sequencing studies is the large number of sequence of unknown genes of unknown organism. This obstacle may be tackled by going back to conventional methods like genomic sequencing of representative pure cultures and the genetic and physiological characterization of strains. Similarly advances in single-cell genome sequencing has hold the promise by enabling the sequencing of yet uncultivated microorganisms (Rinke et al. 2013) which will enable a more specific assignment of metagenome reads to taxa. The analysis of metagenomic data with metatranscriptomic, metaproteomic, and meta-metabolomic data will be one of the very spectacular future perspectives to obtain a more complete view of the activities and the physiological potential of plant-associated microbial communities under given conditions at systems level.

21.5 Conclusion

No doubt sequencing technologies are now blooming and accelerating at the peak speed, but the full exploitation of microbial diversity for the purpose of gene discovery still remains a substantial challenge. The combinations of bioinformatic and next-generation sequencing technology have hugely increased the potential of metagenomic gene discovery to provide novel genes for industry, pharmaceutical production, and agriculture. EDNA strategy, using e-probes in BLAST searches, has the potential of assisting investigation of interactions of multiple microbes with each other and the plant. In conclusion, metagenomics with the help of cutting edge sequencing technologies provides a new window for viewing the microbial world that has the potential to revolutionize understanding of the entire living world. Metagenomics will greatly enhance our knowledge of microbial communities and can lead to major advancements in many areas, including human health, agriculture, energy production, and environmental remediation.

References

Aakvik T, Degnes KF, Dahlsrud R et al (2009) A plasmid RK2-based broad-host-range cloning vector useful for transfer of metagenomic libraries to a variety of bacterial species. FEMS Microbial Let 296:149–158

Alvarez TM, Paiva JH, Ruiz DM et al (2003) Structure and function of a novel cellulase 5 from sugarcane soil metagenome. PLoS One 8(12):e83635. doi:10.1371/journal.pone.0083635

Angelov A, Liebl W (2010) Heterologous gene expression in the hyperthermophilic archaeon Sulfolobus solfataricus. Methods Mol Biol 668:109–116

Arabidopsis Interactome Mapping Consortium (2011) Evidence for network evolution in an Arabidopsis interactome map. Science 333:601–607

Atamna-Ismaeel N, Finkel O, Glaser F, Von Mering C et al (2012) Bacterial anoxygenic photosynthesis on plant leaf surfaces. Environ Microbiol Rep 4:209–216

Bennett S (2004) Solexa Ltd. Pharmacogenomics 5:433–438

Bennett S, Barnes C, Cox A et al (2005) Towards the 1000 dollars human genome. Pharmacogenomics 6:373–382

Bentley DR (2006) Whole-genome re-sequencing. Curr Opin Genet Dev 16:545–552

Berendsen RL, Pieterse CMJ, Bakker PAHM et al (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Bertrand H, Poly F, Van VT et al (2005) High molecular weight DNA recovery from soils prerequisite for biotechnological metagenomic library construction. J Microbiol Method 62:1–11

Binnie C, Cossar JD, Stewart DIH et al (1997) Heterologous biopharmaceutical protein expression in Streptomyces. Trends Biotechnol 15:315–320

Bollmann A, Lewis K, Epstein SS et al (2007) Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates. Appl Environ Microbiol 73:6386–6390

Breuillin F, Schramm J, Hajirezaei M et al (2010) Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. Plant J 64:1002–1017

Brotman Y, Lisec J, Meret M et al (2012) Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. Microbiology 158:139–146

Burke C, Kjelleberg S, Thomas T et al (2009) Selective extraction of bacterial DNA from the surfaces of macroalgae. Appl Environ Microbiol 75:252–256

Canard B, Sarfati S (1994) Novel derivatives usable for the sequencing of nucleic acids. CA Patent 2,158,975, 13 Oct 1994

Chai T, Fadzillah M, Kusnan M et al (2005) Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. Biol Planta 49:153–156

Chaparro JM, Badri DV, Vivanco JM et al (2014) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8:790–803

Chhabra S, Brazil D, Morrissey J et al (2013) Characterization of mineral phosphate solubilization traits from a barley rhizosphere soil functional metagenome. Microbiology 2:717–724

Clarke J, Wu HC, Jayasinghe L et al (2009) Continuous base identification for single-molecule nanopore DNA sequencing. Nat Nanotechnol 4:265–270

Cowan D, Meyer Q, Stafford W et al (2005) Metagenomic gene discovery: past, present and future. Trends Biotechnol 23(6):321–329

Craig JW, Chang FY, Kim JH et al (2010) Expanding small-molecule functional metagenomics through parallel screening of broad-host-range cosmid environmental DNA libraries in diverse proteobacteria. App Environ Microbiol 76:1633–1641

Cullen DW, Hirsch PR (1998) Simple and rapid method for direct extraction of microbial DNA from soil for PCR. Soil Biol Biochem 30:983–993

Demidov VV, Bukanov NO, Frank-Kamenetskii D (2000) Duplex DNA Capture. Curr Issues Mol Biol 2:31–35

Eckardt NA (2000) Sequencing the rice genome. Plant Cell 12:2011–2017

Eid J, Fehr A, Gray J et al (2009) Real-time DNA sequencing from single polymerase molecules. Science 323:133–138
Eyers L, George I, Schuler L et al (2004) Environmental genomics: exploring the unmined richness of microbes to degrade xenobiotics. Appl Microbiol Biotechnol 66:123–130
Fageria NK, Baligar VC, Jones CA et al (2011) Growth and mineral nutrition of field crops, 3rd edn. CRC Press, Boca Raton
Fitter AH, Gilligan CA, Hollingworth K et al (2005) The members of the Nerc soil biodiversity programme. Biodiversity and ecosystem function in soil. Funct Ecol 19: 369–377
Fu YB, Peterson GW (2011) Genetic diversity analysis with 454 pyrosequencing and genomic reduction confirmed the eastern and western division in the cultivated barley gene pool. Plant Genome 4:226–237
Fullwood MJ, Wei CL, Liu ET et al (2009) Next generation DNA sequencing of paired end tags (PET) for transcriptome and genome analyses. Genome Res 19:521–532
Gabor EM, Alkema WB, Janssen DB et al (2004) Quantifying the accessibility of the metagenome by random expression cloning techniques. Environ Microbiol 6:879–886
Goddard ME, Hayes BJ (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nat Rev Genet 10:381–391
Goh CH, Veliz Vallejos DF, Nicotra AB et al (2013) The impact of beneficial plant-associated microbes on plant phenotypic plasticity. J Chem Ecol 39:826–839
Gray JP, Herwig RP (1996) Phylogenetic analysis of the bacterial communities in marine sediments. Appl Environ Microbiol 62:4049–4059
Green BD, Keller M (2006) Capturing the uncultivated majority. Curr Opin Biotechnol 17:236–240
Haldar S, Sengupta S (2015) Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. Open Microbiol J 9:1–7
Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685
Handelsman J, Rondon MR, Brady SF et al (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5:245–249
Healy FG, Ray RM, Aldrich HC et al (1995) Direct isolation of functional genes encoding cellulases from the microbial consortia in a thermophilic, anaerobic digester maintained on lignocellulose. App Microbiol Biotechnol 43:667–674
Hua J (2013) Modulation of plant immunity by light, circadian rhythm, and temperature. Curr Opin Plant Biol 16:406–413
Huse SM, Huber JA, Morrison HG et al (2007) Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biol 8(143):1–9
Ishoey T, Woyke T, Stepanauskas R et al (2008) Genomic sequencing of single microbial cells from environmental samples. Curr Opin Microbiol 11:198–204
Jarvie T, Harkins T (2007) Metagenomics analysis using the genome sequencer FLX system. Nat Method 4:3–5
Johnston CG, Aust SD (1994) Detection of *Phanerochaete chrysosporium* in soil by PCR and restriction enzyme analysis. Appl Environ Microbiol 60:2350–2354
Jonathan M, Rothberg HW et al (2011) An integrated semiconductor device enabling non-optical genome sequencing. Nature 475:348–352
Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329
Kauffmann IM, Schmitt J, Schmid RD (2004) DNA isolation from soil samples for cloning in different hosts. Appl Microbiol Biotechnol 64:665–670
Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front Plant Sci 5(216):1–23
Kojima M, Kamada-Nobusada T, Komatsu H et al (2009) Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography–tandem mass spectrometry: an application for hormone profiling in *Oryza sativa*. Plant Cell Physiol 50:1201–1214
Kothari R, Singh RP, Kothari V et al (2006) Application of next generation sequencing technologies in revealing plant-microbe interactions. J Next Gen Seq App 3(1):1–2

Kozdroj J (2010) Isolation of nucleic acids from the environment. Kosmos 59:141–149
Krsek M, Wellington EMH (1999) Comparison of different methods for the isolation and purification of total community DNA from soil. J Microbiol Method 39:1–16
Lander ES, Linton LM, Birren B et al (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921
Lasken RS (2009) Genomic DNA amplification by the multiple displacement amplification (MDA) method. Biochem Soc Trans 37:450–453
Lee S, Bollinger J, Bezdicek D et al (1996) Estimation of the abundance of an uncultured soil bacterial strain by a competitive quantitative PCR method. Appl Environ Microbiol 62:3787–3793
Levin SA (2006) Fundamental questions in biology. PLoS Biol. doi:10.1371/journal. pbio.0040300
Li X, Qin L (2005) Metagenomics-based drug discovery and marine microbial diversity. Trends Biotechnol 23:539–543
Liesack W, Stackebrandt E (1992) Occurrence of novel groups of the domain bacteria as revealed by analysis of genetic material isolated from an Australian terrestrial environment. J Bacteriol 174:5072–5078
Lister R, Gregory BD, Ecker JR et al (2009) Next is now: new tech- nologies for sequencing of genomes, transcriptomes, and beyond. Curr Opin Plant Biol 12:107–118
Liu L, Li Y, Li S et al (2012) Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012:1–11
Lorenz P, Eck J (2005) Metagenomics and industrial applications. Nat Rev Microbiol 3:510–516
Lorenz P, Schleper C (2002) Metagenome – a challenging source of enzyme discovery. J Mol Cat Ser B-Enzym 19:13–19
Mamanova L, Coffey AJ, Scott EC et al (2010) Target-enrichment strategies for next- generation sequencing. Nat Am 7:111–118
Margulies M, Egholm M, Altman WE et al (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380
McCarthy JJ, McLeod HL, Geoffrey S et al (2013) Ginsburg genomic medicine: a decade of successes, challenges, and opportunities science. Trans Med 5(189):1–4
Memelink J (2009) Regulation of gene expression by jasmonate hormones. Phytochemistry 70:1560–1570
Mendes LW, Kuramae EE, Navarrete AA et al (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 8:1577–1587
Milos P (2008) Helicos BioSciences. Pharmacogenomics 9:477–480
Mosquera G, Giraldo MC, Khang CH et al (2009) Interaction transcriptome analysis identifies *Magnaporthe oryzae* BAS1-4 as biotrophy-associated secreted proteins in rice blast disease. Plant Cell 21:1273–1290
Newton AC, Fitt BD, Atkins SD et al (2010) Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. Trends Microbiol 18:365–373
Nikolaki S, Tsiamis G (2013) Microbial diversity in the era of omic technologies. Biomed Res Int 2013:1–15
Hutchison CA (2007) DNA sequencing: bench to bedside and beyond. Nucleic Acids Res 35:6227–6237
Oldroyd GE (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263
Parachin NS, Gorwa-Grauslund MF (2011) Isolation of xylose isomerases by sequence- and function-based screening from a soil metagenomic library. Biotechnol Biofuels 4:1–9
Poinar HN, Schwarz C, Qi J et al (2006) Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA. Science 20:392–394
Purohit MK, Singh SP (2009) Assessment of various methods for extraction of metagenomic DNA from saline habitats of coastal Gujarat (India) to explore molecular diversity. Lett Appl Microbiol 49:338–344
Rees HC, Grant WD, Jones BE et al (2004) Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. Extremophiles 8:63–71

Riesenfeld CS, Goodman RM, Handelsman J et al (2004) Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environ Microbiol 6:981–989
Rinke C, Schwientek P, Sczyrba A et al (2013) Insights into the phylogeny and coding potential of microbial dark matter. Nature 25:431–437
Robe P, Nalin R, Capellano C et al (2003) Extraction of DNA from soil. Europ J Soil Biol 39:183–190
Ronald PC, Shirasu K (2012) Front-runners in plant-microbe interactions. Curr Opin Plant Biol 15:345–348
Sagerstrom CG, Sun BI, Sive HL et al (1997) Subtractive cloning: past, present, and future. Annu Rev Biochem 66:751–783
Saito K, Matsuda F (2010) Metabolomics for functional genomics, systems biology, and biotechnology. Annu Rev Plant Biol 61:463–489
Sanger F, Nicklen S, Coulson AR et al (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463–5467
Schenk PM, Remans T, Sagi L et al (2001) Promoters for pregenomic RNA of banana streak badnavirus are active for transgene expression in monocot and dicot plants. Plant Mol Biol 47:399–412
Sharma S, Vakhlu J (2014) Metagenomics as advanced screening methods for novel microbial metabolite. In: Harzevili FD, Chen H (eds) Microbial biotechnology progress and trends. CRC Press, Boca Raton, pp 43–62
Shendure J, Porreca GJ, Nikos B et al (2005) Accurate multiplex polony sequencing of an evolved bacterial genome. Science 309:1728–1732
Shizuya H, Birren B, Kim UJ et al (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc Nat Aca Sci USA 89:8794–8797
Simon C, Daniel R (2011) Metagenomic analyses: past and future trends. App Environ Microbial 77:1153–1161
Stacey G, Libault M, Brechenmacher L et al (2006) Genetics and functional genomics of legume nodulation. Curr Opin P Biol 9:110–121
Steele H, Streit WR (2006) Metagenomics for the study of soil microbial communities. In: Cooper JE, Rao JR (eds) Molecular approaches this soil rhizosphere and plant microorganism analysis. CAB Inter-national, Wallingford, pp 42–54
Steele HL, Jaeger KE, Daniel R et al (2009) Advances in recovery of novel biocatalysts from metagenomes. J Mol Microbial Biotechnol 16:25–37
Strohl WR (1992) Compilation and analysis of DNA sequences associated with apparent Streptomyces promoters. Nucleic Acids Res 20:961–974
Suenaga H (2012) Targeted metagenomics: a high-resolution metagenomics approach for specific gene clusters in complex microbial communities. Environ Microbiol 14:13–22
Thomas T, Rusch D, DeMaere MZ et al (2010) Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. ISME J 4:1557–1567
Tringe SG, Rubin EM (2005) Metagenomics: DNA sequencing of environmental samples. Nat Rev Gen 6:805–814
Tsai Y, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediment. Appl Environ Microbiol 57:1070–1074
Tuffin M, Anderson D, Heath C et al (2009) Metagenomic gene discovery: how far have we moved into novel sequence space. Biotechnol J 4:1671–1683
Unno Y, Shinano T (2013) Metagenomic analysis of the rhizosphere soil microbiome with respect to phytic acid utilization. Microbes Environ 28:120–127
Van Borm S, Belak S, Freimanis G et al (2015) Next-generation sequencing in veterinary medicine: how can the massive amount of information arising from high-throughput technologies improve diagnosis, control, and management of infectious diseases? In: Veterinary infection biology: molecular diagnostics and high-throughput strategies. Springer, Berlin, pp 415–36
Venter JC, Adams MD, Myers EW et al (2001) The sequence of the human genome. Science 291:1304–1351

Weber-Lehmann J, Schilling E, Gradl G et al (2014) Finding the needle in the haystack: differentiating "identical" twins in paternity testing and forensics by ultra-deep next generation sequencing. Forensic Sci Inter Genet 9:42–46

Wheeler DA, Srinivasan M, Egholm M et al (2008) The complete genome of an individual by massively parallel DNA sequencing. Nature 452:872–876

Yeates C, Gillings MR, Davison D et al (1998) Methods for microbial DNA extraction from soil for PCR amplification. Biol Proced Online 1:40–47

Zhao J, Ohsumi TK, Kung JT et al (2010) Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol Cell 40(6):939–953

Disease-Induced Resistance and Plant Immunization Using Microbes

22

Miguel O.P. Navarro, Ane S. Simionato, André R. Barazetti, Igor M.O. dos Santos, Martha V.T. Cely, Andreas L. Chryssafidis, and Galdino Andrade

Abstract

The induction of resistance in plants presents as an alternative to be explored in several species. This process involves the activation of defense mechanisms, which are inactive or latent in the plant and do not require alterations in your genome. This activation can be effected by biotic and abiotic agents known as resistance inducers. The use of resistance inducers leads to activation of the systemic resistance, which leads to a marked reduction in symptoms of the disease after subsequent infections, including different species of pathogens. This chapter gathers information about diverse compounds of biological origin that can act as resistance inductors, as well as an interaction between plants and rhizosphere microorganisms that may result in the activation of this resistance system against pathogens.

Keywords

Jasmonic acid • Ethylene • Mycorrhiza • Secondary metabolites

M.O.P. Navarro • A.S. Simionato • A.R. Barazetti • I.M.O. dos Santos • G. Andrade (✉)
Laboratory of Microbial Ecology, Department of Microbiology, State University of Londrina, Londrina, Paraná, Brazil
e-mail: andradeg@uel.br

M.V.T. Cely
Institute of Agrarian and Environmental Sciences, Federal University of Mato Grosso, Sinop, Mato Grosso, Brazil

A.L. Chryssafidis
Laboratory of Veterinary Toxicology, Department of Preventive Veterinary Medicine, State University of Londrina, Londrina, Paraná, Brazil

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_22

22.1 Introduction

Over thousands of years, plants and microorganisms have coevolved together. During this interaction, plants created strategies to delay or prevent the entry of pathogens and subsequent tissue infection. This capacity to recognize potential invading pathogens and to trigger a successful defense response is defined as *disease-induced resistance*. The selective pressure on plants has led to the improvement of their defense mechanisms. The disease resistance is the rule, while the pathogen success to cause disease, far from being the rule, is an exception (Pascholati and Leite 1995; Staskawicz 2001).

The defense of plants against diseases comprises a number of events related to the recognition, signaling, and response, defined as the plant immunity. The PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) mediate the activation of innate immunity. These defense mechanisms induce accumulation of salicylic acid (SA), which has an intermediary role on inhibiting the action of catalase, converting H_2O_2 in H_2O and O_2. This reactive oxygen species (ROS) can act as a secondary messenger, stimulating the expression of genes responsible for leading systemic acquired response (SAR) (Chen et al. 1993).

SAR is induced in plants when they respond to the primary infection by a pathogen, resulting in hypersensitive reaction (HR), which usually leads to local necrotic lesions formed by brown and desiccated tissue (Compant et al. 2005). These lesions are primarily characterized by the accumulation of pathogenesis-related (PR) proteins (Fritig et al. 1998).The PR-protein group contains different classes of proteins, such as hydrolase [β-1,3-glucanase] (PR-2) and quitinase (PR-3), which can inhibit the growth of pathogens (Schlumbaum et al. 1986; van Loon and van Strien 1999). Therefore, SAR gives the plant a higher level of resistance against a subsequent infection by the same pathogen. These effects persist for a long period postinfection and can be induced by chemical or biological methods.

Another partial response mechanism is called induced systemic resistance (ISR), primarily mediated via jasmonic acid (JA) and ethylene (ET). It is a very important phenomenon, which promotes the control of phytopathogens by localized infection or treatment with structurally unrelated microbial products or organic/inorganic compounds (Pascholati and Leite 1995). ISR is effective in protecting plants against many types of pathogens, but the inducing microorganism does not cause disease in the host, differently from what happens in SAR. However, in both SAR and ISR, the plant recognizes the danger and triggers cellular responses through elicitation.

Elicitation is a set of events by which cells subjected to external factors trigger defense mechanisms that activate and regulate the expression of biochemical and molecular responses, increasing the synthesis of specific metabolites (Leite et al. 1997). These factors are called elicitors and are classified according to their biotic nature as JA, SA, chitosan, alginate, and fungal extracts (Dong and Zhong 2002; Wang and Zhong 2002) or abiotic as heavy metals, thermal stress, and osmotic stress (Yu et al. 2005). Likewise, the plant-elicitor interaction can be classified as specific, when it only acts on a particular species, or general, if it triggers a defense response in any plant (Vasconsuelo and Boland 2007). The detection of specific

elicitors by plants allows recognition of pathogens. The signals are transported throughout the plant, activating local and systemic responses (Pascholati 2003).

Currently, several SAR and ISR elicitors – proteins, glycoprotein, peptides, chitin, glucan, polysaccharides, lipids, and secondary metabolites produced by fungus and bacteria – were found to induce the synthesis of compounds, such as phytoalexins, defensins, phenolic, flavonoids, and proteins that directly attack pathogens (Baker et al. 1997). One of the most promising strategies to control plant pathogens is by ISR, using natural microbial compounds (Louws et al. 2001). The elicitor molecules can activate a systemic resistance mechanism related to the synthesis of proteins, mediated by SA, JA, and ET, protecting the plant tissue against a pathogen infection (Hammond-Kosack and Parker 2003). Furthermore, the development of SAR and ISR using root colonization by plant rhizosphere microorganisms, particularly arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), can also suppress plant diseases caused by a range of pathogens through elicitation of physical and chemical changes related to systemic plant defense mechanisms (Sarma et al. 2002).

The present chapter will present and discuss mechanisms involved in plant defense, including ISR using biocompounds and AMF/PGPR that stimulate protective mechanisms against phytopathogens. We will focus on how some commercial products and rhizosphere microbes act for triggering ISR and SAR and show results of natural microbial compounds that are used to induce expression of specific genes in plants.

22.2 Phytohormonal Defense

Plants and phytopathogenic microorganisms evolved together, both pursuing their own development and reproduction. Pathogens developed mechanisms capable of transposing the host defense barriers, while the plants evolved to better identify and prevent the successful attack of possible phytopathogens, through the activation of defense mechanisms. The establishment of pathogenicity occurs only when the pathogen is able to suppress these plant defenses or to prevent, at some point, the signaling pathway of the host, thus avoiding identification and consequent activation of the plant defense system (Muthamilarasan and Prasad 2013). The process of signaling and activating genes responsible for defense is mediated mainly by the action of three phytohormones: salicylic acid (SA), jasmonic acid (JA), and ethylene (ET).

In general, the SA-mediated signaling pathway is related to the activation of defense against biotrophic phytopathogens, those which require a living tissue to complete their development, whereas routes mediated by JA and ET are related to responses against necrotrophic phytopathogens (those which grow in dead tissue) (Loak and Grant 2007). After their synthesis, receptor proteins, even unknown, interact with SA, JA, and ET and activate a transduction signal pathway that culminates on the activation of transcription, or repression, of a large number of genes

involved in important processes for growth, defense, secondary metabolism, and plant senescence (Wasternack 2007).

Biotic and abiotic stresses like pathogen and insect attack or wounding generate signals/elicitors that activate a phosphorylation cascade, regulating SA and JA/ET biosynthesis and signaling. SA is critical for plant defense against a broad spectrum of pathogens, and it is involved in multiple processes, such as basal resistance, gene-mediated resistance, and SAR (Lu et al. 2016). The accumulation of secondary metabolites by the JA/ET signaling pathway includes a wide variety of plant by-products, including terpenoids, flavonoids, alkaloids, phenylpropanoids, and many other types of secondary metabolites. These molecules can also be transported to distant tissues or neighboring plants that have not been directly challenged to activate systemic gene expression. The JA/ET signaling pathway is fundamental for the biosynthesis of many by-products in plants, including ISR (Kazan and Manners 2008).

The ROS production by SA accumulation triggers defense signaling, which depends on the transduction of non-expressor of PR gene1 (NPR1). NPR1 is part of a group of proteins known to mediate protein interactions and is directly linked to the production of PR proteins and induction of plant resistance (Pieterse et al. 2012). Moreover, NPR1 contributes to the antagonistic interaction (cross talk) between SA and JA (Spoel et al. 2007; Yuan et al. 2007), as well as regulates the suppression of JA-dependent genes (Pieterse et al. 2009). The cross talk between JA and SA signaling occurs at multiple points, also modulated by ET. This complex interaction among signaling networks testifies the plant ability to integrate diverse signals, from multiple sources, in a way that a finely tuned output is produced and hence provides adaptation to its environment.

SA signaling through NPR1 is required to establish SAR. Exogenous application of SA is sufficient to bypass the need for an initial infection on inducing the transcription of antimicrobial PR genes and enhancing general resistance. Besides SAR, NPR1 is also required for ISR in leaves triggered by PGPR. The mechanism of how NPR1 regulates ISR is not completely understood. In contrast to SAR, which demands nuclear localization of NPR1, ISR requires cytoplasmic localized NPR1 by PGPR colonization, and it is independent of SA accumulation but requires JA and ET (Withers and Dong 2016).

22.3 Microorganisms on Induced Systemic Resistance (ISR)

The rhizosphere, defined as the zone of soil surrounding the plant roots, is a critical site for interactions between microorganisms and plants (Paul and Clark 1989). It is a complex and diverse microbiome, where microorganisms presenting beneficial or deleterious effects on plant growth can be found (Mendes et al. 2013). Beneficial microorganisms in the rhizosphere include nitrogen-fixing bacteria, mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), and protozoa. Among these, the most prevalent beneficial organisms associated with plants are the mycorrhizal

fungi, which form mutualistic root-fungal associations know as mycorrhiza (Smith and Read 2008).

Much has been studied about microorganisms and their interactions with plants, especially in the rhizosphere. These interactions are highly beneficial to both, especially when they promote growth through increasing the absorption of nutrients, altering microbiological communities, acting on the biocontrol of pathogens, and promoting plant resistance (SAR/ISR).

Plant growth-promoting fungi (PGPF) and PGPR are among these highly beneficial microorganisms, essential for plant maintenance and nutrition, as well as arbuscular mycorrhizal fungi (AMF) and actinomycetes.

Nonpathogenic bacteria present in the rhizosphere can antagonize pathogenic organisms through competition for nutrients and production of antimicrobial compounds and lytic enzymes but also through the activation of plant defenses. JA, its derivatives, and ET induce ISR, and they are directly involved in the response to systemic lesions. However, ET is not always required for triggering defense responses, and it may not accumulate near the lesions of some plants. Despite that, roots of *Arabidopsis thaliana* demonstrated high ability to convert 1-aminocyclopr opane-1-carboxylate (ACC) to ET after treatment with *Pseudomonas fluorescens*, indicating that these bacteria can induce the production of large amounts of ET, playing a regulatory role in plant defense responses, according to the situation (Bent 2006).

The ISR promoted by PGPR only occurs when a minimum number of microorganisms are present, 10^5 CFU/g of plant root, and the time of root colonization by the bacteria is not less than a few days (Jankiewicz and Koltonowicz 2012). Among the PGPR, the genera *Pseudomonas* and *Bacillus* are the most studied, mostly due to their abundance in the soil and their role in the suppression of soil pathogens (Table 22.1).

The genus *Pseudomonas* is widespread in nature, particularly in humid environments with pathogenic potential. It is an important gram-negative soil bacterium, particularly for plants with systemic root, capable of breaking aromatic polycyclic hydrocarbon rings. They are widely studied in relation to the production of a large variety of secondary metabolites that may inhibit the growth or metabolism of other microorganisms. Some strains have the capacity to produce antibiotic, antiparasitic, antitumor, and antifungal compounds, being able to produce only one class or all of them.

Some studies carried out with *P. fluorescens* WCS417 demonstrated an increase in resistance of plants to *P. syringae* pv. *tomato*, *Hyaloperonospora parasitica*, *Alternaria brassicicola*, and *B. cinerea* (Jankiewicz and Koltonowicz 2012). Basically, ET is the first compound to initiate ISR. Studies indicate that *P. fluorescens* WCS417 activates the transcription of MYB72. This gene is essential for the early stages of ISR and belongs to the R2R3-MYB gene family, which results in the formation of the R2R3-MYB protein, with an important role on stress tolerance, regulation of cell death, and resistance to pathogens in *Arabidopsis* (Stracke et al. 2001).

Table 22.1 Examples of microorganisms in induced systemic resistance (ISR) against phytopathogens

Microorganism	Plant	Phytopathogen	Elicitor	References
P. fluorescens CHAO	Arabidopsis	*Peronospora parasítica*	2,4-diacetylphloroglucinol	Iavicoli et al. (2003)
P. fluorescens Q2-87	Arabidopsis	*P. parasítica*	2,4-diacetylphloroglucinol	Iavicoli et al. (2003)
P. fluorescens WCS417	Clove	*Fusarium* sp.	Lipopolysaccharide	Van Wees et al. (1997)
	Radish	*Fusarium* sp.	Lipopolysaccharide, siderophores	Van Wees et al. (1997)
Pseudomonas putida WCS358	Arabidopsis	*Pseudomonas syringae* pv. tomato	Lipopolysaccharide, flagellin, siderophores	Meziane et al. (2005)
	Bean	*Botrytis cinerea*	Lipopolysaccharide, siderophores	Meziane et al. (2005)
	Tomato	*B. cinerea*	Lipopolysaccharide, siderophores	Meziane et al. (2005)
Pseudomonas aeruginosa 7NSK2	Tobacco	TMV	Salicylic acid	De Meyer et al. (1999a)
	Tomato	*B. cinerea*	Salicylic acid	Audenaert et al. (2002)
	Rice	*Magnaporthe grisea*	Salicylic acid, pyocyanin, pyochelin	De Vleesschauwer et al. (2006)
	Rice	*Rhizoctonia solani*	Salicylic acid, pyocyanin, pyochelin	De Vleesschauwer et al. (2006)
Bacillus subtilis GB03	Arabidopsis	*Erwinia carotovora*	2,3-butanediol	Ryu et al. (2004)
Bacillus amyloliquefaciens IN937	Arabidopsis	*E. carotovora*	2,3-butanediol	Ryu et al. (2004)
Serratia liquefaciens	Tomato	*Alternaria alternata*	N-acyl-L-homoserine lactone	Schuhegger et al. (2006)
Trichoderma harzianum T-78	Tomato	*Meloidogyne incógnita*	Xylanase, proteinaceous nonenzymatic elicitor (SM1)	Martínez-Medina et al. (2016)
	Tomato	*Meloidogyne javanica*	Xylanase, SM1	Martínez-Medina et al. (2016)
Fusarium oxysporum 47 (Fo47)	Tomato	*F. oxysporum*		Vos et al. (2014)
Pythium oligandrum	Tomato	*F. oxysporum*		Vos et al. (2014)
Trichoderma virens	Cotton	*Colletotrichum* sp.	SM1	Djonović et al. (2006)
		Magnaporthe sp.		

While ISR is associated with resistance induction by PGPR/PGPF, SAR is usually associated with necrotic phytopathogenic bacteria and fungi. However, this is not a rule. There were frequent reports of PGPR inducing resistance via SAR, suggesting that nonpathogenic bacteria, which commonly promote resistance through ISR, may have acquired genes from pathogenic microorganisms or developed these genes by themselves. As previously explained, induction of resistance via SAR involves a number of plant responses, including accumulation of SA, PR proteins, phytoalexin production, and enzyme expression (Bent 2006).

In *E. coli*, for instance, salicylate exposure inhibits the production of OmpF porins, which act as nonselective pores in the outer wall of bacterium, through which small molecules can be diffused. Reduction of OmpF porin expression results in smaller channels, increasing the selectivity and preventing large, usually toxic, molecules from permeating the bacterial cell. It is believed that the ability of a bacterium to colonize plant tissues and the rhizosphere is influenced by sensitivity to phytoalexins, indicating that the regulation of porin size, in response to plant defense, may help PGPR gram-negative bacteria inducing SAR to overcome the plant defense (Bent 2006).

P. aeruginosa 7NSK2 significantly reduces the diameter of the tobacco mosaic virus (TMV) infection by triggering ISR, through the release of nanograms (10–100 ng) of SA, and similar effect was observed on bean (De Meyer et al. 1999b), tomato (Audenaert et al. 2002), and rice (De Vleesschauwer et al. 2006). This example is an indication of how interactions between pathways of plants signal transduction can occur, which allows adjustments in the defense strategy to different environmental conditions (Verhagen et al. 2010). *P. aeruginosa* 7NSK2 also produces pyochelin, phenazine, and pyoverdine compounds, which may be associated with or combined to SA, in the activation of ISR (Audenaert et al. 2002).

Similar to *Pseudomonas*, the genus *Bacillus* is broadly distributed in nature, being found in soil, plants, water, and others. They stand out for producing a diverse range of bioactive compounds with antimicrobial activity and potential ISR elicitors. Some species of *Bacillus*, such as *B. subtilis* GB03 and *B. amyloliquefaciens* IN937, produce volatile organic compounds (VOCs) that induce ISR in *Arabidopsis* (Ryu et al. 2003), reducing the severity of *E. carotovora*. Of all the VOCs produced by these species, only 2,3-butanediol and 3-hydroxy-2-butanone (acetoin) have inductive activity. They are produced under low atmospheric O_2 partial pressure, in situations where it is not possible to perform the usual breathing (Ryu et al. 2004). Activation of ISR by *B. subtilis* GB03 is uniquely dependent on ET, unlike *B. amyloliquefaciens* IN937, which is not activated by any of the three usual pathways, presenting new ISR trigger pathways to be explored (Choudhary and Johri 2009).

The promotion of plant growth by fungi is performed by groups of nonpathogenic fungi, such as ascomycetes (*Trichoderma*, *Fusarium*) and oomycetes (*Pythium*), but some of them may be strains of hypervirulent fungi (Bent 2006).

Among the major fungi with the capacity to induce ISR in plants, those from the genus *Trichoderma* are among the most studied and well understood. *Trichoderma* spp. are normal constituents of the soil microbiota and are frequently isolated in the rhizosphere, as nonpathogenic and opportunistic microbes that colonize the roots of

many plants. These fungi present great biotechnological potential, which could provide important benefits to agriculture, since they have the capacity to protect large crops against diseases through the biocontrol of pathogenic fungi, oomycetes, and nematodes, besides stimulating ISR (Hermosa et al. 2013; Martínez-Medina et al. 2016).

About parasitic attack, the interaction between roots and nematodes is very dynamic, and the plant response differs significantly between the early and advanced stages of the infestation. Initially, plant defense responses (callose deposition) are related to recognition, invasion, and migration of nematodes. In later stages of infection, plants defenses are directed to the development and reproduction of vesicles. Fungi from the genus *Trichoderma* have been associated with ISR against nematode attack, for instance, against the attack of *M. javanica*. The fungus increased the accumulation of different antagonistic compounds, such as peroxidases and phenol oxidases. In a split-root experiment, *T. harzianum* T-78 protected tomato roots against *Meloidogyne incognita* attack at different stages of infection, in local and systemic root tissues, by eliciting ISR (Martínez-Medina et al. 2016).

Fusarium oxysporum is another of the most representative fungi populations in soil microbiota all over the world. Some strains of *F. oxysporum* are pathogenic, causing damage and disease in many cultures, but other strains are nonpathogenic. Moreover, some of these have the ability to protect plants against the attack of virulent strains. The use of nonpathogenic strains of *F. oxysporum* was proposed for the alternative control of diseases caused by pathogenic species from the same genus. The strain of *F. oxysporum* 47 (Fo47) was efficient in controlling tomato wilt, by inducing the expression of defense genes encoding extracellular pathogenesis-related proteins (PR), potentially by SAR (Aimé et al. 2013).

Another fungus that demonstrated protective potential in tomato plants against many fungal and bacterial pathogens via SAR is the oomycete *Pythium oligandrum*. Tomato plants pretreated with *P. oligandrum* typically react to subsequent infection by pathogenic organisms with a combination of physical and chemical responses, seeking to limit penetration and propagation of the pathogen. Pretreated tomato plants later infected with *F. oxysporum* f. sp. *lycopersici* exhibited a rise in callose deposition, thus preventing the penetration of pathogen in the cell cylinder. Increased amounts of phytoalexins and PR proteins were observed as well. Structural barrier formations also prevented infections of tomato plants by wilt-causing *Ralstonia solanacearum* (Vos et al. 2014).

22.3.1 Arbuscular Mycorrhizal Symbioses

The mycorrhizas are subdivided in two categories, ectomycorrhizas and endomycorrhizas. Ectomycorrhizas form typical structures as mantle and a Hartig net in the roots and are present predominantly in tree species. In endomycorrhizas, the fungal hyphae colonize inside the root cells. The endomycorrhizas category includes arbuscular mycorrhizas, ericoid mycorrhizas, arbutoid mycorrhizas, monotropoid mycorrhizas, and orchid mycorrhizas (Peterson et al. 2004).

Arbuscular mycorrhizal fungi (AMF) belong to the phylum *Glomeromycota*. They are mandatory biotrophic organisms that associate with the roots, forming a symbiotic mutualist relationship called arbuscular mycorrhiza (AM), which occurs in about 80% of plant species (Giovannetti et al. 2010; Gutjahr and Parniske 2013). In this interaction, the AMF is benefited by the transfer of carbon compounds from the host plants, while AMF provides the host plant with mineral nutrients (especially phosphate and nitrogen) and water and enhances the plant defense capacity against pathogens (Smith and Read 2008).

The establishment of AM can be divided in distinct phases, characterized by the degree of AMF hyphae progression during root colonization. The process of colonization begins with the reciprocal chemical signalization between AMF and plants, called pre-symbiotic phase. In pre-symbiotic phase, the hyphae forms branches, induced by the recognition of strigolactones produced by the plant, also called branching factors, which helps the fungi to make contact with the root and to establish symbiosis (Fig. 22.1) (Akiyama and Hayashi 2006; Akiyama et al. 2010).

The appressorium (infection structure) is formed after contact between hyphae and root. During the hyphal penetration into the roots tissues, the epidermal cell nucleus moves toward the appressorium, forming a dense structure consisted of vesicles from the endoplasmic reticulum, actin filaments, and microtubules around the contact point. Consequently, the nucleus migrates to the opposite point of the epidermal cell and forms a cytoplasmic space that contains the pre-penetration apparatus (PPA) and connects the nucleus to its original position, below the appressorium. PPA formation predetermines the pathway of hyphae penetration into the cells. The hyphae elongation into epidermal and cortical cells is facilitated by the oscillation in the concentration of Ca^{2+} in the cell root cytoplasm. This Ca^{2+} oscillation in the cytoplasm induces the expression of genes related to mycorrhizal infection, activated when the AM fungi get in contact with the root (Gutjahr and Parniske 2013). The AMF mycelia grow into the roots, where it establishes highly branched hyphae called arbuscules within the cortical cells. In the arbuscule, mineral nutrients are transferred for host plant (Harrison 2012).

22.3.1.1 Arbuscular Mycorrhizal Symbiosis: Plant Responses and Resistance Induction

The interaction between plant and fungi on AM establishment induces hormonal alterations in host plants, modifying their responses to biotic and abiotic stresses (Pozo et al. 2010). These molecular responses to AM colonization can regulate the host tolerance to AMF colonization; on the other hand, other tissues or organs can develop early and enhanced defense responses to pathogen attack after AM establishment. This process is called "priming" effect (Jung et al. 2012; Selosse et al. 2014).

Hormonal host responses to AM colonization include auxins, cytokinins, abscisic acid (ABA), and JA production (Ludwing-Müller 2010; Pozo et al. 2015). Auxin, especially indole-3-butyric acid (IBA), occurs after AMF colonization in roots (Jentschel et al. 2007) and has been related to modifications in root architecture on lateral root formation (Kaldorf and Ludwig-Müller 2000; Ludwing-Müller 2010).

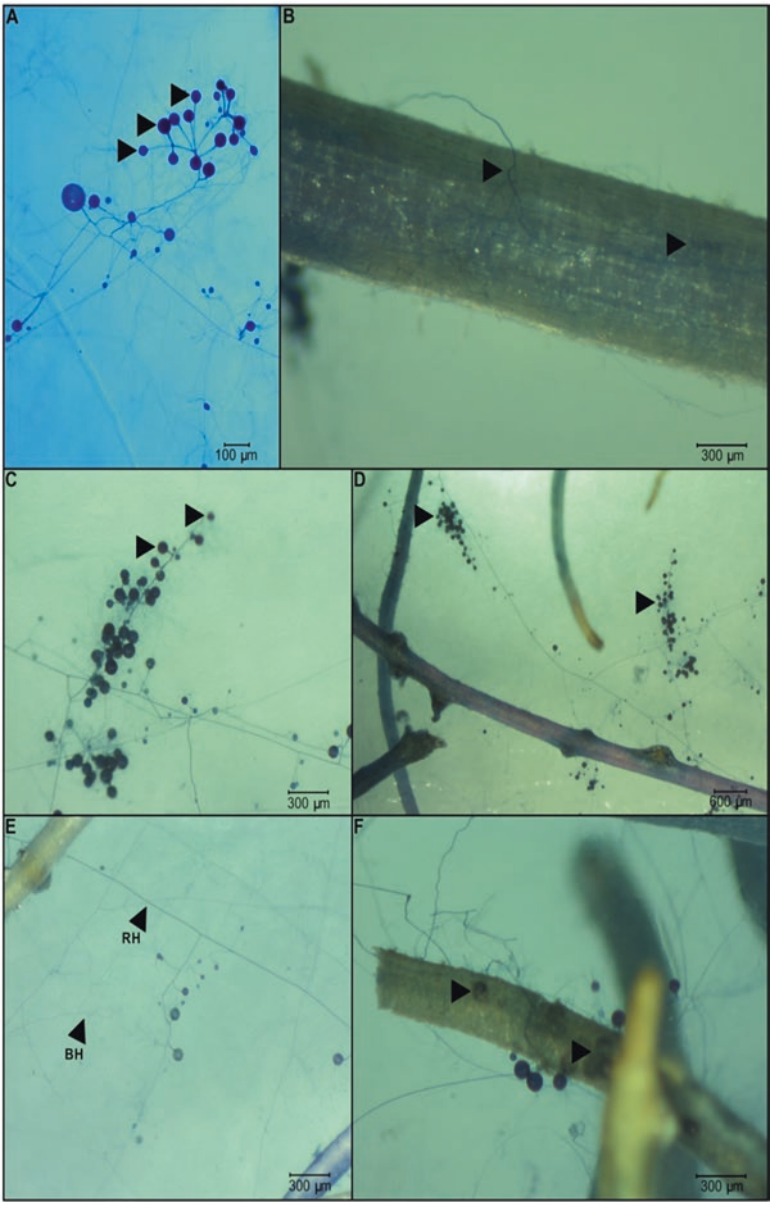


Fig. 22.1 In vitro development of FMA *Rhizophagus clarus*: formation of vesicles in the presymbiotic phase (**a**); hypha contact with the root and formation of infection structure (**b**); spore production (**c**, **d**); extra-radicular mycelium architecture "running hyphae" (*RH*) and "branched hyphae" (*BH*) (**e**); spores production within the root (**f**)

Cytokinins function as receptors in AM symbiosis and help the repression of defensive response of host during colonization (Shaul et al. 1999). ABA levels increase after AM formation and contribute to drought stress tolerance in roots (Asensio et al. 2012). JA is a common regulator in plants. It is a signal to different abiotic and biotic stresses in plant cellular responses and in plant-herbivore/plant-plant interactions. Several studies have related the induction of defense gene expression by JA (Pozo et al. 2005).

It was observed that AMF (*Glomus intraradices*) colonization increased the induction of JA biosynthesis in colonized roots of barley (*Hordeum vulgare* cv. Salome) (Hause et al. 2002) and soybean (Meixner et al. 2005). In *Oryza sativa* sp. *japonica* cv. Nihonmasari, high levels of JA in the roots suppressed AM development, most likely by the induction of defense (Gutjahr et al. 2015). The induction of defense in plants is correlated with the synthesis of PR proteins, such as 1,3-b-glucanases and chitinases. JA, SA, and ET induce PR proteins production, resulting in priming defense effect, which will modulate JA, ET, and SA production (Pozo et al. 2010).

In studies with tomato roots colonized by AMF, the formation of papilla-like structures was observed around cells infected with parasitic *Phytophtora*, with significant deposition of non-esterified pectins and callus, preventing the dissemination of the pathogen. Plants accumulated higher amounts of PR-1α and β-1,3-glucanases when compared to non-mycorrhized plants (Pozo and Azcón-Aguilar 2007).

Other studies evaluated the effects of AMF on diseases observed above the soil line, gaining notorious importance in recent years, as some of them suggested a greater tolerance to pathogen attacks. Campos-Soriano et al. (2012), evaluating the effect of mycorrhizae on rice plants, observed higher resistance against blast fungus *Magnaporthe oryzae*, due to higher expression of defense marker genes (PR genes) in their leaves.

The resistance of plants to herbivorous insects induced by mycorrhizal colonization was reported, although the mechanisms involved are not well understood. Song et al. (2013) evaluated the effect of *Glomus mosseae* colonization on the plant defense response against *Helicoverpa armigera*. They found that mycorrhization adversely affected the caterpillar performance. The effect was caused by a much stronger plant response, induced by the LOXD, AOX, PI-I, and PI-II defense genes in the leaves, when compared to uninoculated plants.

JA and ET interactions can present antagonistic or synergistic effects. In some plants, like tobacco, an antagonistic effect of JA and ET in the biosynthesis of antiherbivore compounds was detected. On the other hand, synergism between JA and ET was observed in the pathogen-induced expression of the defensin gene PDF1.2 in *Arabidopsis*, which required simultaneous activation of the JA and ET signaling pathway for full expression (Pozo et al. 2005).

22.4 Commercial Products

In the search for compounds that mimic the SA action, capable of inducing plant defenses, several screening methods were based on the initial work of Kurc (1982), leading to the discovery of 2,6-dichloroisonicotinic acid and its methyl ester (INA), the first synthetic compound characterized as a potent SAR inducer in plants. After the discovery of INA in 1987 by Syngenta (at time Ciba-Geigy), S-methyl benzo[1,2,3]thiadiazole-7-carbothioate acid (benzothiadiazole, BTH) was identified by the same research group, with demonstrated effects similar to INA. With continued sorting of benzo[1,2,3]thiadiazole-7-carboxylic acid derivatives, acibenzolar-S-methyl acid (ASM or CGA245704) (Bektas and Eulgem 2015) was obtained. Because of its ability to control a broad spectrum of phytopathogens in several species and its different mode of action, CGA245704 (ASM) was selected for commercial production, which led to the registration of the first commercial product with exclusive effect on the plant defense system activation named Bion® in Europe and Actigard® in the United States (Leadbeater and Staub 2014). Several compounds with elicitor and resistance-inducing properties, both of biotic and abiotic origin, are currently known (Walters et al. 2013), with several commercial products already registered for these purposes.

The acibenzolar (ASM) was initially registered as Bion® and Actigard® in the 1990s. It is currently marketed as Blockad® and Boost®, all belonging to Syngenta Crop Protection Ltd. ASM is a compound of low toxicity with fast absorption and translocation in the plant, which can replace the salicylic acid in SAR, capable of inducing resistance without pathogen attack (Ziadi et al. 2001). Their use has already been established for the control of bacterial spot (*Xanthomonas axonopodis* pv. vesicatoria) and bacterial speck (*P. syringae* pv. tomato) (Reglinski et al. 2011; Parkinson et al. 2015). ASM does not cause undesirable effects in the plant, and it has no antimicrobial effect found in exogenous SA application. ASM induces the activation of SAR with the same characteristics as the SA-biologically activated SAR (Friedrich et al. 1996). It is successful in promoting plant defense against the attack of fungal, oomycetes (Patel et al. 2016), bacteria (Abo-Elyousr et al. 2012; Kuźniak et al. 2014), viruses (Mandal et al. 2008; Tripathi and Pappu 2015), and nematodes (Schouteden et al. 2016).

Probenazole (3-allyloxy-1,2-benzisothiazole-1,1-dioxide, PBZ) is another example of commercial product capable of activating plant immune system. Developed and registered by Meiji Seika under the name Oryzemate®, PBZ has been used by Japanese farmers since 1975 to protect rice plants from the rice blast fungus *Magnaporthe grisea* (Roberts and Taylor 2016). Plants treated with PBZ increase the activity of phenylpropanoids biosynthetic pathway, which plays an important role in the plant defense system, increasing the synthesis of lignin, a barrier to the attack of the pathogen, and the production of phytoalexins with antimicrobial properties (Bektas and Eulgem 2015). Although Oryzemate® does not have antimicrobial action, the production of antifungal compounds in rice plants is induced by the application of PBZ (Zhu et al. 2015). PBZ also induces rice plants to produce superoxides (O^{2-}) and defense-related genes (Iwata et al. 2004). Despite its use for

over 30 years, no evidence of the development of fungal resistance to probenazole has been found (Leadbeater and Staub 2014).

The most acknowledged use of a microbial origin compound as a commercial product able to activate the plant's immune system is the application of harpin protein, which induces systemic resistance in plants through different mechanisms, guaranteeing protection against the attack of fungi, bacteria, and viruses (Zhu and Zhang 2016). Harpin is a naturally occurring protein found in phytopathogenic bacteria and is related to pathogenicity and the activation of HR in nonhost plants. Composed of 403 amino acids, it has a mass of approximately 44 kD, also characterized by being thermostable (Dimlioğlu et al. 2015). The first harpin protein was successfully isolated from the bacterium causal agent of apple fire blight, *Erwinia amylovora*, at Cornell University, USA (Wei et al. 1992). Harpins induce the response to plant hypersensitivity (HR), and it is encoded by a group of genes called hypersensitive response and pathogenicity (HRP) (Tang et al. 2015). The capability to induce plant defenses against diseases without causing visible HR lesions, besides promoting considerable plant growth, resulted in a harpin-based commercial product, which was named Messenger® (Eden Bioscience, USA). For commercial production, the DNA fragment responsible for coding harpin in *E. amylovora* was transferred into weakened strains of *E. coli* strain K-12, which produce large amounts of harpin. The protein is subsequently extracted from the culture medium, isolated, and purified, resulting in a protein identical to the one produced in nature. The *E.coli* K-12 strain used for harpin production presents a nutritional deficiency; it is not pathogenic and incapable of growing in the environment (Copping and Duke 2007).

The SA signaling pathway is activated when harpin (Messenger®) is applied exogenously. Similarly, ABA signaling is triggered, inducing tolerance to drought. Finally, ET signaling pathway is modified, inducing resistance to insect attack and promoting plant growth (Li et al. 2014). Activation of the JA signaling pathway can also occur when HR is elicited by harpins, which also induces the formation of reactive oxygen species (ROSs), callose deposition, and increased expression of HR marker genes (Che et al. 2011). The use of harpins has proved a satisfactory control in infection caused by fungi, bacteria, or viruses such as *X. campestris*, *P. syringae*, *P. solanacearum*, *M. salvinii*, *Rhizoctonia solani*, *T. roseum*, *Guignardia bidwellii*, *Penicillium expansum*, *Meloidogyne* spp., and TMV, effectively used in tomato, strawberry, cucumber, tobacco, cotton, pepper, and citrus crops (Copping and Duke 2007).

Different active ingredients with eliciting action are found in commercial products, some of them containing SA (Spotless® and Treet ™), chitosan (ARMOR-Zen® and Chitoplant®), phosphorous acid (Agri-Fos® 600, Aliette® WG, Fostonic® 80WP), lamarine (Iodus 40®, Vacciplant®), prohexadione-calcium (Regalis®), and reactive oxygen (Oxycom ™).

Pseudomonas spp. strains are frequently associated with ISR in plants. However, new studies are finding that low-molecular-weight metabolites secreted by *P. aeruginosa* LV strain may induce SAR in plant, without showing any phytotoxicity to plant and being effective to reduce pathogen population in infected plants under

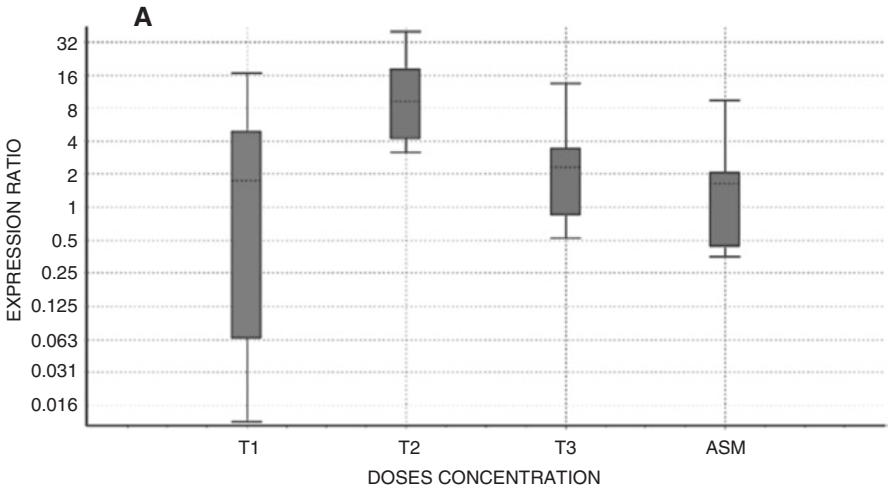


Fig. 22.2 The SAR induction by F4A fraction (*Pseudomonas aeruginosa* LV strain secondary metabolite) to control HLB in *Citrus sinensis* cv. *valencia*

greenhouse conditions (de Oliveira et al. 2011, 2016). Plants treated with a fraction produced by secondary metabolism of *P. aeruginosa* LV strain had a level of expression of PR gene resistance increased when compared to non-treated plants, based on RT-qPCR. The results obtained in these studies revealed that bacterial metabolites also have potential SAR induction (Fig. 22.2).

22.5 Conclusions

The increasing demographic demand for sustainable mechanisms that can improve yield and quality of world food production is a major concern. However, there is an ambiguity about strategies for plant protection in many cultures. Chemicals that pose health risks and cause other undesirable effects overwhelm pests, diseases, and weeds that impair production.

Therefore, a growing concern about control measures has emerged in recent years. Many chemicals have been questioned about their sustainability and are (or will be) banned. There is a current run for the development alternative management tools, with low biological and environmental impact.

ISR with natural products is an alternative to increase the sustainability of agriculture and decrease the impact on nontarget organisms. More research into the strategy of ISR by beneficial microbial agents is necessary, including antagonistic bacteria, plant growth-promoting bacteria, and natural microbial compounds.

There are many challenges and studies to be carried out for the application of microorganisms in the induction of resistance. However, studies with alternative methods are developed every day, and in the near future, they may result in products of biological origin, with potential to be applied in the field.

References

Abo-Elyousr KAM, Ibrahim Y, Balabel NM (2012) Induction of disease defensive enzymes in response to treatment with acibenzolar-s-methyl (ASM) and *Pseudomonas fluorescens* Pf2 and inoculation with *Ralstonia solanacearum* race 3, biovar 2 (phylotype II). J Phytopathol 160:382–389

Aimé S, Alabouvette C, Steinberg C, Olivain C (2013) The endophytic strain *Fusarium oxysporum* Fo47: a good candidate for priming the defense responses in tomato roots. MPMI 26:918–926

Akiyama K, Hayashi H (2006) Strigolactones: chemical signals for fungal symbionts and parasitic weeds in plant roots. Ann Bot 97:925–931

Akiyama K, Ogasawara S, Ito S, Hayashi (2010) Structural requirements of strigolactones for hyphal branching in AM fungi. Plant Cell Physiol 51:1104–1117

Asensio D, Rapparini F, Peñuelas J (2012) AM fungi root colonization increases the production of essential isoprenoids vs. nonessential isoprenoids especially under drought stress conditions or after jasmonic acid application. Phytochemistry 77:149–161

Audenaert K, Pattery T, Cornelis P, Höfte M (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin and pyocyanin. MPMI 15:1147–1156

Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP (1997) Signaling in plant–microbe interactions. Science 276:726–733

Bektas Y, Eulgem T (2015) Synthetic plant defense elicitors. Front Plant Sci 5:804. doi:10.3389/fpls.2014.00804

Bent E (2006) Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and Fungi (PGPF). In: Tuzun S, Bent E (eds) Multigenic and induced systemic resistance in plants. Springer, New York, pp 225–258

Campos-Soriano L, García-Martínez J, San Segundo B (2012) The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. Mol Plant Pathol 13:579–592

Che YZ, Li YR, Zou HS, Zou LF, Zhang B, Chen GY (2011) A novel antimicrobial protein for plant protection consisting of a Xanthomonas oryzae harpin and active domains of cecropin A and melittin. Microb Biotechnol 4(6):777–793

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262:1883–1885

Choudhary DK, Johri BN (2009) Interactions of *Bacillus* spp. and plants – with special reference to induced systemic resistance (ISR). Microbiol Res 164(5):493–513

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Barka EA (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71:1685–1693

Copping LG, Duke SO (2007) Natural products that have been used commercially as crop protection agents. Pest Manag Sci 63:524–554

De Meyer G, Audenaert K, Höfte M (1999a) *Pseudomonas aeruginosa* 7NSK2-induced systemic resistance in tobacco depends on in planta salicylic acid accumulation but is not associated with PR1a expression. Eur J Plant Pathol 105:513–517

De Meyer G, Capieau K, Audenaert K, Buchala A, Métraux J, Höfte M (1999b) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. MPMI 12:450–458

De Oliveira AG, Murate LS, Spago FR, Lopes LP, Beranger JPO, San Martin JAB, Nogueira MA, Mello JCP, Andrade CGTJ, Andrade G (2011) Evaluation of the antibiotic activity of extracellular compounds produced by the *Pseudomonas* strain against the *Xanthomonas citri* pv. *Citri* 306 strain. Biol Control 56:125–131

De Oliveira AG, Spago FR, Simionato AS, Navarro MO, Silva CS, Barazetti AR, Cely MV, Tischer CA, San Martin JA, Andrade CG, Novello CR, Mello JC, Andrade G (2016) Bioactive organocopper compound from *Pseudomonas aeruginosa* inhibits the growth of *Xanthomonas citri* subsp. *citri*. Front Microbiol 7:1–12

De Vleesschauwe D, Cornelis P, Höfte M (2006) Redox-active pyocyanin secreted by *Pseudomonas aeruginosa* 7NSK2 triggers systemic resistance to *Magnaporthe grisea* but enhances *Rhizoctonia solani* susceptibility in rice. MPMI 19:1406–1419

Dimlioğlu G, Daş ZA, Bor M, Özdemir F, Türkan İ (2015) The impact of GABA in harpin-elicited biotic stress responses in *Nicotiana tabaccum*. J Plant Physiol 188:51–57

Djonovic S, Pozo MJ, Dangott LJ, Howell CR, Kenerley CM (2006) Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. Mol Plant-Microbe Interact 19:838–885

Dong HD, Zhong JJ (2002) Enhanced taxane productivity in bioreactor cultivation of *Taxus chinensis* cells by combining elicitation, sucrose and ethylene incorporation. Enzym Microb Technol 31:116–121

Friedrich L, Lawton K, Ruess W, Masne P, Specker N, Gut Rella M, Meier B, Dincher S, Staub T, Uknes S, Metraux JP, Kessmann H, Ryals J (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J 10:61–70

Fritig B, Heitz T, Legrand M (1998) Antimicrobial proteins in induced plant defense. Curr Opin Immunol 10:16–22

Giovannetti M, Avio L, Sbrana C (2010) Fungal spore germination and pre-symbiotic mycelial growth – physiological and genetic aspects. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, New York, pp 3–31

Gutjahr C, Parniske M (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. Ann Rev Cell Dev Biol 29:593–617

Gutjahr C, Siegler H, Haga K, Iino M, Paszkowski (2015) Full establishment of arbuscular mycorrhizal symbiosis in rice occurs independently of enzymatic jasmonate biosynthesis. PLoS One. doi:10.1371/journal.pone.0123422

Hammond-Kosack KE, Parker JE (2003) Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. Curr Opin Biotech 14:177–193

Harrison MJ (2012) Cellular programs for arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 15:691–698

Hause B, Maier W, Miersch O, Kramell R, Strack D (2002) Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiol 130:1213–1220

Hermosa R, Rubio M, Cardoza RE, Nicolas C, Monte E, Gutiérrez S (2013) The contribution of Trichoderma to balancing the costs of plant growth and defense. Int Microbiol 16:69–80

Iavicoli A, Boutet E, Buchala A, Métraux JP (2003) Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHAO. MPMI 16:851–858

Iwata M, Umemura K, Midoh N (2004) Probenazole (Oryzemate®)-A plant defense activator. In: Rice blast: interaction with rice and control. Springer, Dordrecht, pp 163–171

Jankiewicz U, Koltonowicz M (2012) The involvement of *Pseudomonas* bacteria in induced systemic resistance in plants (review). Appl Biochem Microbiol 48:244–249

Jentschel K, Thiel D, Rehn F, Ludwig-Müller J (2007) Arbuscular mycorrhiza enhances auxin levels and alters auxin biosynthesis in *Tropaeolum majus* during early stages of colonization. Physiol Plant 129:320–333

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming of plant defenses. J Chem Ecol 38:651–664

Kaldorf M, Ludwig-Müller J (2000) AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. Physiol Plantarum 109:58–67

Kazan K, Manners JM (2008) Jasmonate signaling: toward an integrated view. Plant Physiol 146:1459–1468

Kurc J (1982) Induced immunity to plant diseases. Bioscience 32:854–860

Kuźniak E, Głowacki R, Chwatko G, Skłodowska M (2014) Involvement of ascorbate, glutathione, protein S-thiolation and salicylic acid in benzothiadiazole-inducible defence response of cucumber against *Pseudomonas syringae pv lachrymans*. Physiol Mol Plant Path 86:89–97

Leadbeater A, Staub T (2014) Exploitation of induced resistance: a commercial perspective. In: Walters DR, Newton AC, Lyon GD (eds) Induced resistance for plant defense: a sustainable approach to crop protection. Wiley-Blackwell, Oxford, pp 300–315

Leite B, Roncato LDB, Pascholati SF, Lambais MR (1997) Reconhecimento e transdução de sinais moleculares em interações planta-fungos fitopatogênicos. RAPP 5:235–280

Li X, Han B, Xu M, Han L, Zhao Y, Liu Z, Dong H, Zhang C (2014) Plant growth enhancement and associated physiological responses are coregulated by ethylene and gibberellin in response to harpin protein Hpa1. Planta 239:831–846

Loake G, Grant M (2007) Salicylic acid in plant defence-the players and protagonists. Curr Opin Plant Biol 10:466–472

Louws FJ, Wilson M, Campbell HL, Cuppels DA, Jones JB, Shoemaker PB, Sahin F, Miller SA (2001) Field control of bacterial spot and bacterial speck of tomato using a plant activator. Plant Dis 85:481–488

Lu H, Greenberg JT, Holuigue L (2016) Editorial: salicylic acid signaling networks. Front Plant Sci. doi:10.3389/fpls.2016.00238

Ludwig-Müller J (2010) Hormonal responses in host plants triggered by arbuscular mycorrhizal fungi. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, New York, pp 3–31

Mandal B, Mandal S, Csinos S, Martinez N, Culbreath AK, Pappu HR (2008) Biological and molecular analyses of the acibenzolar-S-methyl-induced systemic acquired resistance in flue-cured tobacco against tomato spotted wilt virus. Phytopathology 98:196–204

Martínez-Medina A, Fernandez I, Lok GB, Pozo MJ, Pieterse CMJ, Van Wees SCM (2016) Shifting from priming of salicylic acid-to jasmonic acid-regulated defenses by *Trichoderma* protects tomato against the root knot nematode *Meloidogyne incognita*. New Phytol. doi:10.1111/nph.14251

Meixner C, Ludwig-Müller J, Miersch O, Gresshoff P, Staehelin C, Vierheilig H (2005) Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant nts1007. Planta 222(4):709–715

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. doi:10.1111/1574-6976.12028

Meziane H, Van Der SI, Van Loon LC, Höfte M, Bakker PA (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol Plant Pathol 6:177–185

Muthamilarasan M, Prasad M (2013) Plant innate immunity: an updated insight into defense mechanism. J Biosci 38:433–449

Parkinson LE, Crew KS, Thomas JE, Dann EK (2015) Efficacy of acibenzolar-S-methyl (Bion®) treatment of Australian commercial passion fruit, *Passiflora edulis* f. sp. *flavicarpa*, on resistance to *Passionfruit woodiness virus* (PWV) and activities of chitinase & β-1,3-glucanase. Australas Plant Pathol 44:311–318

Pascholati SF (2003) Indução de resistência: opção para o controle de doenças de plantas no século XXI. Summa Phytopathol l29:115–116

Pascholati SF, Leite B (1995) Hospedeiro: Mecanismos de resistência. In: Bergamin Filho A, Kimati H, Amorim L (eds) Manual de fitopatologia: princípios e conceitos. Ed. Agronômica Ceres, São Paulo, pp 193–217

Patel JS, Zhang S, McGrath M (2016) Red light increases suppression of downy mildew in basil by chemical and organic products. Plant Phytopathol 164(11–12):1022–1029

Paul EA, Clark FE (1989) Soil microbiology and biochemistry. Academic, New York

Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas: anatomy and cell biology. National Research Council of Canada, Ottawa

Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM (2012) Hormonal modulation of plant immunity. Ann Rev Cell Dev Biol 28:489–521

Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398

Pozo MJ, Van-Loon LC, Pieterse CMJ (2005) Jasmonates: signals in plant-microbe interactions. J Plant Growth Regul 23:211–222

Pozo MJ, Jung SC, López-Ráez JA, Azcón-Aguilar C (2010) Impact of arbuscular mycorrhizal symbiosis on plant response to biotic stress: the role of plant defense mechanisms. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, New York, pp 3–31

Pozo MJ, Lopez-R JÁ, Azcon-Aguilar C, Garcia-Garrido JM (2015) Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytol 205:1431–1436

Reglinski T, Wurms K, Elmer P (2011) Short report on commercially available elicitors, natural products and microbes for evaluation against *Pseudomonas syringae* pv. *Actinidiae*. Plant & Food Research, Ruakura

Roberts R, Taylor JE (2016) Exploiting plant induced resistance as a route to sustainable crop protection. In: Collige DB (ed) Plant pathogen resistance biotechnology. Wiley-Blackwell, Hoboken, pp 317–339

Ryu C, Farag MA, Hu C, Reddy MS, Wei HX, Paré PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. PNAS 100:4927–4932

Ryu C, Farag MA, Hu C, Reddy MS, Kloepper JW, Paré PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Sarma BK, Mehta S, Singh HB, Singh UP (2002) Plant growth-promoting rhizobacteria elicited alteration in phenolic profile of chickpea (*Cicer arietinum*) infected by *Sclerotium rolfsii*. Phytopathol J 150:277–282

Schlumbaum A, Mauch F, Vogeli U, Boller T (1986) Plant chitinases are potent inhibitors of fungal growth. Nature 324:365–367. acibenzolar-S-methyl against *Meloidogyne incognita*. Nat Prod Res. doi:10.1080/14786419.2016.1230111

Schouteden N, Lemmens E, Stuer N, Curtis R, Panis B, De Waele D (2016) Direct nematicidal effects of methyl jasmonate and acibenzolar-S-methyl against *Meloidogyne incognita*. Nat Prod Res 23:1–4

Schuhegger R et al (2006) Induction of systemic resistance in tomato by N-acyl-L-homoserine lactone-producing rhizosphere bacteria. Plant Cell Environ 29:909–918

Selosse MA, Bessis A, Pozo MJ (2014) Microbial priming of plant and animal immunity: symbionts as developmental signals. Trends Microbiol 22:607–613

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-induced changes in disease severity and pr protein expression in tobacco leaves. Mol Plant Microbe 12(11):1000–1007

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, New York

Song YY, Ye M, Li CY, Wang RL, Wei XC, Luo SM, Zeng RS (2013) Priming of anti-herbivore defense in tomato by arbuscular mycorrhizal fungus and involvement of the jasmonate pathway. J Chem Ecol 39:1036–1044

Spoel SH, Johnson JS, Dong X (2007) Regulation of tradeoffs between plant defenses against pathogens with different life styles. Proc Natl Acad Sci U S A 104:18842–18847

Staskawicz BJ (2001) Genetics of plant-pathogen interactions specifying plant disease resistance. Plant Physiol 125:73–76

Stracke R, Werber M, Weisshaar B (2001) The R2R3-MYB gene family in *Arabidopsis thaliana*. Curr Opin Plant Biol 4:447–456

Tang RJ, Zhao FG, Garcia VJ, Kleist TJ, Yang L, Zhang HX et al (2015) Tonoplast CBL–CIPK calcium signaling network regulates magnesium homeostasis in *Arabidopsis*. Proc Natl Acad Sci U S A 112:3134–3139. doi:10.1073/pnas.1420944112

Tripathi D, Pappu HR (2015) Evaluation of acibenzolar-S-methyl-induced resistance against iris yellow spot tospovirus. Eur J Plant Pathol 142:855–864

Van Loon LC, van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol 55:85–97

Van Wees SCM, Pieterse CMJ, Trijssenaar A, Van'tWestende YAM, Hartog F, Van Loon LC (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. MPMI 10:716–724

Vasconsuelo A, Boland R (2007) Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci 172:861–875

Verhagen BWM, Trotel-Aziz P, Couderchet M, Höfte M, Aziz A (2010) Pseudomonas spp.-induced systemic resistance to *Botrytis cinerea* is associated with induction and priming of defense responses in grapevine. J Exp Bot 61:249–260

Vos CM, Yang Y, de Coninck B, Cammue BPA (2014) Fungal (-like) biocontrol organisms in tomato disease control. Biol Control 74:65–81

Walters DR, Ratsep J, Havis ND (2013) Controlling crop diseases using induced resistance: challenges for the future. J Exp Bot 64:1263–1280. doi:10.1093/jxb/ert026

Wang W, Zhong JJ (2002) Manipulation of ginsenoside heterogeneity in cell cultures of *Panax notoginseng* by addition of jasmonates. J Biosci Bioeng 93:48–53

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 100:681–697

Wei ZM, Laby RJ, Zumoff CH, Bauer DW, He SY, Collmer A, Bee SV (1992) Harpin elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257:85–88

Withers J, Dong X (2016) Posttranslational modifications of NPR1: a single protein playing multiple roles in plant immunity and physiology. PLoS Pathog. doi:10.1371/journal.ppat.1005707

Yu K, Niranjana M, Hahn E, Paek K (2005) Ginsenoside production by hairy root cultures of *Panax ginseng*: influence of temperature and light quality. Biochem Eng J 23:53–56

Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Li Q, Yang D, He Z (2007) Functional analysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. Plant Biotechnol J 5:313–324

Zhu Z, Zhang X (2016) Effect of harpin on control of postharvest decay and resistant responses of tomato fruit. Postharvest Biol Technol 112:241–246

Zhu Z, Gao J, Yang JX, Wang XY, Ren GD, Ding YL, Kuai BK (2015) Synthetic promoters consisting of defined cis-acting elements link multiple signaling pathways to probenazole-inducible system. J Zhejiang Univ Sci B 16:253–263

Ziadi S, Barbedette S, Godard F, Monot C, Le-Corre D, Silue AD (2001) Production of pathogenesis-related proteins in the cauliflower (*Brassica oleracea* var *botrytis*) downy mildew (*Peronospora parasitica)* pathosystem treated with acibenzolar-S-methyl. Plant Pathol 50:579–586

Exploring the Role of Plant-Microbe Interactions in Improving Soil Structure and Function Through Root Exudation: A Key to Sustainable Agriculture

23

Kanchan Vishwakarma, Mitali Mishra, Shruti Jain, Jaspreet Singh, Neha Upadhyay, Rishi Kumar Verma, Pankaj Verma, Durgesh Kumar Tripathi, Vivek Kumar, Rohit Mishra, and Shivesh Sharma

Abstract

The most astonishing feature of plant roots is their capability of secreting a broad variety of compounds ranging from low molecular to high molecular weights into the rhizosphere. These compounds act as signals for establishing and regulating the interactions among plant roots and microorganisms present in rhizosphere through different mechanisms. The mechanism of establishment of these relationships includes complex signaling cascades and involves different transporter proteins. Exudation is an important process that influences the microbial diversity and relevant biological activities. In addition, these secretions mediate the phenomena of mineral uptake in soil with low nutrient content either through chelation directly or by affecting biological activity of microbes. Further,

K. Vishwakarma • J. Singh • N. Upadhyay • R.K. Verma • P. Verma
Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad 211004, Uttar Pradesh, India

M. Mishra • S. Jain • D.K. Tripathi • R. Mishra
Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Allahabad 211004, Uttar Pradesh, India

V. Kumar
Amity Institute of Microbial Technology, AMITY University, Noida 201303, India

S. Sharma (✉)
Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad 211004, Uttar Pradesh, India

Centre for Medical Diagnostic and Research (CMDR), MNNIT Allahabad, Allahabad 211004, Uttar Pradesh, India
e-mail: shiveshs@mnnit.ac.in; ssnvsharma@gmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_23

microbes associated with plants have the potential to upgrade phytoremediation efficiency by facilitating phytoextraction and phytostabilization and through increase in biomass production by plants. Overall these exudation-mediated plant-microbe interactions influence the soil structurally and functionally via orchestrating microbial richness, nutrient acquisition, and phytoremediation. Hence, in light of this, the chapter is intended to provide the perceptivity to comprehend the impact of root exudation-mediated plant-microbe interactions in enriching the structural and functional characteristics of soil.

Keywords
Root exudates • Microbial diversity • Soil • Phytoremediation • Rhizobacteria

23.1 Introduction

Among different metabolic features of plant roots, one of the utmost amazing properties is to secrete out numerous substances into rhizosphere. These secretions are outlined as lightweight permeable excretes which can be eliminated out without any trouble through passive diffusion, and for this process, plants don't have to spend their energy (Bertin et al. 2003; Bais et al. 2006). Root secretions are broadly classified as (i) lightweight molecules like organic acids, amino acids, sugars, and some secondary metabolites, which include most of the excreted products from roots, and (ii) heavyweight secretions that include reminiscent of mucilage (polysaccharides) and proteins, which are large biomolecules in terms of weight Badr and Vivanco 2009. This mechanism involves replenishment of soil with micro- and macronutrients excreted out through roots (Hutsch et al. 2000; Nguyen 2003). The qualitative and quantitative nature of root secretions are reliant on the age and type of the plant, and other physical and biological parameters. Owing to the process of root exudation, various biochemicals excreted from plant roots have the capacity to control microbial growth, allow symbiotic relationship, prevent the development of parasitic and pathogenic species in the surrounding area of roots, and regulate the composition of soil (Nardi et al. 2000; Walker et al. 2003). Around 5–21% of whole photosynthetic carbon is being circulated throughout the rhizosphere by means of root exudation (Nguyen 2003; Derrien et al. 2004). Although the exudates excreted from roots supply biomass and energy to soil, they also help the plant to establish communication with other microbes and regulate their growth. The crops facilitate each positive and negative communication within the rhizosphere by the means of root exudation (Bais et al. 2006; Philippot et al. 2013). The positive communication comprises symbiotic associations with useful microorganisms, similar to rhizobium, mycorrhizae, and plant growth-promoting rhizobacteria (PGPR). The existence of numerous microbes in rhizosphere impacts the soil by performing various processes like transportation of water and nutrients through roots, maintaining fertility of soil, and nodule formation (White 2003). These root secretions symbolize a vital role in plants for

maintaining interactions with rhizosphere-inhabiting microbes. To support communication, many types of substances and signaling substances are secreted from plant roots, known as autoinducers. However, several types of compounds are released from plant roots, and most of them belong to compounds having low molecular weight, referred to as secondary metabolites (Bais et al. 2004). Few of these secondary metabolic products had been recognized earlier, and their roles within the rhizosphere have been studied and explained in detail.

23.2 Root Exudation and Its Mechanism

The mode of secretion of root exudates involve the release of carbon into soil from plant roots (Hutsch et al. 2000; Nguyen 2003; Vishwakarma et al. 2016). Roots usually retort by secretion of particular proteins and small molecules (Stotz et al. 2000). These secretions are utilized by bacteria present in soil for biomass and energy production. Root exudates might exhibit both positive and negative association within the rhizosphere. The investigation of these secretions released from roots help to have in-depth knowledge of communication among plants and microbes (Broeckling et al. 2008; Weir et al. 2004; Bais et al. 2004, 2006). The optimistic interaction includes symbiotic relationship with invaluable microbes, corresponding to PGPR. Rhizobia, mycorrhizae, and negative response incorporate organization with parasitic and pathogenic microbes. Rhizospheric bacteria are responsible to remove these contaminants, while the roots supply nutrients for microbial growth (Bais et al. 2008). Workers have explained the enhanced mechanism for isolation of microorganisms from soil which have the following properties: (1) breakdown of particular contaminant and (2) enriched medium for growth of microorganisms. Shukla et al. (2010) explained the approach "rhizo-remediation" to describe the significance of root exudates and the rhizospheric microorganisms.

23.2.1 Diffusion

The passive process involves transport of natural substances like phenolics, carboxylic acids, sugars, and amino acids according to the formation of gradient of concentrations between cytosol of root cells (high concentration) and soil (low concentration). Due to membrane permeability for natural compounds, it allows movement of compounds through lipid bilayer of the plasma membrane. The factors accountable for permeability are concentration and polar nature of the compounds. This system allows the transport of lipophilic substances. Under a particular cytosolic pH of 7.1–7.4, small polar intracellular molecules together with carboxylic acids and amino acids occur as anions which move slowly through the plasma membrane. However, during the process of K^+ ion diffusion and the transfer of protons with the help of ATPase, there is generation of positive-charge gradient which allows influx of cations and efflux of carboxylate anions by diffusion. Root

secretion of sugars and amino acids occurs through diffusion under stress (Jones and Darrah 1994a, b).

23.2.2 ABC Proteins

They are the proteins that are most widely present in nearly all families. Many substances like metabolic products, anions, and cations are transported by utilizing energy generated by ATP hydrolysis. Hence, they are the primary active transporters having the property to drive substances against the electrochemical gradient (Krattinger et al. 2009). In eukaryotes, these proteins help in export of substances from cytoplasm to apoplast and transfer from cytoplasm to organelles like mitochondria. About more than hundreds of ABC transporters were reported in the genome of rice and *Arabidopsis*, and few of them were observed to be associated in the transportation of compounds like glutathione (Martinoia et al. 2002), auxins (Noh et al. 2001), and anthocyanins (Goodman et al. 2004) and antifungal components. Furthermore, ABCs are assumed to transfer diterpene sclareol from *N. plumbaginifolia* leaves (Jasinski et al. 2002) and the isoflavone genistein (antifungal agent) from the roots of soybean (Sugiyama et al. 2007). These proteins also act as phytoalexin because of their activities against microbes (Geibel 1994). However, around 25 ABC transporter genes showed significant increase in gene expression levels in *Arabidopsis* root which are responsible for exudation processes (Badri et al. 2008). In an experiment, such genes were knocked out and secretions released from wild type and mutants were analyzed. It was observed that the nature of these secretions from wild type and mutants was different. It was concluded that ABC transporter proteins were in regulation of exudation process. The other example in which a gene responsible for powdery mold resistance in *Arabidopsis* codes an ABC transporter is known as PEN3. It is located in the membrane of the cell, and its movement toward infected area on the epidermis and hair of roots is regulated by structures present on pathogens like chitin and flagellin (Stein et al. 2006). This active transporter releases antimicrobial substances including derivative of glucosinolates into the apoplast to stop the pathogenic microbial movement further into the cell. However, PEN3 (¼AtPDR8) also inhibits toxic effect exhibited by heavy metal by transferring cadmium ions from cells of root (Kim et al. 2007) showing that the identical transporter protein is responsible for many functions in the other tissue.

23.2.3 Multidrug and Toxic Compound Extrusion (MATE) Proteins

Interestingly, MATE proteins facilitate the transportation of secondary metabolites. They are expressed in both eukaryotic and prokaryotic species (Hvorup et al. 2003; Magalhaes 2010), and some bacterial species and mammals are accountable for multidrug resistance. Although not much information is reported about these proteins, MATEs act as secondary transporters that transport ions (H+ and sodium ions)

along the electrochemical gradient allowing the transport of substance across the membrane. According to data reported, *Arabidopsis* genome has 58 MATE genes, and description about these transporters has been already reported in detail. ALF5 is expressed in cortical root cells and the epidermis and is suggested to guard plants from xenobiotics by removing them from root cells (Diener et al. 2001). In a study by Li et al. (2002), *Arabidopsis* gene named AtDTX1 is found to encode a protein located in the plasma membrane and helps in the exportation of antibiotics, alkaloids, and toxic components from roots. Some other MATE genes present in different plants like *H. vulgare* (HvAACT1), *Sorghum* (SbMATE1), and *Arabidopsis* (AtMATE1) show Al resistance by allowing an Al-activated outward movement of citrate ions from root tips (Furukawa et al. 2007; Magalhaes et al. 2007; Liu et al. 2009).

23.2.4 Aluminum-Activated Malate Transporter (ALMT) Proteins

ALMT proteins are accountable for discharging malate ions from roots, thereby providing resistance against aluminum toxicity in both dicotyledons and monocotyledons (Ryan et al. 2011). These protein families are found only in plants and not in animals and bacteria. These proteins form anion-transporting networks that create pores with selective nature in membranes and initiate flaccid transport of substrates along electrochemical gradients (Lynch and Whipps 1990). This clearly elucidate that due to movement of anions outward or cations inward along ion channels, there is generation of difference in potential across the cell membrane ranging from −100 to −200 mV or across tonoplast ranging from −10 to −50 mV. They are present on the cell membrane of roots and help in Al resistance. Gene TaALMT1 present in wheat is shown to be expressed in suspension cultures of tobacco (*Nicotiana tabacum* L.), *Arabidopsis*, wheat, and barley and is responsible for malic acid transportation facilitating Al tolerance (Delhaize et al. 2004, 2007; Ryan et al. 2011).

23.2.5 Major Facilitator Superfamily (MFS) Proteins

This family of proteins is among the prevalent class of transporter proteins in biological systems. The release of phytosiderophores (secondary metabolites) displays a substantial part in providing iron (Fe) nutrition to the grasses (Marschner et al. 1987). The produced secondary metabolites remove Fe^{+3} from the rhizospheric soil and form complex with these ions which is further delivered to plant root cells by H^{+}-linked transporters of OPT family (Kim and Guerinot 2007).

MFS proteins are responsible for initial export of the compounds. These are categorized into different classes based on their function such as antiporters, co-transporters, or uniporters. The gene named as TOM1 expanded as “transporter of mugineic acid family phytosiderophores1” in rice discharges avenic acid and deoxymugineic acid from roots of rice plant (deficient in iron) (Nozoye et al. 2011). During decrease in iron supply, the gene expression of TOM1 is enhanced, and

overexpression of TOM1 in transgenic plants exhibited enhanced release of deoxymugineic acid with better Fe tolerance.

23.2.6 Hot Spots of Exudations from Root

The spots of exudation from roots are foremost since they show a predominant impact on the arrangement of microbial communities alongside the plant roots. The most important regions of secretion are the root tip with destructive cells invading from the tip region to the region of death of outer cells (Bowen and Rovira 1991). Utilizing a ^{4}C-labeling manner, McDougali and Rovira (1970) and Rovira (1973) confirmed that both main and lateral roots are among the major areas of root secretion followed by the elongation of roots. Bowen (1979) used *Pseudomonas fluorescens* as a model for identifying areas of secretion on *Pinus radiata* by coating disinfected seedlings with the bacteria and analyzing their spots of progress alongside roots. From this observation, it was elucidated that cell junctions present in longitudinal axis had been the major hot spots. However, the amount of root exudation of several substances from different sites of roots is inadequate.

23.3 Interaction of Root Exudates

23.3.1 Roots and Rhizosphere Interactions

Plants have the capability to adapt to the local environment by perceiving changes in a specific rhizospheric environment. These subsequent changes in a particular rhizosphere include variations in the growth of neighboring plants and microbes invading in close vicinity. Due to presence of any external organism, roots retort by secreting some proteins and molecules (Stotz et al. 2000; Stintzi and Browse 2000). Furthermore, the root exudates can show mutualistic or protective roles in positive or negative interaction, according to other constituents in the rhizosphere. Although numerous reports are present to show plant's association with microbe and insect in the plant organs such as stems and leaves, only a small amount has been concentrated during interaction of root with microbes and soil inside the rhizosphere.

Root exudates are also observed as a mode of interaction among plant roots and PGPR inside the rhizosphere (Hirsch 2003; Bais et al. 2006) and comprise of proteins, phenolics, organic acids (OAs), and sugars (Bais et al. 2006; De Weert et al. 2002). Although root secretions deliver nutrients to PGPR, they also differentiate microbes inhabiting in soil (Badri et al. 2008). As per the data published about the low molecular weight OAs such as malate, fumarate, and citrates exuded from roots hairs, it was observed that it allowed PGPR growth (De Weert et al. 2002; Kamilova 2006; Rudrappa et al. 2008; Ling et al. 2011). Organic acids of tricarboxylic acid cycle are also responsible for playing a key role as molecular signals (Jones 1998).

23.3.2 Root-Root Communication

The occurrence of one root system prevents the invasion to the other by releasing some chemical substances. The mechanism of allelopathy inhibits the progression of other plant species in vicinity by excreting chemical inhibitors. It also has importance in agriculture as it does not allow growth of weeds and acts as natural weedicide (Callaway and Aschehoug 2000). Bais et al. (2002c) found that (±)-catechin prevents growth of knapweed within rhizosphere by releasing phytotoxin from roots. The aforementioned example illustrates how these plant roots interact with neighboring roots. Plants have capability to utilize exuded secondary metabolites to control the rhizospheric conditions for causing damage to neighboring plants. Plants with this nature have the capacity to utilize metabolites released from roots as chemical linkers for haustorial growth needed for heterotrophic development of plant (Keyes et al. 2000). Some parasites of food crops including rice (*O. sativa*), legumes, millet, and sorghum (*S. bicolor*) and from Scrophulariaceae family particularly invade the root of nearby plants to acquire mineral, water, and other beneficial growth-promoting compounds from host plant (Yoder 2001). Some of the allelochemicals like flavonoids, quinones, cytokines, and para-hydroxy acids are reported to facilitate formation of haustoria (Becard et al. 1995; Estabrook and Yoder 1998; Yoder 2001); however, detailed structures of released compounds for formation of haustoria are not still clear.

23.3.3 Root Exudate-Mediated Mutualistic Interactions

Mutualism among plants and microbes is principally mediated by exudation of roots. Generally, three specific microbial groups have been observed, i.e., mycorrhizal fungi, N_2-fixing bacteria, and other beneficial bacteria (Azcon-Aguilar and Barea 1996) (Fig. 23.1). One more mode of communication that characterizes underground zone is root-microbe interactions. The compounds which play an essential part in interaction between roots and microbes are flavonoids existing in the exudation from leguminous roots and stimulate genes of *Rhizobium meliloti* involved in the process of nodulation (Peters et al. 1986). Many molecular signaling mechanisms are included in the process of identifying plant secretions by bacteria. The mutualistic relationship between rhizobia family and their leguminous plant hosts from Fabaceae family is attributed to the signals produced and compounds secreted by both of them. During this process, the exudation by roots generates signals, which further stimulates the genes involved in nodulation process (nod genes) (Hirsch et al. 2001). According to the analysis, flavonoids are accountable for nod gene activation (Wang et al. 2012; Peck et al. 2006). Flavonoids are known to act as agonists for some rhizobia species but inhibitors for other species (Cooper 2007). Chemical compounds like flavonoids are continuously secreted into the rhizospheric soil, but their concentration is considerably enhanced in the presence of a particular *Rhizobium* species (Becard et al. 1992).

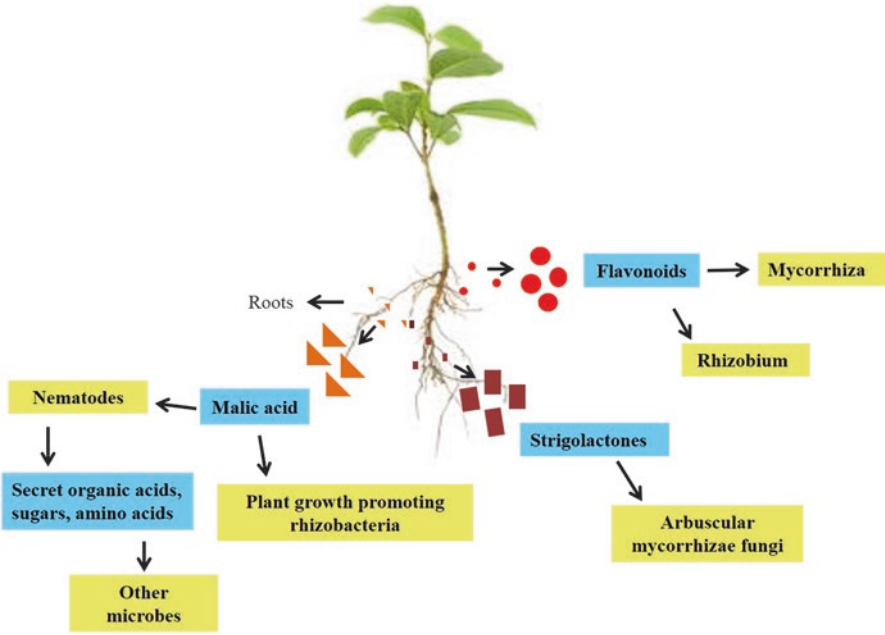


Fig. 23.1 Root exudation-mediated microbial colonization in rhizosphere

The symbiotic interactions between *Frankia* (*Actinobacteria*) and eight families of dicotyledonous plants are known as actinorhizal (Wall 2000). There are series of regulatory events happening during the course of symbiosis and they start with an infection. Further, a common step both in *Rhizobium* and *Frankia* known as curling of the root hair is preceding nodule development. However, the phenomenon of nodule formation is regulated by phenolic compounds (benzoic and cinnamic acids) and flavonoid-like components (flavanone and isoflavanone) (Ishimaru et al. 2011; Benoit and Berry 1997). It was reported that curling of the root hair is improved in *Alnus glutinosa* root filtrate (Van Ghelue et al. 1997; Prin and Rougier 1987). Popovici et al. (2010) observed that plants of Myricaceae family regulate their root secretions in the presence of *Frankia* and that flavonoids might determine its micro-symbiont specificity. The chief plant substances which were modulated by inoculating *Frankia* are hydroxycinnamic acids, flavonoids, and phenols. It was reported that genes accountable for synthesis of flavonoids are stimulated in *A. glutinosa* when co-inoculated with *Frankia* (Kim et al. 2003; Hammad et al. 2003).

Among most of terrestrial plants, arbuscular mycorrhizal fungi (AMF) and plant roots are considered to form symbiotic associations (Van der Heijden and Sanders 2002). These relationships facilitate the nutrient and mineral uptake by plants; nevertheless, fungi exploit the lipids and carbohydrates from host root. By increasing resistance against pathogens and herbivores, AMF have shown to benefit the plants indirectly by modulating its tolerance against pathogens and herbivores in several

known systems (Pozo and Azcon-Aguilar 2007; Cameron et al. 2013; Bennett et al. 2006). Since these are obligate organisms, therefore their survival is governed by their potentiality to develop rapid symbiotic relation with the roots. The branching and hyphae growth prior to infecting the roots need the presence of compounds secreted by plant roots (Giovannetti et al. 1996). A number of researches have performed studies on AMF and exudation process by roots (Nagahashi 2000; Nagahashi and Doudes 2003; Yu et al. 2003; Vierheilig and Bago 2005; Harrison 2005). Root exudates were also shown to involve in the establishment of symbiotic relationships of AMF (Vierheilig and Bago 2005). The signals provided by host plant roots facilitate the development of infection structure and AMF (Czarnota et al. 2003; Smith and Read 1997). As flavonoids persist in root exudates, their connection with signaling in establishing plant-AMF relationships has been explicated. Flavonoids are also regarded as a key compound for transforming nonsymbiotic AMF into symbiotic one (Besserer et al. 2006). Plentiful data have speculated the effects of flavonoids on growth of hyphae, differentiation, and colonization in the roots (Morandi 1996). Flavonoids show chemical structure-dependent stimulatory impact on growth of hyphae in AMF. However, in occurrence of CO_2, the flavonoids' stimulatory effects were found to be more pronounced (Bécard et al. 1992; Chabot et al. 1992; Poulin et al. 1993). Recently, in several studies, it is described that flavonoids show AMF species-specific effects through pre-symbiotic growth and its exposure to plants (Scervino et al. 2005). Colonization of AMF has been observed to amend the qualitative and quantitative nature of root exudates in the host system (Azaizeh et al. 1995) and chemotactic response of soil microbes (Sood 2003; Buee et al. 2000). However, strigolactones (carotenoid-derived terpenoids) were reported to promote branching in *G. margarita*, spore germination in *Glomus intraradices*, and cell proliferation in *Gigaspora rosea* (Akiyama et al. 2005).

23.3.4 Root-Insect Communication

Root-insect interaction has been localized to stems and leaves, but studies relevant to them are very few in numbers because of complex rhizospheric system of and unavailability of proper devices for experimentation (Koricheva et al. 2009). Root-insect interaction by bugs/pests like aphids can result in major damage to crops including *Beta vulgaris* and *Solanum tuberosum* (Hutchison and Campbell 1994). In observations made by Wu et al. (1999) on in vitro simultaneous cultures of aphids and hairy roots, it was elucidated that aphids decreased the vegetative growth and enhanced the polyacetylene synthesis with a similar response to phytoalexin (Flores et al. 1988). Fluorescent-carboline alkaloids were characterized from root secretions of *O. tuberosa* (*oca*) by Bais et al. (2002a). The main fluorescence showing compounds was recognized as harmaline (3,4-dihydroharmine) and harmine (7-methoxy-1-methyl-carboline). These alkaloids possess the fluorescence as well as phototoxic activities against insects (Larson et al. 1988).

23.3.5 Root-Pathogen Communication

The survival of root cells against pathogenic microorganisms depends on the release of chemicals like phytoalexins and defense proteins (Flores et al. 1999). This would have led the scientists to explore the chemodiversity present in root exudates for new biological entities including antimicrobials. Rosmarinic acid (RA) was found to be released from hairy root tips of cultures of *Ocimum basilicum* when stimulated by extracts of the cellular wall of fungi, i.e., *Phytophthora cinnamon* (Bais et al. 2002b). Roots were elicited to secrete rosmarinic acid by incorporation of *Pythium ultimum* in situ. This secondary metabolite exhibited antimicrobial activity against a diverse group of soil microbes including *Pseudomonas aeruginosa* (Bais et al. 2002b). Similarly, in other studies, hairy root cultures of the plant *Lithospermum erythrorhizon* were induced for producing cell-specific pigmented naphthoquinones and other biochemical entities against bacteria and fungi present in the soil (Brigham et al. 1999). The abovementioned examples proved that RA and naphthoquinones released as root exudates had defensive mechanisms against pathogenic microorganisms. Both Gram-positive and Gram-negative bacteria comprising of essential plant pathogens such as *Agrobacterium* and *Erwinia* spp. hold quorum-sensing mechanisms that regulate the transcription of genes needed for their pathogenic activities (Fray 2002). It is the cell-cell interaction between bacteria controlled by autoinducers. They are peptide-signaling molecules for Gram-positive bacteria and acylated homoserine lactones (AHLs) for Gram-negative bacteria. After reaching to saturation level in bacterial growth, it automatically activates certain transcription regulatory proteins which regulate particular genes (Teplitski et al. 2000). Hence, these signals allow bacterial cells to modulate the expression of genes in variation to population. Therefore, roots develop defense mechanism by releasing components in the rhizospheric soil that block quorum-sensing responses in bacteria, like signal blockers and signal-degrading enzymes. Further practices in this direction are still required to aid the isolation and characterization of these compounds.

23.4 Effects of Root Exudates on Soil Structure and Function

It is well known that roots of plants exude a huge number of biochemicals into the rhizosphere. Through this exudation, roots may regulate the microbes available in the vicinity of soil, deal with herbivores, restrain the development of competitors, and promote useful symbiosis (Rougier 1981). Abiotic stress is one of the severe stresses of environment that lowers the growth and yield of any crop even on irrigated land throughout the world (Vishwakarma et al. 2017). Root exudates mediate the beneficial alternations in soil function and structure by promoting microbial richness and facilitating mineral uptake in soil as well as removing of toxic substances from the soil (Nardi et al. 2000).

23.4.1 Maintenance of Microbial Diversity

Plants use the exuded molecules to protect themselves against pathogenic and parasitic organisms and to attract positive ones. These root exudates are used by bacteria present in close vicinity for production of biomass and energy. In addition, 20% of photosynthetic products are released by plants which form the basis for plant-microbe interactions. These interactions support the growth of plants by increasing the accessibility of minerals, promoting synthesis of phytohormones, degrading phytotoxic compounds, and suppressing pathogenic activities of microorganisms (Bais et al. 2006). The reported demonstrations clearly signify the value of understanding the functional attributes of microbial colonies available in the soil and the modes by which root exudates affect activity and microbial diversity.

The bacterial and fungal growth in the rhizospheric soil are selectively influenced by root exudates by altering the soil chemistry and allocating specific substrates for microbial growth. In turn, microorganisms affect the constitution and amount of various root exudates by influencing its secretion from plant root cells along with metabolism and nutrition of plants. Alternations in root exudations and rhizodeposition in distinct zones of roots form the foundation of variation of structure of present microbial communities and other species in different locations of roots (Paterson et al. 2007). In addition, soil type, status of nutrition, and environmental factors are also responsible for variation in rhizospheric microbial communities (Yang and Crowley 2000).

Studies depicting the close connection between root exudation and microbial composition in rhizosphere are increasing dramatically (Broeckling et al. 2008; Badri et al. 2008, 2013a; Chapparro et al. 2012, 2013; Micallef et al. 2009). In these studies, chemical compounds occurring in the exudates were reported as signaling molecules, substrates, or attractants that mediate the variations in microbial community (Shaw et al. 2006; Jain and Nainawatee, 2002; de Weert et al. 2002; Horiuchi et al. 2005; Badri and Vivanco 2009; Bais et al. 2006; Badri et al. 2013a, b; Neal et al. 2012). Moreover, it was explicated that root seedlings secrete sugars as substrates for the early development of extensive types of microbes and antimicrobial compounds for selecting particular microbial populations present in rhizospheric soil (Badri et al. 2013a; Chapparo et al. 2013). Rhizospheric microbial diversity is also affected by different varieties of plants (Smalla et al. 2001; Kowalchuk et al. 2002; Costa et al. 2006). This perhaps can be correlated with the constituents secreted in the form of exudates as it alters with the age, type, and location of plant along the root system (Lupwayi et al. 1998; Hertenberger et al. 2002; Yang and Crowley 2000).

23.4.2 Phytoremediation

Environmental pollution is a problem of concern nowadays and it is harshly affecting the soil-plant systems. Phytoremediation has become the emerging topic in the recent days due to its environmentally safe and cost-effective properties. Root

exudates facilitate phytoremediation by varying the physicochemical characteristics of rhizosphere by affecting absorption of metals (Lebeau et al. 2008). The modes through which root exudates scavenge heavy metals include pH modification of rhizosphere, chelation, complex formation, and alternation of microbial diversity within the rhizosphere. Through these processes, root exudates alter the chemical subsistence of heavy metals, enhance their bioavailability, make soil microbes active, and thus reduce the pollution. Ectoenzymes present in the root exudates mediate the elimination of organic contaminants by either directly degrading the pollutants or indirectly invigorating the microbial activity (Kuang et al. 2002). In general, the microbial activities occurring in the rhizosphere augment the effects of phytoremediation by two pathways:

1. *Direct pathway* in which microbes concomitant with plants increase translocation of metals and hence mediate phytoextraction or decrease mobility of metal pollutants from the rhizosphere contributing in phytostabilization
2. *Indirect pathway* in which microorganisms attribute metal tolerance to the plants or increase the biomass production by plants to arrest/remove the metal pollutants.

The rhizosphere bacteria have gained the special interest among microbes participating in heavy metal removal owing to their capability to improve the process directly by altering availability of metals by changing pH of soil and secreting chelators (e.g., siderophores, organic acids) and by redox reactions (Khan et al. 2009a, b; Gadd 2000; Kidd et al. 2009; Rajkumar et al. 2010; Ma et al. 2001, 2011; Uroz et al. 2009; Wenzel 2009).

There are numerous advantages for using microbe-mediated heavy metal mobilization as compared to chemical methods because metabolites synthesized by microbes are degradable, have low toxicity, and can easily be formed under in situ conditions in rhizosphere. However, plant growth-enhancing substances like plant growth hormones, siderophores, and ACC (1-aminocyclopropane-1-carboxylic acid) deaminase synthesized by microbes can interact with plants to help in plant growth in heavy metal-polluted soils (Wu et al. 2006; Babu and Reddy 2011; Glick 2010, 2012; Glick et al. 2007; Kuffner et al. 2010; Luo et al. 2011; Luo et al. 2012; Ma et al. 2011; Rajkumar et al. 2010; Miransari 2011) (Fig. 23.2).

23.4.3 Mineral Acquisition

As previously mentioned, the compounds secreted as exudates from roots serve as signals for numerous heterogenous, diverse, and active microbial communities available in soil. They make the soil system dynamic for nutrient turnover and sustainable for crop productivity with improved physicochemical structure (Chandler et al. 2008). By modifying physicochemical properties of soil, root exudates control the framework of microbial community present in close proximity of root surface (Dakora and Phillips 2002). Few molecules are metabolized by rhizospheric

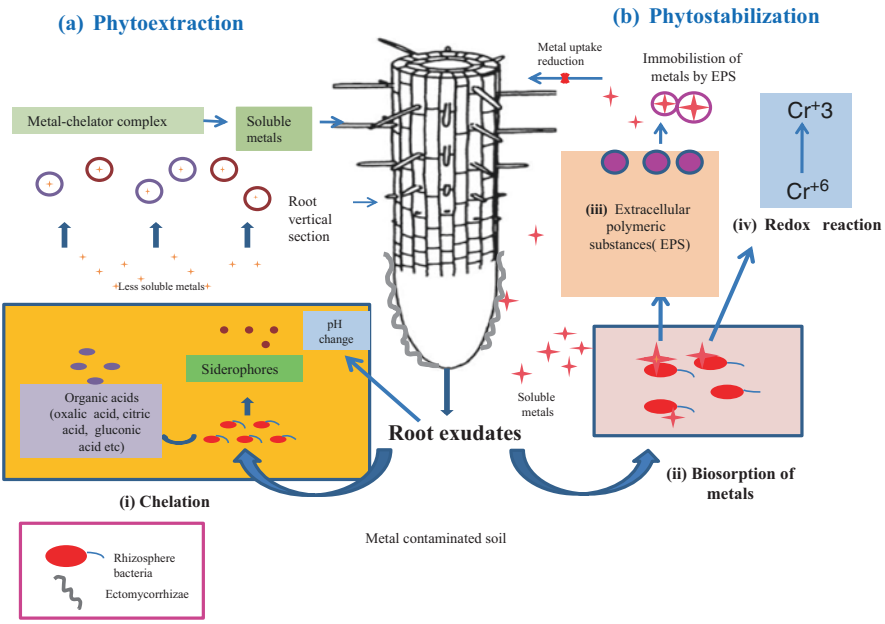


Fig. 23.2 Root exudation-mediated plant-microbe interactions facilitate **a** phytoextraction **b** phytostabilization process in metal-polluted soil by (*i*) chelation, (*ii*) biosorption, (*iii*) immobilization of metals by EPS, and (*iv*) redox reactions (modified from Rajkumar et al. 2012)

microbes as C and N sources, while other molecules which are secreted out by microbes are subsequently utilized by plant species for their development and growth (Kang et al. 2010).

Various agricultural soils lack adequate amount of iron, phosphorus, and nitrogen that results in minimal growth of plants. Majorly, the nutrients are taken up by the plants via rhizosphere when microorganisms interact with compounds in root exudates. They contain the combination of inorganic ions, organic acids, enzymes, vitamins, and amino acids. Aldonic acid and phenolics released by plant roots of N_2-fixing legumes trigger the root-nodule-forming bacteria, i.e., *Rhizobiaceae*. These signals activate nod gene expression in symbiotic bacteria and thus facilitate nitrogen fixation. Biological nitrogen fixation represents economically and environmentally favorable substitutes to the chemical fertilizers (Munees and Kibret 2014).

Root exudates are utilized by plants growing in the low-nutrition condition not only as symbiotic attractants of microbes involved in mineral acquisition but also in other ways. Extracellular enzymes present in root exudates release phosphorus from organic compounds and other molecules (Richardson 2001). Further by chelation, these enzymes make P available to the plants. In addition, organic ions can also mediate the mobilization of phosphorus through decreased sorption of phosphorus by altering soil topological properties, chelation of cations, and desorption of orthophosphates from a particular region (Bar-Yosef 1991; Jones 1998).

23.5 Conclusion

Several researches have elucidated that root exudates act as key factor for establishment of plant-microbe symbiotic relationships. However, there is requirement of investigating other factors to understand these relationships in ecological point of view. Recent advancements in technology have a significant role in knowing multifaceted interactions between plants and microbes. Furthermore, it is also important to study the root exudation phenomenon in specific environmental conditions for exploring many other soil microbes, biological activities, and related genes to demonstrate their applications in acquiring nutrients, scavenging toxins from contaminated soils, attracting plant growth-promoting microbes, and improving the quality of soil. Although significant researches have been carried out in exploring the capability of rhizospheric microbes in heavy metal toxin phytoremediation, more advances in this aspect are still required to be anticipated. In this context, future researches are required to completely study the genomics of rhizospheric microbes, uptake mechanism of metal-chelator complex in plant, signaling cascades involved in activation of microbes under stress induced by heavy metal, and various factors affecting acquisition of minerals. Such studies might provide sufficient knowledge for utilizing these microbes efficiently in scavenging of soil contaminants and improving structural and functional properties of soil to facilitate sustainable agriculture.

Acknowledgment The authors are thankful to Director MNNIT Allahabad and Design and Innovation Centre (DIC) MNNIT Allahabad for providing necessary facilities to carry out the research work.

References

Akiyama K, Matsuzaki KI, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Azaizeh HA, Marschner H, Romheld V, Wittenmayer L (1995) Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants. Mycorrhiza 5:321–327

Azcon-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens—an overview of the mechanisms involved. Mycorrhiza 6:457–464

Babu AG, Reddy S (2011) Dual inoculation of arbuscular mycorrhizal and phosphate solubilizing fungi contributes in sustainable maintenance of plant health in fly ash ponds. Water Air Soil Pollut 219:3–10

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Badri DV, Loyola-Vargas VM, Broeckling CD (2008) Altered profile of secondary metabolites in the root exudates of *Arabidopsis* ATP-binding cassette transporter mutants. Plant Physiol 146:762–771

Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013a) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J Biol Chem 288:4502–4512

Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM (2013b) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytol 198:264–273
Bais HP, Park S-W, Stermitz FR, Halligan KM, Vivanco JM (2002a) Exudation of fluorescent -carbolines from *Oxalis tuberosa* L. roots. Phytochemistry 61:539–543
Bais HP, Walker TS, Schweizer HP, Vivanco JM (2002b) Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of sweet basil (*Ocimum basilicum* L.) Plant Physiol Biochem 40:983–995
Bais HP, Walker TS, Stermitz FR, Hufbauer RA, Vivanco JM (2002c) Enantiomeric dependent phytotoxic and antimicrobial activity of (±)-catechin; a rhizosecreted racemic mixture from *Centaurea maculosa* (spotted knapweed). Plant Physiol 128:1173–1179
Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trend Plant Sci 9:26–32
Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266
Bais HP, Broeckling CD, Vivanco JM (2008) Root exudates modulate plant–microbe interactions in the rhizosphere in secondary metabolites in soil ecology. Soil Biol 14(241):252
Bar-Yosef B (1991) Root excretions and their environmental effects: influence on availability of phosphorus. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half. Marcel Dekker, New York, pp 529–557
Becard G, Douds DD, Pfeffer PE (1992) Extensive in vitro hyphal growth of vesicular-arbuscular mycorrhizal fungi in presence of CO_2 and flavonols. Appl Environ Microbiol 58:821–825
Becard G, Taylor LP, Douds DD, Pfeffer PE, Doner LW (1995) Flavonoids are not necessary plant signal compounds in arbuscular mycorrhizal symbiosis. Mol Plant-Microbe Interact 8:252–258
Bennett AE, Alers-Garcia J, Bever JD (2006) Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: hypotheses and synthesis. Am Nat 167:141–152
Benoit LF, Berry AM (1997) Flavonoid-like compounds from seeds of red alder (*Alnus rubra*) influence host nodulation by Frankia (Actinomycetales). Plant Physiol 99:588–593
Bertin C, Yang X, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83
Besserer A, Puech-Page ‘s V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Be Card G, Sejalon Delmas N (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoSBiol 4:e226
Bowen GD (1979) Integrated and experimental approaches to study the growth of organisms around root and seeds. In: Schippers B, Gams W (eds) Soil-borne pathogens. Academic, London, pp 209–227
Bowen GD, Rovira AD (1991) The rhizosphere: the hidden half of the hidden half. In: Waisel Y, Eshel A, Kalkafi U (eds) Plant Roots: The Hidden Half. Marcel Dekker, New York, pp 641–669
Brigham LA, Michaels PJ, Flores HE (1999) Cell-specific production and antimicrobial activity of naphthoquinones in roots of *Lithospermum erythrorhizon*. Plant Physiol 119:417–428
Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744
Buee M, Rossigno M, Jauneaul A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Am Phyto-Pathol Soc, MPMI 13(6):693–698
Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 90:521–523
Cameron DD, Neal AL, Van Wees SCM, Ton J (2013) Mycorrhiza induced resistance: more than the sum of its parts? Trends Plant Sci 18:539–545
Chabot S, Bel-Rhlid R, Chênevert R, Piché Y (1992) Hyphal growth promotion in vitro of the VA mycorrhizal fungus, Gigaspora margarita Becker & Hall, by the activity of structurally specific flavonoids compounds under CO_2-enriched conditions. New Phytol 122:461–467
Chandler D, Davidson G, Grant WP, Greaves J, Tatchell GM (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Tech 19:275–283

Chaparro JM, SheflinAM MDK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499
Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One 8:e5573
Cooper JE (2007) Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. J Appl Microbiol 103:1355–1365
Costa R, Gotz M, Mrotzek N, Lottmann J, Berg G, Smalla K (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol Ecol 56:236–249
Czarnota MA, Rimando AM, Weston LA (2003) Evaluation of root exudates of seven sorghum accessions. J Chem Ecol 29:2073–2083
Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47
de Weert S, Vermeiren H, Mulders IHM, Kuiper I, Hendrickx N, Bloemberg GV, Vanderleyden J, De Mot R, Lugtenberg BJJ (2002) Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by pseudomonas fluorescens. Mol Plant-Microbe Interact 15:1173–1180
Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H (2004) Engineering high-level aluminum tolerance in barley with the ALMT1 gene. Proc Natl Acad Sci U S A 101:15249–15254
Delhaize E, Gruber BD, Ryan PR (2007) The roles of organic anion permeases in aluminium tolerance and mineral nutrition. FEBS Lett 581:2255–2262
Derrien D, Marol C, Balesdent J (2004) The dynamics of neutral sugars in the rhizosphere of wheat. An approach by 13C pulse-labelling and GC/C/IRMS. Plant Soil 267:243–253
Diener AC, Gaxiola RA, Fink GR (2001) Arabidopsis ALF5, a multidrug efflux transporter gene family member, confers resistance to toxins. Plant Cell 13:1625–1637
Estabrook EM, Yoder JI (1998) Plant-plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. Plant Physiol 116:1–7
Flores HE, Pickard JJ, Hoy MW (1988) Production of polyacetylenes and thiophenes in heterotrophic and photosynthetic root cultures of *Asteraceae*. Biol Mol 7:233–254
Flores HE, Vivanco JM, Loyola-Vargas VM (1999) "Radicle" biochemistry: the biology of root-specific metabolism. Trends Plant Sci 4:220–226
Fray RG (2002) Altering plant-microbe interaction through artificially manipulating bacterial quorum sensing. Ann Bot 89:245–253
Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y, Sato K, Katsuhara M, Takeda K, Ma JF (2007) An aluminum-activated citrate transporter in barley. Plant Cell Physiol 48:1081–1091
Gadd GM (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr Opin Biotechnol 11:271–279
Geibel M (1994) Sensitivity of the fungus *Cytospora persoonii* to the flavonoids of *Prunus cerasus*. Phytochemistry 38:599–601
Giovannetti M, Sbrana C, Citernesi AS, Avio L (1996) Analysis of factors involved in fungal recognition responses to host-derived signals by arbuscular mycorrhizal fungi. New Phytol 133:65–71
Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374
Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Hindawi Publishing Corporation, Scientifica
Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242
Goodman CD, Casati P, Walbot V (2004) A multidrug resistance associated protein involved in anthocyanin transport in *Zea mays*. Plant Cell 16:1812–1826
Hammad Y, Nalin R, Marechal K, Fiasson K, Pepin R, Berry AM, Normand P, Domenach AM (2003) A possible role for phenylacetic acid (PAA) in *Alnus glutinosa* nodulation by Frankia. Plant Soil 254:193–205

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42
Hertenberger G, Zampach P, Bachmann G (2002) Plant species affect the concentration of free sugars and free amino acids in different types of soil. J Plant Nutr Soil Sci 165:557–565
Hirsch AM (2003) Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms. Ecology 84:858–868
Hirsch AM, Lum MR, Downie JA (2001) What makes rhizobia-legume symbiosis so special? Plant Physiol 127:1484–1492
Horiuchi J, Prithiviraj B, Bais HP, Kimball BA, Vivanco JM (2005) Soil nematodes mediate positive interactions between legume plants and rhizobium bacteria. Planta 222:848–857
Hutchison WD, Campbell CD (1994) Economic impact of sugarbeet root aphid (Homoptera: Aphididae) on sugarbeet yield and quality in Southern Minnesota restricted access. J Eco Entmo 28:465–475
Hutsch BW, Augustin J, Merbach W (2000) Plant rhizodeposition an important source for carbon turnover in soils. J Plant Nut Soil Sci 165:397–407
Hvorup RN, Winnen B, Chang AB, Jiang Y, Zhou XF, Saier MH (2003) The multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) exporter superfamily. European J Biochem 270:799–813
Ishimaru Y, Kakei Y, Shimo H, Bashir K, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK (2011) A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele. J Biol Chem 286:24649–24655
Jain V, Nainawatee HS (2002) Plant flavonoids: signals to legume nodulation and soil microorganisms. J Plant Biochem Biotechnol 11:1–10
Jasinski M, Stukkens Y, Degand H, Purnell B (2002) A plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion. Plant Cell 13:1095–1107
Jones DL (1998) Organic acids in the rhizosphere – a critical review. Plant Soil 205:25–44
Jones DL, Darrah PR (1994a) Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. Plant Soil 163:1–12
Jones DL, Darrah PR (1994b) Role of root derived organic acids in the mobilization of nutrients in the rhizosphere. Plant Soil 166:247–257
Kamilova F (2006) Organic acids, sugars, and L-tryptophan in exudates of vegetables growing on stone wool and their effects on activities of rhizosphere bacteria. Mol Plant Microbe 19:250–256
Kang BG, Kim WT, Yun HS, Chang SC (2010) Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol Rep 4:179–183
Keyes WJ, O'Malley RC, Kim D, Lynn DG (2000) Signaling organogenesis in parasitic angiosperms: xenognosin generation, perception, and response. J Plant Growth Regul 19:217–231
Khan MS, Zaidi A, Musarrat J (eds) (2009a) Microbes in sustainable agriculture, Nova science Publisher. USA, New York
Khan MS, Zaidi A, Wani PA, Oves M (2009b) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19
Kidd P, Barcelo J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S et al (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259
Kim SA, Guerinot ML (2007) Mining iron: iron uptake and transport in plants. FEBS Lett 581:2273–2280
Kim HB, Oh CJ, Lee H, Sun An C (2003) A type-i chalcone isomerase mRNA is highly expressed in the root nodules of *Elaeagnus umbellata*. J Plant Biol 46:263–270
Kim D-Y, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J 50(207):218
Koricheva J, Gange AC, Jones T (2009) Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. Ecology 90:2088–2097
Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PGL, vanVeen JA (2002) Effects of above-ground species composition and diversity on the diversity of soil borne microorganisms. Antonie Van Leeuwenhoek 81:509–520

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) Putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Kuang Y, Wen D, Zhong C, Zhou G (2002) Root exudates and their roles in phytoremediation. Acta Phyto Ecol Sinica 27(5):709–717

Kuffner M, De Maria S, Puschenreiter M, Fallmann K, Wieshammer G, Gorfer M et al (2010) Culturable bacteria from Zn- and cd accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability. J Appl Microbiol 108(4):1471–1484

Larson RA, Marley KA, Tuveson RW, Berenbaum MR (1988) Carboline alkaloids: mechanisms of phototoxicity to bacteria and insects. Photochem Photobiol 48:665–674

Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153:497–522

Li L, He Z, Pandey GK, Tsuchiya T, Luan S (2002) Functional cloning and characterisation of a plant efflux carrier for multidrug and heavy metal detoxification. J Biol Chem 277:5360–5368

Ling N, Raza W, Ma JH, Huang QW, Shen QR (2011) Identification and role of organic acids in watermelon root exudates for recruiting *Paenibacillus polymyxa* SQR-21 in the rhizosphere. Eur J Soil Biol 47:374–379

Liu JP, Magalhaes JV, Shaff J, Kochian LV (2009) Aluminum activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminium tolerance. Plant J 57:389–399

Luo SL, Chen L, Chen JI, Xiao X, Xu TY, Wan Y et al (2011) Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd hyperaccumulator *Solanum nigrum* L and their potential use for phytoremediation. Chemosphere 85:1130–1138

Luo S, Xu T, Chen L, Chen J, Rao C, Xiao X et al (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte Bacillus sp. SLS18. Appl Microbiol Biotechnol 93:1745–1753

Lupwayi NZ, Rice WA, Clayton GW (1998) Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biol Biochem 30:1733–1741

Lynch JM, Whipps JM (1990) Substrate flows in the rhizosphere. Plant Soil 129:1–10

Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273–278

Ma Y, Rajkumar M, Luo Y, Freitas H (2011) Inoculation of endophytic bacteria on host and non-host plants – effects on plant growth and Ni uptake. J Hazard Mater 196:230–237

Magalhaes JV (2010) How a microbial drug transporter became essential for crop cultivation on acid soils: aluminium tolerance conferred by the multidrug and toxic compound extrusion (MATE) family. Ann Bot 106:199–203

Magalhaes JV, Liu J, Guimaraes CT (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat Genet 39:1156–1161

Marschner H, Romheld V, Kissel M (1987) Localization of phytosiderophore release and of iron uptake along intact barley roots. Plant Physiol 71:157–162

Martinoia E, Klein M, Geisler M, Bovet L, Forestier C, Kolukisaoglu U, Muller-Rober B, Schulz B (2002) Multifunctionality of plant ABC transporters: more than just detoxifiers. Planta 214:345–355

McDougali BM, Rovira AD (1970) Sites of exudation of 14C-labelled compounds from wheat roots. New Phytol 69:999–1003

Micallef SA, Shiaris MP, Colon-Carmona A (2009) Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. J Exp Bot 60:1729–1742

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. BiotechnolAdv 29:645–653

Morandi D (1996) Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interaction, and their potential role in biology control. Plant Soil 185:241–251

Munees A, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ-Sci 26(1):1–20

Nagahashi G (2000) In vitro and in situ techniques to examination the role of roots and root exudates during AM fungus-host interactions. In: Kapulink Y, DoudsJr D (eds) Arbuscular mycorrhizas: physiology and function. Kluwer Academic Publishers, Netherlands, pp 278–300
Nagahashi G, Jr-Douds DD (2003) Action spectrum for the induction of hyphal branches of an arbuscular mycorrhizal fungus: exposure sites versus branching sites. Mycol Res 107:1075–1082
Nardi S, Concheri G, Pizzeghello D, Sturaro A, Rella R, Parvoli G (2000) Soil organic matter mobilization by root exudates. Chemosphere 5:653–658
Neal AL, Ahmad S, Gordon-Weeks R, Ton J (2012) Benzoxazinoids in root exudates of maize attract pseudomonas putida to the rhizosphere. PLoS One 7:e35498
Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396
Noh B, Murphy AS, Spalding EP (2001) Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. Plant Cell 13:2441–2454
Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK (2011) Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. J Biol Chem 286:5446–5454
Paterson E, Gebbing T, Abel C, Sim A, Telfer G (2007) Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytol 173:600–610
Peck MC, Fisher RF, Long SR (2006) Diverse flavonoids stimulate NodD1 binding to nod gene promoters in *Sinorhizobium meliloti*. J Bacteriol 188:5417–5427
Peters NK, Frost JW, Long SR (1986) A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science 233:977–980
Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones CM, Sarr A, Maron PA (2013) Loss in microbial diversity affects nitrogen cycling in soil. ISME J 7:1609–1619
Popovici J, Comte G, Bagnarol E, Alloisio N, Fournier P, Bellvert F, Bertrand C, Fernandez MP (2010) Differential effects of rare specific flavonoids on compatible and incompatible strains in the Myrica gale-Frankia actinorhizal symbiosis. Appl Environ Microbiol 76:2451–2460
Poulin MJ, Bel-Rhlid R, Piché Y, Chênevert R (1993) Flavonoids released by carrot (*Daucus carota*) seedlings stimulate hyphal development of vesicular-arbuscular mycorrhizal fungi in the presence of optimal CO2 enrichment. J Chem Ecol 19:2317–2327
Pozo MJ, Azcon-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398
Prin Y, Rougier M (1987) Preinfection events in the establishment of Alnus-Frankia symbiosis: study of the root haïrs deformation step. Plant Physiol 6:99–106
Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149
Rajkumar M, Sandhya S, Prasad MN, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574
Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol 28:897–906
Rougier M (1981) Secretory activity at the root cap. In: Tanner W, Loews FA (eds) Encyclopedia of Plant Physiology, New Series. Springer Verlag, Berlin. 13B, Plant Carbohydrates 2:542–574
Rovira AD (1973) Zones of exudation along plant roots and spatial distribution of micro-organisms in the rhizosphere. Science 4:361–366
Rudrappa T, Czymmek KJ, Pare PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556
Ryan PR, Tyerman SD, Sasaki T, Yamamoto Y, Zhang WH, Delhaize E (2011) Identification of aluminium-resistance genes in plants provides an opportunity for enhancing the acid-soil tolerance of crop species. J Exp Bot 62:9–20
Scervino JM, PonceMA E-BR, Vierheilig H, Ocampo JA, Godeas A (2005) Flavonoids exclusively present in mycorrhizal roots of white clover exhibit different effects on arbuscular mycorrhizal fungi than flavonoids exclusively present in non-mycorrhizal roots of white clover. J Plant Interact 15:22–30

Shaw LJ, Morris P, Hooker JE (2006) Perception and modification of plant flavonoid signals by rhizosphere microorganisms. Environ Microbiol 8:1867–1880

Shukla KP, Singh NK, Sharma S (2010) Bioremediation: developments, current practices and perspectives. Gen Eng Biotechnol J 3:1–20

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser Set al. (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Sood SG (2003) Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiol Ecol 45:219–227

Stein M, Dittgen J, Sanchez-Rodrıguez C, Hou BH, Molina A, Schulze-Lefert P, Lipka V, Shauna Somervillea S (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to non-host resistance to inappropriate pathogens that enter by direct penetration. Plant Cell 18:731–746

Stintzi A, Browse J (2000) The Arabidopsis male-sterile mutant, opr3, lacks the 12 oxophytodienoic acid reductase required for jasmonate synthesis. Proc Natl Acad Sci 97:10625–10630

Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, Mitchell OT (2000) Induced plant defense responses against chewing insects, ethylene signaling reduces resistance of *Arabidopsis* against Egyptian cotton worm but not diamond back moth. Plant Physiol 124:1007–1018

Sugiyama A, Shitan N, Yazaki K (2007) Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume–rhizobium symbiosis. Plant Physiol 144:2000–2008

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant-Microbe Interact 13:637–648

Uroz S, Calvaruso C, Turpault MP, Frey-Klett P (2009) Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol 17:378–378

Van der Heijden MGA, Sanders IR (eds) (2002) Mycorrhizal ecology. Springer-Verlag, Berlin

Van Ghelue M, Lovaas E, Ringo E, Solheim B (1997) Early interactions between *Alnus glutinosa* and *Frankia* strain ArI3. Production and specificity of root hair deformation factor(s). Plant Physiol 99:579–587

Vierheilig H, Bago B (2005) Host and non-host impact on the physiology of the AM symbiosis. In: Declerck S, Strullu DG, Fortin JA (eds) In vitro culture of mycorrhizas. Springer, Heidelberg, pp 139–158

Vishwakarma K, Sharma S, Kumar N, Upadhyay N, Devi S, Tiwari A (2016) Contribution of microbial inoculants to soil carbon sequestration and sustainable agriculture. In: Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 101–113

Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra RK, Kumar V, Verma R, Upadhyay RG, Pandey M, Sharma S (2017) Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. Front Plant Sci 8:161

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132:44–51

Wall LG (2000) The actinorhizal symbiosis. J Plant Growth Regul 19:167–182

Wang E, Schornack S, Marsh JF, Gobbato E, Schwessinger B, Eastmond P, Schultze M, Kamoun S, Oldroyd GE (2012) A common signaling process that promotes mycorrhizal and oomycete colonization of plants. Curr Biol 22:2242–2246

Weir TL, Park SW, Vivanco JM (2004) Biochemical and physiological mechanisms mediated by allelochemicals. Curr Opin Plant Biol 7:472–479

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408

White PJ (2003) Ion transport. In: Thomas B, Murphy DJ, Murray DJ (eds) Encyclopedia of applied plant sciences. Academic, London, pp 625–634

Wu T, Wittkamper J, Flores HE (1999) Root herbivory In vitro: interactions between roots and aphids grown in aseptic coculture. In vitro. Cell Dev Biol-Plant 35:259–264

Wu SC, Cheung KC, Luo YM, Wong MH (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ Pollut 140:124–135

Yang CH, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl Environ Microbiol 66:345–351

Yoder JI (2001) Host-plant recognition by parasitic Scrophulariaceae. Curr Opin Plant Bio l4:359–365

Yu JQ, Ye SF, Zhang MF, Hu WH (2003) Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochem Syst Ecol 31:129–139

Understanding Functional Genomics of PTGS Silencing Mechanisms for *Tobacco Streak Virus* and Other Ilarviruses Mediated by RNAi and VIGS

24

Avinash Marwal and R.K. Gaur

Abstract

Post-transcriptional gene silencing (PTGS) is a successful technology for the investigation of functions of gene in plants. In general, this phrase refers to the capability of a cell to avert the expression of a definite gene. PTGS can be achieved either by RNA interference (RNAi) or virus-induced gene silencing (VIGS). *Tobacco Streak Virus* (genus *Ilarvirus* and family *Bromoviridae*) consists of a tripartite genome and infects plants by causing symptoms like necrosis and leaf puckering. *Ilarvirus* are the most imperative viruses, thus causing enormous economic losses worldwide by plummeting crop production by its quantity and quality. Virus infection in plants is known to activate the silencing pathway in which siRNAs are produced. There are numerous reports for the genus *Ilarvirus*, which have confirmed that RNAi is engineered to target viral RNA in plants. RNA silencing is a high-throughput tool for restraining gene expression carried out by sequence-specific manner, chiefly via transcriptional repression or RNA degradation. As a retort to this defence mechanism, many ilarviruses programme gene silencing suppressor proteins performing at diverse stages in the silencing pathway.

Keywords

PTGS • RNAi • VIGS • *Tobacco Streak Virus* • *Ilarvirus* • Silencing

A. Marwal • R.K. Gaur (✉)
Department of Biosciences, College of Arts, Science and Humanities, Mody University, Lakshmangarh, Sikar, Rajasthan 332311, India
e-mail: gaurrajarshi@hotmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_24

24.1 Introduction

A number of plant species are vulnerable to a variety of plant viruses (Marwal et al. 2013a). One of such kind is the genus *Ilarvirus*, belonging to *Bromoviridae* family (Bujarski et al. 2012). The genus *Ilarvirus* comprises of 19 virus species. *Tobacco Streak Virus* (Johnson 1936) is the main species of this genus prevailing in the world. The rest of the species of *Ilarvirus* are divided into six subgroups: subgroup 1, subgroup 2, subgroup 3, subgroup 4, subgroup 5 and subgroup 6. *Tobacco Streak Virus* and *Parietaria Mottle Virus* belong to subgroup 1. *Citrus Leaf Rugose Virus*, *Asparagus Virus 2*, *Elm Mottle Virus*, *Citrus Variegation Virus*, *Tulare Apple Mosaic Virus* and *Spinach Latent Virus* fall in subgroup 2. Whereas subgroup 3 comprises of *Apple Mosaic Virus* (Fenner 1976), *Humulus japonicus Latent Virus* (Francki et al. 1991) and *Prunus Necrotic Ringspot Virus* (Candresse et al. 1998), *Fragaria chiloensis Latent Virus* (van Regenmortel et al. 2000) and *Prune Dwarf Virus* (Boari et al. 1998) belong to subgroup 4. Only one species, i.e. *American Plum Line Pattern Virus* (Matthews 1982; Alayasa et al. 2003), is in the subgroup 5. Finally subgroup 6 contains *Lilac Leaf Chlorosis Virus* (James et al. 2010). The four species, viz. *Blackberry Chlorotic Ringspot Virus* (Tzanetakis et al. 2004), *Blueberry Shock Virus* (Jones et al. 2006), *Lilac Ring Mottle Virus* (Matthews 1979; Scott and Zimmerman 2008) and *Strawberry Necrotic Shock Virus* (Tzanetakis et al. 2010), are unassigned ilarviruses. *Tobacco Streak Virus* causes serious crop production losses and decreases in the product quality as well (Walter et al. 1995).

These viruses are isometric in shape and acquire a single-stranded, mainly tripartite RNA genome. *Tobacco Streak Virus* has been studied the most, and similarly a good deal of knowledge has been attained for *Prunus Necrotic Ringspot Virus* (Sharman et al. 2009). Developing new plant varieties with resistance to *Tobacco Streak Virus* (Ladhalakshmi et al. 2009) and other viral pathogens is considered highly necessary for farmers. Even if procreation for virus-resistant varieties has been followed for a long time by simple breeding techniques, the advancement remains sluggish because of the inherent genetic complexity of resistance (Barba et al. 1992; Waterhouse and Helliwell 2003). Wherein molecular biology makes it promising to manoeuvre and improve plant resistance to *Tobacco Streak Virus*, such molecular biology skill highlights post-transcriptional gene silencing (PTGS) (Baulcomb 2004). The 2b protein of *Tobacco Streak Virus* and other ilarviruses are responsible for RNA silencing and the viral movement for long distances in the plant (Guo and Ding 2002). Here, we are presenting a mini review on silencing of *Tobacco Streak Virus* and other ilarviruses mediated via RNAi (RNA interference) (Watson et al. 2005) and understanding the effect of various genes through VIGS (virus-induced gene silencing) (Burch-Smith et al. 2004; Constantin et al. 2004) from the agronomical and horticultural point of view.

24.2 Optimization Through RNAi-Mediated Silencing

RNA interference (RNAi) takes place in a broad range of living beings; this includes plants, fungi and animals (Bass 2000; Saunders et al. 2004). RNA degradation progression is a sequence-specific RNA silencing mechanism that is activated either by the formation of dsRNA or otherwise by unusual RNAs associated with transgenes viruses and transposons (Vaucheret 2006; Marwal et al. 2013b). Double-stranded RNA (dsRNA) is generally cleaved in plants by the cellular machinery into short interfering RNAs (siRNAs), which are efficient inducers of gene silencing (Fusaro et al. 2006; Kerschen et al. 2004). RNAs with hairpin with a loop structures are particularly actual inducers of PTGS in plants (Ikegami et al. 2011; Yoshikawa et al. 2013). The dsRNAs trigger an RNA-mediated defence system resulting in their cleavage into small-interfering RNAs (siRNAs) by Dicer-like enzymes. In RNA-induced silencing complex, the siRNAs further act upon the degradation of RNAs, which has identical sequences to those of the inserted fragment and viral genome (Baulcomb 2004; Lecellier and Voinnet 2004; Marwal et al. 2013c; Meister and Tuschi 2004).

Prunus species are harmfully pretentious by a major pollen scattered *Ilarvirus*, i.e. *Prunus Necrotic Ringspot* (PNRSV) (Amari et al. 2007). RNA interference (RNAi) vector pART27–PNRSV was created, enclosed with an inverted repeat (IR) region consisting of PNRSV. This construct was then inoculated into two hybrid cherry rootstocks [‘Gisela 6’ (GI 148–1) and ‘Gisela 7’ (GI 148–8)] which were tolerant and sensitive, respectively, to PNRSV infection (Lacomme et al. 2003). After 1 year of inoculation with PNRSV plus *Prune Dwarf Virus*, nontransgenic ‘Gisela 6’ doesn’t exhibit any indication of virus disease but does possessed a noteworthy PNRSV titre. The transgenic ‘Gisela 6’ was devoid of symptoms and encountered with negligible PNRSV titre. In the course of this experiment, the nontransgenic ‘Gisela 7’ trees don’t survive, while the transgenic ones, i.e. ‘Gisela 7’ trees, continue to exist (Song et al. 2013).

A number of leading viruses critically impinge on *Prunus* L. fruit production (Aparicio et al. 2010). It is exceedingly required by growers and breeders that the expansions of new varieties resistant to these viruses are quite exigent. For engineering multivirus resistance in plants, a post-transcriptional gene silencing foundation was accounted. For this approach, a solo chimeric transgene, i.e. PTRAP6, was fashioned by the amalgam of around 400–500-base pair (bp) gene fragments from six major *Prunus* fruit viruses, consisting of *Peach Mosaic Virus*, *American Plum Line Pattern Virus*, *Prunus Necrotic Ringspot Virus*, *Prune Dwarf Virus* (*PDV*), *Plum Pox Virus* (*PPV*) and *Tomato Ringspot Virus* (ToRSV). Devoid of any splicing intrusion, it was found that the two strands of PTRAP6 created a 2.5 kb transcript in plant when being transcribed.

PTRAP6i was shaped by insertion of two copies of PTRAP6 in an inverted repeat under the command of the *Cauliflower Mosaic Virus* 35S promoter and divides by an intron spacer fragment for inducing gene silencing/virus resistance. Out of 28 R0 PTRAP6i transgenic lines, only 12 were resistant to ToRSV which were earlier inoculated in *Nicotiana benthamiana* plants. The symptoms range from mild

visualization to phenotypes which were devoid of any symptoms. Detailed analysis of two of the three highly resistant homozygous R3 generation lines demonstrated that they were resistant to PPV, PDV and ToRSV. The rest of the three viruses targeted by PTRAP6i were either unavailable for this study or were unable to systemically infect *N. benthamiana* (Lui et al. 2007).

In another incident, *Prune Dwarf Virus* (PDV) was found causing systemic infection in some almond trees and other *Prunus* sp. which were spread by means of pollen grains (Abou-Jawdah et al. 2004). An approach that was focused on the coat protein (cp) gene subjected to restrict PDV replication in host plant cells has been studied. To construct the cDNA of the cp gene, a Portuguese isolate of PDV was acquired from infected almond leaves. To seek for the transgenic expression of the new or customized *Prune Dwarf Virus* coat protein (cpPDVSense and cpPDVMutated), a range of constructs was organized based on this sequence. Similar aspects were made in case of cpPDV RNA (cpPDVAntisense and cpPDV without start codon) for its expression. Widespread molecular characterization and controlled infections were achieved on transformants and their offspring, where all constructs were tested in a PDV host model, *Nicotiana benthamiana*.

As evaluated by DAS-ELISA on newly developed leaves, transgenic plants exhibiting cp RNA were capable of blocking the propagation of *Prune Dwarf Virus* isolate, thus contributing nearly 91% homology with the isolate used for cpPDV cloning. With cp expression, the obstruction of PDV propagation in lately formed leaves was only accomplished due to the mutated construct of cpPDV, where arginine was replaced by alanine due to substitution in the coat protein at the 14th aa residue position. The experiment emphasizes the possible responsibility of the mutated amino acid in the virus capability to replicate and proliferate. The following study expressed the likelihood for accomplishing defence against *Prune Dwarf Virus* via mutated cp sequence or by coat protein RNA (Raquel et al. 2008).

Prunus domestica L were transformed with the *Plum Pox Virus* coat protein gene (PPV-CP). Transgenic plums were extremely challenging to PPV infection since it exhibits post-transcriptional gene silencing (PTGS). In order to test the consequence of heterologous viruses on the usefulness and constancy of PTGS against PPV, transgenic C5 trees were graft inoculated with diverse amalgamation of *Prunus Necrotic Ringspot Virus* (PNRSV), *Apple Chlorotic Leaf Spot Virus* (ACLSV), *Prune Dwarf Virus* (PDV) and PPV-D strain (Sasaki et al. 2011).

The possibility for suppression of the silencing system mediated by these viruses was evaluated. Confront experiments were performed under greenhouse, nursery and field conditions in Romania and Spain, including two different environments, continental and Mediterranean, respectively. Virus infections were appraised by visual supervises of symptom and by molecular and serological study. Resistance against *Plum Pox Virus* for C5 transgenic plums was engineered, which was firm and was not obscured by the occurrence of the challenging heterologous viruses. This study was carried over a period of 3 year in all trials (Zagrai et al. 2008).

Three experiments were undertaken in Romania, where transgenic plums of *Prunus domestica* L. as the subjects were inoculated with PPV-CP (coat protein gene of *Plum Pox Virus*). With the influence of natural infection, the transgenic

clones such as C2, C3, C4, C5, C6 and PT3 were assessed for sharka resistance. The highest resistance was observed in transgenic clone C5 (named as 'HoneySweet'). Up to 10 years, transgenic C5 trees were devoid of any visible symptoms caused by naturally infected aphids. This is due to post-transcriptional gene silencing (PTGS) exhibited by the resistant C5 lines. The second study evaluated the consequence of two heterologous viruses (i.e. *Prune Dwarf Virus* and *Prunus Necrotic Ringspot Virus*) based on the effectiveness and stability of PTGS-mediated resistance to *Plum Pox Virus* demonstrated by the C5 plum. This engineered resistance to *Plum Pox Virus* in the C5 transgenic plums was firm and doesn't concealed by the existence of the examined heterologous viruses (Zagrai et al. 2011).

One of the most efficiently important viruses infecting several crop plants in India is the *Tobacco Streak Virus* (TSV). RT-PCR with TSV replicase gene-specific primers was carried on indicative samples collected from sunflower and okra fields. In order to build up tobacco transgenic plants resistance to *Tobacco Streak Virus* (TSV) by articulating hairpin RNA transcript (hpRNA), the replicase (Rep) genes of these isolates were sequenced. A 99% nucleotide sequence identity of replicase gene of these isolates with Tamil Nadu okra isolate was revealed. The position 3065–3405 of the TSV replicase gene was used for building of pHANNIBAL vector, i.e. a conserved nucleotide sequence having a hairpin construct.

The Rep hairpin construct was cloned into pART27 and congregate into *Agrobacterium tumefaciens* LBA4404 and commenced into tobacco by *Agrobacterium*-mediated transformation. Taking the genomic DNA from transformed tobacco plants, the T0 plants produced were subjected to PCR and Southern blot examination. Corresponding to nptII gene and Rep gene, the transformants produced ~299 bp and 340 bp amplicons, respectively. The single- and multiple-copy integration of the transgenes was confirmed by Southern blot analysis. Upon mechanical inoculation of TSV, the transgenic T0 tobacco plants illustrate resistance against TSV without showing any visible symptoms; resistance was also confirmed by DAC-ELISA (Suppaiah et al. 2015).

24.3 Engineering by VIGS: A Versatile Tool

Viruses that derived small-interfering RNAs (siRNAs) are the hallmarks of an innate immune response in plants that targets invading viruses through post-transcriptional gene silencing (PTGS). Virus-induced gene silencing (VIGS) has a great potential as a reverse genetic tool in plant genomics (Burch-Smith et al. 2004; Marwal et al. 2014; Robertson 2004). In plants, PTGS has been widely studied, and like PTGS that is distinguished by sequence-specific resistance against virus infection, viruses also induced an RNA-mediated defence system in plants. *Tobacco Mosaic Virus* (TMV) was the first RNA virus used as silencing vectors (Godge et al. 2008).

VIGS involves using a vector containing the piece of gene of interest that causes the silencing of specific gene expression (Gleba et al. 2007). siRNA is an important method for evaluating gene functionality and is being exploited for the development of new approaches to control plant viruses (Mourrain et al. 2000; Covey et al. 1997;

Marwal et al. 2012; Ratcliff et al. 1997; Lu et al. 2003). VIGS engross the release of a recombinant virus to plants containing a portion of the plant gene that is proposed to be silenced. The plant defence mechanism system then diminishes not only the virus but also the targeted endogenous plant gene expression through post-transcriptional gene silencing (Robertson 2004).

Asparagus Virus 2 (AV-2) is another member of the genus *Ilarvirus*. The coat protein (CP) and the 2b protein (2b) genes of AV-2 isolates were cloned from *Asparagus* plants from a variety of province, and it was established that the sequence for CP and for 2b was extremely conserved among the isolates, signifying that AV-2 from around the world is almost indistinguishable (Xin et al. 1998). Later an AV-2 infectious clone was created by instantaneous inoculation with in vitro transcripts of RNAs 1–3 of AV-2 and in vitro-synthesized CP, which is obligatory for initial infection. Because 2b of cucumoviruses in *Bromoviridae* can hold back systemic silencing as well as confined silencing, it was analysed whether there is practical synteny of 2b protein between AV-2 and *Cucumovirus*. By means of the AV-2 infectious clone, the *Ilarvirus* 2b job as an RNA silencing suppressor is now evident; AV-2 2b has suppressor bustle against systemic silencing but not confined silencing (Shimura et al. 2013).

For molecular characterization of gene functions in plants, RNA silencing is a dominant skill. Genetic transformation is a generally used method for the introduction of RNA silencing. The best potent substitute is to use a customized viral vector for virus-induced gene silencing (VIGS) to demean RNA molecules partaking similar nucleotide sequence. Due to a long immature stage and intractable to genetic transformation, unfortunately genomic studies in many allogamous woody perennials such as peach are sternly delayed. The construction of a viral vector imitative from *Prunus Necrotic Ringspot Virus* (PNRSV), a prevalent fruit tree virus that is endemic in all *Prunus* fruit production countries and regions in the world, was reported.

It was affirmed that the modified PNRSV vector, an anchor ageing the sense-orientated objective gene sequence of 100–200 bp in length in genomic RNA 3, could impressively trigger the silencing of a transgene or an endogenous gene in the model plant *Nicotiana benthamiana*. It was further demonstrated that vector formed by *Prunus Necrotic Ringspot Virus* can be easily manoeuvre to cause silencing of endogenous genes in peach similar to translation initiation factor 4E isoform (eIF(iso)4E) of eukaryotic, a host factor of many potyviruses including *Plum Pox Virus* (PPV). Moreover, the eIF(iso)4E-knocked down peach plants were resistant to PPV (Cui and Wang 2016).

Functional genomics authorize knockdown of expression of individual genes or closely linked gene families through virus-based gene silencing systems which is a well thought-out influential tool. TSV shows recovery from initial symptoms and efficiently invades both meristems and developing embryos in soybean making it an excellent candidate for a virus-based silencing system for those tissues. TSV RNAs 1, 2, 3 and 4 were cloned into pHST40, a pUC-based plasmid vector, and pCASS-4RZ, an *Agrobacterium tumefaciens*-compatible binary vector. Both sets of clones were infectious in soybean and tobacco. 2b gene of pHST40-RNA2 was truncated,

and multicloning site was introduced, and the clone was stably transmitted in soybean seed.

Obvious leaf yellowing typical for silencing of MgCh mRNA was exhibited, when magnesium chelatase (MgCh) gene parts of 105 nt and 175 nt were put into the truncated 2b vector and were stable in systemic leaves of inoculated 'Williams82' plants. RNA 3 of the pCASS-4RZ clone was partitioned between two RNAs, one with only the movement protein (pCASS-R3Mp) and the other expressing only the coat protein (pCASS-R3Cp). Full-length green fluorescent protein (GFP) and phytoene desaturase (PDS) coding regions were inserted into pCASS-R3Mp and pCASS-R3Cp, respectively. Tobacco plants illustrated steady expression of GFP and photo bleaching symptoms when inoculated, which is reliable with silencing of PDS mRNA (Jossey et al. 2011).

Dahlia (*Dahlia variabilis*) flower colour has been credited with black, due to the elevated levels of anthocyanins which are cyaniding compounds (Chen et al. 2004). This pattern transpires because of flavone synthesis, as it is reduced for the reason that of post-transcriptional gene silencing (PTGS) of flavone synthase II (DvFNS). Apart from the black colour, purple-coloured flowers are also known, which has appeared from a black cultivar 'Kokucho'. It was found that the purple colour of flower is not the result of mutation but due to the infection of *Tobacco Streak Virus*, which suppresses the PTGS of DvFNS. When *Tobacco Streak Virus* was eradicated from the purple flowering 'Kokucho' by leaf primordia-free shoot apical meristem culture, the resultant flowers again restore their black colour (Deguchi et al. 2015).

It was portentous that *Tobacco Streak Virus* has a silencing suppressor, as due to *Tobacco Streak Virus* which was infecting purple flowers showed lower numbers of siRNAs than black flowers. *Tobacco Streak Virus*-infected dahlia distorted the flower colour severely by the graft inoculation of other black cultivars apart from 'Fidalgo Blacky', which is a very deep black cultivar with the highest amount of cyaniding-based anthocyanins. The flowers of all six *Tobacco Streak Virus*-infected *Dahlia* cultivars mount up augmented quantity of flavones and reduced quantity of cyaniding-based anthocyanins. There was no change in the accumulation of pigments in 'Fidalgo Blacky' and thus remained black whereas in *Dahlia* plants infected with *Tobacco Streak Virus* still had higher level of cyaniding-based anthocyanins than other cultivars.

24.4 Conclusion

This review makes noticeable that the RNAi tactic is beneficial for developing viral resistance in plants and such transgenics have imminent to augment production of customized varieties (nongenetically) thus evading concern regarding transgene flow. Transgene-wide and siRNA species were detected along with vanishing of transgene transcript in the resistant lines, representing that PTGS underlies the method of resistance. This review presents confirmation that RNAi is able to bestow gene silencing-based resistance to multiple ilarviruses (Hamilton and Baulcombe 1999).

Virus-induced gene silencing (VIGS) is a successful technology for the investigation of functions of gene in plants (Gronlund et al. 2008; vanKammen 1997; Zhang and Ghabrial 2006). This work opens a potential avenue for the control of virus diseases in plants via viral vector-mediated silencing of host factors, and vector may serve as a powerful molecular tool for functional genomic studies. Ultimately, the two approaches discussed above are used to produce virus-resistant cultivars. Researchers around the world are currently developing approaches to engineer multivirus resistance in plants to address the serious virus problems encountered in agricultural practice.

Acknowledgements The authors are thankful to the Science and Engineering Research Board – Department of Science and Technology, New Delhi, India, for the financial assistance (File No. YSS/2015/000265) and also to the University Grants Commission, New Delhi, for providing financial assistantship under Research Award for Teacher (F.30-1/2014/RA-2014-16-GE-RAJ-4696 (SA-II).

References

Abou-Jawdah Y, Sobh H, Cordahi N, Kawtharani H, Nemer G, Maxwell DP, Nakhla MK (2004) Immunodiagnosis of *Prune Dwarf Virus* using antiserum produced to its recombinant coat protein. J Virol Methods 121:31–38

Alayasa N, Al Rwahnih M, Myrta A, Herranz MC, Minafra A, Boscia D, Pallás V (2003) Identification and characterization of an *American Plum Line Pattern Virus* isolate from Palestine. J Plant Pathol 85:3–7

Amari K, Burgos L, Pallás V, Sánchez-Pina MA (2007) *Prunus Necrotic Ringspot Virus* early invasion and its effects on apricot pollen grains performance. Phytopathology 97:892–899

Aparicio F, Sánchez-Navarro JA, Pallás V (2010) Implication of the C terminus of the *Prunus Necrotic Ringspot Virus* movement protein in cell-to-cell transport and in its interaction with the coat protein. J Gen Virol 91:1865–1870

Barba M, Martino L, Lauretti F (1992) Comparison of different methods to produce virus free stone fruits. Acta Hortic 309:385–392

Bass BL (2000) Double-stranded RNA as a template for gene silencing. Cell 101:235–238

Baulcomb D (2004) RNA silencing in plants. Nature 431:356–363

Boari A, Boscia D, Di Terlizzi B, Savino V (1998) Study on seed transmission of *Prune Dwarf Virus* (PDV) in Prunus mahaleb L. Adv Hortic Sci 12:89–92

Bujarski J, Figlerowicz M, Gallitelli D, Roossinck MJ, Scott SW (2012) Family Bromoviridae. In: Virus taxonomy. Ninth report of the international committee on taxonomy of viruses. Elsevier Academic, San Diego, pp 965–976

Burch-Smith TM, Anderson JC, Martin GB, Dinesh-Kumar SP (2004) Applications and advantages of virus-induced gene silencing for gene function studies in plants. Plant J 39:734–746

Candresse T, Kofalvi SA, Lanneau M, Dunez J (1998) A PCRELISA for the simultaneous detection and identification of Prunus Necrotic Ringspot (PNRSV) and Apple mosaic (APMV) ilarviruses. Acta Hortic 472:219–224

Chen J-C, Jiang C-Z, Gookin TE, Hunter DA, Clark DG, Reid MS (2004) Chalcone synthase as a reporter in virus-induced gene silencing studies of flower senescence. Plant Mol Biol 55:521–530

Constantin GD, Krath BN, MacFarlane SA, Nicolaisen M, Johansen IE, Lund OS (2004) Virus-induced gene silencing as a tool for functional genomics in a legume species. Plant J 40:622–631

Covey SN, Al-Kaff NS, La ´ngara A, Turner DS (1997) Plants combat infection by gene silencing. Nature (London) 385:781–782

Cui H, Wang A (2016) An efficient viral vector for functional genomic studies of Prunus fruit trees and its induced resistance to *Plum Pox Virus* via silencing of a host factor gene. Plant Biotechnol J 15(3):344–356

Deguchi A, Tatsuzawa F, Hosokawa M, Doi M, Ohno S (2015) *Tobacco Streak Virus* (strain dahlia) suppresses posttranscriptional gene silencing of flavone synthase II in black dahlia cultivars and causes a drastic flower color change. Planta 242:663–675

Fenner F (1976) Classification and nomenclature of viruses. Second report of the international committee on taxonomy of viruses. Intervirology 7:1–115

Francki RIB, Fauquet CM, Knudson DL, Brown F (1991) Classification and nomenclature of viruses. Fifth report of the international committee on taxonomy of viruses. Arch Virol Suppl 2:452

Fusaro AF, Matthew L, Smith NA, Curtin SJ, Dedic-Hagan J, Ellacott GA, Watson JM, Wang MB, Brosnan C, Carroll BJ, Waterhouse PM (2006) RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway. EMBO Rep 7:1168–1175

Gleba Y, Klimyuk V, Marillonnet S (2007) Viral vectors for the expression of proteins in plants. Curr Opin Biotechnol 18:134–141

Godge MR, Purkayastha A, Dasgupta I, Kumar PP (2008) Virus-induced gene silencing for functional analysis of selected genes. Plant Cell Rep 27:209–219

Gronlund M, Constantin G, Piednoir E, Kovacev J, Johansen IE, Lund OS (2008) Virus-induced gene silencing in *Medicago truncatula* and *Lathyrus odorata*. Virus Res 135:345–349

Guo HS, Ding SW (2002) A viral protein inhibits the long range signalling activity of the gene silencing signal. EMBO J 21:398–407

Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286:950–952

Ikegami M, Kon T, Sharma P (2011) RNA silencing and viral encoded silencing suppressors. In: Gaur RK, Gafni Y, Gupta VK, Sharma P (eds) RNAi technology. CRC Press, Boca Raton, pp 209–240

James D, Varga A, Leippi L, Godkin S, Masters C (2010) Sequence analysis of RNA 2 and RNA 3 of *Lilac Leaf Chlorosis Virus*: a putative new member of the genus Ilarvirus. Arch Virol 155:993–998

Johnson J (1936) Tobacco streak, a virus disease. Phytopathology 26:285–292

Jones AT, McGavin WJ, Gepp V, Scott SW, Zimmerman MT (2006) Purification and properties of *Blackberry Chlorotic Ringspot*, a new virus species in Su bgroup 1 of the genus Ilarvirus found naturally infecting blackberry in the UK. Ann Appl Biol 149:125–135

Jossey S, Singh AK, Ghabrial SA, Domier LL (2011) Development of a Tobacco Streak Virus (TSV) based gene silencing vector for soybean seed development. APS IPPC Joint Meeting, Honolulu

Kerschen A, Napoli C, Jorgensen R, Muller A (2004) Effectiveness of RNA interference in transgenic plants. FEBS Lett 566:223–228

Lacomme C, Hrubikova K, Hein I (2003) Enhancement of virus induced gene silencing through viral-based production of inverted-repeats. Plant J 34(4):543–553

Ladhalakshmi D, Ramaiah M, Ganapathy T, Krishna Reddy M, Khabbaz SE, Babu M, Kamalakannan A (2009) First report of the natural occurrence of *Tobacco Streak Virus* on blackgram (Vigna mungo). Plant Pathol 12:55

Lecellier CH, Voinnet O (2004) RNA silencing: no mercy for viruses? Immunol Rev 198:285–303

Lu R, Martin-Hernandez AM, Peart JR, Malcuit I, Baulcombe DC (2003) Virus-induced gene silencing in plants. Methods 30:296–303

Lui Z, Scorza S, Hily JM, Scott SW, James D (2007) Engineering resistance to multiple Prunus Fruit Viruses through expression of chimeric hairpins. J Am Soc Hortic Sci 132(3):407–414

Marwal A, Sahu A, Prajapat R, Choudhary DK, Gaur RK (2012) First report of association of begomovirus with the leaf curl disease of a common weed, *Datura inoxia*. Virus Dis 23(1):83–84

Marwal A, Sahu A, Sharma P, Gaur RK (2013a) Transmission and host interaction of geminivirus in weeds. Plant virus-host interaction: molecular approaches and viral evolution. Elsevier Chapter 7:143–161

Marwal A, Sahu A, Sharma P, Gaur RK (2013b) Molecular characterizations of two begomoviruses infecting *Vinca rosea* and *Raphanus sativus* in India. Virol Sin Virol Sin 28(1):053–056
Marwal A, Sahu A, Choudhary DK, Gaur RK (2013c) Complete nucleotide sequence of a begomovirus associated with satellites molecules infecting a new host *Tagetes patula* in India. Virus Genes 47(1):194–198
Marwal A, Sahu A, Gaur RK (2014) First report of airborne begomovirus infection in *Melia azedarach* (pride of India), an ornamental tree in India. Aerobiologia 30(2):211–215
Matthews REF (1979) Classification and nomenclature of viruses. Third report of the international committee on taxonomy of viruses. Intervirology 12:129–296
Matthews REF (1982) Classification and nomenclature of viruses. Fourth report of the international committee on taxonomy of viruses. Intervirology 17:1–199
Meister G, Tuschi T (2004) Mechanisms of gene silencing by double stranded RNA. Nature 431:343–349
Mourrain P, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Jouette D, Lacombe AM, Nikic S, Picault N, Remoue K, Sanial M, Vo TA, Vaucheret H (2000) Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. Cell 101:533–542
Raquel H, Lourenco T¸ Moita C, Oliveira MM (2008) Expression of prune dwarf Ilarvirus coat protein sequences in *Nicotiana benthamiana* plants interferes with PDV systemic proliferation. Plant Biotechnol Rep 2:75–85
Ratcliff FG, Harrison BD, Baulcombe DC (1997) A similarity between viral defence and gene silencing in plants. Science 276:1558–1560
Robertson D (2004) VIGS vectors for gene silencing: many targets, many tools. Annu Rev Plant Biol 55:495–519
Sasaki S, Yamagishi N, Yoshikawa N (2011) Efficient virus-induced gene silencing in apple, pear and Japanese pear using apple latent spherical virus vectors. Plant Methods 7:15
Saunders K, Norman A, Gucciardo S, Stanley J (2004) The DNA- b satellite component associated with ageratum yellow vein disease encodes an essential pathogenicity protein (bC1). Virology 324:37–47
Scott SW, Zimmerman MT (2008) Partial nucleotide sequences of the RNA 1 and RNA 2 of *Lilac Ring Mottle Virus* confirm that this virus should be considered a member of subgroup 2 of the genus Ilarvirus. Arch Virol 153:2169–2172
Sharman M, Persley DM, Thomas JE (2009) Distribution in Australia and seed transmission of *Tobacco Streak Virus* in Parthenium hysterophorus. Plant Dis 93:708–712
Shimura H, Masuta C, Yoshida N, Sueda K, Suzuki M (2013) The 2b protein of *Asparagus virus 2* functions as an RNA silencing suppress or against systemic silencing to prove functional synteny with related cucumoviruses. Virology 442:180–188
Song GQ, Sink KC, Walworth AE, Cook MA, Allison RF, Lang GA (2013) Engineering cherry rootstocks with resistance to *Prunus Necrotic Ring Spot Virus* through RNAi-mediated silencing. Plant Biotechnol J 11(6):702–708
Suppaiah R, Muthuraj R, Gandhi K (2015) Conserved sequence of replicase gene mediated resistance in *Nicotiana tabacum* L. cv Abirami through RNA silencing. Eur J Plant Pathol. doi:10.1007/s10658-015-0658-z
Tzanetakis IE, Mackey IC, Martin RR (2004) *Strawberry Necrotic Shock Virus* is a distinct virus and not a strain of *Tobacco Streak Virus*. Arch Virol 149:2001–2011
Tzanetakis IE, Martin RR, Scott SW (2010) Genomic sequences of *Blackberry Chlorotic Ringspot Virus* and *Strawberry Necrotic Shock Virus* and the phylogeny of viruses in subgroup 1 of the genus Ilarvirus. Arch Virol 155:557–561
vanKammen A (1997) Virus-induced gene silencing in infected and transgenic plants. Trends Plant Sci 2:409–411
van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (2000) Virus taxonomy, Seventh report of the International Committee on Taxonomy of Viruses. Academic, San Diego. p1162

Vaucheret H (2006) Post-transcriptional small RNA pathways in plants: mechanisms and regulations. Genes Dev 20:759–771

Walter MH, Wyatt SD, Kaiser WJ (1995) Comparison of the RNAs and some physiochemical properties of the seed transmitted *Tobacco Streak Virus* isolate Mel 40 and the infrequently seed-transmitted isolate Mel F. Phytopathology 85:1394–1399

Waterhouse PM, Helliwell CA (2003) Exploring plant genomes by RNA-induced gene silencing. Nat Rev Genet 4:29–38

Watson JM, Fusaro AF, Wang MB, Waterhouse PM (2005) RNA silencing platforms in plants. FEBS Lett 579:5982–5987

Xin HW, Ji LH, Scott SW, Symons RH, Ding SH (1998) Ilarviruses encode a Cucumovirus-like 2b gene that is absent in other genera within the *Bromoviridae*. J Virol 72(8):6956–6959

Yoshikawa M, Iki T, Tsutsui Y, Miyashita K, Poethig RS, Habu Y, Ishikawa M (2013) 3′ fragment of miR173 programmed RISC cleaved RNA is protected from degradation in a complex with RISC and SGS3. Proc Natl Acad Sci U S A 110:4117–4122

Zagrai I, Capote N, Ravelonandro M, Cambra M, Zagrai L, Scorza R (2008) *Plum Pox Virus* silencing of c5 transgenic plums is stable under challenge inoculation with heterologous viruses. PT J Plant Pathol 90(1):S1.63–S1.71

Zagrai I, Ravelonandro M, Zagrai L, Scorza R, Minoiu N (2011) Overview of the investigations of transgenic plums in Romania. Acta Horticult ISHS 899:153–158

Zhang C, Ghabrial SA (2006) Development of *Bean Pod Mottle Virus* based vectors for stable protein expression and sequence-specific virus-induced gene silencing in soybean. Virology 344:401–411

Rhizocompetence of Applied Bioinoculants

25

Chandandeep Kaur, G. Selvakumar, and A.N. Ganeshamurthy

Abstract

Concomitant with the demand for chemical free food, the demand for bioinoculants for plant growth promotion and protection against pests and disease causing organisms has also seen a phenomenal increase. This has led to the mushrooming of several products in the market that have met with varying degrees of success. Very often it has been observed that inoculant strains that perform exceedingly well under laboratory conditions fail under field conditions. This can be primarily attributed to the utilization of non-rhizocompetent strains. Since the inoculated strain has to compete with a multitude of native microbes in the rhizospheric region, strains lacking rhizocompetence traits often fail to establish and perform in the rhizosphere. Rhizocompetence traits such as biofilm formation, siderophore production, antagonism, ability to utilize root exudates, motility, and protease activity can prove to be game changers under field conditions. This chapter attempts to highlight the importance of rhizocompetence traits in inoculant selection and development, in order to harness the benefit of applied inoculants.

Keywords

Bioinoculants • Rhizosphere • Rhizocompetence • Root colonization • Traits

C. Kaur • G. Selvakumar (✉) • A. Ganeshamurthy
ICAR- Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru 560089, India
e-mail: gselva74@rediffmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_25

25.1 Introduction

The rhizosphere is a hub of microbial activity that influences plant growth and development in a myriad fashion. Some rhizospheric microbial associations are beneficial to plant growth, while some are detrimental and few others are neutral in nature. Though the term rhizosphere was coined in the previous century (Hiltner 1904), its importance in sustaining microbial activity and subsequently plant growth and development came to the forefront with the coining of the term plant growth promoting rhizobacteria (PGPR) by Kloepper and Schroth (1978). Considering the harmful effects of chemical fertilizers and pesticides, environment friendly bioinoculants have received widespread acceptance worldwide when introduced into soil or on seeds, roots, and bulbs for decades. Many bacterial and fungal inoculants have been commercialized and are currently available in the market. But root colonization remains the fundamental element that determines the persistence of the bioinoculant and its subsequent success. Most commercial inoculants are either coated onto the seed or applied in furrows; the organism has to first multiply in the spermosphere and subsequently colonize the emerging roots (Nelson 2004; Kloepper et al. 1985). Both these processes are fraught with several obstacles for the applied microbial strains. These factors include competition from other native microbes; presence of grazers; edaphic factors like soil pH, concentration of mineral nutrients, soil type, and moisture content; and ultimately the presence of a suitable host plant root, all of which determine the success of the applied inoculant. It is said that a true root colonizer is a bacterial/fungal strain which can colonize the roots under competitive conditions, i.e., in natural field conditions (Kloepper and Beauchamp 1992). But there are many practical constraints, since the applied inoculant population is usually not sufficient to compete with the indigenous microflora. An earlier approach to solve this was proposed by Rao (1993), who advocated the application of higher concentrations of microbial preparations of interest in order to ensure better functionality. But very often this approach fails due to the overriding competitive factors and the high costs of blanket inoculation. An alternative to this is the assessment of the rhizocompetence of the strains along with their functional traits during the screening stages in order to select rhizocompetent strains for microbial inoculant preparation. Rhizocompetence is endowed upon a microbial strain by a number of functional traits like the ability to form biofilms and produce siderophores, antibiosis, motility, utilization of root exudates, and ability to exhibit protease activity, all of which collectively confer upon a strain the ability to survive and function in the highly competitive rhizosphere.

25.2 Significance of Rhizocompetence in Microbial Inoculant Technology

While the importance of microbial inoculants and their benefits have been overemphasized by numerous workers, the issues related to survival of inoculants over extended periods in the rhizosphere have been largely overlooked. The primary

reasons for inoculant failure are the unpredictable soil environment and insufficient adherence of the applied inoculant strain to the root surface. The heterogenous soil environment can throw up surprises in terms of soil microbial community structure, even if different types of soils are exposed to the same cropping patterns over a period of time. This is primarily due to the prevalence of stress factors such as soil salinity, acidity, heavy metal accumulation and water stress, which have a significant bearing on soil microbial community structure and the fate of the applied inoculant. This problem has been compounded the "one-size-fits-all approach" that has been widely followed in microbial inoculant technology, wherein the same microbial inoculant strain has been recommended across geographies and soil types, resulting in the lack of desirable results. It is not uncommon to come across microbial strains that perform very well under laboratory conditions but fail miserably under field conditions thereby defeating the very purpose of the inoculation exercise. There are plentiful commercial products of either single strains or consortia, but there is dearth of peer-reviewed publications on validation of such products (Owen et al. 2015; Bowen and Rovira 1999). The advocacy of non-rhizocompetent stains has led to the decline in the interest and usage of microbial inoculants across the world. Hence there is an urgent need to select rhizocompetent strains alone for commercial inoculant production.

25.3 Rhizocompetence Traits

25.3.1 Biofilm Formation

Biofilms are a thin mucilaginous layers or films that adhere to biotic or abiotic surfaces. Biofilms primarily comprise a community of bacteria and other microorganisms which are enclosed in extracellular polymeric substances (Rossi and Roberto 2015). Life within a biofilm offers the inhabitants various advantages, viz., protection against harsh environmental conditions like high salinity, tannin concentrations, low pH, heavy metals, predation by earthworms, competition by native soil populations (Seneviratne et al. 2008), and resistance to grazing by protozoans (Weitere et al. 2005). Since most rhizospheric microorganisms exist as biofilms rather than in the planktonic mode (O'Toole et al. 1999; Davey and O'Toole 2000), the ability of inoculated strains to form biofilms can confer upon them a distinct advantage in terms of their survival and competition with indigenous microflora. Bacterial biofilms are known to protect plants against pathogens as well as against abiotic stress conditions (Haggag and Timmusk 2008; Timmusk et al. 2009; Timmusk and Wagner 1999). Timmusk et al. (2005) demonstrated that the endospore forming bacterium *Paenibacillus polymyxa* colonizes the root tips as biofilms. Members of the genus *Pseudomonas* form biofilms on roots, leaves, and soil (Ude et al. 2006), which greatly enhances their functionality in the rhizosphere. A novel approach has been the development of biofilmed inoculants in order to enhance the survival and functionality of the applied strains. The cyanobacterial exocellular polymeric matrix (EPM) is an excellent niche for biofilm formation which confers

structural stability to the biofilm, besides improving surface adhesion and nutrient uptake (Mager and Thomas 2011). Biofilmed bacterial inoculants involving the cyanobacterium *Anabaena torulosa* have been observed to accumulate nitrogen, carbon and phosphorous and increase plant growth by defense enzyme production and enhanced micronutrient concentrations during pathogen attack (Prasanna et al. 2012, 2013). Jayasinghhearachchi and Seneviratne (2004) developed a fungal biofilmed inoculant of rhizobia which increased nitrogen fixation by 30% as compared to conventional *Rhizobium* inoculant when applied to soybean. But it is surprising that the basic and crucial aspect like biofilm formation ability of the formulated strains has not received sufficient attention of the microbial inoculant industry.

25.3.2 Siderophore Production

The estimated concentration of total iron in soil is 10^{-17} M which is too scanty for microbial growth. Most of the available iron in the aerobic soil environment occurs in the insoluble form (Fe^{3+}) and not freely accessible to plants or microbes, though it is crucial for the major physiological processes like N fixation, photosynthesis, respiration, etc. (Dudeja et al. 1997). To sequester this crucial element, several PGPR produce siderophores, which are low molecular weight and extracellular compounds with high affinity for iron. The siderophore-bound Fe^{3+} ions get transported into microbial cells and are made available for microbial growth (Sharma and Johri 2003). The transportation into the cell is mediated through membrane receptor molecules regulated by an operon (Crosa 1989). The ability to produce siderophores enhances the survival of inoculant strains in a highly competitive soil environment. Members of *Pseudomonas*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Serratia*, *Azospirillum*, *and Rhizobium* produce siderophores in large quantities under iron limited conditions (Sharma et al. 2003; Loper et al. 1999; Leong and Neilands 1981). Fluorescent *Pseudomonads* have been extensively examined for their capacity to produce siderophore under iron stress conditions. Siderophores pigments like pyoverdine (fluorescent), pyocyanin (non-fluorescent), and pseudobactin (fluorescent chelator of iron) possess plant growth promoting properties (Rovira and Campbell 1975). Though siderophore production is one unique trait that greatly enhances the survivability of the applied inoculant strain in the highly competitive root zone, this aspect has received attention only in the case of biological control strains, while biofertilizer strains have largely remained outside the ambit of this important trait.

25.3.3 Motility

Root colonization is a complicated process where ecological parameters such as motility and chemotaxis play an important role in the early stages. Flagellar motility is considered to be an important trait for successful plant root colonization by bacteria. The applied bioinoculant reaches the root surfaces by active motility

facilitated by flagellar locomotion and guided by chemotactic responses (Pinton et al. 2007). Several studies have revealed that chemotaxis intensifies the capacity of soil bacteria to colonize the roots of diverse plant hosts (Ames and Bergman 1981; Bais et al. 2006; Bauer and Caetano-Anollés 1990; Berendsen et al. 2012; Caetano-Anollés et al. 1988; Dharmatilake and Bauer 1992; Gulash et al. 1984). Flagellar movement plays an early role in attachment to surfaces by surface adhesins but also plays a role in biofilm development and maturation in bacterial species like *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *E. coli* (Nogales et al. 2016). The movement of bacteria usually involves several mechanisms like swarming, swimming, twitching, gliding, and sliding (Kearns 2010). Swarming motility across a surface is propelled by the rotating flagella (Henrichsen 1972). Swimming motility involves the swift movement of individual cells in liquid environments, guided by chemotaxis and powered by rotating flagella. Twitching, gliding and sliding motilities involve the surface agitation at minor velocities that do not require flagella. Bacterial motility provides aided superiority in acquiring nutrients, escaping from toxic matter, approaching the desired host, and colonizing preferred sites within them (Macnab and Aizawa 1984). The whole genome sequences of plant-associated bacterial species demonstrate the existence of heterogenous chemotaxis systems and chemoreceptors. The flagellar formation requires multiple genes for regulating and expressing the movement in an organized manner (Macnab 2003). The chemotaxis systems of beneficial rhizospheric microorganisms are diverse, and the response is guided by root exudate compounds such as sugars, organic acids, and amino acids (Barbour et al. 1991; Heinrich and Hess 1985; Mandimba et al. 1986). At the laboratory level, the screening of elite bioinoculant strains for motility through simple tests can go a long way in ensuring that the most motile strains are promoted for inoculant formulation.

25.3.4 Antagonism

Antagonism is the single most important trait for the biocontrol strains, in order to exclude plant pathogens and safeguard the plant roots from pathogen attack. Antagonism is achieved by the synthesis of antibiotic compounds and synthesis of hydrolytic enzymes such as chitinases, glucanases, proteases, and lipases (Glick and Bashan 1997; Van Loon 2007). But very often the antagonistic activity exhibited by elite strains under laboratory conditions is not observed under soil conditions in order to achieve desired levels of biological control. This is mainly due to the influence of various edaphic factors and influence of indigenous microbial communities (Van Veen et al. 1997). While antagonism toward pathogenic microbes is a preferred rhizocompetence trait, it has to be ensured that the inoculated strain does not alter the microbial diversity of the rhizosphere and its immediate vicinity; therefore a careful evaluation of strains for antagonism has to be carried out while arriving at the threshold levels of this trait.

25.3.5 Ability to Utilize Root Exudates

The discharge of exudates from living plant roots to the rhizosphere is a universal process. The root mucilage comprises polysaccharides released from the root cap in the form of oxidized organic compounds like sugars, organic acids, and amino acids (Jones et al. 1995). There are two classes of organic compounds, viz., low-molecular-weight compounds (amino acids, organic acids, sugars, phenolics, and variety of secondary metabolites) and high-molecular-weight compounds like mucilage and proteins. Based on the nature of the exudates, the plant-microbe interaction may be either positive or negative. The positive interactions are symbiotic associations with beneficial microbes, such as mycorrhizae, rhizobia, and plant growth promoting rhizobacteria (PGPR), which provide defense against pathogenic microorganisms and form grounds for chemotaxis to attract and repel a particular microbial species or populations (Abbott and Murphy 2003; Kumar et al. 2007). Negative interactions are the partnerships with parasitic plants, pathogenic microbes and invertebrate herbivores. Several environmental factors affect the root exudate composition qualitatively and quantitatively (Singh et al. 2006), the most important being the growth stage of the plant since the composition of root exudates varies at different stages of growth of the plant (Li et al. 2013). The nature of the inoculants also influences the root exudate pattern of the plant; Raja et al. (2006) performed the biochemical analysis of root exudates, orienting from plants inoculated with a consortium of inoculants and observed that the total sugars, reducing sugars, and amino nitrogen content were higher compared to the root exudates pattern of plants inoculated with a single inoculant. Considering the ability of a bioinoculant stain to utilize different types of available exudates available in soil would therefore determine its rhizospheric competence to a great extent. Among the root exudate compounds, organic acids act as significant metabolic regulators for rhizospheric survival (Robinson and Bauer 1993). Many beneficial soil bacterial species possess specific receptors for a wide variety of structurally different organic acids (Sampedro et al. 2015). It is postulated that since the concentration of bioinoculant is always higher in comparison to the indigenous beneficial microflora, it would catabolize the organic compounds that are present in the root exudates quite efficiently (Barraquiro et al. 2000). But this has to factor the influence of culturable and nonculturable microflora and different soil and climatic conditions. Therefore, profiling of bioinoculant strains for their root exudate utilization ability should form a crucial step in bioinoculant technology.

25.3.6 Protease Activity

The protease group of enzymes performs proteolysis by the hydrolysis of the peptide bonds and is therefore an intrinsic component of all microorganisms. Based on their mode of secretion, proteases have been classified as exopeptidases and endopeptidases, while based on their pH preference, they have been classified as acidic, neutral, and alkaline proteases (Banerjee et al. 1993). Acid proteases function at a

pH range of 2.0–5.0 and are derived from fungi. Proteases having optimum pH in the range of 7.0 are known as neutral proteases and are mainly derived from plants, whereas alkaline proteases have pH optima of 8 or above (Alnahdi 2012). Among the various microbial groups, bacteria are leading producers of alkaline proteases, with the genus *Bacillus* being the predominant source (Gupta et al. 2002). Some of the potential alkaline protease-producing bacterial species are *B. licheniformis, B. subtilis B. amyloliquefaciens. Pseudomonas*, *Flavobacterium*, *Halobacterium*, *Vibrio*, *Serratia*, *Staphylococcus Brevibacterium*, and *Alcaligenes* (Gupta et al. 2005). Among Actinobacteria, strains of *Streptomyces*, *Nocardia* and *Nocardiopsis* are potential producers, while *Aspergillus spp.* remain the most studied group among the fungi. Other fungal genera include strains of *Neurospora*, *Penicillium*, *Ophiostoma*, *Myxococcus*, and *Rhizopus* (Gupta et al. 2005). Protease being a lytic enzyme hydrolyzes N compounds to NH_4 (Tabatabai 1982). Most commonly protease enzymes are coupled with inorganic and organic colloids in soil (Burns 1982; Ladd et al. 1996). The presence of this extracellular enzyme not only reflects the biological capacity of a soil for the enzymatic conversion of the substrate but also plays an important role in ecosystem sustainability (Burns 1982). From the point of view of rhizospheric competence, inoculants with significant protease producing abilities have a distinct edge over inoculants with weak activities, owing to their ability to hydrolyze nitrogenous compounds that are plentifully available in the rhizospheric region. Apart from conferring rhizospheric competence, protease enzymes can also serve as excellent indicators of the shelf life of the microbial inoculants since protease activity is a direct indicator of microbial viability (Kumar and Takagi 1999).

25.4 Visual Demarcation of Inoculated Microbial Strains from Native Microflora

The complicated correlation between the applied inoculants, plant, and its associated ecological factors is vital for understanding the rhizosphere competence of microbial strains. The major factors that influence rhizospheric microbial community composition are the root exudate patterns, the specific native microbial population of different soil types, and the metabolic profile of the inoculant strains (Neumann 2014). A distinctive scheme is therefore needed to discriminate between the inoculated strain and the indigenous rhizosphere communities. The earlier methods used to distinguish the inoculated strain and indigenous rhizosphere included antibiotic resistance, immunological approaches, and insertion of foreign DNA sequences all which possessed advantages and disadvantages in equal measures. Later, a lot of emphasis was placed on statistical methods to determine the colonization levels (Kloepper 1992). Methods to quantify the inoculant densities in rhizosphere of field grown crops have been described by Scherwinski et al. (2008), Lottmann et al. (2000), Chowdhury et al. (2013), Scher et al. (1984), Ahmad and Baker (1987), and Sivan and Chet (1989). The denaturing gradient gel electrophoresis (DGGE) can reveal the multilevel association between plant, inoculant,

pathogen, and indigenous microbial community (Schreiter et al. 2014). Cardinale (2015) used the fluorescence in situ hybridization methods with oligonucleotide probes and specific *gfp*-tagging for inoculants *Pseudomonas* strains in combination with confocal laser scanning microscopy, with a certain degree of success. But it is highly desirous to quantify the bacterial density of viable nonculturable cells because there are chances that the introduced microbe undergoes transformation to nonculturable state and is therefore not culturable by conventional means. The drawbacks of the methods listed above are the costs involved and the technical expertise needed to perform these studies. It would be therefore ideal to devise strategies that overcome these drawbacks and are commercially viable.

25.5 Conclusion

The term bioinoculant is self explanatory in nature, i.e., "live microorganism" that will be beneficial to soil and plant in terms of better plant growth, yield, nutrient acquisition, and biocontrol, while continuing to multiply in soil for unspecified periods. The primary way to achieve this is by the establishment of the inoculant strain in the rhizosphere. This requires a certain degree of functional rhizocompetence, conferred by multiple traits that need to operate in in a synchronous fashion at various stages of the associated plant growth. While choosing rhizocompetent strains, we need to keep in mind that the inoculated strain should not alter the soil population dynamics to a great extent while simultaneously establishing its functional superiority in the rhizosphere. This requires screening of elite strains additionally for rhizocompetence traits. But unfortunately, this aspect has received scant attention at present and has led to multiple instances of bioinoculant failure in the field level, thereby diminishing the confidence of growers in the technology itself. To overcome this, suitable screening strategy that identifies rhizocompetent elite bacterial strains needs to be developed. Another crucial factor is the determination of the proportion of inoculated strains in the soil system by inexpensive, commercially viable technologies. If both these issues are addressed, the popularity and usage of bioinoculants can be boosted to a great extent.

Acknowledgment Chandandeep Kaur was supported by a grant from the Department of Science and Technology, Ministry of Science and Technology, Government of India, under the WOS-A scheme.

References

Abbott LK, Murphy DV (2003) Soil biological fertility: a key to sustainable land use in agriculture. Kluwer Academic Publishers, Dordrecht, pp 1–15

Ahmad JS, Baker R (1987) Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182–189

Alnahdi HS (2012) Isolation and screening of extracellular proteases produced by new isolated *Bacillus* sp. J Appl Pharma Sci 2:071–074

Ames P, Bergman K (1981) Competitive advantage provided by bacterial motility in the formation of nodules by *Rhizobium meliloti*. J Bacteriol 148:728–729
Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266
Banerjee R, Agnihotri R, Bhattacharyya BC (1993) Purification of alkaline protease of *Rhizopus oryzae* by foam fractionation. Bioprocess Biosyst Eng 9(6):245–248
Barbour WM, Hattermann DR, Stacey G (1991) Chemotaxis of *Bradyrhizobium japonicum* to soybean exudates. Appl Environ Microbiol 57:2635–2639
Barraquio WL, Segubre EM, Gonzalez MS et al (2000) Diazotrophic enterobacteria: what is their role in the rhizosphere? In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in rice. IRRI, Manila, pp 93–118
Bauer WD, Caetano-Anollés G (1990) Chemotaxis, induced gene expression and competitiveness in the rhizosphere. Plant Soil 129:45–52
Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486
Bowen GD, Rovira AD (1999) The rhizosphere and its management to improve plant growth. Adv Agron 66:1–02
Burns RG (1982) Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol Biochem 14:423–427
Caetano-Anolles G, Crist-Estes DK, Bauer WD (1988) Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. J Bacteriol 170:3164–3169
Cardinale M (2015) Scanning a microhabitat: plant-microbe interactions revealed by confocal laser microscopy. Front Microbiol doi.org/10.3389/fmicb.2014.00094
Chowdhury SP, Dietel K, Rändler M et al (2013) Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. PLoS One 8:68818
Crosa JH (1989) Genetics and molecular biology of siderophore-mediated iron transport in bacteria. Microbiol Rev 53:517–530
Davey ME, O'Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867
Dharmatilake AJ, Bauer WD (1992) Chemotaxis of *Rhizobium meliloti* towards nodulation gene-inducing compounds from alfalfa roots. Appl Environ Microbiol 58:1153–1158
Dudeja SS, Suneja S, Khurana AL (1997) Iron acquisition system and its role in legume-Rhizobium symbiosis. Indian J Microbiol 37:1–2
Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15:353–378
Gulash M, Ames P, Larosiliere RC et al (1984) Rhizobia are attracted to localized sites on legume roots. Appl Environ Microbiol 48:149–152
Gupta R, Beg Q, Lorenz P (2002) Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol 59:15–32
Gupta A, Roy I, Khare SK et al (2005) Purification and characterization of a solvent stable protease from *Pseudomonas aeruginosa* PseA. J Chromatogr A1069(2):155–161
Haggag WM, Timmusk S (2008) Colonization of peanut roots by biofilm forming Paenibacillus polymyxa initiates biocontrol against crown rot disease. J Appl Microbiol 4:961–969
Heinrich D, Hess D (1985) Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. Can J Microbiol 31:26–31
Henrichsen J (1972) Gliding and twitching motility of bacteria unaffected by cytochalasin B. Acta Pathol Microbiol Scand Sect B Microbiol Immunol 80:623–624
Hiltner L (1904) Überneuere Erfahrungen und Probleme auf demGebiete der BodenbakteriologieunterbesondererBerücksichtigung der Gründüngung und Brache. Arb DLG 98:59–78
Jayasinghearachchi HS, Seneviratne GA (2004) Bradyrhizobial-*Penicillium* spp. biofilm with nitrogenase activity improves N_2 fixing symbiosis of soybean. Biol Fert Soils 40:432–434

Jones DL, Darrah PR (1995) Influx and efflux of organic acids across the soil-root interface of *Zea mays* L. and its implications in rhizosphere C flow. Plant Soil 173:103–109
Kearns DB (2010) A field guide to bacterial swarming motility. Nature Rev Microbiol 8:634–644
Kloepper JW, Beauchamp CJ (1992) A review of issues related to measuring colonization of plant roots by bacteria. Can J Microbiol 12:1219–1232
Kloepper JW, Schroth MN (1978) Plant growth promoting rhizobacteria on radishes. Proc 4th Int Conf Plant Pathogenic Bacteria 2:879–882
Kloepper JW, Scher FM, Laliberte M et al (1985) Measuring the spermosphere colonizing capacity (spermosphere competence) of bacterial inoculants. Can J Microbiol 1:926–929
Kumar CG, Takagi H (1999) Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnol Adv 17(7):561–594
Kumar R, Bhatia R, Kukreja K et al (2007) Establishment of *Azotobacter* on plant roots: chemotactic response, development and analysis of root exudates of cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.) J Basic Microbiol 47:436–439
Ladd JN, Foster RC, Nannipieri P et al (1996) Soil structure and biological activity. Soil Biol Biochem 9:23–78
Leong SA, Neilands JB (1981) Relationship of siderophore-mediated iron assimilation to virulence in crown gall disease. J Bacteriol 147:482–491
Li XG, Zhang TL, Wang XX et al (2013) The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. J Biol Sci 9(2):164–173
Loper JE, Henkels MD (1999) Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Appl Environ Microbiol 65:5357–5363
Lottmann J, Heuer H, de Vries J et al (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 1:41–49
Macnab RM (2003) How bacteria assemble flagella. Annu Rev Microbiol 57:77–100
Macnab RM, Aizawa SI (1984) Bacterial motility and the bacterial flagellar motor. Annu Rev Biophys Bioeng 13(1):51–83
Mager DM, Thomas AD (2011) Extracellular polysaccharides from cyanobacterial soil crusts: a review of their role in dryland soil processes. J Arid Environ 75(2):91–97
Mandimba G, Heulin T, Bally R et al (1986) Chemotaxis of free-living nitrogen-fixing bacteria towards maize mucilage. Plant Soil 90:129–139
Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Crop Manag 3(1)0–0
Neumann G, Bott S, Ohler MA et al (2014) Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as affected by different soils. Front Microbiol 5:82–92
Nogales J, Pérez-Mendoza D, Gallegos MT et al (2016) Importance of motile and biofilm lifestyles of rhizobia for the establishment of symbiosis with legumes. Beneficial Plant-microbial Interactions: Ecology and Applications. (in press) p 47–69
O'Toole GA, Pratt LA, Watnick PI et al (1999) Genetic approaches to study of biofilms. Methods Enzymol 310:91–109
Owen D, Williams AP, Griffith GW et al (2015) Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. Appl Soil Ecol 86:41–54
Pinton R, Varanini Z, Nannipieri P (2007) The rhizosphere: biochemistry and organic substances at the soil-plant interface. Marcel-Dekker, New York
Prasanna R, Joshi M, Rana A et al (2012) Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C–N sequestration in soil under rice crop. World J Microbiol Biotechnol 28(3):1223–1235
Prasanna R, Chaudhary V, Gupta V et al (2013) Cyanobacteria mediated plant growth promotion and bioprotection against *Fusarium* wilt in tomato. Eur J Plant Pathol 136(2):337–353
Raja P, Uma S, Gopal H et al (2006) Impact of bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth. J Biol Sci 6:815–823

Rao NSS (1993) Biofertilizers in agriculture and forestry. International Science Publishers, New Delhi

Robinson JB, Bauer WD (1993) Relationships between C4 dicarboxylic acid transport and chemotaxis in *Rhizobium meliloti*. J Bacteriol 175:2284–2291

Rossi F, Roberto De P (2015) Role of cyanobacterial exopolysaccharides in phototrophic biofilms and in complex microbial mats. Life 5(2):1218–1238

Rovira AD, Campbell R (1975) A scanning electron microscope study of interactions between microorganisms and *Gaeumannomyces graminis* (Syn. *Ophiobolus graminis*) on wheat roots. Microb Ecol 2(3):177–185

Sampedro I, Parales RE, Krell T et al (2015) *Pseudomonas* chemotaxis. FEMS Microbiol Rev 39:17–46

Scher FM, Ziegle JS, Kloepper JW (1984) A method for assessing the root-colonizing capacity of bacteria on maize. Can J Microbiol 30:151–157

Scherwinski K, Grosch R, Berg G (2008) Effect of bacterial antagonists on lettuce: active biocontrol of *Rhizoctonia solani* and negligible, short-term effects on non-target microorganisms. FEMS Microbial Ecol 1:106–116

Schreiter S, Sandmann M, Smalla K et al (2014) Soil type dependent rhizosphere competence and biocontrol of two bacterial inoculant strains and their effects on the rhizosphere microbial community of field-grown lettuce. PLoS One 9:103726

Seneviratne CJ, Jin L, Samaranayake LP (2008) Biofilm lifestyle of *Candida*: a mini review. Oral Dis 14:582–590

Sharma A, Johri BN (2003) Growth promoting influence of siderophore producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiol Res 158:243–248

Sharma A, Johri BN, Sharma AK et al (2003) Plant growth-promoting bacterium *Pseudomonas sp*. strain GRP 3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzcek). Soil Biol Biochem 35:887–894

Singh G, Mukerji KG (2006) Root exudates as determinant of rhizospheric microbial biodiversity. In: Mukerji KG, Manoharachary C, Singh J (eds) Soil biology: microbial activity in the rhizosphere. Springer, Berlin/Heidelberg, pp 39–53

Sivan A, Chet I (1989) Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum*. Microbiology 135:675–682

Tabatabai MA (1982) Soil enzymes – methods of soil analysis. Part 2 Chemical and microbiological properties. Agronomy monograph no. 9, 2nd edn. American Society of Agronomy, Madison, pp 903–947

Timmusk S, Wagner EG (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant-Microbe Interact 12:951–959

Timmusk S, Grantcharova N, Wagner EG (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl Environ Microbiol 71:7292–7300

Timmusk S, Van West P, Gow NA et al (2009) *Paenibacillus polymyxa* antagonizes oomycete plant pathogens *Phytophthora palmivora* and *Pythium aphanidermatum*. J Appl Microbiol 106:1473–1481

Ude S, Arnold DL, Moon CD et al (2006) Biofilm formation and cellulose expression among diverse environmental *Pseudomonas* isolates. Environ Microbiol 8:1997–2011

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Van Veen JA, Van Overbeek LS, Van Elsas JD (1997) Fate and activity of microorganisms introduced into soil. Microbiol Mol Biol Rev 61:121–135

Weitere M, Bergfeld T, Rice SA et al (2005) Grazing resistance of *Pseudomonas aeruginosa* biofilms depends on type of protective mechanism, developmental stage and protozoan feeding mode. Environ Microbiol 7:1593–1601

Beneficial Bacteria for Disease Suppression and Plant Growth Promotion

26

Ying Ma

Abstract

Beneficial plant-microbe interactions in the rhizosphere are major factors in determining soil fertility and thus plant productivity and health. Plant growth-promoting bacteria (PGPB) can establish intimate associations with host plants via a great variety of mechanisms, which enhance plant growth and protect them from various biotic (e.g., phytopathogens) and abiotic stresses (e.g., drought, extreme temperature, salinity, and heavy metals). To meet the needs of sustainable and eco-friendly approach of agriculture, the use of transgenic plants and PGPB has been recommended as a part of mainstream agricultural applications. Since PGPB inoculation is more easily manipulated compared to transgenic plants, the application of PGPB in agriculture has attracted increasing attention. This article reviews the importance of plant-microbe interactions to the development of efficient PGPB inoculants and progresses of the recent researches on the role of PGPB to improve plant growth and health for sustainable agriculture.

Keywords

Plant-microbe interactions • Plant growth-promoting bacteria (PGPB) • Bacterial colonization • Sustainable agriculture

Y. Ma (✉)
Centre for Functional Ecology, Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
e-mail: cathymaying@gmail.com

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_26

26.1 Plant Growth-Promoting Bacteria

Bacteria that beneficially affect plant growth, known as abundant symbiotic partners of plants, are defined as plant growth-promoting bacteria (PGPB). The PGPB usually migrate from bulk soil to plant rhizosphere and then colonize the rhizosphere (rhizobacteria) and tissues interior (endophytic bacteria) of host plants in an aggressive manner (Kloepper and Schroth 1978). In general, bacteria are in far greater abundance in the rhizosphere than in bulk soil, due to the presence of various nutrients (e.g., sugars, amino and organic acids) from plant root exudates (Badri et al. 2009). Regardless of the abundance of bacterial communities in a particular soil domain, soil bacteria can affect plants in a beneficial, harmful, or neutral way, and bacterial effects could vary as soil conditions change (Lynch 1990). For instance, a bacterium that increases plant growth by facilitating nutrient uptake via nitrogen (N_2) fixation and phosphate (P) and potassium (K) solubilization could not exert beneficial effects on plants when the soil is rich in chemical nutrients, such as N_2, P, and K fertilizers. Likewise, bacteria that can protect plant from various biotic and abiotic stresses are improbable to positively affect plant growth under favorable conditions (Ma et al. 2011a).

The PGPB include those that are free living in the rhizosphere/rhizoplane [plant growth-promoting rhizobacteria (PGPR)] and those that inhabit plant tissue interior and establish an intrinsic endophytic relationship with host plants [plant growth-promoting endophytic bacteria (PGPE)]. All these PGPB utilize the same plant growth promotion (PGP) mechanisms, despite the difference between these types of bacteria. PGPB can enhance plant growth directly by facilitating nutrient acquisition or producing phytohormones (as biofertilizers) or indirectly by suppressing diseases caused by phytopathogens via synthesis of allelochemicals and/or induced systemic resistance (ISR) (as biocontrol agents/bioprotectants) (Fig. 26.1). The basic requirement of the effective of PGPB is their capacity to colonize the rhizosphere, rhizoplane, or tissue interior of host plants.

26.2 Bacterial Colonization

Notwithstanding the fact that PGPB have great potential as bioinoculants to enhance crop production and health, application of PGPB fails to induce the desired performance in field tests due to their poor rhizosphere competence (Compant et al. 2010). As a prerequisite of active biocontrol (Fig. 26.1), rhizosphere competence of PGPB over indigenous microbes, such as effective root colonization, survival, and proliferation along living plant roots over a considerable period of time, contributes to enhance PGPB efficiency and reliability (Lugtenberg and Dekkers 1999).

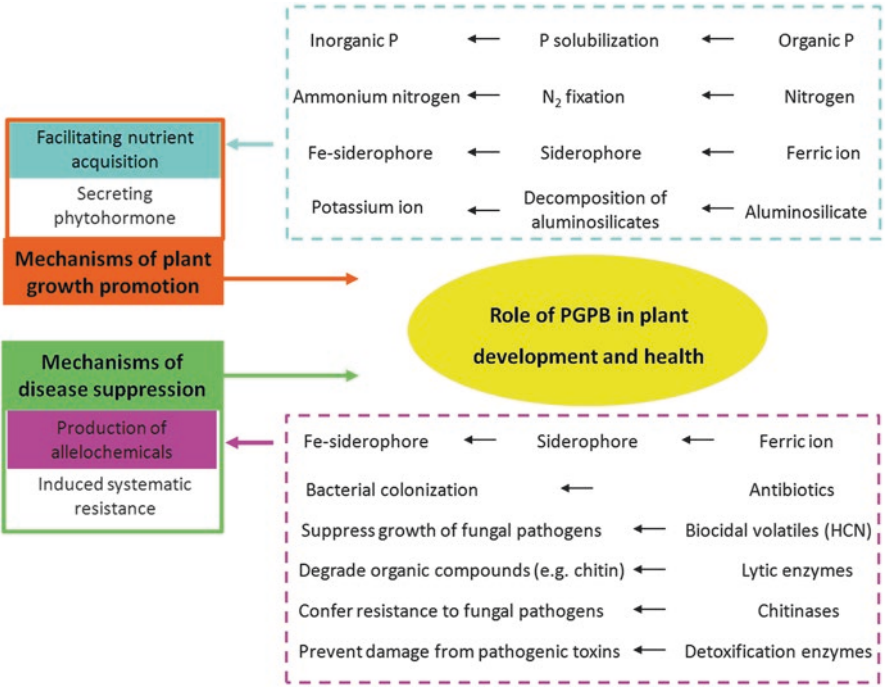


Fig. 26.1 Role of PGPB in plant development and health

26.2.1 Competition for Nutrient-Rich Niches

Various suitable nutrient-rich niches existing along root surface and surrounding rhizosphere attract a great diversity of microbes, including phytopathogens. Competition for appropriate nutrient-rich niches is a major mechanism by which PGPB utilize to protect host plants against phytopathogens (Duffy 2001). In general, PGPB reach root surfaces by flagellum-mediated bacterial motility (Szurmant and Ordal 2004). Among chemoattractants from root exudates (e.g., specific sugars, organic and amino acids), some function as antimicrobial agents and provide ecological niche advantage to those microbes possessing adequate enzymatic detoxification machinery. Since the nature of chemoattractants in root exudates is under environmental control, PGPB competence is greatly dependent on their abilities to either exploit a particular environment or acclimate to changing conditions (Bais et al. 2004). PGPB may be uniquely equipped to sense chemoattractants, because of their capacity to synthesize various metabolites, such as lipopolysaccharides (LPS), fibers, vitamins, and nicotinamide adenine dinucleotide (NADH) dehydrogenase (Compant et al. 2005). For instance, root exudates of *Oryza sativa* (e.g., amino acids and carbohydrates) induced a greater chemotactic response for bacterial endophytes than for non-PGPR (Bacilio-Jiménez et al. 2003). Similarly,

a surface-exposed fiber type IV pili produced by N_2-fixing bacterium *Azospirillum* sp. plays a significant role in the colonization of plant tissues (Steenhoudt and Vanderleyden 2000).

26.2.2 Chemotaxis Toward Root Exudates

Root exudates (such as carbohydrates, sugars, amino and organic acids, etc.) are utilized as the primary nutrient sources by soil microbes for their rhizosphere competence (Walker et al. 2003). Microbes can be chemoattracted and move toward plant root exudates, empowering them to colonize and reproduce in the rhizosphere and/or rhizoplane (Lugtenberg and Kamilova 2009). In addition, root mucilage is known to stimulate soil bacteria as it is secreted from growing root cap cells. For instance, a complex polysaccharide mucilage secreted by roots of *Pisum sativum* provided a carbon source for PGPB *Rhizobium leguminosarum* to use for the competitive rhizosphere colonization (Knee et al. 2001). Dissimilarly, it was investigated that root mucilage from *Zea mays* inhibits root colonization by *Pseudomonas fluorescens* (Humphris et al. 2005). As mentioned above, the polyamines (such as putrescine, spermine, and spermidine) from root exudates have a bacteriostatic effect; thus, uptake rate of polyamines must be effectively regulated by PGPB for ensuring bacterial growth and competitive root colonization (Kuiper et al. 2001). Hence, different responses to root exudates or mucilage can be attributed to the temporal and spatial differences of bacterial colonization along plant root system.

26.2.3 Root Colonization

In general, root exudates and mucilage attract beneficial, neutral, and deleterious soil microorganisms (Walker et al. 2003). PGPB are highly competitive to successfully colonize the root zone, as the secondary metabolites synthesized by PGPB confer themselves a selective and competitive advantage against other microbes and thus enhance their competence and root and/or rhizosphere colonization (Compant et al. 2005, 2010; Lugtenberg and Kamilova 2009; Ma et al. 2011a). The secondary metabolites such as siderophores, lytic enzymes, antibiotics, and biocidal volatiles are major biocontrol mechanisms involved in growth inhibition of rhizosphere phytopathogens and deleterious microbes (van Loon and Bakker 2005). Certain PGPB may produce one or more of these metabolites facilitating better competition with indigenous microflora in root environment (Haas and Défago 2005). Paulsen et al. (2005) reported that metabolite gene clusters possessed by *P. fluorescens*, which are responsible for siderophore production and detoxification, contributed to efficient bacterial colonization and biocontrol properties.

26.2.4 Endophytic Colonization

Some PGPB deriving from the rhizosphere soil can penetrate the roots to establish endophytic populations, thereafter colonizing xylem vessels and move through the plant parts (Gray and Smith 2005; Compant et al. 2010). The success of endophyte colonization depends on compatibility of plant host. Miche et al. (2006) dissected responses of rice roots to N_2-fixing endophytic bacterial colonization and jasmonic acid application by using a proteomic approach. Data imply that induced plant defense responses may contribute to restricting endophytic colonization. Moreover, plant cell wall-hydrolyzing enzymes (such as cellulase and pectinase) play a key role in the mechanisms by which endophytic bacteria penetrate into, persist, and then colonize the tissues of host plant (Hung et al. 2007; Ma et al. 2011b). It has been observed that *Alcaligenes faecalis* and *Bacillus cereus*, possessing endophytic colonization ability, were pectinase-producing isolates (Abdallah et al. 2016).

26.3 Plant Growth Promotion Mechanisms

Plants are constantly confronted to various abiotic and biotic stresses that cause major losses in their productivity. Nevertheless, PGPB are found to help the host plants to avoid or partially overcome such environmental stresses by various PGP mechanisms as discussed below (Fig. 26.1).

26.3.1 Facilitating Nutrient Acquisition

Plants usually confront significant challenges in obtaining adequate supplies of nutrients to meet the demands of basic cellular processes. PGPB are known to convert the insoluble mineral nutrients into soluble forms in soil and make them available for plants to uptake (Ma et al. 2011a).

26.3.1.1 Nitrogen Fixation

Nitrogen is an important element and a major limiting factor for plant development. In many symbiotic or mutualistic relationships, plants generally provide an ecological niche for carbon fixation to bacteria and exchange for fixed N_2. In previous studies, PGPB possessing N_2-fixing ability facilitate plants to survive or adapt to N_2-poor environment and contribute majorly to improve plant growth and health (Hurek and Reinhold-Hurek 2003). For instance, N_2-fixing PGPB *Azotobacter chroococcum* was found to ameliorate saline stress and promote growth of *Z. mays* by improving plant antioxidative capacity and mineral nutrition (Rojas-Tapias et al. 2012). Recently, Gupta et al. (2013) also reported that N_2-fixing endophytic bacteria increased fixation rate and accumulation of N_2 in plants exposed to long-term N_2-poor ecosystems.

26.3.1.2 Phosphate Solubilization

As a major essential macronutrient, phosphorus plays a key role in the biological growth and development (Ehrlich 1990). Only P in monobasic ($H_2PO_4^-$) or dibasic (HPO_4^{2-}) soluble forms can be taken up by plants for biomass production (Glass 1989). PGPB are known to enhance P bioavailability by solubilizing inorganic phosphates and/or mineralizing organic P in soil (Pradhan and Sukla 2005; Chen et al. 2006). The inoculation of P-solubilizing bacteria has been found responsible for the enhancement observed in growth and production of several crops such as *Arachis hypogaea* (Bhatia et al. 2008), *Glycine max* (Yasmeen and Bano 2014), *O. sativa* (Trivedi et al. 2007), and *Phaseolus vulgaris* (Kumar et al. 2016) in either pot or field trials. Recently, Kang et al. (2014) demonstrated the ability of P-solubilizing *Bacillus megaterium* to improve the growth of *Brassica juncea* by regulating carbohydrates and amino acids concentrations.

26.3.1.3 Iron Sequestration

Iron (Fe) is an essential nutrient element for plant, the limited bioavailable Fe in soil provokes a serious competition. Under such stressful Fe-limiting conditions, PGPB can synthesize low-molecular-weight siderophores (Fe-chelating agents) to help plant acquire sufficient Fe^{3+} for its uptake, as phytosiderophores released from plants normally have lower affinity (Ma et al. 2011a). In general, plant root can take up Fe from siderophores-Fe complexes via direct uptake, chelate degradation, and a ligand exchange reaction (Rajkumar et al. 2010). Several studies have detected the enhanced plant Fe uptake with concurrent plant growth stimulation as a consequence of PGPB inoculations (Ma et al. 2010; Scagliola et al. 2016). Bacterial siderophore synthesis can be affected by a variety of environmental factors, such as pH, concentration and form of Fe, and content of other trace and nutrient elements in soil (Duffy and Défago 1999).

26.3.2 Phytohormone Production

Phytohormones produced by PGPB mainly including indole-3-acetic acid (IAA), cytokinins, gibberellins, and ethylene can stimulate plant establishment, growth, and development and and protect plants from various environmental stresses (Taghavi et al. 2009).

26.3.2.1 Indole-3-Acetic Acid

IAA plays an important role in plant growth and development. It is able to stimulate both rapid (cell elongation) and long-term (cell division and tissue differentiation) physiological responses in plants (Kumar et al. 2016). Approximately 80% of rhizobacteria can produce IAA to modulate plant growth and defense (Patten and Glick 1996; Navarro et al. 2006). Several studies have demonstrated that IAA-producing PGPB are capable of regulating root development, morphology, and other growth mechanism, such as ethylene biosynthesis, vascular tissue differentiation, and phototropism (Aloni et al. 2006; Ferrara et al. 2012). The IAA biosynthesis

pathways play a crucial role in the plant-microbe interactions. For example, beneficial bacteria (e.g., PGPB) synthesize IAA via indole-3-pyruvate pathway, while pathogenic bacteria mostly use indole-3-acetamide pathway (Hardoim et al. 2008).

26.3.2.2 Cytokinins

As adenine derivative phytohormones, cytokinins can stimulate cell division, cycle and nucleotide synthesis, and developmental and environmental response of plants (Srivastava 2002). Numerous studies have demonstrated stimulatory effects of cytokinins produced by various PGPB (such as *Halomonas desiderata*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Bacillus licheniformis, B. cereus*, and *B. subtilis*) on the growth and morphology (e.g., shoot branching, apical dominance, chloroplast development, and leaf senescence) of various plants (e.g., *Cucumis sativus*, *Lactuca sativa*, and *Solanum lycopersicum*) under environmental stress and non-stressed conditions (Arkhipova et al. 2007; Hussain and Hasnain 2009). Moreover, cytokinins synthesized by PGPB may stimulate amino acid rhizodeposition and enhance rhizoplane colonization (Kudoyarova et al. 2014).

26.3.2.3 Gibberellins

Gibberellins are tetracyclic diterpenoid acids that are essential for plant growth and development (e.g., germination and emergence, seedling establishment, tissue growth, flowering, and fruiting) (King and Evans 2003). Gibberellins assist plants to absorb nutrients and water for their development by acting cooperatively with other phytohormones (e.g., IAA) via highly integrated signaling pathways (Radhakrishnan and Lee 2016). Recently, a numbers of gibberellins were identified in several bacterial species (such as *Bacillus amyloliquefaciens*, *B. licheniformis, B. pumilus*, and *Penicillium* sp.), which assisted to enhance plant establishments and development under harsh environmental stress conditions (Gutierrez-Manero et al. 2001; Leitão and Enguita 2016; Shahzad et al. 2016).

26.3.2.4 Ethylene

Plant stress hormone ethylene can modulate plant growth and cellular metabolism, which is involved in plant stress resistance, plant-microbe interactions, and nutrient cycle (Ping and Boland 2004). PGPB are known to alleviate ethylene stress-induced impact on plants (e.g., inhibition of root elongation, lateral root growth, and root hair formation) (Mayak et al. 2004) by enzymatic hydrolysis of 1-aminocyclopropane-1-carboxylic acid (ACC) (Glick et al. 2007). PGPB can take up ACC (a precursor of ethylene) from root exudates, cleave it to α-ketobutyrate and ammonia by bacterial ACC deaminase before its oxidation, and then utilize the ammonia as a sole N_2 source, thereby lowering ethylene level in plant (Penrose and Glick 2001). Many studies have demonstrated the ACC deaminase produced by PGPB is involved in the stress (such as salinity, flood, extreme temperature, and heavy metals) alleviation and plant productivity enhancement (Grichko and Glick 2001; Cheng et al. 2007; Dell'Amico et al. 2008; Yim et al. 2013; Ali et al. 2014; Subramanian et al. 2015; Khan et al. 2016).

26.4 Mechanisms of Disease Suppression

In addition to the above-described PGP mechanisms, PGPB can protect plants against pathogens and harmful microbes by disease suppression mechanisms, which comprise synthesis of allelochemicals and ISR (Fig. 26.1).

26.4.1 Synthesis of Allelochemicals

Efficient root colonization and defensive retention of rhizosphere niches by PGPB are enabled by synthesis of various bacterial allelochemicals, such as siderophores, antibiotics, and lytic enzymes (Compant et al. 2005).

26.4.2 Siderophores

Apart from the use of siderophores as nutrient uptake agents, siderophore produced by PGPB can out-compete fungal phytopathogens by depriving Fe from them, since fungal siderophores possess lower affinity (Loper and Henkels 1999). The competition for Fe by PGPB siderophores has been considered as an important antagonistic activity against phytopathogens (de Boer et al. 2003). Yu et al. (2011) found that catecholic siderophore produced by *Bacillus subtilis* significantly reduced the incidence of *Fusarium* wilt and enhanced pepper yield. Similarly, Kumar et al. (2016) reported the growth and production enhancement of *Phaseolus vulgaris* by application of siderophore-producing *Bacillus* sp., *Pseudomonas* sp., *Rhizobium leguminosarum*, and their combination. Therefore, siderophore-producing PGPB may serve as effective biological control agents.

26.4.3 Antibiotics

The antibiotics synthesized by PGPB, such as amphisin, 2,4-diacetylphloroglucinol (DAPG), hydrogen cyanide, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, cyclic lipopeptides, oligomycin A, kanosamine, zwittermicin A, and xanthobaccin have been considered as major biocontrol traits (Compant et al. 2005). Antibiotic production mainly depends on the metabolic status of cells, which in turn is dominated by plant host genotype and environmental stimuli, such as pH, temperature, nutrient availability, carbon content, and minerals (Nielsen and Sørensen 2003). For instance, antibiotic macrosphelide A could be produced by *Coniothyrium minitans* at 10–30 °C or at pH 3–5; however, the optimum temperature and pH for bacterial antibiotic production and biocontrol capacity was at approximately 15–20 °C and at pH 3 (Tomprefa et al. 2011). Moreover, biotic stresses can also affect antibiotic biosynthesis (Compant et al. 2005). The bacterial metabolites, e.g., salicylates and pyoluteorin have been found to repress antimicrobial metabolite DAPG biosynthesis by *P. fluorescens* CHA0 (Schnider-Keel et al. 2000). Furthermore, antibiotic

synthesis can be affected by plant development stage. Picard et al. (2000) have reported that the frequency and bioactivity of DAPG-producing bacterial population in the rhizosphere soil were very low in the first stage of maize growth and increased over time.

26.4.4 Lytic Enzymes

The microbial hyperparasitic activity is considered as an important component of disease suppression mechanisms. PGPB are known to attack phytopathogens or inhibit their growth through secretion of cell wall hydrolases and production of various enzymes such as chitinases, dehydrogenase, β-glucanase, lipases, phosphatases, proteases, etc. (Nagarajkumar et al. 2004; Anitha and Rebeeth 2010; Lanteigne et al. 2012; Kumar and Dubey 2012; Gupta et al. 2015). For instance, the production of extracellular lytic enzymes (e.g., chitinase and β-1,3-glucanases) by *P. fluorescens* has been found to be responsible for degrading fungal cell wall (Tewari and Arora 2016) and digesting mycelium of fungal phytopathogens (Khabbaz et al. 2015). Numerous studies indicated the effectiveness of PGPB (such as *A. chroococcum*, *P. fluorescens*, *P. putida*, etc.) as biocontrol agents in protecting plant hosts from a variety of biotic and abiotic stresses by suppression of pathogenic fungi including *Botrytis cinerea*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Phytophthora capsici*, *Pythium ultimum*, *Sclerotium rolfsii*, *Sesamum indicum*, *Rhizoctonia solani*, and *Thielaviopsis basicola* (Arora et al. 2007; Maheshwari et al. 2012; Upadyay et al. 2012; Nadeem et al. 2013). Recently, *P. fluorescens* has been suggested as an efficient biocontrol agent, due to its ability to colonize the rhizosphere and protect plants against various fungal diseases, such as damping off, root rot, and black root rot (Arora et al. 2007, 2008; Khabbaz et al. 2015). Similarly, it has been demonstrated that inoculation of *Trichoderma* sp. with plants was a perfect choice for being a biocontrol agent against pathogenic fungi (Kottb et al. 2015; Samuelian 2016). Moreover, Singh et al. (2013) recently reported that synergistic effects of triple microbial consortium (e.g., *Pseudomonas* sp., *Trichoderma* sp., and *Rhizobium*) on the alleviation of stress response of *Cicer arietinum* to *S. rolfsii*. Thus, the application of multiple PGPB inoculants in a synergistic manner could be a good option enabling microbes to successfully survive and reproduce in the rhizosphere.

26.5 Induced Systemic Resistance

ISR is a physiological state of improved defensive capacity triggered by specific environmental stimuli, and consequently the plant innate defenses are induced or potentiated against pathogen attack (van Loon 2000). The capacity of PGPB to act as bioprotectants via ISR has been demonstrated in the protection of plants from soilborne phytopathogens (e.g., fungi, bacteria, insects, and nematodes) (Avis et al. 2008). Several bacterial traits, such as flagellation; production of siderophores,

LPS, and cyclic lipopeptides; and secretion of volatile organic compounds (VOCs) (e.g., acetoin and 2, 3-butanediol), are involved in triggering ISR (Compant et al. 2005). For instance, VOCs such as salicylic acid and jasmonic acid secreted by *B. subtilis* and *B. amyloliquefaciens* were found to activate an ISR pathway in seedlings of *Arabidopsis thaliana* under biotic stress induced by *Erwinia carotovora* subsp. carotovora (Ryu et al. 2004). In general, most of PGPB activate ISR via a sialic acid-independent pathway involving jasmonate and ethylene signals within plants and these phytohormones stimulate the plant's defense responses against various phytopathogens (Compant et al. 2005).

26.6 Conclusions

The use of PGPB as microbial inoculants in agriculture offers considerable advantages, due to their competitive colonization and their abilities to suppress phytopathogens and to enhance plant growth. Therefore, PGPB hold the prospect of reducing the input of chemical fertilizers, pesticides, and artificial growth regulators, and their inoculation can be regarded as an eco-friendly approach and biotechnological tool for sustainable agricultural applications. The productive efficiency of a specific PGPB can be further enhanced with the optimization and acclimatization considering the prevailing soil conditions. The important advances on plant-PGPB association will be brought in the future by combining ecological and functional biological approaches (Table 26.1).

Table 26.1 Plant growth-promoting bacteria enhanced plant productivity

PGPB strain	Plant growth-promoting traits	Mechanisms	References
Enterobacter cloacae, E. cloacae, Pseudomonas putida	ACC deaminase production	Enhanced tolerance to flooding stress, plant growth, ACC deaminase activity, and leaf chlorophyll concentration	Grichko and Glick (2001)
Bacillus licheniformis, Bacillus subtilis, Pseudomonas aeruginosa	Cytokinin production	Enhanced cell division, fresh weight, and cotyledon size of *Cucumis sativus*	Hussain and Hasnain (2009)
Pseudomonas fluorescens	Production of antibiotic 2,4-diacetylphloroglucinol	Inhibited plant-parasitic nematodes on *Gossypium hirsutum*, *Zea mays*, *Arachis hypogaea*, and *Glycine max*	Timper et al. (2009)
Azotobacter chroococcum	Production of IAA, HCN, siderophore, N_2 fixation, P solubilization, antagonistic activity	Increased plant growth parameters and yield, oil and protein yield in *Sesamum indicum*	Maheshwari et al. (2012)

(continued)

Table 26.1 (continued)

PGPB strain	Plant growth-promoting traits	Mechanisms	References
Azotobacter chroococcum	IAA production, N_2 fixation, P solubilization	Alleviated saline stress and promoted growth of *Zea mays*	Rojas-Tapias et al. (2012)
Methylobacterium spp.	ACC deaminase production	Reduced disease symptom, lowered stress ethylene level, and increased PR proteins in *Solanum lycopersicum*	Yim et al. (2013)
Pseudomonas fluorescens sp., *Pseudomonas migulae*	ACC deaminase production	Alleviated saline stress and enhanced fresh and dry biomass, chlorophyll contents, number of flowers, and buds of *Solanum lycopersicum*	Ali et al. (2014)
Pseudomonas fluorescens, *Pseudomonas* sp., *Bacillus subtilis*	Production of antibiotics, metabolites, volatiles, phytohormones, and lytic enzymes	Inhibited pathogen growth and suppressed seedling diseases of *Cucumis sativus* (*Phytophthora capsici*) and *Raphanus sativus* (*Rhizoctonia solani*)	Khabbaz et al. (2015)
Pseudomonas brassicacearum, P. veronii, Pseudomonas fluorescens	ACC deaminase production	Increased length and biomass of wheat seedlings under in vitro conditions	Magnucka and Pietr (2015)
Flavobacterium sp., *Pseudomonas frederiksbergensis*	ACC deaminase production	Induced ethylene emission, ACC content, and ACO activity in inoculated *Solanum lycopersicum* under chilling stress	Subramanian et al. (2015)
Sphingomonas sp., *Bacillus* sp., *Methylobacterium* sp.	Production of IAA and ACC deaminase, P solubilization	Promoted growth of *Solanum lycopersicum* (*B. subtilis*)	Khan et al. (2016)
Bacillus sp., *Halobacillus* sp., *Staphylococcus succinus, Zhihengliuella halotolerans*, *Oceanobacillus oncorhynchus*, *Exiguobacterium aurantiacum*, *Bacillus atrophaeus*, *Zhihengliuella* sp., *Halomonas* sp., *Virgibacillus picturae*, *Oceanobacillus* sp., *Thalassobacillus* sp.	Production IAA, ammonia and ACC deaminase, N_2 fixation, P solubilization	Increased the root and shoot length and fresh weight of *Triticum aestivum*	Orhan (2016)

(continued)

Table 26.1 (continued)

PGPB strain	Plant growth-promoting traits	Mechanisms	References
Bacillus methylotrophicus	Production of gibberellins and IAA	Increased plant biomass, nutritional and pigment contents in *Lactuca sativa*	Radhakrishnana and Lee (2016)
Trichoderma spp.	Antagonistic activity	Protected *Musa acuminata* against the leaf pathogens *Mycosphaerella musicola*, *Cordana musae*, and *Deightoniella torulosa*	Samuelian (2016)
Pseudomonas sp., *Enterobacter* sp., *Azotobacter* sp., *Stenotrophomonas* sp., *Chryseobacterium* sp., *Rhizobium* sp.	Production of IAA and siderophores, P solubilization	Modulated plant Fe-chelate reductase, affecting Fe bioavailability	Scagliola et al. (2016)
Bacillus amyloliquefaciens	Gibberellins production	Increased biomass of *Oryza sativa*	Shahzad et al. (2016)
Pseudomonas fluorescens	Production of IAA, siderophore, pyocyanin, HCN, chitinase, β-1,3 glucanase, P solubilization	Enhanced growth of *Helianthus annuus* and protected plant against phytopathogen, *Macrophomina phaseolina* under saline conditions	Tewari and Arora (2016)

ACC 1-aminocyclopropane-1-carboxylic acid, *P* phosphate, *IAA* indole-3-acetic acid, N_2 nitrogen, *HCN* hydrogen cyanide

Acknowledgments Y. Ma thankfully acknowledges the Portuguese Foundation for Science and Technology (FCT) for awarding a postdoctoral research grant (SFRH/BPD/76028/2011). This work is financed by National Funds through the FCT within the project UID/BIA/04004/2013.

References

Abdallah RAB, Mokni-Tlili S, Nefzi A, Jabnoun-Khiareddine H, Daami-Remadi M (2016) Biocontrol of fusarium wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Nicotiana glauca* organs. Biol Control 97:80–88

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Anitha A, Rebeeth M (2010) Degradation of fungal cell walls of phytopathogenic fungi by lytic enzyme of *Streptomyces griseus*. African J Plant Sci 4(3):61–66

Arkhipova TN, Prinsen E, Veselov SU, Martinenko EV, Melentiev AI, Kudoyarova GR (2007) Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292:305–315

Arora NK, Kang SC, Kim MJ, Maheshwari DK (2007) Role of chitinases and beta 1.3-glucanases produced by fluorescent *Pseudomonas* and in vitro inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. Can J Microbiol 53:207–212

Arora NK, Khare E, Verma A, Sahu RK (2008) In vivo control of *Macrophomina phaseolina* by a chitinase and β-1, 3-glucanase- producing pseudomonad NDN_1. Symbiosis 46:129–135

Avis TJ, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem 40:1733–1740

Bacilio-Jiménez M, Aguilar-Flores S, Ventura-Zapata E, Pérez-Campos E, Bouquelet S, Zenteno E (2003) Chemical characterization of root exudates from rice (*Oryza sativa*) and their effects on the chemotactic response of endophytic bacteria. Plant Soil 249:271–277

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. Curr Opin Biotechnol 20:642–650

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway? Trends Plant Sci 9:26–32

Bhatia S, Maheshwari DK, Dubey RC, Arora DS, Bajpai VK, Kang SC (2008) Beneficial effects of fluorescent *Pseudomonas* on seed germination, growth promotion and suppression of charcoal rot in groundnut. (*Arachis hypogaea* L). J Microbiol Biotechnol 18:1578–1583

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Cheng Z, Park E, Glick BR (2007) 1-aminocyclopropane-1-carboxylate deaminase from pseudomonas putida UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

de Boer M, Born P, Kindt F, Keurentjes JJB, van der Sluis I, van Loon LC, Bakker PAHM (2003) Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different diseases-suppressive mechanisms. Phytopathology 93:626–632

Dell'Amico E, Cavalca L, Andreoni V (2008) Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. Soil Biol Biochem 40:74–84

Duffy BK (2001) Competition. In: Maloy OC, Murray TD (eds) Encyclopedia of plant pathology. Wiley, New York, pp 243–244

Duffy BK, Défago G (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. Appl Environ Microbiol 65:2429–2438

Ehrlich HL (1990) Mikrobiologische und biochemische Verfahren stechnik. In: Einsele A, Finn RK, Samhaber W (eds) Geomicrobiology, Second edn. VCH Verlagsgesellschaft, Weinheim

Ferrara FIS, Oliveira ZM, Gonzales HHS, Floh EIS, Barbosa HR (2012) Endophytic and rhizospheric enterobacteria isolated from sugar cane have different potentials for producing plant growth-promoting substances. Plant Soil 353:409–417

Glass ADM (1989) Plant nutrition: an introduction to current concepts. Jones and Bartlett Publishers, Boston, p 234

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39:11–17

Gupta G, Panwar J, Jha PN (2013) Natural occurrence of Pseudomonas aeruginosa, a dominant cultivable diazotrophic endophytic bacterium colonizing Pennisetum glaucum (L.) R. Br. Appl Soil Ecol 64:252–261

Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7:96–102

Gutierrez-Manero FJ, Ramos B, Probanza A, Mehouachi J, Talon M (2001) The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plant 111:206–211

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471

Humphris SN, Bengough AG, Griffiths BS, Kilham K, Rodger S, Stubbs V, Valentine TA, Young IM (2005) Root cap influences root colonization by *Pseudomonas fluorescens* SBW25 on maize. FEMS Microbiol Ecol 54:123–130

Hung PQ, Kumar SM, Govindsamy V, Annapurna K (2007) Isolation and characterization of endophytic bacteria. Biol Fertil Soils 44:155–162

Hurek T, Reinhold-Hurek B (2003) *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. J Biotechnol 106:169–178

Hussain A, Hasnain S (2009) Cytokinin production by some bacteria: its impact on cell division in cucumber cotyledons. Afr J Microbiol Res 3(11):704–712

Kang SM, Radhakrishnan R, You YH, Joo GJ, Lee IJ, Lee KE, Kim JH (2014) Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. Indian J Microbiol 54(4):427–433

Khabbaz SE, Zhang L, Cáceres LA, Sumarah M, Wang A, Abbas PA (2015) Characterisation of antagonistic bacillus and pseudomonas strains for biocontrol potential and suppression of damping-off and root rot diseases. Ann App Biol 166:456–471

Khan AL, Halo BA, Elyassi A, Ali S, Al-Hosni K, Hussain J, Al-Harrasi A, Lee IJ (2016) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64

King RW, Evans LT (2003) Gibberellins and flowering of grasses and cereals: prizing open the lid of the "florigen" black box. Annu Rev Plant Biol 54:307–328

Kloepper, J.W., Schroth, M.N. (1978). Plant growth-promoting rhizobacteria on radishes. Proceedings of the 4th International Conference on Plant Pathogen Bacteria 2:879–882

Knee EM, Gong FC, Gao M, Teplitski M, Jones AR, Foxworthy A, Mort AJ, Bauer WD (2001) Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. Mol Plant-Microbe Interact 14:775–784

Kottb M, Gigolashvili T, Großkinsky DK, Piechulla B (2015) Trichoderma volatiles effecting *Arabidopsis*: from inhibition to protection against phytopathogenic fungi. Front Microbiol 6:995

Kudoyarova GR, Melentiev AI, Martynenko EV, Timergalina LN, Arkhipova TN, Shendel GV, Kuz'mina LY, Dodd IC, Veselov SY (2014) Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. Plant Physiol Biochem 83:285–291

Kuiper I, Bloemberg GV, Noreen S, Thomas-Oates JE, Lugtenberg BJJ (2001) Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by *Pseudomonas fluorescens* strain WCS365. Mol Plant-Microbe Interact 14:1096–1104

Kumar P, Dubey RC (2012) Plant growth promoting rhizobacteria for biocontrol of phytopathogens and yield enhancement of *Phaseolus vulgaris*. J Curr Pers Appl Microbiol 1:6–38

Kumar P, Pandey P, Dubey RC, Maheshwari DK (2016) Bacteria consortium optimization improves nutrient uptake, nodulation, disease suppression and growth of the common bean (*Phaseous vulgaris*) in both pot and field studies. Rhizosphere 2:13–23

Lanteigne C, Gadkar VJ, Wallon T, Novinscak A, Filion M (2012) Production of DAPG and HCN by *Pseudomonas* sp. LBUM3s00 contributes to the biological control of bacterial canker of tomato. Phytopathology 102:967–973

Leitão AL, Enguita FJ (2016) Gibberellins in *Penicillium* strains: challenges for endophyte-plant host interactions under salinity stress. Microbiol Res 183:8–18

Loper JE, Henkel MD (1999) Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Appl Environ Microbiol 65:5357–5363

Lugtenberg BJJ, Dekkers LC (1999) What make *Pseudomonas* bacteria rhizosphere competent? Environ Microbiol 1:9–13

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Lynch JM (1990) The rhizosphere. Wiley-Interscience, Chichester

Ma Y, Rajkumar M, Vicente J, Freitas H (2010) Inoculation of Ni-resistant plant growth promoting bacterium Psychrobacter sp. strain SRS8 for the improvement of nickel phytoextraction by energy crops. Int J Phytoremediation 13:126–139

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011a) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258

Ma Y, Rajkumar M, Luo YM, Freitas H (2011b) Inoculation of endophytic bacteria on host and non-host plants – effects on plant growth and Ni uptake. J Hazard Mater 196:230–237

Maheshwari DK, Dubey RC, Aeron A, Kumar B, Kumar S, Tewari S, Arora NK (2012) Integrated approach for disease management and growth enhancement of Sesamum indicum L. utilizing Azotobacter chroococcum TRA2 and chemical fertilizer. World J Microbiol Biotechnol 28:3015–3024

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Miche L, Battistoni F, Gemmer S, Belghazi M, Reinhold-Hurek B (2006) Up regulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the endophyte *Azoarcus* sp. Mol Plant-Microbe Interact 19:502–511

Nadeem SM, Naveed M, Zahir ZA, Asghar HN (2013) Plant-microbe interactions for sustainable agriculture: fundamentals and recent advances. In: Arora NK (ed) Plant microbe symbiosis: fundamentals and advances. Springer, India, pp 51–103

Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescensin* inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res 159:73–81

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312:436–439

Nielsen TH, Sørensen J (2003) Production of cyclic lipopeptides by *Pseudomonas fluorescens*-strains in bulk soil and in the sugar beet rhizosphere. Appl Environ Microb 69:861–868

Orhan F (2016) Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). Braz J Microbiol 47(3):621–627

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GSA, Mavrodi DV, DeBoy RT, Seshadri R, Ren Q, Madupu R, Dodson RJ, Durkin AS, Brinkac LM, Daugherty SC, Sullivan SA, Rosovitz MJ, Gwinn ML, Zhou L, Schneider DJ, Cartinhour SW, Nelson WC, Weidman J, Watkins K, Tran K, Khouri H, Pierson EA, Pierson LS III, Thomashow LS, Loper JE (2005) Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. Nat Biotechnol 23:873–878

Penrose DM, Glick BR (2001) Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. Can J Microbiol 47:368–372

Picard C, Di Cello F, Ventura M, Fani R, Guckert A (2000) Frequency and biodiversity of 2, 4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. Appl Environ Microbiol 66(3):948–955

Ping L, Boland W (2004) Signals from the underground: bacterial volatiles promote growth in *Arabidopsis*. Trends Plant Sci 9:263–266

Pradhan N, Sukla LB (2005) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afri J Biotechnol 5:850–854

Radhakrishnan R, Lee IJ (2016) Gibberellins producing *Bacillus methylotrophicus* KE2 supports plant growth and enhances nutritional metabolites and food values of lettuce. Plant Physiol Biochem 109:181–189

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Rojas-Tapias D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R (2012) Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). Appl Soil Ecol 61:264–272

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Paré PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Samuelian S (2016) Potential of Trichoderma harzianum for control of banana leaf fungal pathogens when applied with a food source and an organic adjuvant. 3 Biotech 6(1):8

Scagliola M, Pii Y, Mimmo T, Cesco S, Ricciuti P, Crecchio C (2016) Characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (Hordeum vulgare L.) and tomato (Solanum lycopersicon L.) grown under Fe sufficiency and deficiency. Plant Physiol Biochem 107:187–196

Schnider-Keel U, Seematter A, Maurhofer M, Blumer C, Duffy B, Gigot-Bonnefoy C, Reimmann C, Notz R, Défago G, Haas D, Keel C (2000) Autoinduction of 2, 4-diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. J Bacteriol 182:1215–1225

Shahzad R, Waqas M, Khan AL, Asaf S, Khan MA, Kang SM, Yun BW, Lee IJ (2016) Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. Plant Physiol Biochem 106:236–243

Singh A, Sarma BK, Upadhyay RS, Singh HB (2013) Compatible rhizosphere microbes mediated alleviation of biotic stress in chickpea through enhanced antioxidant and phenylpropanoid activities. Microbiol Res 168:33–40

Srivastava LM (2002) Plant growth and development. hormones and the environment. Academic, Oxford

Steenhoudt O, Vanderleyden J (2000) *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506

Subramanian P, Krishnamoorthy R, Chanratana M, Kim K, Sa T (2015) Expression of an exogenous 1-aminocyclopropane-1-carboxylate deaminase gene in psychrotolerant bacteria modulates ethylene metabolism and cold induced genes in tomato under chilling stress. Plant Physiol Biochem 89:18–23

Szurmant H, Ordal GW (2004) Diversity in chemotaxis mechanisms among the bacteria and archaea. Microbiol Mol Biol Rev 68:301–319

Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar. Appl Environ Microbiol 75:748–757

Tewari S, Arora NK (2016) Fluorescent *Pseudomonas* sp. PF17 as an efficient plant growth regulator and biocontrol agent for sunflower crop under saline conditions. Symbiosis 68:99–108

Timper P, Koné D, Yin J, Ji P, McSpadden Gardener BB (2009) Evaluation of an antibiotic-producing strain of *Pseudomonas fluorescens* for suppression of plant-parasitic nematodes. J Nematol 41(3):234–240

Tomprefa N, Hill R, Whipps J, McQuilken M (2011) Some environmental factors affect growth and antibiotic production by the mycoparasite *Coniothyrium minitans*. Biocontrol Sci Tech 21:721–731

Trivedi P, Kumar B, Pandey A, Palni LMS (2007) Growth promotion of rice by phosphate solubilizing bioinoculants in a Himalayan location. In: Velazquez E, Rodriguez-barrueco C (eds) Plant and soil, developments in plant and soil sciences, first international meeting on microbial phosphate solubilization. Springer, Salamanca, pp 291–299

Upadyay SK, Maurya SK, Singh DP (2012) Salinity tolerance in free living plant growth promoting rhizobacteria. Ind J Sci Res 3:73–78

van Loon LC (2000) Systemic induced resistance. In: Slusarenko AJ, Fraser RSS, van Loon LC (eds) Mechanisms of resistance to plant diseases. Kluwer, Dordrecht, pp 521–574

van Loon LC, Bakker PAHM (2005) Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 39–66

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132:44–51

Yasmeen S, Bano A (2014) Combined effect of phosphate-solubilizing microorganisms, rhizobium and enterobacter on root nodulation and physiology of soybean (Glycine max L.) Soil Sci Plan 45:2373–2384

Yim W, Seshadri S, Kim K, Lee G, Sa T (2013) Ethylene emission and PR protein synthesis in ACC deaminase producing *Methylobacterium* spp. inoculated tomato plants (*Lycopersicon esculentum* Mill.) challenged with *Ralstonia solanacearum* under greenhouse conditions. Plant Physiol Biochem 67:95–104

Yu X, Ai C, Xin L, Zhou G (2011) The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on fusarium wilt and promotes the growth of pepper. Eur J Soil Biol 47:138–145

Bacterial Strains with Nutrient Mobilisation Ability from Ciuc Mountains (Transylvania Region, Romania)

27

Éva Laslo, Éva György, Beáta Ábrahám, and Gyöngyvér Mara

Abstract

This chapter presents the study about some wild leguminous plant's nodule and rhizosphere bacteria from Ciuc Mountains with beneficial traits related to mineral nutrition and their multiple plant growth-promoting activities as a part of plant-bacteria interaction.

The isolated bacterial strains have nutrient mobilisation abilities and plant growth stimulation effect as phosphate solubilisation, nitrogen fixation and siderophore and indole-3-acetic acid production. During this study, we identified, on the basis of 16S rDNA sequence, 21 bacterial strains originated from different leguminous plants nodules and rhizosphere. These bacterial strains belong to diverse bacterial genus and were identified as *Rhizobium leguminosarum* (CM2, CM3, CM9, CM11, CM13, CM14, CM15), *Rhizobium yanglingense* CM1, *Bacillus* sp. CM4, *Mitsuaria chitosanitabida* CM5, *Variovorax paradoxus* CM6 and CM8, *Rhizobium rhizogenes* CM7, *Sinorhizobium meliloti* CM10, *Rhizobium etli* CM12, *Pseudomonas abietaniphila* CM16, *Pseudomonas brassicacearum* CM17, *Acinetobacter johnsonii* CM18, *Ensifer* (*Sinorhizobium*) sp. CM19, *Serratia proteamaculans* CM20 and *Serratia* sp. CM21. The present study revealed two interesting strains, *Mitsuaria chitosanitabida* CM5 and *Acinetobacter johnsonii* CM18 with beneficial characteristics as improving the availability of different nutrients.

É. Laslo (✉) • B. Ábrahám • G. Mara
Faculty of Economics and Socio-Human Sciences and Engineering, Department of Bioengineering, Sapientia Hungarian University of Transylvania,
Libertății Sq. Nr. 1, 530104 Miercurea Ciuc, Romania
e-mail: lasloeva@yahoo.com

É. György
Faculty of Economics and Socio-Human Sciences and Engineering, Department of Food Science, Sapientia Hungarian University of Transylvania,
Libertății Sq. Nr. 1, 530104 Miercurea Ciuc, Romania

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_27

The identified and denotated allochthonous bacterial strains with beneficial characteristics confer beneficial effects to plants as a part of the plant functional diversity and microbial composition and are important in sustainable agriculture.

Keywords
Beneficial strains • Nodule bacteria • Rhizospheric bacteria • Nutrient mobilisation

27.1 Introduction

Ciuc Mountains belongs to Eastern Carpathians, as a part of the Carpathian flysch. The altitude of the Ciuc Mountains varies between 680 and 1553 m. The forest cover is relatively low, representing about 50% of the area. The higher altitude grasslands are represented by hay meadows with specific diversity and secondary grasslands created due to the traditional agriculture in the past two to three centuries. The vegetation is represented by an outstanding plant species richness including rare and endemic plant species as Elder-flowered orchid (*Dactylorhiza sambucina*), red vanilla orchid (*Nigritella rubra*), lady's-slipper orchid (*Cypripedium calceolus*), narcissus-flowered anemone (*Anemone narcissiflora*), a Carpathian endemic primrose species (*Primula leucophylla*) and stemless and spring gentian (*Gentiana acaulis*, *G. verna*) (Demeter et al. 2011). The region is characterised also by special climate, like an annual average temperature between 6 and 7 °C and 700 and 1000 mm precipitation. The high biodiversity of the region favours the development of a diverse microbial community (Grigulis et al. 2013), being adapted to the specific climate conditions.

Microorganisms have a key role in ecosystem function, maintaining healthy soil. They take part in the biogeochemical cycling of the nutrients, thus enhancing the soil productivity and structure (Parmar and Dufresne 2011; Trivedi et al. 2011). Application of *Bacillus megaterium* or *Rhizobium* species besides the plant growth promotion caused an increase in the soil organic matter correlated with untreated soil (Sharma et al. 2012). Soil bacteria also influence plant nutrient availability, and hence plant productivity, through nutrient mineralisation, whereby soil microbes convert organic matter into inorganic plant-available form (van der Heijden et al. 2008).

Thus, in terrestrial and agroecosystems, plant productivity is in correlation with soils with high microbial diversity (van der Heijden and Wagg 2013). To predict the response of the agroecosystems to the continuous environmental changes and various biotechnological applications, functional diversity analysis of microorganisms is crucial (Prosser 2002; Parmar and Dufresne 2011).

Bacteria-plant interactions are defined as complex, intimate associations that rely on signalling events at the molecular level. Specificity of plant-bacteria relationship is based on two fundamentals: (a) bacterial strain-specific adaptation to a

non-specific trait of host plant and (b) bacterial non-specific adaptation to a genotype specific trait of the host plant (Drogue et al. 2012; Bulgarelli et al. 2013).

The bacterial cooperation with plants indicates a specific chemoattraction, based on specific molecular and genetic patterns. Through the combination of these patterns results the plant-bacteria relationship, for example, supplying nutrients and phytohormones. Thus, the beneficial effects of the plant growth-promoting bacteria (PGPR) are related to specific plant compounds or exudates. As example, the indole-3-acetic acid (IAA) production is regulated by the presence of tryptophan or tyrosine, nitrogen fixation by wheat germ agglutinin and phosphate mobilisation by glucose or glycerol. The main step of the establishment of plant-bacteria relationship is the colonisation of plant root surface by bacteria. This process is based on plant-bacteria surface components, molecular interactions. Specialised, strain-specific molecules are involved, as outer membrane proteins, lipopolysaccharides, exopolysaccharides and capsular polysaccharides (Drogue et al. 2012; Drogue et al. 2013).

Soil microbes facilitate plant growth and development through different direct mechanisms as mediating nutrient accessibility and assimilation (e.g. nitrogen and phosphorus) and producing iron-binding molecules and phytohormones (Young et al. 2012). These beneficial bacteria are called as plant growth-promoting bacteria (PGPR) (Babalola 2010) and include different species from the genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Serratia* and *Rhizobium* (Parmar and Dufresne 2011; Verma et al. 2013).

The biological nitrogen fixation contributes to the nitrogen acquisition of plants. Bacteria belonging to different taxonomic groups – able to provide for plants one of the limiting macronutrients – are called diazotrophs. They include symbiotic, associative and endophytic bacteria (Franche et al. 2009). The expression of nitrogenase, necessarily for the reduction of the nitrogen, is rigorously regulated and based on molecular signalling (Glick 2012).

The phosphorus mobilisation from organic and inorganic phosphates by the bacteria is realised through various mechanisms: increasing the root growth, secreting metabolically different organic acids, producing phosphatases and realising proton extrusion (Richardson and Simpson 2011; Singh and Satyanarayana 2012; Glick 2012). These beneficial bacterial traits are manifested due to different processes as acidification of the environment, metal complexing and reduction and direct (enzymatic) and indirect phosphate dissolution (Bashan et al. 2013). Phosphorus mobilisation also influences the symbiotic interaction between rhizobia and legumes contributing in this way to nitrogen acquisition (Castagno et al. 2011).

Beneficial bacteria owing to the production of iron-binding molecules as a response to iron deficiency called siderophores improve the plant growth. The plant growth-promoting bacteria contribute to the iron nutrition of plants and also suppress phytopathogenic microorganisms due to the competition for the limiting micronutrient, iron (Lemanceau et al. 2009).

Another mechanism of PGPR bacteria to increase plant growth is phytostimulation. Production of auxin like IAA is detected in different bacterial species. These plant hormones are synthesised in bacteria through different pathways, are involved

in physiological processes and have a role in bacteria-plant interactions and colonisations (Spaepen et al. 2007). The biosynthesis of the phytohormone can be one of the driving forces for the selection of effective PGPR bacteria. IAA is implicated in the plant growth due to the stimulation of rapid and long-term responses in plants like enhanced elongation, cell division and differentiation (Chaiharn and Lumyong 2011). The IAA biosynthesised by bacteria is involved in the growth and development of root length and surface area. This facilitates the accessibility of plants to the soil nutrients (Bhattacharyya and Jha 2012; Glick 2012).

In meadow and grassland ecosystems from Ciuc Mountains, the biological productivity remains high even if nutrient supply is not provided. Therefore, a nutrient recycling and supply is realised by rhizosphere bacteria. A strong relationship between plants and soil microbes was observed (Bardgett et al. 2005). It is shown that agricultural management has an impact on PGPR bacteria and has a lot of research reports on diversity of cultivated plants' rhizosphere microbial spectrum and their biotechnological use in agricultural practices (Yuan et al. 2011; Martínez et al. 2011).

27.2 Isolation of the Rhizobacteria

Sample prelevation of rhizospheric soils was realised in Ciuc Mountains. The soil of this region is umbric podzols that have the following characters: skeletal character has 30–90 % detrital sandstones, the humus content is very high (2.87–7.39%) and the pH values are low with acidic properties (5.0–5.2). The base saturation grade is very low (37–51%), the phosphorus (P) content is low (3.03–4.50 ppm) and the potassium (K) content is very high (522–421 ppm) (Pásztohy 2013).

A number of 101 bacterial strains were isolated from 14 leguminous plant root nodule and rhizosphere, belonging to the spontaneous flora of Ciuc Mountains, as follows: *Anthyllis vulneraria* L., *Trifolium pannonicum* L., *Trifolium medium* L., *Cytisus hirsutus* L., *Lathyrus transsilvanicus* (Spreng.) Fritsch, *Trifolium montanum* L., *Onobrychis montana* DC. ssp. *transsilvanica*, *Medicago lupulina* L., *Trifolium repens* L., *Vicia sepium* L., *Lotus corniculatus* L., *Trifolium alpestre* L., *Vicia cracca* L. and *Tetragonolobus maritimus* L. (Roth).

The nodules were surface-disinfected with sodium hypochlorite solution (Merck, 6–14% active chlorine) and rinsed with sterilised water. Surface-sterilised tissues were smashed in a sterile mortar and homogenised with 9 ml sterilised water. An amount of 0.1 ml of the homogenised extract was spread on the surface of yeast extract mannitol agar (YEM medium) (10.00 g mannitol, 0.50 g K_2HPO_4, 0.20 g $MgSO_4 \cdot 7H_2O$, 0.10 g NaCl, 1.00 g yeast extract, 0.20 g $CaCl_2 \cdot 2H_2O$, 0.01 g $FeCl_3 \cdot 6H_2O$, 20.00 g agar, 25.00 mg bromothymol blue in 1 l of distilled water).

From the soil samples, dilution series were prepared and 0.1 ml of each solution was plated on YEM. The plates were incubated at 28 °C for 48 h. The isolates were maintained on YMA agar slants (10.0 g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.4 g yeast extract, 15.0 g agar in 1 l of distilled water).

As the result of the isolation of rhizobacteria from an ecosystem in Ciuc Mountains, we obtained a total number of 101 bacterial isolates. On the basis of biochemical and phenotypic characterisation, 21 bacterial strains were selected. Among the 21 strains studied, 13 (61.9%) bacterial strains were isolated from the rhizosphere, whereas 8 (38.1%) strains were isolated from the nodule of 14 indigenous leguminous plant species.

27.3 Genotypic Characterisation and Identification of the Selected Bacteria

Identification at the species level of the 21 bacterial isolates was realised using 16S rDNA gene sequence analysis. Genomic DNA was isolated using Promega wizard genomic DNA isolation kit, following the manufacturer's protocol. The amplification of partial sequence of bacterial 16S rDNA gene was realised with the universal oligonucleotides 27f 5′ AGAGTTTGATCMTGGCTCAG 3′ and 1492r 5′TACGGYTACCTTGTTACGACTT3′ primers flanking the bacterial 16S rDNA region. The amplification reaction was completed in an ESCO Swift mini-thermocycler and included an initial denaturation at 94 °C for 5 min, which was followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min.

In order to check for the presence of false-positive samples due to reagent contamination, negative controls were used. For separation of amplified products, 1% agarose gels were run in 1x TAE buffer at 100 V/cm, using ethidium bromide staining. Amplification products were observed with a Bio-Rad UV transilluminator (Gel Doc XR software). The purification of amplification products was realised using PCR purification kit (Fermentas), and the 16S rDNA fragments were analysed by sequencing. The sequences were edited and aligned with Chromas and MEGA 4 system. The comparison of the sequences with those found in the NCBI database was done with a BLAST algorithm.

The 16S rDNA BLAST data resulted in bacterial isolates belonging to eight genera, showing 98–100% similarity with species described in the GenBank (Table 27.1). 16S rDNA gene BLAST data suggest that ten of the strains belong to the *Rhizobium* genus, with seven *Rhizobium leguminosarum* strains: CM2, CM3, CM9, CM11, CM13, CM14 and CM15, showing similarity between 99 and 100% with previously described strains (Table 27.1). The other *Rhizobium* sp. strains isolated from Ciuc Mountain hay meadows were as follows: *Rhizobium yanglingense* CM1, *Rhizobium rhizogenes* CM7 and *Rhizobium etli* CM12.

Two isolates were identified as belonging to genus *Pseudomonas*, *Pseudomonas abietaniphila* CM16 and *Pseudomonas brassicacearum* CM17. Two of the characterised 21 bacterial strains are *Serratia* species, *Serratia proteamaculans* CM20 and *Serratia* sp. CM21. Two isolates according to the 16S rDNA BLAST data were identified as *Ensifer* (*Sinorhizobium*) sp. CM19 (99.1% similarity) and *Sinorhizobium meliloti* CM10 (99.8% similarity), belonging to genus *Sinorhizobium*. Other two isolates CM6 and CM8 were identified as *Variovorax paradoxus* with 100%

Table 27.1 Identification of the beneficial strains isolated from different indigenous leguminous plants

Isolates code	Origin	Most closely related organism Species (strain)	% Gene identity
CM1	*Onobrychis montana* DC ssp. *transsilvanica,* rhizosphere	*Rhizobium yanglingense* (SH 22623(T))	100
CM2	*Onobrychis montana* DC ssp. *transsilvanica,* nodule	*Rhizobium leguminosarum* (CCBAU65673)	99.8
CM3	*Onobrychis montana* DC ssp. *transsilvanica,* rhizosphere	*Rhizobium leguminosarum* (USDA 2370(T))	99.2
CM4	*Trifolium montanum* L., rhizosphere	*Bacillus* sp.	99.2
CM5	*Medicago lupulina* L., rhizosphere	*Mitsuaria chitosanitabida* (3001(T))	98
CM6	*Lathyrus transsilvanicus* (Spreng.) Fritsch, rhizosphere	*Variovorax paradoxus* (12373(T))	100
CM7	*Trifolium montanum* L., rhizosphere	*Rhizobium rhizogenes* (13257(T))	100
CM8	*Lathyrus transsilvanicus* (Spreng.) Fritsch, rhizosphere	*Variovorax paradoxus* (12373(T))	100
CM9	*Trifolium medium* L., nodule	*Rhizobium leguminosarum* (USDA 2370(T))	99.3
CM10	*Trifolium hybridum*, rhizosphere	*Sinorhizobium meliloti* (CCNWXJ203)	99.8
CM11	*Trifolium pannonicum* L., nodule	*Rhizobium leguminosarum* (USDA 2370(T))	99.2
CM12	*Trifolium alpestre* L., nodule	*Rhizobium etli* (CCBAU 85027)	99.8
CM13	*Vicia cracca* L., nodule	*Rhizobium leguminosarum* (CCBAU 43229)	99.3
CM14	*Vicia sepium* L., rhizosphere	*Rhizobium leguminosarum* (CCNWXJ0177)	100
CM15	*Trifolium pannonicum* L., nodule	*Rhizobium leguminosarum* M14	99
CM16	*Cytisus hirsutus* L., rhizosphere	*Pseudomonas abietaniphila* (ATCC 700689 (T))	100
CM17	*Vicia sepium* L., rhizosphere	*Pseudomonas brassicacearum* (MA250)	100
CM18	*Lotus corniculatus* L., nodule	*Acinetobacter johnsonii* (DSM 6963(T))	99
CM19	*Vicia cracca* L., nodule	*Ensifer* (*Sinorhizobium*) sp.	99.1
CM20	*Vicia sepium* L., rhizosphere	*Serratia proteamaculans* wg-2 16S	99.8
CM21	*Trifolium montanum* L., rhizosphere	*Serratia* sp.	100

similarity. One bacterial isolate CM5 was annotated to belong to *Mitsuaria* genus showing 98% similarity with *Mitsuaria chitosanitabida*. One isolated strain CM4 was more than 99.2% matched with the *Bacillus* sp. (Table 27.1).

27.4 Nitrogen Fixation Capacity

The ability of the isolated bacteria to fix molecular nitrogen was tested using acetylene reduction assay. Five millilitre of YMA liquid medium was inoculated with the bacterial isolates in an air-tight bottle, and 10% of the air was replaced with acetylene gas. To realise the reduction of acetylene, the bottles were incubated at 28 °C for 24 h. After incubation, 25 µl of gas mixture was transferred in gas chromatograph (Varian CP-3380) using 100 °C for injection temperature and 27 °C for detector temperature (Laslo et al. 2012). Ethylene peaks were detected using FID detector, fitted with fused silica WCOT (25 m × 0.25 mm) CP-Sil 5 CB-coated column. Out of twenty-one studied strains 13 (61.9%) gave positive result, whereas eight strains (38.1%) gave negative result for acetylene reduction. The 13 strains able to reduce the acetylene possess active nitrogenase enzyme; therefore, they can contribute to the atmospheric nitrogen fixing in order to supply the N necessary for host plants.

27.5 Siderophore Production

The basis of siderophore production analysis lies on the competition for iron between the ferric complex of an indicator dye (chromeazurol) and a chelator or siderophore produced by microorganisms. The iron is removed from the complex by the siderophore, which apparently has a higher affinity for iron (III). The positive reaction results in a colour change of the dye reagent (usually from *blue* to *orange*). The siderophore production capacity of the bacterial isolates was evaluated qualitatively using chromazurol-S medium (Oldal et al. 2002). A 10 µl quantity of overnight bacterial culture in YMA and nutrient medium was spotted onto a CAS agar plate in triplicate and incubated at 28 °C for 5 days.

From the assayed bacterial isolates at 17 (80.95%), the siderophore production capacity was detected. The siderophore production index (SPI) in case of the studied isolates varied between 2.1 and 2.69. The majority of the isolates showed medium siderophore production ability, with an index value between 2.3 and 2.5. Three from the assayed bacterial strains (*Rhizobium leguminosarum* CM9, *Rhizobium leguminosarum* CM11 from the nodule and *Sinorhizobium meliloti* CM10 from rhizosphere) were found as having a maximal siderophore production capacity. Seven isolates resulted in low siderophore production index (values between 2.1 and 2.3).

27.6 Inorganic Phosphate Solubilisation

The phosphate solubilisation capacity of isolated strains was determined using Pikovskaya's agar with $Ca_3(PO_4)_2$. Each bacterial culture was spot-inoculated and incubated at 28 °C for 48 h. A clear zone around the colony indicated inorganic phosphate solubilisation. The bacterial isolates were ranked based on the phosphate mobilisation capacity using the measurement of the halo zone diameter (Laslo et al. 2012). From the assayed isolates, seven (33.33%) were able to solubilise the calcium phosphate, showing measurable halo around the colony. The inorganic phosphate solubilisation efficiency of the isolates was compared on the basis of the solubilisation index (SI) that ranged from 2.08 to 2.83. Three of the strains have higher SI, *Pseudomonas abietaniphila* CM16 (2.83), *Mitsuaria chitosanitabida* CM5 (2.80) and *Acinetobacter johnsonii* CM18 (2.67). In the case of two bacterial strains, *Pseudomonas abietaniphila* CM16 and *Mitsuaria chitosanitabida* CM5 are isolated from the rhizosphere of *Cytisus hirsutus* L. and *Medicago lupulina* L., and the solubilisation index was above average, compared to others.

27.7 Organic Phosphate Mobilisation Capacity

The selected isolated bacteria were screened for organic phosphate mobilisation capacity. The bacterial strains were spot-inoculated on phytase-specific medium, containing sodium phytate, and incubated at 28 °C for 5 days. A transparent halo generated around the bacterial colonies indicated phytase production and activity (Hosseinkhani et al. 2009).

In case of 14 (66.66%) strains from the isolated and characterised strains, the organic phosphate mobilisation capacity was detected. The phytate mobilisation index values varied between 2.49 and 4.49 for the studied bacterial strains. The highest value was detected in the case of *Bacillus* sp. CM4. Three bacterial strains from the 21 showed outstanding performance; they are *Bacillus* sp. CM4, *Serratia* sp. CM21 and *Mitsuaria chitosanitabida* CM5 strains. The index value was above three in the case of nine (42.85%) bacterial strains. Our results showed week phosphate mobilisation ability of the rhizobial strains.

27.8 Indole-3-Acetic Acid Production

The qualitative assay of IAA was realised on LB agar containing 100 µg/ml L-tryptophan. After the spot inoculation of the bacterial strains, nitrocellulose membranes were placed on agar plates. After 2 day incubation on 28 °C, the nitrocellulose membranes were removed and placed in Salkowski reagent. The IAA production is indicated by the development of a *pink* colour.

The amount of the IAA produced by the selected bacterial strains was determined using colorimetric method. The bacterial strains were inoculated in soy peptone liquid media, with the exception of *Rhizobium* strains (inoculated in YMA),

Table 27.2 Beneficial traits of bacteria isolated from plant nodules and rhizosphere

Bacterial strains	Ac.R.	Sid. PrI	IP SI	OP SI	IAA (μg/ml)
Rhizobium yanglingense CM1	+	2.14 ± 0.032	–	–	9.61 ± 4.15
Rhizobium leguminosarum CM2	–	–	–	–	–
Rhizobium leguminosarum CM3	+	–	–	–	–
Bacillus sp. CM4	+	2.45 ± 0.144	2.27 ± 0.063	4.49	16.00 ± 0.28
Mitsuaria chitosanitabida CM5	+	2.44 ± 0.097	2.80 ± 0.068	3.90	10.48 ± 0.145
Variovorax paradoxus CM6	+	2.28 ± 0.096	–	–	6.25 ± 0.86
Rhizobium rhizogenes CM7	+	2.30 ± 0.089	–	3.21	4.77 ± 1.12
Variovorax paradoxus CM8	–	2.31 ± 0.026	–	2.52	6.78 ± 3.33
Rhizobium leguminosarum CM9	–	2.69 ± 0.194	–	3.26	–
Sinorhizobium meliloti CM10	+	2.62 ± 0.072	–	2.57	5.69 ± 0.22
Rhizobium leguminosarum CM11	+	2.58 ± 0.012	–	–	4.37 ± 1.42
Rhizobium etli CM12	–	2.10 ± 0.053	2.08 ± 0.026	2.78	3.55 ± 0.00
Rhizobium leguminosarum CM13	+	–	–	–	–
Rhizobium leguminosarum CM14	+	2.31 ± 0.051	2.09 ± 0.014	–	4.71 ± 1.397
Rhizobium leguminosarum CM15	–	2.46 ± 0.026	–	2.78	5.56 ± 1.56
Pseudomonas abietaniphila CM16	–	2.21 ± 0.034	2.83 ± 0.118	2.49	12.52 ± 0.435
Pseudomonas brassicacearum CM17	–	2.12 ± 0,055	–	3.28	15.88 ± 0.87
Acinetobacter johnsonii CM18	+	2.13 ± 0.043	2.67 ± 0.124	3.26	14.31 ± 1.60
Ensifer (*Sinorhizobium*) sp. CM19	–	2.31 ± 0.144	–	3.16	3.09 ± 0.02
Serratia proteamaculans CM20	+	2.37 ± 0.030	–	3.77	9.67 ± 0.465
Serratia sp.CM21	+	–	2.07 ± 0.049	3.95	11.06 ± 0.69

AcR acetylene reduction, *Sid.* siderophore/PrI production index, *IP* inorganic phosphate, *OP* organic phosphate, *SI* solubilisation index

containing 100 μg/ml L-tryptophan, and incubated at 28 °C for 72 h. After incubation, the cultures were centrifuged at 5000 rpm for 15 min, and the supernatant was mixed with Salkowski reagent using 1:2 ratio. The mixture was incubated for 25 min in dark on room temperature before absorbance was measured at 530 nm. The concentration of each sample was calculated using a calibration curve equation, with the help of standard IAA solution with concentration varying from 2.5 to 30 μg/ml (Bric et al. 1991).

From the assayed bacterial strains, 17 (80.95%) have IAA production ability. The amount of IAA released in culture media varied from 3.09 to16 μg/ml (Table 27.2). A remarkable amount of IAA was released in case of seven (33.33%) bacterial strains. An amount of 9.67 μg/ml of IAA was released by *Serratia proteamaculans* CM20, whereas 11.06 μg/ml IAA was released by *Serratia* sp. CM21. From *Pseudomonas* genus, two bacterial strains with high IAA production ability (*Pseudomonas brassicacearum* CM17 (15.88 μg/ml) and *Pseudomonas abietaniphila* CM16 (12.52 μg/ml)) were detected. The highest value was measured in case of *Bacillus* sp. CM4 (16.00 μg/ml), whereas *Acinetobacter johnsonii* CM18 and *Mitsuaria chitosanitabida* CM5 bacterial strains produced 14.31 μg/ml and 10.48 μg/ml IAA.

27.9 Plant Growth Promotion Activity

The selected two bacterial strains (*Acinetobacter johnsonii* CM18 and *Mitsuaria chitosanitabida* CM5) with the highest PGPR potential were tested for their effect on wheat and pea seedling growth under controlled conditions. The surface sterilisation of wheat and pea seeds was realised by soaking in sodium hypochlorite solution (Merck, 6–14% active chlorine), followed by germination under gnotobiotic conditions. The germinated seeds were sown in a plastic box (34 × 23 × 16 cm) containing 5 L sterilised soil placed in a growth chamber with controlled conditions of light (16/8 h *light/dark* cycle), temperature (22 ± 2 °C) and relative humidity (70%), for 3 weeks. During this period, the seedlings were treated once with 1 ml bacterial suspension (0.8 transmittance, 10^8 CFU/ml), whether the control plants were treated with sterile distilled water.

The plants were harvested after 3 weeks of growing under controlled condition. The following morphological data were recorded: total weight, shoot length, wet shoot and root weights, dry shoot and root weights. In order to determine the dry weights of the separated root and shoot systems for each plant, samples were dried to constant mass in an oven at 105 °C.

Each of the two bacterial strains, *Acinetobacter johnsonii* CM18 and *Mitsuaria chitosanitabida* CM5, has significant effect on different plant growth parameters in pea and wheat (Tables 27.3 and 27.4).

In the case of pea, the inoculation with bacterial strains significantly ($p < 0.001$) increased biomass (26.67%) and shoot dry weight (29.9%). The individual inoculation was more effective than the co-inoculation. *Mitsuaria chitosanitabida* CM5 was the most effective strain, where the biomass increased up to 50% and the shoot dry weight up to 46%. Another bacterial strain, *Acinetobacter johnsonii* CM18, increased the shoot dry weight up to 35.5%.

In the case of wheat inoculation with the single strains and also in co-inoculation conferred enhanced plant growth in comparison to the control. In all cases, inoculation resulted in higher plant biomass, shoot length and root and shoot dry weight. Treatment with *Acinetobacter johnsonii* CM18 on wheat seedlings resulted in increased biomass up to 44.7%, shoot length up to 25%, root dry weight up to 40% and shoot dry weight up to 22.9%. In the case of *Mitsuaria chitosanitabida* CM5 inoculation, the biomass of the wheat increased up to 42.8%, shoot length up to 19%, root dry weight up to 9.4% and shoot dry weight up to 20%.

27.10 Isolated Bacterial Strains Efficiency

This study revealed that the bacterial strains that originated from nodules and rhizosphere of different plant species from mountain hay meadows show phenotypic diversity. The isolated bacterial strains play a crucial role in the soil ecosystem being involved in biogeochemical cycles. Due to the nutrient availability improvement and other beneficial traits, the bacterial strains were able to exert direct effect on the growth of wheat and pea plants. The cooperation of microorganisms with

Table 27.3 Effect of the two bacterial strains *Acinetobacter johnsonii* CM18 and *Mitsuaria chitosanitabida* CM5 and the co-inoculation on pea seedling growth parameters under in vitro conditions

Samples/pea	Total weight (mg)	Root wet weight (mg)	Shoot wet weight (mg)	Root dry weight (mg)	Shoot dry weight (mg)	Shoot length (cm)
Control	810 ± 207.5	218.8 ± 73.16	555.8 ± 179.93	20.9 ± 15.50	48.7 ± 13.29	8.90 ± 1.32
Mitsuaria chitosanitabida CM5	1220 ± 274.6*	326.82 ± 79.96	892.27 ± 218.24*	19.2 ± 5.25*	71.14 ± 17.78*	11.99 ± 1.76*
Acinetobacter johnsonii CM18	1092.6 ± 225.5*	329.13 ± 64.94*	763.91 ± 195.67*	19.89 ± 4.37	66.00 ± 16.23*	9.56 ± 1.57
Co-inoculant	1026.95 ± 149.31*	277.83 ± 56.24*	750.00 ± 127.03*	19.26 ± 3.88	63.26 ± 11.83*	9.03 ± 1.54

*Significantly different from the control for $p < 0.001$

Table 27.4 Effect of the two bacterial strains *Acinetobacter johnsonii* CM18 and *Mitsuaria chitosanitabida* CM5 and the co-inoculation on wheat seedling growth parameters under in vitro conditions

Samples/wheat	Total weight (mg)	Root wet weight (mg)	Shoot wet weight (mg)	Root dry weight (mg)	Shoot dry weight (mg)	Shoot length (cm)
Control	170.8 ± 48.16	31.20 ± 11.07	139.6 ± 45.65	11.38 ± 5.14	19.36 ± 4.18	23.03 ± 4.46
Mitsuaria chitosanitabida CM5	244.00 ± 43.50*	48.00 ± 11.90*	196.8 ± 42.98*	12.46 ± 2.84	23.22 ± 3.15*	27.51 ± 4.93
Acinetobacter johnsonii CM18	247.2 ± 40.78*	40.8 ± 10.38	205.2 ± 37.43*	15.94 ± 14.58	23.8 ± 3.47*	28.83 ± 3.88*
Co-inoculant	224.40 ± 55.31*	45.6 ± 19.81	178.8 ± 43.14	19.50 ± 11.19*	24.22 ± 4.47*	27.89 ± 3.65*

Data were statistically analysed using F- and student's t-test for detection of significant differences in variance. Treatment effects were considered significant at level of $P < 0.001$

*Significantly different from the control for $p < 0.001$

host plants can result in multiple positive patterns for both parties. The plant growth-promoting processes can be realised through direct and indirect mechanisms. The enhancement of plant nutrition was detected and evaluated in many studies (Adesemoye et al. 2008; Luna et al. 2012; Souza et al. 2013). Complex processes, with specialised proteins, are responsible for molecular interactions between plants and bacteria (Drogue et al. 2012).

Here we report eight genera of PGPR bacteria (*Rhizobium* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Ensifer* (*Sinorhizobium*) sp., *Variovorax* sp., *Serratia* sp., *Bacillus* sp., *Mitsuaria* sp.) and 21 strains, respectively, which originated from spontaneous mountain flora. In the case of bacterial isolates, at least one beneficial characteristic, either related to phytostimulation or to nutrient mobilisation, was detected. Among the tested bacterial strains, 80% possessed at least three beneficial traits. Due to this pattern, they contribute to the increased accessibility of plant nutrients such as the N detected in many cases (Anand and Chanway 2013).

From the 21 characterised bacterial strains, ten belong to *Rhizobium* genus. We detected in case of isolated rhizobia strains the following beneficial properties: siderophore and IAA production in the case of *Rhizobium yanglingense* CM1 and *Rhizobium rhizogenes* CM7; the last one also possesses the ability to solubilise the organic phosphorus. The most efficient strain from this group was *Rhizobium etli* CM12 that possesses four beneficial properties from the five studied ones. Seven bacterial strains identified as *Rhizobium leguminosarum* were isolated from different host plants and differ in the beneficial characteristics that can be explained by the specific plant-bacteria interaction. It was reported in other studies that symbiotic rhizobia delivered from leguminous plants can promote plant growth through other beneficial traits beside the biological N fixation capacity (Biswas et al. 2000; Granada et al. 2014). Our findings agree with those of Souza et al. (2016) that rhizobia strains were weak or non-IC producers.

Two *Pseudomonas* strains were identified from the isolated ones, as follows: *Pseudomonas abietaniphila* CM16 and *Pseudomonas brassicacearum* CM17. The PGPR traits identified for *Pseudomonas* strains in this study were siderophore production, organic phosphate solubilisation and IAA production in both strains, whereas in case of *Pseudomonas abietaniphila* CM16 strain, the inorganic phosphate solubilisation trait was also detected. Our data show concordance with literature data, reporting bacterial strains belonging to *Pseudomonas* genera as having phytostimulation and biofertilisation PGPR abilities (Pérez-Montaño et al. 2014). Here we report new PGPR traits of *Pseudomonas abietaniphila*, with previously known role in bioaugmentation and biodegradation processes (Rico-Martínez et al. 2014).

Two bacterial strains belonging to *Serratia* genera were identified in our study annotated as CM20 and CM21, the first one identified as *Serratia proteamaculans*. PGPR traits as acetylene reduction, organic phosphate solubilisation and IAA production were identified in both strains, whereas *Serratia* sp. CM21 showed inorganic phosphate and *Serratia proteamaculans* CM20 siderophore production. *Serratia* sp. strains isolated from leguminous plants were reported as having

biofertilisation and phytostimulation ability due to phosphate mobilisation and phytohormone production (Pérez-Montaño et al. 2014).

From the 21 isolated strains, two were identified as belonging to *Variovorax paradoxus* species, CM6 and CM8, showing PGPR traits as siderophore and IAA production. *Variovorax paradoxus* CM6 was able to reduce acetylene, whereas CM8 was able to solubilise organic phosphate. *Variovorax paradoxus* strains were previously reported as having PGPR traits (Zhao et al. 2016), phosphate solubilisation and N fixation (Yolcu et al. 2011).

One *Acinetobacter johnsonii* CM18 strain was isolated from the nodule of *Lotus corniculatus* L. plant showing positive results for all the assayed beneficial traits. The plant growth stimulation effect of *Acinetobacter johnsonii* strain was reported previously by Shi et al. (2009). Strains of a closely related species *Acinetobacter pittii* are known as PGPR bacteria having phosphate solubilisation (Liu et al. 2014) or *Acinetobacter calcoaceticus* bacteria having siderophorogenic activity, phytohormone production (ABA and gibberellins) and inorganic phosphate solubilisation (Kang et al. 2009; Patel et al. 2015).

One of the most promising strain isolated from mountain hay meadows of Ciuc Mountains was identified as *Mitsuaria chitosanitabida* CM5, being positive for all studied PGPR traits. *Mitsuaria chitosanitabida* strains were previously described as biofungicide, showing inhibition against *Pythium aphanidermatum*, *Phytophthora sojae*, *Rhizoctonia solani* and *Alternaria solani* and the least against *Pythium sylvaticum* (Benitez and McSpadden Gardener 2009). The *Mitsuaria chitosanitabida* CM5 showed positive result for siderophore production, a compound that besides iron nutrition contributes also to the suppression of deleterious microbes affecting the health of the plants (Sayyed et al. 2013), conferring biocontrol property.

One of the most frequently studied PGPR bacterial genera is *Bacillus* sp. The CM4 isolate was identified as *Bacillus* sp., possessing all the assayed beneficial traits. *Bacillus* sp. strains were previously described having PGPR traits as nitrogen fixing capacity, phytohormone production (gibberellic acid, cytokinins), phosphate mobilisation and antagonistic against a wide range of phytopathogens (Pérez-Montaño et al. 2014).

In the case of three strains, *Mitsuaria chitosanitabida* CM5, *Acinetobacter johnsonii* CM18 and *Bacillus* sp. CM4, all the assayed beneficial nutrient mobilising and phytostimulating traits were positive. The bacterial strain *Mitsuaria chitosanitabida* CM5 isolated from the mountain pasture ecosystem was detected as showing PGPR traits as acetylene reduction, siderophore and IAA production and organic and inorganic phosphate mobilisation. This bacterial strain was not previously mentioned as PGPR bacteria. PGPR attributes of *Acinetobacter johnsonii* CM18 reported in this study were as follows: acetylene reduction, siderophore and IAA production and organic and inorganic phosphate mobilisation. Bacteria from genera *Acinetobacter* were previously described as having PGPR characteristics.

Mitsuaria chitosanitabida as a novel species was first described by Amakata et al. (2005), and the draft genome sequence was described for one *Mitsuaria* sp. strain only (Rong et al. 2012). *Mitsuaria chitosanitabida* was isolated also by Nascimento et al. (2015) as endophytic bacteria from *Piper tuberculatum* Jacq.,

whereas *Acinetobacter johnsonii* was isolated from economical important crops as maize and sugar beet as endophytic bacteria (Shi et al. 2009; Liu et al. 2013).

The difference in the beneficial traits of bacterial isolates originated from plants rhizosphere and nodule can be attributed to their rhizosphere competence, including place, nutrition and plant growth cycle in the ecological niche (Barret et al. 2011; Ghirardi et al. 2012).

27.11 Conclusion

This study shows that the inoculation with the bacterial strains originated from the pasture plants confers benefits to the crops, showing enhanced plant growth in both pea and wheat. In the case of wheat root and shoot dry weight, our results are similar to those observed for winter wheat treated with different phyllospheric and rhizospheric beneficial bacteria (Egamberdieva 2008).

Here we report two bacterial strains *Acinetobacter johnsonii* CM18 and *Mitsuaria chitosanitabida* CM5 that were able to promote plant growth of pea and wheat plants under controlled conditions. Due to the novel PGPR characters and adaptations to local conditions, the above-mentioned bacterial strains are promising for sustainable agriculture.

References

Adesemoye AO, Torbert HA, Kloepper JW (2008) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can J Microbiol 54:876–886. doi:10.1139/w08-081

Amakata D, Matsuo Y, Shimono K et al (2005) *Mitsuaria chitosanitabida* gen. nov., sp. nov., an aerobic, chitosanase-producing member of the "Betaproteobacteria". Int J Syst Evol Microbiol 55:1927–1932. doi:10.1099/ijs.0.63629-0

Anand R, Chanway C (2013) N_2-fixation and growth promotion in cedar colonized by an endophytic strain of *Paenibacillus polymyxa*. Biol Fertil Soils 49:235–239. doi:10.1007/s00374-012-0735-9

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570. doi:10.1007/s10529-010-0347-0

Bardgett RD, Bowman WD, Kaufmann R, Schmidt SK (2005) A temporal approach to linking aboveground and belowground ecology. Trends Ecol Evol 20:634–641. doi:10.1016/j.tree.2005.08.005

Barret M, Morrissey JP, O'Gara F (2011) Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. Biol Fertil Soils 47:729–743. doi:10.1007/s00374-011-0605-x

Bashan Y, Kamnev AA, de-Bashan LE (2013) Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. Biol Fertil Soils 49:465–479. doi:10.1007/s00374-012-0737-7

Benitez M-S, McSpadden Gardener BB (2009) Linking sequence to function in soil bacteria: sequence–directed isolation of novel bacteria contributing to soilborne plant disease suppression. Appl Environ Microbiol 75:915–924. doi:10.1128/AEM.01296-08

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350. doi:10.1007/s11274-011-0979-9

Biswas JC, Ladha JK, Dazzo FB (2000) Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci Soc Am J 64:1644–1650

Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Bulgarelli D, Schlaeppi K, Spaepen S et al (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838. doi:10.1146/annurev-arplant-050312-120106

Castagno LN, Estrella MJ, Sannazzaro AI et al (2011) Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by pantoea eucalypti isolated from lotus tenuis rhizosphere in the Salado River basin (Argentina). J Appl Microbiol 110:1151–1165. doi:10.1111/j.1365-2672.2011.04968.x

Chaiharn M, Lumyong S (2011) Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. Curr Microbiol 62:173–181. doi:10.1007/s00284-010-9674-6

de Souza R, Beneduzi A, Ambrosini A et al (2013) The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. Plant Soil 366:585–603. doi:10.1007/s11104-012-1430-1

de Souza E, Bassani V, Sperotto RA, Granada CE (2016) Inoculation of new rhizobial isolates improve nutrient uptake and growth of bean (*Phaseolus vulgaris*) and arugula (*Eruca sativa*). J Sci Food Agric 96:3446–3453. doi:10.1002/jsfa.7527

Demeter L, Csergő AM, Sándor A, Imecs I, TCs V (2011) Natural treasures of the Csík basin (Depresiunea ciculu) and Csík mountains (Munţii ciucului). In: Knowles B (ed) Mountain hay meadows – hotspots of biodiversity and traditional culture. Society of Biology, London

Drogue B, Doré H, Borland S et al (2012) Which specificity in cooperation between phytostimulating rhizobacteria and plants? Res Microbiol 163:500–510. doi:10.1016/j.resmic.2012.08.006

Drogue B, Combes-Meynet E, Moënne-Loccoz Y et al (2013) Control of the cooperation between plant growth-promoting Rhizobacteria and crops by rhizosphere signals. In: de Bruijn FJ (ed) Molecular microbial ecology of the rhizosphere, vol 1. Wiley, Hoboken, pp 279–293

Egamberdieva D (2008) Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. Turk J Biol 32:9–15

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi:10.1007/s11104-008-9833-8

Ghirardi S, Dessaint F, Mazurier S et al (2012) Identification of traits shared by rhizosphere-competent strains of fluorescent pseudomonads. Microb Ecol 64:725–737. doi:10.1007/s00248-012-0065-3

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:e963401. doi:10.6064/2012/963401

Granada CE, Arruda L, Lisboa BB et al (2014) Diversity of native rhizobia isolated in south Brazil and their growth promotion effect on white clover (*Trifolium repens*) and rice (*Oryza sativa*) plants. Biol Fertil Soils 50:123–132. doi:10.1007/s00374-013-0840-4

Grigulis K, Lavorel S, Krainer U et al (2013) Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. J Ecol 101:47–57. doi:10.1111/1365-2745.12014

Hosseinkhani B, Emtiazi G, Nahvi I (2009) Analysis of phytase producing bacteria (*Pseudomonas* sp.) from poultry faeces and optimization of this enzyme production. Afr J Biotechnol 8(17):4229–4232

Kang S-M, Joo G-J, Hamayun M et al (2009) Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. Biotechnol Lett 31:277–281. doi:10.1007/s10529-008-9867-2

Laslo É, György É, Gy M, Szentes S, Salamon RV, András Cs D, Sz L (2012) The management of N and P nutrition of plants using nitrogen fixing and phosphorus solubilizing bacteria. Environ Eng Manag J 11(2):371–375

Lemanceau P, Expert D, Gaymard F et al (2009) Role of iron in plant–microbe interactions. In: Van Loon LC (ed) Advances in botanical research, vol 51. Elsevier. Academic Press, London, pp 491–549

Liu Y, Zuo S, Zou Y et al (2013) Investigation on diversity and population succession dynamics of endophytic bacteria from seeds of maize (*Zea mays* L., Nongda108) at different growth stages. Ann Microbiol 63:71–79. doi:10.1007/s13213-012-0446-3

Liu FP, Liu HQ, Zhou HL, Dong ZG, Bai XH, Bai P, Qiao JJ (2014) Isolation and characterization of phosphate-solubilizing bacteria from betel nut (Areca Catechu) and their effects on plant growth and phosphorus mobilization in tropical soils. Biol Fertil Soils 50:927–937. doi:10.1007/s00374-014-0913-z

Luna MF, Aprea J, Crespo JM, Boiardi JL (2012) Colonization and yield promotion of tomato by *Gluconacetobacter diazotrophicus*. Appl Soil Ecol 61:225–229. doi:10.1016/j.apsoil.2011.09.002

Martínez OA, Jorquera MA, Crowley DE, Mora ML (2011) Influence of nitrogen fertilisation on pasture culturable rhizobacteria occurrence and the role of environmental factors on their potential PGPR activities. Biol Fertil Soils 47:875–885. doi:10.1007/s00374-011-0593-x

Nascimento SB, Lima AM, Borges BN, de Souza CRB (2015) Endophytic bacteria from Piper tuberculatum Jacq.: isolation, molecular characterization, and in vitro screening for the control of fusarium solani f. sp. piperis, the causal agent of root rot disease in black pepper (Piper nigrum L.) Genet Mol Res 14(3):7567–7577

Oldal B, Jevcsák I, Kecskés M (2002) A sziderofortermelő képesség szerepe Pseudomonas-törzsek növénypatogén-antagonista hatásának biológiai vizsgálatában. Biokémia 26:57–63

Parmar N, Dufresne J (2011) Beneficial interactions of plant growth promoting rhizosphere microorganisms. In: Singh A, Parmar N, Kuhad RC (eds) Bioaugmentation biostimulation biocontrol. Springer, Berlin, pp 27–42

Pásztohy Z (2013) The soils and the biological diversity of the Pogány-havasmicroregion. In:International conference papers: mountain hay meadows – economic, social and environmental value.Gyimesközéplok, Romania23–24 May 2013

Patel K, Goswami D, Dhandhukia D, Thakker J (2015) Techniques to study microbial phytohormones. In: Maheshwari DK (ed) Bacterial metabolites in sustainable agroecosystem. Springer, Switzerland, pp 1–27

Pérez-Montaño F, Alías-Villegas C, Bellogín RA et al (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169:325–336. doi:10.1016/j.micres.2013.09.011

Prosser JI (2002) Molecular and functional diversity in soil micro-organisms. Plant Soil 244:9–17. doi:10.1023/A:1020208100281

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiol 156:989–996. doi:10.1104/pp.111.175448

Rico-Martínez M, Medina FG, Marrero JG, Osegueda-Robles S (2014) Biotransformation of diterpenes. RSC Adv 4:10627–10647. doi:10.1039/C3RA45146A

Rong X, Gurel FB, Meulia T, Gardener BBM (2012) Draft genome sequences of the biocontrol bacterium *Mitsuaria sp. strain H24L5A*. J Bacteriol 194:734–735. doi:10.1128/JB.06537-11

Sayyed RZ, Chincholkar SB, Reddy MS, Gangurde NS, Patel PR (2013) Siderophore producing PGPR for crop nutrition and phytopathogen suppression. In: Maheshwari DK (ed) Bacteria in agrobiology: disease management. Springer, Heidelberg, pp 449–471

Sharma S, Gupta R, Dugar G, Srivastava AK (2012) Impact of application of biofertilizers on soil structure and resident microbial community structure and function. In: Maheshwari DK (ed) Bacteria in agrobiology: plant probiotics. Springer, Heidelberg, pp 65–77

Shi Y, Lou K, Li C (2009) Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol Fertil Soils 45:645–653. doi:10.1007/s00374-009-0376-9

Singh B, Satyanarayana T (2012) Plant growth promotion by phytases and phytase-producing microbes due to amelioration in phosphorus availability. In: Satyanarayana T, Johri BN, Prakash A (eds) Microorganisms in sustainable agriculture and biotechnology. Springer, Dordrecht, pp 3–15

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. doi:10.1111/j.1574-6976.2007.00072.x

Trivedi P, Spann T, Wang N (2011) Isolation and characterization of beneficial bacteria associated with citrus roots in Florida. Microb Ecol 62:324–336. doi:10.1007/s00248-011-9822-y

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. doi:10.1111/j.1461-0248.2007.01139.x

van derHeijden MGA, Wagg C (2013) Soil microbial diversity and agro-ecosystem functioning. Plant Soil 363:1–5. doi:10.1007/s11104-012-1545-4

Verma JP, Yadav J, Tiwari KN, Kumar A (2013) Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecol Eng 51:282–286. doi:10.1016/j.ecoleng.2012.12.022

Yolcu H, Turan M, Lithourgidis A et al (2011) Effect of plant growth-promoting rhizobacteria and manure on yield and quality characteristics of Italian ryegrass under semi arid conditions. Aust J Crop Sci 5(13):1730–1736

Young C-C, Shen F-T, Singh S (2012) Strategies for the exploration and development of biofertilizer. In: Maheshwari DK (ed) Bacteria in agrobiology: plant probiotics. Plant probiotics. Springer, Berlin, pp 127–139

Yuan C-L, Mou C-X, Wu W-L, Guo Y-B (2011) Effect of different fertilization treatments on indole-3-acetic acid producing bacteria in soil. J Soils Sediments 11:322–329. doi:10.1007/s11368-010-0315-2

Zhao S, Zhou N, Zhao Z-Y, Zhang K, Wu G-H, Tian C-Y (2016) Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. Curr Microbiol 73(4):574–581. doi:10.1007/s00284-016-1096-7

Ameliorating Salt Stress in Crops Through Plant Growth-Promoting Bacteria

28

Sana Ullah, Muhammad Baqir Hussain, Muhammad Yahya Khan, and Hafiz Naeem Asghar

Abstract

Abiotic stresses are emerging vicious environmental factors limiting agricultural productivity around the world, while food demand is increasing with growing population. Among these abiotic stresses, salt stress is a serious threat to put down crop production especially in arid and semiarid regions of the world. Therefore, some serious steps are required to stop or slow down the lethal effects of salinity for ensuring food security. Various strategies are adopted to tackle the deleterious impacts of salinity to crops including breeding techniques and genetic engineering, but these techniques have their level of significance and cannot satisfy the whole demand. However, some biological strategies are cost-effective, environment friendly, and easy to adopt/operate. In this scenario, the use of various microorganisms (bacteria, fungi, algae) to enhance salinity resilience in crops is encouraged due to their vital interactions with each other and crop plants. Bacteria are widely used to mitigate deleterious impacts of high salinity on crop plants because they possess various direct and indirect plant beneficial characteristics including exopolysaccharide and siderophore production, biofilm formation, phosphate solubilization, induced systemic resistance, and enhanced nutrient uptake, and they act as biocontrol agents to protect crop plants from many diseases by killing pathogens. This chapter focuses on the negative effects of high salinity on plants, bacterial survival in salt stress, and their mechanisms to mitigate salinity stress and the role of beneficial microbes to enhance crop tolerance against salinity stress.

S. Ullah • M.B. Hussain • H.N. Asghar (✉)
Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
e-mail: naeemasghar@yahoo.com

M.Y. Khan
University of Agriculture, Faisalabad, Sub-Campus Burewala, Vehari, Pakistan

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_28

Keywords
Salinity • Rhizobacteria • Crop production • Stress amelioration

28.1 Introduction

The world agriculture is confronting to various threats and challenges like additional demand of 70% food for 2.3 billion of people by the year 2050 with consumption of scarce natural resources, hunger, and poverty (FAO 2009), but crop production is not increasing in pace with the demand of food. About 14.6 million hectares (Mha) of agriculture land has been degraded in 75 countries of the world in the last 20 years (Qadir et al. 2014; Fan et al. 2016). There are various types of environmental stresses which leave hazardous impacts on plants including salinity, drought, alkalinity, low and high temperature, pathogen infections, and ultraviolet stress (Van et al. 2001). These stresses are the main factors which lower agriculture production; therefore, management of stresses is required to meet increasing food demands (Shanker and Venkateswarlu 2011). Salinity in water and/or soil is harmful which lowers the crop production especially in arid and semiarid regions (Allakhverdiev et al. 2000; Koca et al. 2007; Parvaiz and Satyawati 2008) and affects 6% of the total cultivable area (Munns and Tester 2008). A soil with electrical conductivity of saturated extract more than 4 dS m^{-1} at 25 °C and 15% exchangeable sodium contents is regarded as saline sodic soil (Munns 2005). It is a main environmental threat for our agriculture around the world which is indicated by surplus inorganic salts. Primary or natural salinity occurs under climatic conditions such as high rate of evaporation and less leaching which are prominent in arid and semiarid regions (Jouyban 2012). Accumulation of salt due to weathering of rocks, seawater, aerosol deposits, and rainfall in the world especially in Pakistan, India, America, Iran, and China leads to natural salinization; moreover, approximately 4 Mha of land becomes uncultivable annually because of excess salt accumulation globally (Beresford et al. 2001; Hulsebusch et al. 2007). Secondary salinity occurs due to anthropogenic activities such as inefficient and/or excessive irrigation, growing shallow-rooted vegetation in place of native vegetation, and adopting modern practices of land use. It affects about 74 Mha of land all over the world (Beresford et al. 2001). Salinity affects the ecosystem, deteriorates productive lands, stimulates erosion and growth of salt bushes, and causes discharge of saline surface and groundwater (Beresford et al. 2001; Bridgman et al. 2008).

28.2 Salinity Impacts on Plant

Soil degradation due to excess salts is common in arid and semiarid areas of the world. Salinity is increasing because of inappropriate agricultural practices like the use of brackish or saline water for irrigation of farmlands (Cao et al. 2016: Fan et al. 2016). Soil salinization is a main threat to yield loss of major crops in countries like

Pakistan, where average losses in wheat and rice yields are 32% and 48%, respectively (Qadir et al. 2014). Other crops like onion, corn, and bean show 50% reduction in yield when soil electrical conductivity (EC) exceeds 5 dS m^{-1} (Horneck et al. 2007; Metwali et al. 2015). Keeping in mind the salt sensitivity and/or tolerance, plants are categorized into glycophytes and halophytes. Glycophytes are those plants which are unable to tolerate high salinity (Sairam and Tyagi 2004; Parvaiz and Satyawati 2008). In salt-affected soils, higher concentration of sodium ions deteriorates soil structure by deflocculating the clay particles which cause poor or no aggregation and compaction leading to decreased porosity, permeability, and drainage (McDowell 2008). Basic deleterious impacts of salinity stress on plants include (1) salt stress or specific ion effect, (2) decrease in soil solution osmotic potential leading to water-deficit stress, (3) nutrient imbalance, and/ or (4) combination or any two of them. Firstly, salinity stress affects plant by disrupting photosynthesis, lipid, energy metabolism, and protein synthesis. Plants reduce leaf expansion with increase in salinity as initial response to salt stress, but expansion is resumed as salinity is relieved (Parida and Das 2005; Singh and Jha 2016). Soil salinity induces metabolic and physiological changes in plant body, affecting seed germination, morphological characters, survival percentage, growth, development, and grain/plant yield (Jouyban 2012). Salinity causes reduction in stomatal conductance, chlorophyll contents, leaf area, and efficiency of photosystem II; ultimately, the process of photosynthesis is hampered (Netondo et al. 2004). It inhibits stamen elongation and microsporogenesis, enhances ovule abortion and cell death, and weakens fertilized embryo leaving hazardous impacts on reproductive growth. Overall, salinity stress affects growth and development of plant through osmotic stress, nutrient imbalance, salt stress, and/ or combined effect of all these factors (Ashraf 2004; Shrivastava and Kumar 2015). Under salt stress, production of reactive oxygen species (ROS) increases (Hasegawa et al. 2000) which damages proteins, lipids, nucleic acid, and cell structure, i.e., hydroxyl ions disrupt DNA by damaging pyrimidine and purine bases (Halliwell and Gutteridge 1999; Valko et al. 2006), and lipid peroxidation in intracellular organelles and plasmalemma occurs when ROS react with unsaturated fatty acids (Karabal et al. 2003; Sen and Chandrasekhar 2015). High sodium ion concentration in leaves causes necrosis, while in soil solution, it restricts nutrient uptake by hampering entry of essential nutrients, affecting potassium ion channels, and reducing root growth (Tester and Davenport 2003). Under salt stress, restricted supply of hormones and photosynthetic products toward growing parts of plant (Ashraf 2004) can badly affect the growth and development of plant. Ionic potassium (K^+) acts as cofactor of certain enzymes and vital for protein synthesis, but its replacement by Na^+ (Zhu 2002; Shrivastava and Kumar 2015) may cause its deficiency and/or imbalance, hence damaging plant's developmental processes. Elements like boron, sodium, and chlorine have toxic impacts on plant growth and development under salinity as excessive sodium accumulation causes cell death (necrosis) and osmotic stress (Munns 2002). Salinity reduces uptake of essential nutrients (such as phosphorus, potassium, calcium, nitrogen, zinc, and iron) in plants; moreover, it reduces phosphorus uptake significantly due to binding or precipitation with calcium ions (Bano and Fatima

2009; Shrivastava and Kumar 2015). High concentration of salt may disturb the uptake and balance of other essential ions (Blaylock 1994). Among phytohormones, ethylene regulates plant's physiological processes but regarded as stress hormone because 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene) is enhanced under abiotic stress to inhibit root growth (Morgan and Drew 1997; Wang et al. 1990; Arshad and Frankenberger 2002). Osmotic stress with ion toxicity causes imbalance in plant metabolic reactions or activities and stimulates oxidative stress (Chinnusamy et al. 2006). Salinity affects plants at vegetative and seedling stage which is obvious in *Oryza sativa* plants where salt stress decreased the plant height, leaf area, and number of tillers (Hasanuzzaman et al. 2009), while in wheat, it reduces fertility, decreases the number of spikelets, delays spike emergence, and causes reduction in grain yield (Munns and Rawson 1999). High salinity levels inhibit nodulation in legumes by affecting signal transduction between two partners (legume and rhizobia) to reduce nodulation (Miransaria and Smith 2009) and decrease the legume crop yield. The abovementioned deleterious effects of salinity can reduce crop yield at large scale; therefore, plausible approaches are required to dilute the effect of salinity on crops to achieve the desired yield potential.

28.3 Strategies to Tackle Salt Stress

Various strategies are adopted by the researchers to ameliorate the impact of salinity on plants such as breeding techniques, agronomic approaches, and use of plant growth-promoting microbes described as following.

28.3.1 Agronomic Approaches

These may include using of salinity-tolerant plant, improved management practices, salt leaching from the root zone, and suitable irrigation method like micro-jet or drip irrigation, growing of perennial plants with deeper roots which grow throughout the season, and using water which is extra from annual and/or desired crop. These perennial crops prevent salt movement toward the soil surface by reducing water table rise. Precision farming, intercropping, alley cropping, and crop rotation are also adopted to ameliorate salt stress, but all these are limited due to cost and shortage of good quality irrigation water (Munns et al. 2002; Manchanda and Garg 2008; Shrivastava and Kumar 2015).

Usually, the soils which have heaps of salt on the surface are treated physically where no organic amendment or inorganic chemicals are applied. For the purpose, heavy machinery is required for inverting the subsoil to the surface and surface soil to the depth, plowing deeply, sand mixing, profile mixing, and using drip irrigation system. As salinity reduces the soil permeability, these treatments tend to increase soil physical condition for water infiltration which is a prerequisite for salinity remediation. In soils where gypsum is present in subsoil, deep plowing is proven to be a very useful strategy (Ahmed and Qamar 2004). Irrigation with good quality

water or high rainfall may also be helpful in leaching or draining the excess soluble salts from the upper layers of soil (root zone) to the lower soil layers (Qadir and Schubert 2002; Zhang et al. 2008). Drip irrigation is considered a very effective technique for reclaiming saline soils. Bresler et al. (1982) recorded a higher rate of leaching with drip irrigation system as compared to traditional irrigation schemes. Cultivation of crops along with drip irrigation system accelerates the reclamation process of salt-affected soils particularly in the root zone (0–5 cm). The most limiting factor in the physical reclamation/remediation of salt-affected soils is the availability and cost of good quality irrigation water in the region (Qadir and Schubert 2002; Zhang et al. 2006). Therefore, to make saline soils workable for crop production, the integration of physical approach with plantation is recommended (Li et al. 2008; Qadir and Schubert 2002; Zhang et al. 2006). Various seedbed preparation strategies have been devised to grow crops in saline soils while avoiding or reducing the harmful impacts of salt stress such as furrow irrigation to crops where seedbeds are shaped to remove salts away/at a distance from the germinating seeds (Horneck et al. 2007). Physical approach is expensive because it requires heavy machinery, fuel, and irrigation costs where initial plantation is lost to be mixed in the soil.

The approach also includes the application of elemental sulfur (S), gypsum ($CaSO_4.2H_2O$), sulfuric acid (H_2SO_4), and hydrochloric acid (HCl) to the saline and saline sodic soils. These amendments reduce the impact of salinity stress on plants by replacing Na^+ ions from the exchange sites and lower the pH to increase the solubility of salts in soil. These soluble salts can be moved/removed either by leaching or drainage after dissolution in the irrigation water (Ahmed and Qamar 2004). These amendments are also in use with the standing field crops to enhance nutrient use efficiency and ameliorate the damaging impacts on plants. Application of gypsum improves the physicochemical properties (Ayers and Westcot 1985), porosity (Shainberg and Letey 1984; Oster et al. 1996), and hydraulic conductivity (Scotter 1978) of salt-affected soils. Similarly, Southard and Buol (1988) observed a significant decline in the soil bulk density with the application of phosphor gypsum. Gypsum application also produced significant increases in wheat crop grain yield (Ghafoor et al. 1985).

28.3.2 Breeding and Engineering Techniques

Planting salt-tolerant (halophytes) crops on saline soils decreases the water evaporation from the surface significantly which is considered the main cause of salinity (Qadir and Schubert 2002). Many of the field trials have demonstrated improvement in soil physical characteristics by the plantation of forages, where thick and extensive plant root system penetrates the deeper layers of soil to facilitate the leaching of excess soluble salts. For this reason, genes of salinity-tolerant (halophytes) crop species are identified and tried to be transferred in the desired high-yielding crop plants using breeding or genetic engineering tools. Plants obtained using these techniques could be beneficial for sustainable agriculture at marginal lands. In the same way, plant growth-promoting rhizobacteria or bacteria may play

a key role for improving or sustaining growth and production of crops under salt-affected conditions.

Salt stress limits crop growth, yield, and utilization of land resource leading to decrease in agricultural productivity. Plant breeding techniques have served as a tool to the scientists since a long time and play an important role for the improvement of salt-tolerant varieties. Conventional breeding techniques have been reported at intergenic, interspecific, and intraspecific level to get the desired salt-resistant lines but remained less successful due to little variation in the gene pool and reproductive barriers while transforming the desired gene(s) from wild relative to domesticated cultivar (Turan et al. 2012). Genetic engineering is a technique related to the insertion or transfer of desired gene(s) to generate new salt-tolerant plants (Turan et al. 2012). In quantitative trait loci (QTL) engineering technique, segments of genetic material associated with salt tolerance are studied to understand the stress response of salt-tolerant plants. New techniques including gene mapping and transcriptional and expression profiling help in the identification of gene linked with QTLs (Salvi and Tuberosa 2005; Sahi et al. 2006; Walia et al. 2007; Marino et al. 2009; Pandit et al. 2010). Furthermore, in molecular marker technique, markers are used to analyze the quantitative and inherited traits and identify individual gene in the genome set which is controlling the trait of interest (Turan et al. 2012). Another engineering approach is a single gene level management for salt tolerance to design transcription factors. A variety of transcription factors (TF) belonging to different families such as *WRKY*, *MYC*, *DREB*, *MYB*, *NAC*, *Bzip*, and *Cys2His2* zinc finger are involved in salinity stress tolerance. These TF control stress tolerance by binding to respective genes linked in stress response. This approach is not an easy and effective management because the same factors may associate with different kinds of stress or different groups which are linked to the same and/or single response (Turan et al. 2012). For example, *ONAC045* encodes an NAC transcription factor gene which is involved in salinity and drought tolerance in rice plants (Zheng et al. 2009). Engineering of molecular chaperones is also used for enhancing salinity tolerance in plants. Chaperones are different groups of proteins which are involved in protein synthesis or degradation through folding or unfolding and assembling or disassembling of these proteins under stress condition (Boston et al. 1996). *aspgHsc70* encoding for HSP70 isolated from *Pennisetum glaucum* has to be known for its role in salinity tolerance (Reddy et al. 2011). Other genetic engineering approaches include the engineering of genes closely linked to osmoprotectants. Osmoprotectants are the compounds which are produced by plants under stress condition to avoid/reduce the water loss and to maintain cell turgidity such as soluble sugars, amino acids, organic acids, lipids, and polyamines (Guy 1990). Overexpression of the gene *OsTPS1* (encoding trehalose-6-phosphate synthase) improves salinity tolerance and tolerance to other abiotic stresses in rice (Li et al. 2011). Different breeding techniques including conventional selection and transgenic approach are used to improve salt stress resilience in crop plants, but these techniques are time-consuming, expensive, and successful for target species only (Ashraf 2002; Sairam and Tyagi 2004; Singh and Jha 2016; Habib et al. 2016).

28.3.3 Microbial Strategies

The use of microbes for improving crop growth, development, and yield is considered a cost-effective and environment-friendly approach (Vejan et al. 2016); as such application of mycorrhizae and bacteria dilutes the adversities of salinity and improves plant growth (Kohler et al. 2010) by ameliorating the disturbed soil environment. Stress-tolerant microbes such as cyanobacteria, diazotrophs, polymer-releasing algae, and phosphate solubilizers from arid regions can convert saline and barren soils into arable lands (Bhatnagar and Bhatnagar 2001).

28.4 Mechanisms of Bacterial Survival Under Salt Stress

Bacteria which can endure wide and/or high range of NaCl concentration are called as halotolerant bacteria. These bacteria are categorized into extremely, moderately, slightly, and non-tolerant groups on the basis of their growth on media containing 32%, 18–22%, 6–8%, and 1% NaCl salt (w/v), respectively (Hezayen et al. 2010; Hassan and Mahgoub 2011). Literature has confirmed a variety of bacteria isolated or purified from saline environments which are capable of tolerating extreme salinity in soil, for example, *Pseudomonas*, *Actinobacterium*, *Azospirillum*, *Flavobacterium*, *Alcaligenes*, *Virgibacillus*, *Thalassobacillus*, *Planococcus*, *Sporosarcina*, *Staphylococcus*, *Halomonas*, *Halobacillus*, *Brevibacterium*, *Enterobacter*, *Terribacillus*, and *Oceanobacillus* (Ventosa et al., 1983; Moral et al., 1988; Upadhyay et al., 2009; Ilyas et al., 2012; Roohi et al., 2012; Hossain et al., 2016). Potent traits and/or silent features of bacteria which enable them to survive under salinity stress are given in Table 28.1. For influencing growth and development of plants, PGPB should colonize the plant roots, survive, and multiply inside the plants or rhizosphere (Barea et al. 2005).

28.4.1 Organic Osmolytes and Inorganic Osmoprotectants

Salt-tolerant species of bacteria adopt two major mechanisms, accumulation of stress protectant or osmoprotectant organic compounds (ectoins, polyols, betaines, sugars, amino acids) and selective influx of inorganic ions like K^+ which support their survival (Santos and da Costa 2002) through osmotic adjustment and proper cell functioning/metabolism under high salt concentration. Bacteria produce low molecular weight, hydrophilic molecules which help them to compensate osmolarity (osmotic difference) outside the cell; moreover, bacteria also uptake certain compounds from their external environments (Da Costa et al. 1998). Salt-resistant rhizobia bring some metabolic, morphological, and structural modifications to survive and adopt to saline environment (Ahmad et al. 2011). Likewise, under hypo-osmotic environment, *Agrobacterium tumefaciens* and *Rhizobium meliloti* release

Table 28.1 Potent traits and/or salient features of bacteria which enable them to survive under salinity stress

Bacteria	Salinity	Survival mechanism	References
Mesorhizobium alhagi CCNWXJ12-2	0.4 M NaCl	Upregulation of gene encoding YadA domain containing protein (yadA)	Liu et al. (2014)
Tistlia consotensis	0.5%, 4% NaCl	Greater amounts of the HpnM protein for biosynthesis of hopanoids	Rubiano-Labrador et al. (2015)
		Exclusion of Na^+	
		Transport of glycine betaine or proline	
Oceanobacillus profundus (Pmt2), *Staphylococcus saprophyticus* (ST1)	0.5 M NaCl	Biofilm formation, exopolysaccharide production, proline and glycine betaine accumulation	Qurashi and Sabri (2011)
Bacillus spp., *Halobacillus* spp.	20% NaCl	–	Ramadoss et al. (2013)
Pseudomonas fluorescens, *Bacillus megaterium*, *Variovorax paradoxus*	2%, 5% NaCl	–	Nadeem et al. (2016)

certain compounds called cyclic β-(1,2)-glucans which mediate stress resilience. Sucrose, glycine betaine, glucose, and trehalose are found to be very effective to stimulate the cyclic glucan synthesis (Ingram-smith and Miller 1998). These specific glucans are found in cell periplasm (Breedveld and Miller 1994) and serve as principal osmoprotectants. Moreover, they are modified with succinyl substitute and/or phosphoglycerol functional groups (Breedveld and Miller 1995). Accumulation and synthesis of glycine betaine during osmotic stress have been observed and studied in bacteria such as *R. meliloti* (Geremia et al., 1987). These types of bacteria may play a prominent role in salt stress adaptation of plants. Hua et al. (1982) observed that *Rhizobium* sp. (strain WR1001) produces glutamate under salinity which helps to survive or grow up to 500 mM sodium chloride media. Literature has confirmed that *B. subtilis* produce certain proteins which help in their survival under abiotic stresses including oxidative stress, heat shock, and salt stress. These proteins are known as general stress proteins such as Rsbw, Ctc, and GsiB (Volker et al. 1994). Paul and Nair (2008) carried out a proteome analysis of *Pseudomonas fluorescens* (strain MSP-393); the result revealed that salinity facilitated the production of osmolytes and stress proteins which nullified the deleterious impacts of high osmolarity. In this strain, buildup of compatible solutes, i.e., glutamic acid and aspartic acid, enhances with increasing levels of salt concentration. Further, amino acids synthesized in cytosol which act as osmolytes under high salt concentration include Ala (alanine), Gly (glycine), Ser (serine), and Thr (threonine).

28.4.2 Exopolysaccharides and Biofilm Formation

A variety of bacteria are capable of synthesizing and releasing extracellular biopolymeric compounds known as exopolysaccharides (EPS) which help them to resist extreme environmental conditions (Pereira et al. 2009; Jittawuttipoka et al. 2013), such as *Planococcus rifietoensis* (RT4) and *Halomonas variabilis* (HT1) which produce EPS during salt stress which help in their survival under varying conditions of the ecosystem (Qurashi and Sabri 2012). In EPS synthesis, different steps are involved; firstly monosaccharides are produced and then converted into sugar nucleotides in cytoplasm; then glycosyltransferase adds sugars sequentially on the lipid carrier for the assemblage of repeated units; these repeated units pass through polymerization process in plasma membrane; and finally polymers are exported toward the cell surface (Jittawuttipoka et al. 2013). Bacteria form well-structured microcolonies attached to the nonliving or living material to survive adverse conditions called "biofilms" (Salta et al. 2013; Qurashi and Sabri 2016) which gives functional and physical protection to residing bacteria (Ashraf et al. 2005). These microcolonies are made of proteinaceous surface structures called curli, outer membrane proteins, fimbriae, flagella, and EPS matrix having voids/water channels/canals through which flow of liquids occurs assisting diffusion and exchange of gasses like oxygen, ions, antimicrobial agents, and nutrients. Curli structures and membrane proteins help in adhesive attachment to the surface and between bacterial cells, while flagella aid in motility toward surface attachment against repulsive forces (Pratt and Kolter 1998; Lewandowski 2000) which could be helpful under adverse environments like salinity, heavy metal, and drought stress. EPS keep the layers hydrated around biofilms which protect bacterial colonies or biofilms from desiccation stress (Sutherland 2001).

28.4.3 Fatty Acids

Phospholipid fatty acid (PLFA) profile acts as an indicator for the survivability potential of various microbial groups/communities (bacteria, fungi, archaea) under different abiotic stresses (salinity, heavy metal, flooding). Thereby, physiology of microbial group can be perceived by using PLFA profile technique (Baath et al. 2005; Azarbada et al. 2016). The principal component analysis of bacteria showed an increased production of both straight-chain and long-chain fatty acids under increasing salt levels. This increasing fatty acid production and accumulation reduce the permeability and fluidity of the cell membrane under high salt concentration which would make microorganisms compatible to saline environment by preventing solute leakage and controlling osmotic pressure of the living cells (Nicolaus et al. 2001; Azarbada et al. 2016).

28.5 Salt Stress Mitigation by Salt-Tolerant PGPB

Bacteria possessing plant growth promotion potential residing in the rhizosphere are used as biofertilizer to enhance soil fertility and eventually the crop yield for more sustainability in the field of forestry and agriculture (Garcia-Fraile et al. 2015). Beneficial microbes ubiquitously colonize the plant roots and assist plant growth (Hussain et al. 2009, 2014a, b, 2016) in horticulture, silviculture, and agriculture and in cleaning of polluted environment such as phytoremediation (Santoyoa et al. 2016).

Various bacterial species including *Serratia*, *Pseudomonas*, *Flavobacterium*, *Burkholderia*, *Rhodospirillum*, *Agrobacterium*, *Rhodobacter*, *Clostridium*, *Acetobacter*, *Azotobacter*, *Acinetobacter*, *Aerobacter*, *Achromobacter*, *Arthrobacter*, *Erwinia*, *Alcaligenes*, *Rhizobium*, *Xanthomonas*, *Bacillus*, *Azospirillum*, and *Enterobacter* can act as PGPB (Kloepper et al. 1989; Kim and Kim 2008; Joshi and Bhatt 2011; Hossain et al. 2016). These PGPB can be applied either through seed inoculation or directly into soil when there is danger of antagonistic or inhibitory microbes on and in the plant body, whereas seed priming is preferred generally because bacteria can adhere, enter, and acclimate with/within the seeds in prevalent environment. Moreover, it ensures uniform germination and rapid and more crop establishment resulting in higher yields of crops (Mahmood et al. 2016). They possess various properties which may be beneficial for growth and development of crop under salinity stress (Table 28.2). These properties or mechanisms include resistance against salinity, interaction with respective crop, biocontrol agents against diseases, production of phytohormones, and compatible solute (Shrivastava and Kumar 2015) as given in Fig. 28.1. Bacteria increase growth and development of plant through direct and indirect mechanisms; direct mechanisms and/or processes include resistance against stresses, increased availability and uptake of nutrients, phosphate solubilization, and production of useful substances like phytohormones (auxin, cytokinin, gibberellins, abscisic acid), siderophores, and enzymes (1-amino cyclopropane-1-carboxylate (ACC) deaminase), while indirect mechanisms include biocontrol or protection against pathogens and diseases by the production of hydrogen cyanide, parasitism, antibiosis, and nutrient competition (Jha and Saraf 2015), increasing rhizosphere exploring area, and interaction with microbes that are also beneficial for plant health (Hussain et al. 2009, 2014a, b, 2016). After inoculation of stress-tolerant bacterial strains, the plant shows increase in growth, physiological, and yield parameters including biomass, proteins, chlorophyll contents, and shoot and root length (Tiwari et al. 2011). Besides rhizobial functioning under normal condition, rhizobia performance with their partner has vital importance especially under stress environments; for this reason, rhizobia must be resistant to promote plant growth and development under stress condition. So, treating legumes with salt-tolerant microbes can improve nitrogen fixation efficiency of plants in saline environments (Zou et al. 1995). Rhizobia of woody legumes such as *Prosopis rhizobia* selected and then isolated from desert soils facing adverse environmental conditions like drought, salinity, and heat stress could be used as stress-tolerant inocula for legume crops (Jenkins et al. 1987; Zahran 1999). PGPB also interact with other

Table 28.2 Impact of plant growth-promoting bacteria on the growth and yield parameters of different crops under salinity

Test crop	Salinity level	Bacteria	Increase over control	References
Soybean	NaCl = 20 dS m^{-1} (50 mM)	PGPB strains Rkh1, Rkh2, Rkh3, and Rkh4	Shoot length (54%)	Naz et al. (2009)
			Root length (75%)	
			Root weight (227%)	
			Shoot weight (90%)	
Tomato	NaCl = 60 mM	PGPB (plant growth-promoting bacteria)	Germination index (60%)	Chookietwattana and Maneewan (2012)
			Root length (61%)	
Basil	EC = 6 dS m^{-1}	*Bacillus lentus* and *Pseudomonades* sp.	Dry weight (12.3%)	Golpayegani and Tilebeni (2011)
Wheat	NaCl = 320 mM	*Bacillus halodenitrificans* PU62 and *Halobacillus* sp. SL3	Root length (90%)	Ramadoss et al. (2013)
			Dry weight (17.4%)	
Wheat	EC_e = 8 dS m^{-1}	*Pseudomonas syringae* (strain MAS129), *Bacillus amyloliquefaciens* (strains MAS526 and MAS4), *Microbacterium* sp. (strain MAS133), and *B. insolitus* (strains MAS26 and MAS10)	Root length (167%)	Ashraf et al. (2006)
			Roots dry matter (380%)	
Wheat	EC_e = 8 dS m^{-1}	*Bacillus insolitus* (strain MAS17), *Aeromonas hydrophila/caviae* (strain MAS765), and *Bacillus* sp. (strains MAS617, MAS620, and MAS820)	Shoot dry matter (85–281%)	Ashraf et al. (2004)
			Root dry matter (149–522%)	
			Mass of rhizospheric soil (176–790%)	
Maize	EC_e = 11.8–13.6 dS m^{-1}	*Enterobacter* and *Pseudomonas* sp.	Plant height (29%)	Nadeem et al. (2009)
			Grain yield (60%)	
Rice	NaCl = 1.5%	*Bacillus pumilus* and *Pseudomonas pseudoalcaligenes*	Root length (18%)	Jha and Subramanian (2014)
			Shoot length (8.3%)	
			Dry biomass (39%)	

(continued)

Table 28.2 (continued)

Test crop	Salinity level	Bacteria	Increase over control	References
Rice	NaCl = 1.5–2.5%	*B. pumilus* and *P. pseudoalcaligenes*	Plant height (70%)	Jha et al. (2011)
			Dry biomass (11–22%)	
			Glycine betaine (3.5%)	
Wheat	EC_w = 12 dS m^{-1}	*Azospirillum*	Plant height (35%)	Nia et al. (2012)
			Tiller per plant (104%)	
			Proline conc. (84%)	
Wheat	NaCl = 320 mM	*Azospirillum brasilense* sp. 245	RWC (18%)	Creus et al. (1997)
			Shoot dry weight (30%)	
Mung bean	EC_e = 5.59–6.56 dS m^{-1}	*Pseudomonas* and *Rhizobium*	Stomatal conductance (105%)	Ahmad et al. (2013)
			SPAD chlorophyll conc. (38.73%)	
			Photosynthetic rate (83%)	
			WUE (37%)	
			Leaf K^+ (28%)	
			Leaf Na^+ reduction (50%)	
			Grain N (61%)	
			Grain P (64%)	
Mung bean	EC_w = 6 dS m^{-1}	*Pseudomonas syringae* Mk1, *Pseudomonas fluorescens* biotype GMk25, *Pseudomonas fluorescens* Mk20, *Rhizobium phaseoli* strains M1, M6, and M9	Shoot length (51%)	Ahmad et al. (2011)
			Root length (62%)	
			Shoot fresh biomass (234%)	
			Root fresh biomass (196%)	
Cotton	Total salts = 3.50 g kg^{-1} soil	*Pseudomonas putida* Rs-198	IAA (51%)	Yao et al. (2010)
			ABA reduction (23.25%)	
			Plant height (12.8%)	

(continued)

Table 28.2 (continued)

Test crop	Salinity level	Bacteria	Increase over control	References
Chickpea	NaCl = 100 and 200 mM	*Planococcus rifietoensis* (RT4) and *Halomonas variabilis* (HT1)	Germination (178%)	Qurashi and Sabri (2012)
			Seedling length (114%)	
			Seedling fresh weight (177%)	
			Total soluble sugar contents (256%)	
			Protein contents (219%)	
			Mass of aggregated soil (808%)	
Cotton	NaCl = 0.7%	*Pseudomonas putida* RS-198	Germination rate (42.35%)	He et al. (2015)
			Plant height (21%)	
			Soluble proteins (500%)	
Vicia pannonica	NaCl = 60 mM	*Agrobacterium rubi* A1, *Bacillus megaterium* M3, *Bacillus subtilis* OSU-142	Plant N (97%), plant P (19%), plant K (69%), plant Ca (69%), plant Mg (57%), proline reduction (93.6%)	Esringua et al. (2016)
Cucumber	NaCl = 10 dS m^{-1}	*Bacillus megaterium*, *Pseudomonas fluorescens*, and *Variovorax paradoxus*	Shoot growth (55%),	Nadeem et al. (2016)
			Root length (67%)	
			Total biomass (118%)	
Lettuce	EC_w = 7 dS m^{-1}	*Serratia proteamaculans* and *Rhizobium leguminosarum*	Dry weight (7.86%)	Han and Lee (2005)
			Total chlorophyll (14.78%)	
			P (17%), K (9.25%), Ca (25.3%)	
Rice	NaCl = 100 mM	*Pseudomonas* strains TDK1 and PF1	Nitrate reductase activity (11%)	Sen and Chandrasekhar (2015)
			Catalase activity (31%)	
			Peroxidase activity (20%)	

(continued)

Table 28.2 (continued)

Test crop	Salinity level	Bacteria	Increase over control	References
Sunflower	EC_e = 9.42 and 7.51 dS m^{-1}	PGPB strains KS 7, KS 41, KS 42, and KS 44	Grain yield (110%), plant height (56%)	Kiani et al. (2016)
			Shoot dry weight (182%)	
Wheat	EC_e = 11.8 and 14.2 dS m^{-1}	*Enterobacter cloacae*, *Pseudomonas fluorescens*, *Serratia ficaria*, and *Pseudomonas putida*	Plant height (29.6%)	Nadeem et al. (2013)
			No. of tillers (21%), grain yield (24%)	
			P uptake (92%), K uptake (17%)	
			K^+/Na^+ ratio (31%)	
Wheat	NaCl = 150–200 mM	*Serratia* sp. SL- 12	K uptake (39%)	Singh and Jha (2016)
			Na uptake reduction (65%)	
			Proline reduction (65%)	
			MDA reduction (63%)	
			Ch "a" (76%), Ch "b" (24%)	
			Auxins (58%), total proteins (43%)	
Wheat	NaCl = 200 mM	*Klebsiella* sp. SBP-8	Shoot length (47%)	Singh et al. (2015)
			Root length (36%)	
Pistachio	NaCl = 2000 mg kg^{-1} soil	PGPB	Zn conc. in shoot (48%)	Azarmi et al. (2016)
			H_2O_2 reduction (12%)	
			Carotenoids (35%), SOD in leaf (18%), PPO in leaf (62%), POX in shoot (15%)	

(continued)

Table 28.2 (continued)

Test crop	Salinity level	Bacteria	Increase over control	References
Pea	NaCl = 70 and 130 mM	*Variovorax paradoxus* 5C-2	Total biomass (54%)	Wang et al. (2016)
			Ca (52%), Mg (167%), P (23%)	
			Root to shoot Na^+ supply reduction (9%), shoot Na^+ reduction (13%)	
Okra	NaCl = 100 mM	*Enterobacter* sp. (UPMR18)	Seed germination (100%)	Habib et al. (2016)
		Bacillus megaterium (UPMR2)	Root fresh weight (91%)	
			Root dry weight (50%)	
			Shoot fresh weight (68%)	
			Shoot dry weight (57%)	

bacteria, fungi, and algae in the rhizosphere, showing synergistic or antagonistic effect which augment plant growth indirectly (Vejan et al. 2016), such as coculture of bacterial strains Rs-198 (*Pseudomonas* sp.) and Rs-5 (*Klebsiella* sp.) having synergistic relation which can be used as biofertilizer for plant growth promotion under salt stress (Yuan-yuan et al. 2008; Zhong-hong et al. 2009). Dynamic interactions among plant roots, soil, water, and microbes occurring in the rhizosphere bring physicochemical and structural changes in soil (Haynes and Swift 1990) which are very beneficial for plant health especially under stress environment, for example, microbial-produced exopolysaccharides improve soil structure by forming micro- and macroaggregates of soil particles. For example, EPS-producing strains of bacteria enhance plant tolerance against drought and salinity stress by improving soil structure (Sandhya et al. 2009). In salt-affected soils, increasing population of EPS-producing bacteria can ameliorate hazardous effects of salinity on crops because they restrict influx or uptake of sodium ions due to cation bindings (Chen et al. 2007). It can be speculated from previous studies that EPS production and formation of biofilm augment the fertility of soil and significantly improve plant growth (Ashraf and Harris 2004; Ashraf et al. 2005; Liaqat et al. 2009; Davey and Toole 2000). Moreover, biofilm formation and EPS production ability of PGPB increase with increase in salinity levels which facilitate aggregation of the soil particles in the vicinity of plant roots (Qurashi and Sabri 2012). Biofilms are assemblage of microbes like bacteria with their extracellular released compounds such as EPS at biotic or abiotic surface; these biofilms help in soil aggregation near plant roots which facilitate plant growth (Batool and Hasnain 2005).With the aid of biofilms,

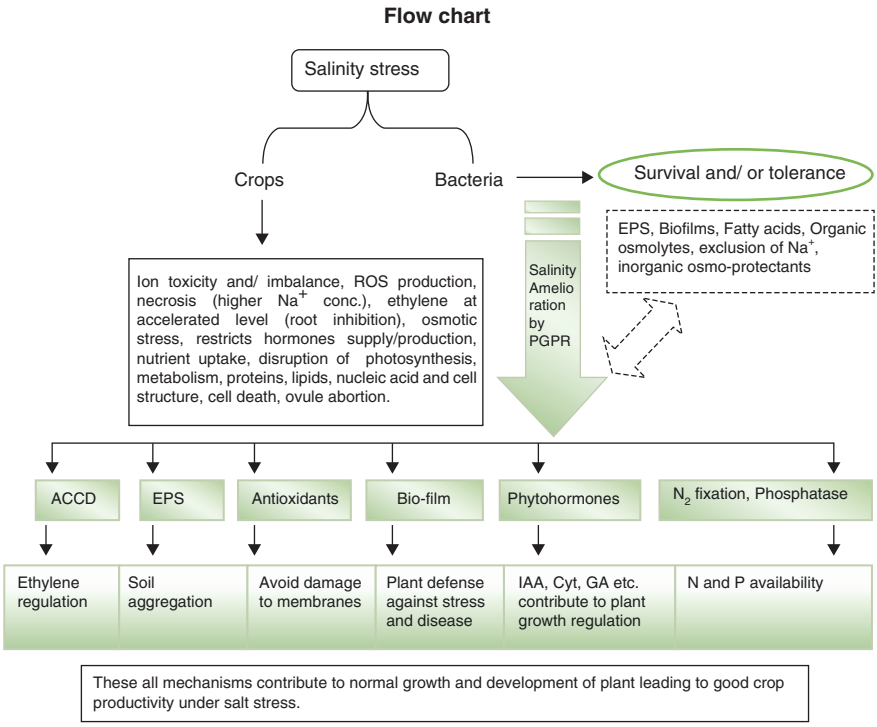


Fig. 28.1 The negative influences of salt stress on crop plants, i.e., nutrient imbalance, root inhibition, necrosis, ROS production, rupturing of lipid and proteins, and cell death. The potent traits (EPS, biofilms, osmoprotectants) of PGPR to resist salinity and salt stress amelioration through various mechanisms include ACCD, EPS, antioxidants, phytohormones, biocontrol, ISR, and nutrient availability

bacteria form microcolonies on root or soil particle surfaces, assisting soil particle cementation (soil aggregation) which improve physicochemical properties of soil like water retention capacity (Batool and Hasnain 2005; Ashraf et al. 2005) and develop favorable environment for plant growth. Bacterial inoculation enhanced the uptake of macronutrients such as potassium (K), nitrogen (N), phosphorus (P), and calcium (Ca) and improved K^+/Na^+ ratio by restricting the entry of sodium ions in the plant body under salinity stress (Alamri and Mostafa 2009; Nadeem et al. 2013). PGPB solubilize P from P complex and make it available for plants under salt stress (Hamdali et al. 2008; Palaniyandi et al. 2014). They also enhance the availability of essential micronutrients like magnesium (Mg^{+2}) and calcium (Ca^{+2}) ions with reduced uptake of sodium ions under saline conditions, ultimately increasing growth and development of crop plants under stress (Yao et al. 2010). In the literature, it has been observed that K^+/Na^+ ratio plays a significant role in sustaining osmotic potential of the plant body; hence, restricted sodium ion uptake is beneficial for maintaining plant turgor under salinity (Song and Fujiyama 1996; Ashraf et al. 2004, 2006). *Planococcus rifietoensis* $M8^T$ is a bacterium which can withstand saline conditions

(halotolerant) and possesses phosphate metabolism, potassium homeostasis genes, glutamine synthetase, glutamate dehydrogenase, hydroxyl methyltransferase, and glycine dehydrogenase (See-Too et al. 2016). *Planococcus rifietoensis* is capable of improving wheat growth by enhancing soil fertility and nitrogen availability and metabolizing potassium under saline conditions (Rajput et al. 2013). Plants can maintain this balance under high salinity through HKT transporters also called K^+ transporters which control Na^+ uptake (Zhang et al. 2008). Production of osmoprotectants (proline, betaine, glycine, glutamate, trehalose, ectoin, and K^+) to sustain osmolarity of plant cytoplasm and EPS to make Na^+ unavailable for plant through binding mechanism is of vital significance under salinity and other stresses (Grover et al. 2011; Nishma et al. 2014). Production of antioxidants includes enzymatic (ascorbate peroxidase, glutathione reductase, catalase peroxidase, superoxide dismutase) and nonenzymatic compounds (carotenoids, α-tocopherol, ascorbate) which facilitate plant protection against the dangerous effects of reactive oxygen species (ROS) (Baniaghil et al. 2013; Nishma et al. 2014). PGPB dilute salinity effects by enhancing activities of different antioxidant enzymes such as catalase, peroxidase, and nitrate reductase (Sen and Chandrasekhar 2015).

Plant beneficial bacteria induce systemic tolerance in plants which refers to chemical and physical changes in the plant body to withstand different stresses or enhance stress resilience (Yang et al. 2009; Shrivastava and Kumar 2015). Plant beneficial bacteria containing ACC deaminase stimulate plant growth under salinity by restricting Na^+ uptake (Wang et al. 2016) and alleviating negative effects of higher ethylene level by hydrolyzing ACC into ammonia and α-ketobutyrate which are later used by bacteria as food; this process is done by an enzyme called ACC deaminase released by bacteria (Glick et al. 1998). ACC deaminase producing PGPB strain *A. piechaudii* ARV8 induced systemic tolerance in pepper and tomato against drought and salinity stress (Mayak et al. 2004). Growth-promoting bacteria produce antioxidants and cytokinins which neutralize ROS and stimulate abscisic acid (ABA) accumulation, while antioxidant enzyme production also confers oxidative stress (Stajner et al. 1997). Colonization ability of PGPB determines their potential whether they can efficiently enhance salt tolerance and increase the plant root growth. Free release into the soil and/or direct inoculation of PGPB to plant seed/root like *Klebsiella oxytoca* Rs-5 may face numerous difficulties in survival and better colonization because bacterial strains are susceptible to various environmental factors including temperature and pH fluctuations and competitive indigenous microbes (Vassileva et al. 1999; Rekha et al. 2007; Wu et al. 2011, 2012). Therefore, encapsulation form of desired inocula can be an alternative to free dispersal/release of bacteria into rhizosphere. Due to controlled dispersal of bacteria, they can grow and survive more efficiently; hence, better results or positive influences can be attained for a long period (Wu et al. 2011, 2014).

Auxins (IAA) can mitigate effect of salinity stress and enhance plant growth by lessening ABA levels which act as growth inhibitors especially under stress condition (Patten and Glick 2002; Yao et al. 2010). It has been confirmed that PGPB like IAA producing *Pseudomonas* sp. Rs-198 can help the growth of plants under salt stress (Yuan-yuan et al. 2008). PGPB enhance the release of lipopolysaccharides

and flavonoids and produce IAA in rhizosphere which could assist root growth in saline condition (Dardanelli et al. 2008; Metwali et al. 2015). Due to chemical nature, siderophores act as chelating agents and play a vital role in phytoremediation, biocontrol, biosensor, and weathering of soil minerals to support plant growth (Ahmedm and Holmstrom 2014). Generally, iron is made available in the form of Fe^{+3}-siderophore complex at the mineral surface which is later transferred to solution phase of soil where it is taken up by plants or microbes (Kalinowski et al. 2000; Kraemer 2004).

Bacterial isolates *B. subtilis*, *P. putida*, and *P. fluorescens* have a great potential for inducing salinity tolerance in faba bean under salinity up to 8000 ppm (Metwali et al. 2015). However, *P. fluorescens* has significantly increased germination percentage (96%), plant length (10.66%), shoot fresh weight (9.52%), and leaf area (61.86%) in faba bean cultivar Wadi-1 under salinity (Metwali et al. 2015). Inoculation of pea plants with ACC deaminase producing *Variovorax paradoxus* (strain 5C-2) under NaCl salinity (70 and 130 mM) depicted a significant increase in plant biomass, photosynthetic efficiency, and K uptake and decreased Na^+ deposition in shoot by 54% and 25%, 19% and 12%, 28% and 26%, and 6% and 13% at 130 and 70 mM, respectively, as compared to uninoculated plants (Wang et al. 2016). Similarly, the inoculation of salt-tolerant and EPS-producing rhizobacterial strains (*Bacillus insolitus* strain MAS17; *Bacillus* sp. strains MAS820, MAS617, and MAS620; and *Aeromonas caviae/hydrophila* strain MAS-765) in wheat showed increases in the dry matter of shoot, root, and mass of rhizospheric soil up to 281%, 527%, and 790%, respectively, as compared to control (Ashraf et al. 2004). These EPS-producing bacteria significantly reduced the concentration of Na^+ ion in roots and shoots of inoculated plants up to 61% and 60%, respectively (Ashraf et al. 2004). Singh and Jha (2016) demonstrated a significant decrease in the proline and MDA contents of wheat leaves by 65% and 63%, respectively, with the inoculation of ACC deaminase containing bacterium (*Serratia* strain SL-12) at 200 mM NaCl salinity. They also recorded a significant increase in length of root, shoot, dry weight, fresh weight, and chlorophyll a and b, by 27%, 35%, 34%, 31%, 76%, and 24%, respectively, over uninoculated control at 200 mM NaCl salt stress. *Pseudomonas putida* Rs-198 (isolated from alkaline soil) modulated the impact of salinity on cotton seed and increased fresh weight (30.7%), dry weight (10%), plant height (12.8%), and IAA contents as compared to uninoculated plants (Yao et al. 2010). ACC deaminase containing *Klebsiella* sp. SBP-8 induced salinity tolerance in wheat at 200 mM NaCl salinity modulating K^+ content in plant tissues (Singh et al. 2015). Similarly, Nadeem et al. (2016) elucidated *Variovorax paradoxus*, *Bacillus megaterium*, and *Pseudomonas fluorescens* on cucumber for inducing salinity tolerance. They suggested *Pseudomonas fluorescens* as a potential inoculant for cucumber as it increased root weight, root length, shoot weight, and total biomass by 87%, 67%, 73%, and 118%, respectively. However, Qurashi and Sabri (2016) described that osmolyte accumulation and biofilm formation by *Staphylococcus sciuri* HP3 augmented soluble sugar and soluble protein contents by 60% and 11% of *Lens esculenta* under stress (200 mM of NaCl).

28.6 Conclusions and Future Prospects

Maintenance of a sustainable agriculture system is the dire need for upholding the economic stature of the growers, safeguarding the environment, and producing sufficient quality food for the ever-increasing population of the world. But abiotic stress conditions are causing a major threat to the world's agriculture production where salinity carries the highest importance. Salinity has injurious impacts on plant, disrupting its normal functioning at physiological, biochemical, and molecular levels, which ultimately reduces the yield. Understanding the plant's response to salinity from cell to organism levels has been studied using the tools of breeding and genetics which lead to the development of cultivars or transgenes capable of growing under high salinity. But these breeding and engineering tools are very expensive and time intensive. Similarly, various physical and chemical approaches have also been tried to reduce or remove the impacts of high salinity on plant growth and productivity which proved to be unsustainable in the long run. These physical and chemical approaches have mostly environmental consequences and also expensive to adopt. Therefore, the use of plant growth-promoting bacteria seems to be a good opportunity for sustaining the production of crops under high salinity. These bacteria could be rhizospheric, phyllospheric, or endophytic. In either case of association, they perform plant beneficial attributes which induce salinity tolerance in plants. PGPB are specific to specific crops for particular conditions, which can be formulated as a biofertilizer and conveyed to the farmers in an easy-to-use formulation. Though beneficial relationship of plants with PGPB has been elaborated in several studies, still there is no sound clue for plant-microbe cross talk leading to that beneficial association which needs to be studied. Moreover, the integrated approach utilizing molecular techniques and genetic engineering tools along with biochemical modifications may precisely describe the mechanisms involved in plant-microbe interaction.

Acknowledgments The authors are thankful for the support and encouragement by the colleagues from the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.

References

Ahmad M, Zahir ZA, Asghar HN et al (2011) Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate-deaminase. Can J Microbiol 57:578–589

Ahmad M, Zahir ZA, Khalid M (2013) Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiol Biochem 63:170–176

Ahmed M, Qamar I (2004) Productive rehabilitation and use of salt-affected land through afforestation (a review). Sci Vis 9:1–14

Ahmedm E, Holmstrom SJ (2014) Siderophores in environmental research: roles and applications. Microb Biotechnol 7:196–208

Alamri SA, Mostafa YS (2009) Effect of nitrogen supply and *Azospirillum brasilense* Sp-248 on the response of wheat to seawater irrigation. Saudi J Biol Sci 16:101–107
Allakhverdiev SI, Sakamoto A, Nishiyama Y et al (2000) Ionic and osmotic effects of NaCl induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiol 123:1047–1056
Arshad M, Frankenberger WT Jr (2002) Ethylene: agricultural sources and applications. Kluwer Academic Publishers, New York, p 342
Ashraf M (2002) Salt tolerance of cotton: some new advances. Crit Rev Plant Sci 21:1–30
Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. Flora 199:361–376
Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16
Ashraf M, Hasnain S, Berge O et al (2004) Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40:157–162
Ashraf M, Hassan S, Hussain F (2005) Exo-polysaccharides (EPS) producing bacteria in improving physic-chemical characteristics of the salt-affected soil. In: Iftikhar AR et al (eds) Proc int conf environmentally sustainable development (ESDew-2005), Abbottabad, Pakistan. COMSAT Institute of Information Technology, Abbottabad. 2005
Ashraf M, Hasnain S, Berge O (2006) Effect of exo-polysaccharides producing bacterial inoculation on growth of roots of wheat (*Triticum aestivum* L.) plants grown in a salt-affected soil. Int J Environ Sci Technol 3:43–51
Ayers RS, Westcot DW (1985) Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome
Azarbada H, Straalen NMV, Laskowskia R et al (2016) Susceptibility to additional stressors in metal-tolerant soil microbial communities from two pollution gradients. Appl Soil Ecol 98:233–242
Azarmi F, Mozafari V, Dahaji PA et al (2016) Biochemical, physiological and antioxidant enzymatic activity responses of pistachio seedlings treated with plant growth promoting rhizobacteria and Zn to salinity stress. Acta Physiol Plant 38:21
Baath E, Diaz-Ravina M, Bakken LR (2005) Microbial biomass, community structure and metal tolerance of a naturally Pb-enriched forest soil. Microb Ecol 50:496–505
Baniaghil N, Arzanesh MH, Ghorbanli M et al (2013) The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. J Appl Environ Biol Sci 3:17–27
Bano A, Fatima M (2009) Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. Biol Fertil Soils 45:405–413
Barea JM, Pozo M, Azcon R et al (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778
Batool R, Hasnain S (2005) Growth stimulatory effects of *Enterobacter* and *Serratia* isolated from biofilms on plant growth and soil aggregation. Biotechnology 4:347–353
Beresford Q, Bekle H, Phillips H et al (2001) The salinity crisis: landscapes, communities and politics. University of Western Australia Press, Crawley
Bhatnagar M, Bhatnagar A (2001) Biotechnological potential of desert algae. In: Trivedi PC (ed) Algal biotechnology. Pointer Publ, Jaipur, pp 338–356
Blaylock AD (1994) Soil salinity, salt tolerance and growth potential of horticultural and landscape plants. Co-operative Extension Service, University of Wyoming, Department of Plant, Soil and Insect Sciences, College of Agriculture, Laramie
Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32:191–222
Breedveld MW, Miller KJ (1994) Cyclic b-glucans of members of the family *Rhizobiaceae*. Microbiol Rev 58:145–161
Breedveld MW, Miller KJ (1995) Synthesis of glycerophosphorylated cyclic (1,2)-b-glucans in *Rhizobium meliloti* strain 1021 after osmotic shock. Microbiology 141:583–588

Bresler E, Dagan G, Hanks RJ (1982) Statistical analysis of crop yield under controlled line-source irrigation. Soil Sci Soc Am J 46:841–847

Bridgman H, Dragovish D, Dodson J (eds) (2008) The Australian physical environment. Oxford University Press, South Melbourne

Cao Y, Tian Y, Gao L et al (2016) Attenuating the negative effects of irrigation with saline water on cucumber (*Cucumis sativus* L.) by application of straw biological-reactor. Agric Water Manag 163:169–179

Chen M, Wei H, Cao J et al (2007) Expression of *Bacillus subtilis* proAB genes and reduction of feedback inhibition of proline synthesis increases proline production and confers osmotolerance in transgenic *Arabidopsis*. J Biochem Mol Biol 40:396–403

Chinnusamy V, Zhu J, Zhu et al (2006) Gene regulation during cold acclimation in plants. Physiol Plant 126:52–61

Chookietwattana K, Maneewan K (2012) Selection of efficient salt-tolerant bacteria containing ACC deaminase for promotion of tomato growth under salinity stress. Soil Environ 31:30–36

Creus CM, Sueldo RJ, Barassi CA (1997) Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. Plant Physiol Biochem 35:939–944

Da Costa MS, Santos H, Gallinski EA (1998) An overview of the role and diversity of compatible solutes in Bacteria and Archaea. Adv Biochem Eng Biotechnol 61:117–153

Dardanelli MS, Cordoba FJF, Espuny MR et al (2008) Effect of *Azospirillum brasilense* coinoculated with *Rhizobium* on *Phaseolus vulgaris* flavonoids and nod factor production under salt stress. Soil Biol Biochem 40:2713–2721

Davey ME, Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867

Esringua A, Kaynarb D, Turanc M et al (2016) Ameliorative effect of humic acid and plant growth-promoting rhizobacteria (PGPR) on hungarian vetch plants under salinity stress. Commun Soil Sci Plant Anal 47:602–618

Fan P, Chen D, He Y et al (2016) Alleviating salt stress in tomato seedlings using *Arthrobacter* and *Bacillus megaterium* isolated from the rhizosphere of wild plants grown on saline-alkaline lands. Int J Phytoremediation 18:1113–1121

FAO (2009) High level expert forum – how to feed the world in 2050. Economic and Social Development Department, Food and Agricultural Organization of the United Nations, Rome

Garcia-Fraile P, Menendez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng 2:183–205

Geremia RA, Cavaignac S, Zorreguieta A et al (1987) A *Rhizobium meliloti* mutant that forms ineffective pseudonodules in alfalfa produces exopolysaccharide but fails to form b-(1,2)-glucan. J Bacteriol 169:880–884

Ghafoor A, Muhammed S, Rauf A (1985) Field studies on the reclamation of the Gandhra saline-sodic soil. Pak J Agric Sci 22:154–162

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentration by plant growth promoting bacteria. J Theory Biol 190:63–68

Golpayegani A, Tilebeni HG (2011) Effect of biological fertilizers on biochemical and physiological parameters of basil (*Ociumum basilicm* L.) medicine plant. Am Eurasian J Agric Environ Sci 11:445–450

Grover M, Ali SZ, Sandhya V et al (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240

Guy CL (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu Rev Plant Physiol Plant Mol Biol 41:187–223

Habib SH, Kausar H, Saud HM (2016) Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ros-scavenging enzymes. Bio Med Res Int. doi.org/10.1155/2016/6284547

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Oxford University Press, Oxford

Hamdali H, Bouizgarne B, Hafidi M (2008) Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Appl Soil Ecol 38:12–19

Han HS, Lee KD (2005) Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res J Agric Biol Sci 1:210–215

Hasanuzzaman M, Fujita M, Islam MN (2009) Performance of four irrigated rice varieties under different levels of salinity stress. Int J Integr Biol 6:85–90

Hasegawa P, Bressan RA, Zhu JK (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499

Hassan AA, Mahgoub SAM (2011) Salt inducible-proteins and conjugal gene transfer of halotolerant staphylococcus isolated from salinity soil. Egypt J Genet Cytol 40:263–280

Haynes RJ, Swift RS (1990) Stability of soil aggregates in relation to organic constituents and soil water content. J Soil Sci 41:73–83

He YH, Peng YJ, Wu ZS (2015) Survivability of *Pseudomonas putida* RS-198 in liquid formulations and evaluation its growth-promoting abilities on cotton. Dang J Anim Plant Sci 3:180–189

Hezayen FF, Younis MAM, Hagaggi NSA (2010) *Oceanobacillus aswanensis* strain FS10 sp. Nov., an extremely halotolerant bacterium isolated from salted fish sauce in Aswan City, Egypt. Glob J Mol Sci 5:1–6

Horneck DA, Ellsworth JW, Hopkins BG (2007) Managing salt-affected soils for crop production. A Pacific Northwest Extension Publication. Oregon State University, Corvallis

Hossain MM, Das KC, Yesmin S et al (2016) Effect of plant growth promoting rhizobacteria (PGPR) in seed germination and root-shoot development of chickpea (*Cicer arietinum* L.) under different salinity condition. Res Agric Livest Fish 3:105–113

Hua SST, Tsai VY, Lichens MGM et al (1982) Accumulation of amino acids in *Rhizobium* sp. strain wr1001 in response to sodium chloride salinity. Appl Environ Microbiol 44:135–140

Hulsebusch C, Wichern F, Hemann H et al (2007) Organic agriculture in the tropics and subtropics-status and perspectives, Supplement No. 89 to the Journal of Agriculture and Rural Development in the Tropics and Subtropics. Kassel University Press, Kassel

Hussain MB, Mehboob I, Zahir ZA et al (2009) Potential of *Rhizobium* spp. for improving growth and yield of rice. Soil Environ 28:49–55

Hussain MB, Zahir ZA, Asghar HN et al (2014a) Can catalase and EPS producing rhizobia ameliorate drought in wheat. Int J Agric Biol 16:3–13

Hussain MB, Zahir ZA, Asghar HN et al (2014b) Scrutinizing rhizobia to rescue maize growth under reduced water conditions. Soil Sci Soc Am J 78:538–545

Hussain MB, Zahir ZA, Asghar HN et al (2016) Efficacy of rhizobia for improving photosynthesis, productivity and mineral nutrition of maize. Clean Soil Air Water 44:1–8

Ilyas N, Bano A, Iqbal S (2012) Physiological, biochemical and molecular characterization of *Azospirillum* spp. isolated from maize under water stress. Pak J Bot 44:71–80

Ingram-smith C, Miller KJ (1998) Effects of ionic and osmotic strength on the glucosyltransferase of *Rhizobium meliloti* responsible for cyclic b-(1,2)-glucan biosynthesis. Appl Environ Microbiol 64:1290–1297

Jenkins MB, Virginia RA, Jarrel WM (1987) Rhizobial ecology of the woody legume mesquite (*Prosopis glandulosa*) in the Sonoran desert. Appl Environ Microbiol 33:36–40

Jha CK, Saraf M (2015) Plant growth promoting Rhizobacteria (PGPR): a review. J Agric Res Dev 5:108–119

Jha Y, Subramanian RB (2014) PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. Physiol Mol Biol Plants 20:201–207

Jha Y, Subramanian RB, Patel S (2011) Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiol Plant 33:797–802

Jittawuttipoka T, Planchon M, Spalla O et al (2013) Multidisciplinary evidences that *Synechocystis* PCC6803 exopolysaccharides operate in cell sedimentation and protection against salt and metal stresses. PLoS One 8:e55564

Joshi P, Bhatt AB (2011) Diversity and function of plant growth promoting rhizobacteria associated with wheat rhizosphere in North Himalayan region. Int J Environ Sci 1:1135–1143

Jouyban Z (2012) The effects of salt stress on plant growth. Tech J Eng Appl Sci 2:7–10
Kalinowski BE, Liermann LJ, Brantley SL et al (2000) X-ray photoelectron evidence for bacteria-enhanced dissolution of hornblende. Geochim Cosmochim Acta 64:1331–1343
Karabal E, Yucel M, Oktem HA (2003) Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci 164:925–933
Kiani MZ, Sultan T, Ali A et al (2016) Application of ACC-deaminase containing PGPR improves sunflower yield under natural salinity stress. Pak J Bot 1:53–56
Kim JT, Kim SD (2008) Suppression of bacterial wilt with *Bacillus subtilis* SKU48-2 strain. Korean J Microbiol Biotechnol 36:115–120
Kloepper JW, Lifshitz R, Zablotwicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43
Koca M, Bor M, Ozdemir F (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp Bot 60:344–351
Kohler J, Caravaca F, Roldan A (2010) An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*. Soil Biol Biochem 42:429–434
Kraemer SM (2004) Iron oxide dissolution and solubility in the presence of siderophores. Aquat Sci 66:3–18
Lewandowski Z (2000) In: Evans LV (ed) Biofilms: recent advances in their study and control. Harwood Academic Publishers, Amsterdam., 2000, pp 1–17
Li P, Song A, Li Z et al (2008) Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.) Plant Soil 354:407–419
Li HW, Zang BS, Deng XW et al (2011) Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. Planta 234:1007–1018
Liaqat I, Sumbal F, Sabri AN (2009) Tetracycline and chloramphenicol efficiency against selected biofilm forming bacteria. Curr Microbiol 59:212–220
Liu X, Luo Y, Mohamed OA et al (2014) Global transcriptome analysis of *Mesorhizobium alhagi* CCNWXJ12-2 under salt stress. BMC Microbiol. doi:10.1186/s12866-014-0319-y
Mahmood A, Turgay OC, Farooq M et al (2016) Seed biopriming with plant growth promoting rhizobacteria: a review. FEMS Microbiol Ecol 92:fiw112
Manchanda G, Garg N (2008) Salinity and its effects on the functional biology of legumes. Acta Physiol Plant 30:595–618
Marino R, Ponnaiah M, Krajewski P et al (2009) Addressing drought tolerance in maize by transcriptional profiling and mapping. Mol Gen Genomics 218:163–179
Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530
McDowell RW (ed) (2008) Environmental impacts of pasture-based farming. CAB International, Oxfordshire
Metwali EMR, Abdelmoneim TS, Bakheit MA (2015) Alleviation of salinity stress in Faba bean (*Vicia faba* L.) plants by inoculation with plant growth promoting rhizobacteria (PGPR). Plant Omics J 8:449–460
Miransaria M, Smith DL (2009) Alleviating salt stress on soybean (*Glycine max* L. Merr.) *Bradyrhizobium japonicum* symbiosis, using signal molecule genistein. Eur J Soil Biol 45:146–152
Moral AD, Prado B, Quesda E et al (1988) Numerical taxonomy of moderately halophilic Gram negative rods from an inland saltern. J Gen Microbiol 134:733–741
Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. Plant Physiol 100:620–630
Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250
Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663
Munns R, Rawson HM (1999) Effect of salinity on salt accumulation and reproductive development in the apical meristem of wheat and barley. Aust J Plant Physiol 26:459–464
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Munns R, Husain S, Rivelli AR et al (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant Soil 247:93–105
Nadeem SM, Zahir ZA, Naveed M et al (2009) Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Can J Microbiol 55:1302–1309
Nadeem SM, Zahir ZA, Naveed M, Nawaz S (2013) Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions. Ann Microbiol 63(1):225–232
Nadeem SM, Ahmad M, Naveed M et al (2016) Relationship between in vitro characterization and comparative efficacy of plant growth-promoting rhizobacteria for improving cucumber salt tolerance. Arch Microbiol 198:379–387
Naveed M, Hussain MB, Zahir ZA et al (2014a) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73:121–131
Naveed M, Mitter B, Thomas G et al (2014b) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD 17. Environ Exp Bot 97:30–39
Naz I, Bano A, Tamoor-ul-Hassan (2009) Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. Afr J Biotechnol 8:5762–5766
Netondo GW, Onyango JC, Beck E (2004) Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci 44:806–811
Nia SH, Zarea MJ, Rejali F et al (2012) Yield and yield components of wheat as affected by salinity and inoculation with *Azospirillum* strains from saline or non-saline soil. J Saudi Soc Agric Sci 11:113–121
Nicolaus B, Manca M, Lama L et al (2001) Lipid modulation by environmental stresses in two models of extremophiles isolated from Antarctica. Polar Biol 24:1–8
Nishma KS, Adrisyanti B, Anusha SH et al (2014) Induced growth promotion under in vitro salt stress tolerance on *Solanum lycopersicum* by *Fluorescent pseudomonads* associated with rhizosphere. Int J Appl Sci Eng Res 3:422–430
Oster JD, Shainberg I, Abrol IP (1996) Reclamation of salt-affected soil. In: Agassi M (ed) Soil erosion, conservation, and rehabilitation, vol 414. Marcel Dekker, New York, pp 315–352
Palaniyandi SA, Damodharan K, Yang SH et al (2014) *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of 'Micro Tom' tomato plants. J Appl Microbiol 117:766–773
Pandit A, Rai V, Bal S et al (2010) Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.) Mol Gen Genomics 284:121–136
Parida AK, Das AB (2005) Salt tolerance and salinity effect on plants: a review. Ecotoxicol Environ Saf 60:324–349
Parvaiz A, Satyawati S (2008) Salt stress and phytobiochemical responses of plants: a review. Plant Soil Environ 54:89–99
Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801
Paul D, Nair S (2008) Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. J Basic Microbiol 48:378–384
Pereira S, Zille A, Micheletti E et al (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941
Pratt LA, Kolter R (1998) Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 30:285–293
Qadir M, Schubert S (2002) Degradation processes and nutrient constraints in sodic soils. Land Degrad Dev 13:275–294
Qadir M, Quillerou, Nangia V et al (2014) Economics of salt-induced land degradation and restoration. Nat Res Forum 38:282–295

Qurashi AW, Sabri AN (2011) Osmoadaptation and plant growth promotion by salt tolerant bacteria under salt stress. Afr J Microbiol Res 5:3546–3554
Qurashi AW, Sabri AN (2012) Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. Braz J Microbiol:1183–1191. ISSN 1517-8382
Qurashi AW, Sabri AN (2016) Induction of Osmotolerance by *Staphylococcus sciuri* HP3 in Lens esculenta Var. Masoor 93 under NaCl stress. Pak J Life Soc Sci 14:42–51
Rajput L, Imran A, Mubeen F et al (2013) Salt-tolerant PGPR strain *Planococcus rifietoensis* promotes the growth and yield of wheat (*Triticum aestivum* L.) cultivated in saline soil. Pak J Bot 45:1955–1962
Ramadoss D, Lakkineni VK, Bose P (2013) Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springer Plus 2:1–7
Reddy PS, Thirulogachandar V, Vaishnavi CS et al (2011) Molecular characterization and expression of a gene encoding cytosolic Hsp90 from *Pennisetum glaucum* and its role in abiotic stress adaptation. Gene 474:29–38
Rekha PD, Lai WA, Arun AB et al (2007) Effect of free and encapsulated *Pseudomonas putida* CC-FR2-4 and *Bacillus subtilis* CC-pg104 on plant growth under gnotobiotic conditions. Bioresour Technol 98:447–451
Roohi A, Ahmed I, Iqbal M et al (2012) Preliminary isolation and characterization of halotolerant and halophilic bacteria from salt mines of Karak. Pakistan. Pak J Bot 44:365–370
Rubiano-Labrador C, Bland C, Miotello G (2015) Salt stress induced changes in the exoproteome of the halotolerant bacterium *Tistlia consotensis* deciphered by proteogenomics. PLoS One. doi:10.1371/journal.pone.0135065
Sahi C, Singh A, Kumar K et al (2006) Salt stress response in rice: genetics, molecular biology, and comparative genomics. Funct Integr Genomics 6:263–284
Sairam RK, Tyagi A (2004) Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci 86:407–421
Salta M, Warton JA, Blache Y et al (2013) Marine biofilms on artificial surfaces: structure and dynamics. Environ Microbiol 15:2879–2893
Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10:297–304
Sandhya V, Ali SZ, Grover M et al (2009) Alleviation of drought stress effects in sunflower seedlings by exopolysaccharides producing *Pseudomonas putida* strain P45. Biol Fertil Soils 46:17–26
Santos H, da Costa MS (2002) Compatible solutes of organisms that live in hot saline environments. Environ Microbiol 4:501–509
Santoyoa G, Hagelsiebb GM, Mosquedac MDCO et al (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99
Scotter DR (1978) Preferential solute movement through larger soil voids. I. Some computations using simple theory. Soil Res 16:257–267
See-Too WS, Convey P, Pearce DA et al (2016) Complete genome of *Planococcus rifietoensis* M8T, a halotolerant and potentially plant growth promoting bacterium. J Biotechnol 221:114–115
Sen S, Chandrasekhar CN (2015) Effect of PGPR on enzymatic activities of rice (*Oryza sativa* L.) under salt stress. Asian J Plant Sci Res 5:44–48
Shainberg I, Letey J (1984) Response of soils to sodic and saline conditions. Hilgardia 52:1–57
Shanker AK, Venkateswarlu B (2011) Abiotic stress in plants–mechanisms and adaptations. In Tech, Rijeka, p ix
Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131
Singh RP, Jha PN (2016) Alleviation of salinity-induced damage on wheat plant by an ACC deaminase-producing halophilic bacterium *Serratia* sp. SL-12 isolated from a salt lake. Symbiosis 69:101–111

Singh RP, Jha P, Jha PN (2015) The plant-growth-promoting bacterium *Klebsiella* sp. SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. J Plant Physiol 184:57–67

Song JQ, Fujiyama H (1996) Ameliorative effects of potassium on rice and tomato subjected to sodium salinization. Soil Sci Plant Nutr 42:493–501

Southard RJ, Buol SW (1988) Subsoil saturated hydraulic conductivity in relation to soil properties in the North Carolina Coastal Plain. Soil Sci Soc Am J 52:1091–1094

Stajner D, Kevresan S, Gasic O et al (1997) Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. Biol Plant 39:441–445

Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9

Tester M, Davenport R (2003) Na^+ tolerance and Na+ transport in higher plants. Ann Bot 91:503–527

Tiwari S, Singh P, Tiwari R et al (2011) Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. Biol Fertil Soils 47:907–916

Turan S, Cornish K, Kumar S (2012) Salinity tolerance in plants: breeding and genetic engineering. AJCS 6:1337–1348

Upadhyay SK, Singh DP, Saikia R (2009) Genetic diversity of plant growth promoting rhizobacteria isolated from rhizosphere soils of wheat under saline condition. Curr Microbiol 59:489–496

Valko M, Rhodes CJ, Moncol J et al (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40

Van BF, Vranova E, Dat JF et al (2001) The role of active oxygen species in plant signal transduction. Plant Sci 161:405–414

Vassileva M, Azcon R, Barea JM et al (1999) Effect of encapsulated cells of *Enterobacter* sp. on plant growth and phosphate uptake. Bioresour Technol 67:229–232

Vejan P, Abdullah R, Khadiran T et al (2016) Role of plant growth promoting rhizobacteria in agricultural sustainability-a review. Molecules 21:1–17

Ventosa A, Ramose A, Kocur M (1983) Moderately halophilic gram-positive cocci from hypersaline environment. Syst Appl Microbiol 4:564–570

Volker U, Engelmann S, Maul B et al (1994) Analysis of the induction of general stress proteins of *Bacillus subtilis*. Microbiology 140:741–752

Walia H, Wilson C, Zeng L et al (2007) Genome-wide transcriptional analysis of salinity stressed japonica and indica rice genotypes during panicle initiation stage. Plant Mol Biol 63:609–623

Wang SY, Wang CY, Welburn AR (1990) Role of ethylene under stress conditions. In: Alscher R, Cumming J (eds) Stress responses in plants adaptation and acclimation mechanisms. Wiley-Liss, New York, pp 147–173

Wang Q, Dodd IC, Belimov AA et al (2016) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting Na^+ accumulation. Funct Plant Biol 43:161–172

Wu ZS, Zhao YF, Kaleem I et al (2011) Preparation of calcium-alginate microcapsuled microbial fertilizer coating *Klebsiella oxytoca* Rs-5 and its performance under salinity stress. Eur J Soil Biol 47:152–159

Wu ZS, Guo LN, Qin SH et al (2012) Encapsulation of *R. planticola* Rs-2 from alginate-starch-bentonite and its controlled release and swelling behavior under simulated soil conditions. J Ind Microbiol Biotechnol 39:317–327

Wu Z, Peng Y, Guo L et al (2014) Root colonization of encapsulated *Klebsiella oxytoca* Rs-5 on cotton plants and its promoting growth performance under salinity stress. Eur J Soil Biol 60:81–87

Yang J, Kloepper, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yao L, Wu Z, Zheng Y et al (2010) Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. Eur J Soil Biol 46:49–54

Yuan-yuan Z, Hai-tao Y, Zai-qiang S et al (2008) Physiochemical characters and ability to promote cotton germination of bacteria strains Rs-5 and Rs-198 under salt stress. Sci Agric Sin 41:1326–1332

Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Zhang JH, Liu YP, Pan QH et al (2006) Changes in membrane associated H^+ − ATPase activities and amounts in young grape plants during the cross adaptation to temperature stresses. Plant Sci 170:768–777

Zhang H, Kim MS, Sun Y et al (2008) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mol Plant-Microbe Interact 21:737–744

Zheng X, Chen B, Lu G et al (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem Biophys Res Commun 379:985–989

Zhong-hong W, Ma J et al (2009) Identification of salt tolerant promoting growth bacteria Rs-198 and study on co-culture with Rs-5. Biotechnology 19:63–66

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Bol 53:247–273

Zou N, Dort PJ, Marcar NE (1995) Interaction of salinity and rhizobial strains on growth and N_2 fixation by *Acacia ampliceps*. Soil Biol Biochem 27:409–413

Improvement of Soilborne Pests Control with Agronomical Practices Exploiting the Interaction of Entomophagous Fungi

29

E. Malusá, L. Canfora, F. Pinzari, M. Tartanus, and B.H. Łabanowska

Abstract

The application of biological control agents (BCAs) is considered as an effective alternative for pest control. However, factors such as the formulation of the product, whose quality can affect the inoculant viability and persistence in soil, the stabilisation of the biocontrol effect under field conditions and the influence of agronomical practices as well as of the environmental conditions (weather and soil) are hampering a wider use of BCAs. After a brief review of these factors, we present some results concerning agronomical and ecological aspects from a case study carried out using different entomopathogenic fungi on organic strawberry plantations, which underline the possibility of improving BCAs efficacy, particularly when integrated into a more general strategy of pest control.

Keywords

Biocontrol • Pests • Entomophagous fungi • Soilborne • Agronomy

29.1 Introduction

Biological control of arthropods was defined as 'the study and uses of parasites, predators and pathogens for the regulation of host (pest) densities' (DeBach 1964). Even though a number of important crops' pests can be kept at a low population density by biological control over long periods of time, a more efficient approach is that of integrated pest management (IPM; Stern et al. 1959) where additional

E. Malusá (✉) • M. Tartanus • B.H. Łabanowska
Research Institute of Horticulture, Skierniewice, Poland
e-mail: malusa@inrete.it

L. Canfora • F. Pinzari
CREA-Research Center Agriculture and Environment, Rome, Italy

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_29

agronomical methods, including resistant plants, cultural techniques, physical barriers and semiochemicals, are applied to achieve an adequate level of control.

Fungal entomopathogens have been known as important mortality factors of insects for more than 100 years and are employed in pest biocontrol strategies throughout the world (Vega et al. 2009). Over 170 products have been developed based on at least 12 species of these fungi and are used as inundative biological control agents of insects, mites and ticks (de Faria and Wraight 2007).

In the last decade, the application of biological control agents (BCAs) has started to be considered as an effective alternative for pest control (Jackson et al. 2010; Mazid et al. 2011). Different microorganisms, belonging to several species of bacteria and fungi, are being used as inoculants (Malusá et al. 2016). However, the utilisation of BCAs in agricultural practice is still hampered by factors such as the formulation of the product, whose quality can affect the inoculant viability and persistence in soil, the stabilisation of the biocontrol effect under field conditions, the use of single BCA strains and the development of appropriate methods for risk assessment studies including tracing the inoculant in the environment (Berg et al. 2013; Canfora et al. 2016).

The impact of 'inoculative' BCAs is conditioned by several factors, particularly agronomical practices, the environmental conditions (weather and soil) as well as the plant holobiome (Bordenstein and Theis 2015). For example, DGGE and pyrosequencing analyses revealed significant modifications of bacterial community composition of lettuce rhizosphere following application of the BCA *P. jessenii* RU47 in alluvial loam, but not in sand or loess loam (Schreiter et al. 2014).These effects were much less distinct in comparison with the influence of soil types: different taxonomic groups responded to the BCA application depending on the soil type, particularly in alluvial loam. It is thus deemed important to devise methods which can be sensitive enough to detect the changes after BCA application on the microbiome to assess their impact on the environment. The development and use of next-generation molecular methods for the characterisation of soil microbial diversity and structure is expected to enable a more rapid and thorough assessment of the impact of 'inoculative BCA' (Trabelsi and Mhamdi 2013; Schwieger and Tebbe 2000; Hirsch et al. 2010; Canfora et al. 2015). However, the same techniques can be used to assess the persistence of a single strain or that of a consortium of the applied BCA.

The application of BCA consortia has been considered in order to gain a better control of plant pests and diseases (Chandler et al. 1993; Inglis et al. 1997, 1999). For instance, the combined application of *Beauveria bassiana* and *Metarhizium flavoviride* overcame some of the temperature constraints encountered in the use of entomopathogenic Hyphomycetes to control grasshoppers' populations, especially in countries where temperatures fluctuate or are high for a significant period of time (Inglis et al. 1997). However, particularly in the case of microorganisms showing different mechanisms of action or growth behaviours, it is important to analyse their compatibility when co-inoculated.

Furthermore, considering that the penetration and lysis of the host insect cell wall has been demonstrated to be an important step in the mycoparasitic attack

(Fang et al. 2005), in order to enhance the overall efficacy of BCA consortia, it could be important to evaluate the effect of the combined inoculum on the chitinolytic activities of the strains. Indeed, the improvement of entomopathogenic fungi virulence can be achieved by understanding mechanisms of pathogenesis and virulence factors. Chitinolytic genes, encoding chitinases and b-N-acetylglucosaminidases, represent markers for fungal activity against host insects (Fan et al. 2007; Sahai and Manocha 1993). Since chitin is often used in commercial formulations to enhance conidia production and fungal virulence (Gupta et al. 1994; Boldo et al. 2009), the role of chitin and other compounds, as well as the use of mixed inocula in triggering or depressing the eso- and endo-chitinolytic activity of biocontrol fungi, could be a method to assess the potential efficacy of new BCA strains or consortia.

29.2 Evaluation of Entomopathogenic Fungi

May beetle *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) is an important soilborne pest damaging different crops in several European countries (Dolci et al. 2006; Łabanowska and Olszak 2003; Łabanowska and Bednarek 2011; Mayerhofer et al. 2015). In several European countries, the entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Beauveria brongniartii* (Saccardo) Petch (De Hoog 1972) are used for specifically controlling soilborne pests, particularly *M. melolontha* and other pests such as strawberry root weevil, *Otiorhynchus sulcatus*, and wireworms (Elateridae), since no chemical active substances are registered for such purpose in fruit crops. Therefore, we have been looking for alternative methods to control these pests, mainly based on the use of BCAs. Several trials have been carried out under different soil conditions and in association with diverse agronomical practices. Two trials are considered in the following headings, both carried on strawberry cv. Polka plantations conducted according to organic farming practices in Poland. Trial A (location Nowa Wola) was established in spring 2014, on a 2-year-old plantation which experienced up to 50% damage from *M. melolontha* larvae; the BCAs were applied twice during the 2014 and 2015 growing seasons with a total dose of 7.5 and 10 $kg \cdot ha^{-1}$, respectively. The field of trial B (location Brzostówka) was treated in August 2014 with soil steam disinfection (Tesi et al. 2007) and the plantation established in late summer applying the BCA at planting and once more before the end of the growing season, as well as twice in 2015, with the same doses as above.

29.2.1 Efficacy Under Different Soil Conditions and Agronomical Practices

The efficacy of BCAs was checked by evaluating the number of damaged plants carried out at the end of the 2014 season and at the beginning and end of the following season.

Table 29.1 Effect of soil inoculation with *B. bassiana* and *B. brongniartii* on the number of strawberry plants cv. Polka damaged by *M. melolontha*

Treatment	Damaged plants (%)		
	September 2014	June 2015	October 2015
Trial A (Nowa Wola)			
Control	22.5 a	7.2 a	29.0 b
B. bassiana	15.0 a	2.2 a	12.7 a
B .brongniartii	22.7 a	2.2 a	13.7 a
Trial B (Brzostòwka)			
Control	21,5 b	5,2 a	29,5 b
B. bassiana	8,5 a	1,5 a	13,7 a
B. brongniartii	11,7 a	2,0 a	13,7 a

Both trials showed that the two entomopathogenic fungi require a certain period of time and several applications to pursue a substantial effect in controlling the pest (Table 29.1). After the first assessment, in September 2014, a very low efficacy was noted in trial A; however, a significant reduction of plant damage was recorded in trial B. Such outcome could support the hypothesis of increasing the efficacy of BCA treatment through the creation of favourable soil microbiological conditions: the reduction of population pressure from autochthonous microorganisms and of fungistasis (Pereira et al. 1993), as obtained by the soil steaming, would prompt a faster development of the inoculated strains. A rapid increase in *Trichoderma* spp. and other bacteria and fungi was indeed recorded after soil steaming (Triolo et al. 2004; Meszka et al. 2014).

The effect of the treatment was maintained also at the beginning of the following season, though not statistically significant. However, at the end of the 2015 growing season, the percentage of damaged plants in the plots treated with BCAs was significantly reduced in comparison to the not treated control. The plant damage reduction was between 43% and 62% compared to the control plants, depending on the BCA used: the highest efficacy was recorded for *B. bassiana*.

Efficacy of BCAs incorporated into the soil depends on several ecological factors that are difficult to be controlled. Soil texture, temperature and moisture are the most relevant among those related to the soil environment (Jaronski 2007; Kabaluk et al. 2007). Beside microbial flora, also microfauna can affect the efficacy of entomopathogenic fungi (Jaronski 2010 and references therein). Therefore, considering the very high level of initial infestation by *M. melolontha*, the reduction in damaged plants achieved in only two seasons has been deemed a very good result by the farmers themselves; producing under organic management has indeed taught them that re-equilibrating the agro-environment requires time and continuous application of BCAs.

Table 29.2 Average number of microbial operational taxonomic units (OTUs) and diversity index (H′) of microbial populations of the soil from the two trials

Trial A (Nowa Wola)				
Treatments	Bacteria		Fungi	
Bulk soil	OTU number	H′ index	OTU number	H′ index
Control	9	1.9	11	1.1
B. bassiana	14	1.3	30	2.0
B. brongniartii	37	2.1	20	1.8
Root zone soil				
Control	21	1.3	14	1.6
B. bassiana	15	1.3	29	1.9
B. brongniartii	35	2.1	17	1.8
Trial B (Brzostòwka)				
Treatments	Bacteria		Fungi	
Not steamed	OTU number	H′ index	OTU number	H′ index
Control	19	2.0	16	1.8
B. bassiana	23	1.9	23	2.6
B. brongniartii	5	1.1	11	0.9
Steamed				
Control	5	0.8	15	1.9
B. bassiana	11	1.2	4	0.6
B. brongniartii	21	1.9	6	5.0

Soil samples were collected from the root zone or the bulk soil (trial A) or only from the root zone (trial B) from the field where the soil was treated before the application of BCAs with stem disinfection or not steamed

29.2.2 Effect Entomopathogenic Fungi on Soil Bacterial and Fungal Communities

To compare the effect of the treatments on the soil microbial population, a molecular analysis using two sequences specific for bacteria and fungi from the genes encoding 16S rRNA and ITS region was carried out on samples of soil from the two trials. Table 29.2 showed data obtained in T-RFLP analysis of fungal and bacterial operational taxonomic unit (OTU) number. The application of the two BCAs induced a general increase in the bacterial and fungal OTU number both in the bulk and root zone soil (Table 29.2 – trial A). The only exception to this trend was for the bacteria in the root zone soil treated with *B. bassiana*. It is worthy to note that while the inoculation with *B. bassiana* induced a higher increase of bacterial OTUs in comparison to *B. brongniartii*, the latter induced a higher number of OTUs in the fungal population. The diversity H′ index for both bacterial and fungal populations was always increased by *B. brongniartii*, while *B. bassiana* had a small impact in case of bacterial, high for fungal populations. It is interesting to underline the impact of the plant on the behaviour of the soil microbial populations. The root zone had, as expected, a higher number of OTUs and H′ index in comparison to the bulk soil, which balanced, to a certain degree, the effect of the two BCAs, differentiating their

impact on bacterial and fungal populations. These differences could be ascribed to the different behaviours of the two entomopathogenic fungi (Vega et al. 2009). The ability of endophytic development of *B. bassiana* (Vidal 2015) could also be a feature inducing the observed differences in soil bacterial and fungal populations after its application. Nevertheless, the recent finding that *B. brongniartii* can also function as plant growth promoter (Jaber and Enkerli 2016) could also account for its impact on bacterial populations.

When considering the application of BCAs under different soil management conditions (Table 29.2 – trial B), it should be underlined that the effect of soil steaming, an agronomical practice to control soilborne pathogen alternative to chemical fumigation (Tesi et al. 2007), has resulted into a strong reduction of bacterial OTUs, and consequently on the H′ diversity index, but to a limited reduction of fungal population. This confirms the outcomes of previous studies on the overall effect of this method on soil microbiology (Triolo et al. 2004). The successive application of the two BCAs impacted in a different way on the two kinds of microbial populations. *B. bassiana* induced an increase of bacterial and fungal OTUs in not-steamed soil and doubled the genetic diversity, while only the bacteria population was modified in the soil previously treated with the steam. On the contrary, the application of *B. brongniartii* induced a decrease in both kinds of OTUs in not-steamed soil while increased bacterial OTUs and decreased fungal OTUs in the steam-disinfected soil.

Monitoring of the soil microbial genetic diversity for both trials in the following season, after additional application of the BCAs, has shown no negative impact on OTUs number and H′ diversity index, even when the two entomopathogenic fungi were co-inoculated (Tartanus et al. 2016).

The monitoring of the structure and diversity of soil microbial communities is a key task to comply with ecotoxicological requirements of the registration process. Indeed, considering that the soil microbial biodiversity is pivotal and crucial for crop productivity, the structure and diversity of soil microorganisms are important indicators of soil health and fertility (Sparling 1997; Yao et al. 2000; Canfora et al. 2015). Consequently, changes in structure and diversity of fungal and bacterial communities may indicate changes in soil quality and functions. Cultivation-independent monitoring analyses offer a feasible approach supporting risk assessment of microbial biological control agent.

The application of the two BCAs in different soil conditions, where different cultivars were grown and different agronomical practices (steam fumigation) were applied, showed that the control strategy did not negatively affect soil fungal and bacterial communities. Schwarzenbach et al. (2009) in a microcosm study reported significant changes in the soil fungal communities for treatments that contained BCA but showed a smaller and transient effect in comparison with chemical control agent. Hirsch et al. (2013) in a study performed to evaluate the potential effect of a treatment with *B. bassiana* on the diversity of soil fungal communities in an agricultural field in India showed that the overall fungal diversity was not influenced by application of BCA during the 7 weeks of investigation. Registration authorities in the European Union require information on long-term nontarget effects on soil quality, such as effects on native soil microbial communities which may face a potential

Table 29.3 Average values of 490 nm optical density after 176 h of incubation of the substrates that among the 95 tested triggered the respiration of the co-inoculum of the two strains in comparison to single strain

	BA	BR	BA+BR
G10 L-asparagine	1.133	0.421	2.276
B4 i-erythritol	0.481	0.538	1.879
H6 L-serine	1.768	1.470	1.878
D3 D-melezitose	0.815	0.149	1.874
B5 D-fructose	1.601	0.661	1.708
D9 palatinose	0.287	0.225	0.346

The data are all significant for $p<0.05$ (ANOVA and post hoc test)

competition by the BCA. Our long-term study in Poland showed that the overall fungal and bacterial genetic diversity was only transiently influenced by application of BCAs during the 2 years of monitoring in two agricultural fields.

29.2.3 Metabolic Behaviour of Single and Co-inoculum

Based on the interest in the potential effect of co-inoculation and nutrition on fungal virulence maintenance under saprotrophic growth conditions, we analysed in vitro the compatibility and degree of niche overlap between *B. bassiana* and *B. brongniartii* on 95 different carbon sources, in order to reveal which substrate could affect or trigger fungal metabolism and co-inoculum success (Read and Taylor 2001). To this aim, we utilised the Biolog® Phenotype MicroArrays™ (PMs) system, which represents an integration to molecular techniques related to gene expression (Bochner 2011; Borglin et al. 2012; Pinzari et al. 2016). The analysis was aimed at (1) evaluating the overall differences in metabolism of the two fungal isolates and (2) determining the carbon sources differently catabolised by the mixed inoculum.

For some C-source, differences were observed between the three inocula and in particular a greater efficiency of the catabolism by the co-inoculum with respect to the single strains (Table 29.3). *B. bassiana* resulted to be more active on all substrates with respect to *B. brongniartii*. The joint inoculum of the two fungal strains showed an overlap with the strain of *B. bassiana*, thus indicating the prevalence of the latter on *B. brongniartii*. The carbon sources that triggered the metabolic activity (respiration) of the joint fungal inoculum were some of the sugars that can have a role in the natural mechanism of infection. This is the case of melezitose, which is a non-reducing trisaccharide that is produced by many plant sap-eating insects. This sugar along with fructose and palatinose (a disaccharide carbohydrate composed of glucose and fructose, naturally present in some plant extracts) can act as attractants for insect larvae in soil and thus has a role in the activation of virulence in entomopathogenic fungi (Hsiao and Khachatourians 1997). Other compounds, like serine, could be involved in stimulating the production of proteases that showed to play a role in the infection process (Xu et al. 2006).

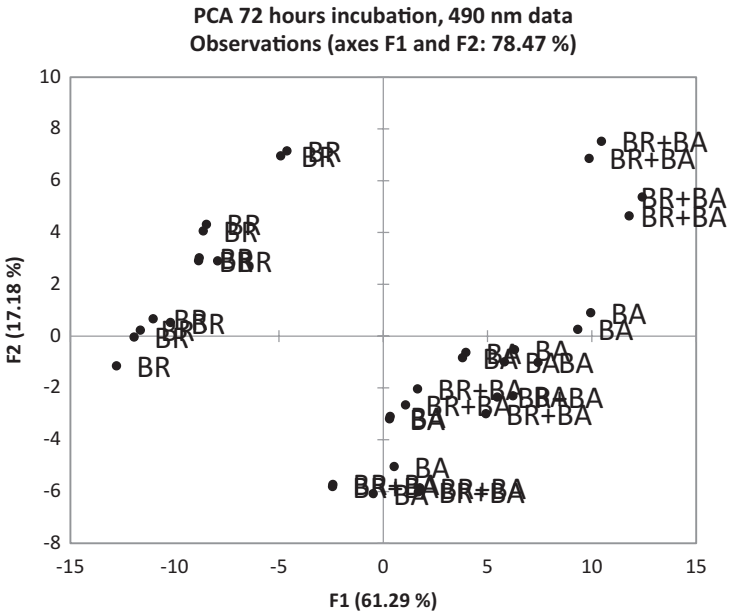


Fig. 29.1 Principal component analysis of respiration data obtained after 72 h of incubation. *BA Beauveria bassiana*, *BR Beauveria brongniartii*, *BA + BR* co-inoculum

Even though some differences were recorded, especially after a long incubation period, the principal component analysis (PCA) of these data showed that the metabolic pattern of *B. bassiana* is very close to that of the co-inoculum, while that of *B. brongniartii* is markedly different from that of the *B. bassiana* (Fig. 29.1). Such behaviour could derive from the possibility that *B. bassiana* has a stronger metabolism when growing as a saprotroph in comparison to *B. brongniartii* and thus overgrow the latter in vitro, on most of the carbon sources. The higher performance of the co-inoculum on some carbon sources suggests a competition or stress-related reaction between the two fungal species that in nature have a different ecological niche, with *B. bassiana*, capable of living free in soil as saprophytic species but also found in a wide host range of nearly 750 insect species (Ghikas et al. 2010) and *B. brongniartii* showing a narrower host specificity, being a selective pathogen of *M. melolontha* (St Leger et al. 1992), and scarcely capable of a saprophytic lifestyle. However, once forced to overlap in vitro (and in the field when artificially co-inoculated), the two fungal species could interfere at the presence of some C-sources and exhibit both a cooperative behaviour and a mutual repression.

29.2.4 In Vitro Chitinolytic Activity of the Single and Co-inoculum

Chitinases are widely distributed in plants, bacteria, fungi, insects and vertebrates (Seidl 2008). They synergistically act with proteases in order to degrade the insect's

Table 29.4 Comparison of different chitinase enzymes' activity (chitobiosidase, b-N-acetylglucosaminidase and endochitinase) when single strains or co-inoculum were grown in vitro with or without chitin

Enzyme	BR	BA	BABR	BR + ch	BA + ch	BABR + ch
Endochitinase	69.42 ± 59.42	31.60 ± 11.60	67.90 ± 47.92	53.52 ± 44.52	17.39 ± 6.50	26.73 ± 12.82
NAGase	**523.77 ± 288.09**	471.75 ± 231.27	165.90 ± 221.93	**88.74 ± 58.02**	300.41 ± 233.05	124.51 ± 95.23
Chitobiosidase	**202.06 ± 122.35**	59.25 ± 107.20	20.28 ± 14.27	**26.04 ± 7.26**	17.86 ± 19.17	17.41 ± 11.09

Labels: *BA B. bassiana*, *BR B. brongniartii*, *BABR* co-inoculum of BA and BR, *ch* grown on agar chitin. Two-sample t-test, *bold* values within each enzymatic activity are significantly different for $p<0.05$. Data are expressed as μM of units of enzymatic activity for each μL of fungal spores' suspension (one unit of chitinase activity released μM of 4-methylumbelliferone from the appropriate substrate at pH 5.0 at 37 °C)

cuticle and are associated to the different stages of entomopathogenic fungi cycles and virulence. The filamentous fungi genome contains between 10 and 25 chitinases for different physiological functions (Li 2006).

Chitinases catalyses the hydrolytic cleavage of the b(1-4)-glycoside bonds present in biopolymers of N-acetylglucosamine, primarily in chitin. The chitinolytic enzymes are categorised based on their enzymatic activity on chitin substrates (Seidl 2008). Endochitinases are the enzymes catalysing the random cleavage at internal points in the chitin chain. Exochitinases catalyse the progressive release of acetylchitobiose or N-acetylglucosamine from the non-reducing end of chitin, thus referred to as chitobiosidase and b-N-acetylglucosaminidase, respectively (Seidl 2008).

The assay used to determine chitinolytic activity of *B. brongniartii* and *B. bassiana* was based on the enzymatic hydrolysis of fluorescent chitinase substrates (Miller et al. 1998). Moreover, the ability of chitin as a growth media to elicit the expression of chitinolytic enzymes in fungal spores was also investigated. Conidia of the two fungal isolates were obtained by cultivation in the dark for 10 days at 25 °C on both Czapek agar and on Chitin-Czapek agar (Czapek medium added with 5% chitin) in separate trials.

Three substrates were used to determine the activity of different chitinases:

1. 4-Methylumbelliferyl N,N-diacetyl-b-D-chitobioside for exochitinase activity detection (chitobiosidase activity)
2. 4-Methylumbelliferyl N-acetyl-b-D-glucosaminide for exochitinase activity detection (b-N-acetylglucosaminidase activity)
3. 4-Methylumbelliferyl b-D-N,N,N-triacetylchitotriose for endochitinase activity detection

The exochitinolytic NAGase activity (b-N-acetylglucosaminidase) resulted on average higher in all inocula with respect to the chitobiosidase and endochitinase activities (Table 29.4). The presence of chitin in the medium depressed on average the chitinolytic activities of all the inocula, although data were statistically significant only for *B. brongniartii* strain. The latter strain showed the highest chitinolytic activity, in particular of NAGase and chitobiosidases activities with respect to both *B. bassiana* and the co-inoculum. Nevertheless, *B. brongniartii* showed a significantly lower NAGase and chitobiosidases activity when the fungus was grown on agar chitin.

Gupta et al. (1994) have shown a correlation between the virulence of *B. bassiana* against *Galleria mellonela* L and the production of high levels of chitinases and proteases. Furthermore, Fang et al. (2005) proved that an overexpression of a chitinase gene (Bbchit1) enhanced the virulence of *B. bassiana* to aphids, compared with a wild-type strain. Besides a positive direct correlation between chitinolytic activity and virulence, the mechanisms of regulation of chitinase expression during fungal growth have been shown to be complex. The synthesis of chitinolytic enzymes by some entomopathogenic fungi, like *Metarhizium anisopliae*, has been found being regulated by products of chitosan and chitin degradation through an inducer-repressor

mechanism (St Leger et al. 1986). The most effective regulators of chitinase and chitosanase synthesis were the principal monomeric constituents of chitin (N-acetylglucosamine) and chitosan (glucosamine). Increasing the release rate of N-acetylglucosamine decreased chitinase synthesis by about 87% and was paralleled by an increase in fungal growth.

The results of our study showed that the conidia of the fungus *B. brongniartii*, which has a narrower host specificity than *B. bassiana*, being a selective pathogen of the European cockchafer (*M. melolontha*) (St Leger et al. 1992), had a significantly lower expression of chitobiosidase and b-N-acetylglucosaminidase activities when grown on chitin agar. On the other hand, the fungus *B. bassiana*, a worldwide diffuse genus of soilborne entomopathogenic filamentous fungus, produced conidia that showed a significantly lower endo-chitinolytic activity when grown on chitin agar. The co-inoculum of the two fungi showed, as a general trend not confirmed statistically, a lower chitinolytic activity of the conidia obtained from mycelia grown in the presence of chitin.

When entomopathogenic fungi are given to crops to control pests, a high dosage of conidia/spores is generally used (Jackson et al. 2010; Kim et al. 2014). The mass production of spores of these fungi is thus important for commercial use of the entomopathogenic species, and an adequate yield of spores is important for the practical application of fungi as BCA. A factor that is also preventing the wider application and use of fungal entomopathogens is a generally slow or low activity against target pests. Therefore, the enhancement of conidia yield and maintenance of virulence during the production process are important to improve the efficacy of entomopathogenic fungi for insect biocontrol. The yield of *B. brongniartii* spores, for example, was found to vary depending on the combination and doses of additives, such as chitin, used for their production (Srikanth and Santhalakshmi 2012). This study provided evidence that *Beauveria* species can produce conidia with different enzymatic potentiality, and therefore a variable degree of virulence, according to the presence or absence of chitin in the nutritive medium. Our results are supporting those of Rodrıguez-Gomez et al. (2009) who showed, for larvae of the insect *Tenebrio molitor*, a mortality of 80% reached after 11 days with *B. bassiana* conidia collected from a commercial Sabouraud-dextrose agar medium and between 15% and 35% with conidia from other media like colloidal chitin or wheat bran. Subculturing of the fungus directly on chitin or insect larvae negatively affected its virulence (Loesch et al. 2010). On the other hand, an inoculum produced on naturally infected host insects resulted highly infective, whereas that produced on artificial media often had a lower virulence (Hallsworth and Magan 1994).

29.3 Final Remarks

The best use of BCAs for pest control, and for pathogen control as well, is for the prevention of outbreaks rather than curative of infestations. Indeed, the length of time needed to reduce the pest population below the economic threshold level is among the main limitations of biological control (Bale et al. 2008). However, even

when used for prevention, to increase BCA efficacy, it is necessary to integrate their application with other biological tools and/or with cultural practices that are favouring their action. In our approach we have developed an innovative strategy based on the adoption of an array of integrated treatments tested in association with different BCAs (different species of entomopathogenic fungi and nematodes): soil steam disinfection, pre-planting cultivation of phytosanitary plants, mechanical cultivation, mass trapping of adults, attractant or repellent plant extracts. Such approach allowed to drastically reduce the population of European cockchafer affecting strawberry plantations (Tartanus et al. 2016).

When applying a BCA, we should also consider the complex network of interactions that can affect its efficacy in the field. Among the biotic ones, there is the need to consider that BCAs can have an impact on the plant microbiome (Bruck 2010). The host-parasite interactions are crucial to understand the functioning of BCAs and thus to improve their efficacy (Baverstock et al. 2010; Engel and Moran 2013). This is particularly true when more than one BCA is applied or when microbial consortia with strains having different functions (e.g. plant nutrition and protection) are utilised. The complex chemical signalling among all 'actors' is also playing a key role in determining the efficacy of a BCA application (Cory and Ericsson 2010; Watrous et al. 2012; Badri et al. 2013). Furthermore, efforts must aim also at designing appropriate application technologies, considering the agronomical practices utilised by the farmers (e.g. use of chemical pesticides and fertilisers), which can have a dramatic effect on the BCA persistence in soil as well as its overall efficacy in the biological control of pests. Despite the complexity of all the biotic, technical and abiotic variables that could interfere or modulate the efficacy and performance of BCAs in pests control, the valuable results obtained with an integrated approach are very tempting and worthy of further studies. What emerged is that there is no single standard strategy, but a set of measures should be considered, which require a deep knowledge of both the agricultural system and the host-pathogens ecology, with their main drivers and variables.

Acknowledgements The work was supported by a grant from the Polish Ministry of Agriculture and Rural Development 'Organic fruit production – Definition of good practices of plant protection against pests and diseases in organic crops', nr. HORre-029-31-29/14(103).

References

Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic related compounds predominantly modulate the soil microbiome. J Biol Chem 288:4502–4512. doi:10.1074/jbc.M112.433300

Bale JS, van Lenteren JC, Bigler F (2008) Biological control and sustainable food production. Phil Trans R Soc B 363:761–776. doi:10.1098/rstb.2007.2182

Baverstock J, Roy HE, Pell JK (2010) Entomopathogenic fungi and insect behaviour: from unsuspecting hosts to targeted vectors. Bio Control 55:89–102. doi:10.1007/s10526-009-9238-5

Berg G, Zachow C, Müller H, Philipps J, Tilcher R (2013) Next-generation bio-products sowing the seeds of success for sustainable agriculture. Agronomy 3:648–656. doi:10.3390/agronomy3040648

Bochner BR (2011) Phenomics and phenotype microarrays: applications complementing metagenomics. In: de Bruijn FJ (ed) Handbook of molecular microbial ecology I: metagenomics and complementary approaches. Wiley, Hoboken, pp 533–540

Boldo JT, Junges A, Amaral KB, Staats CC, Vainstein MH, Schrank A (2009) Endochitinase CHI2 of the biocontrol fungus *Metarhizium anisopliae* affects its virulence toward the cotton stainer bug *Dysdercus peruvianus*. Curr Genet 55:551–560

Bordenstein SR, Theis KR (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol 13(8):e1002226. doi:10.1371/journal.pbio.1002226

Borglin S, Joyner D, DeAngelis KM, Khudyakov J, D'Haeseleer P, Joachimiak MP, Hazen T (2012) Application of phenotypic microarrays to environmental microbiology. Curr Opin Biotechnol 23:41–48

Bruck DJ (2010) Fungal entomopathogens in the rhizosphere. BioControl 55:103–112. doi:10.1007/s10526-009-9236-7

Canfora L, Malusà E, Salvati L, Renzi G, Petrarulo M, Benedetti A (2015) Short-term impact of two liquid organic fertilizers on *Solanum lycopersicum* L. rhizosphere Eubacteria and Archaea diversity. Appl Soil Ecol 88:50–59. doi:10.1016/j.apsoil.2014.11.017

Canfora L, Malusà E, Tkaczuk C, Tartanus M, Łabanowska BH, Pinzari F (2016) Development of a method for detection and quantification of *B. brongniartii* and *B. bassiana* in soil. Sci Rep 6:22933. doi:10.1038/srep22933

Chandler D, Heale JB, Gillespie AT (1993) Competitive interaction between strains of *Verticillium lecanii* on two insect hosts. Ann Appl Biol 122:435–440

Cory JS, Ericsson JD (2010) Fungal entomopathogens in a tritrophic context. BioControl 55:75–88. doi:10.1007/s10526-009-9247-4

de Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 43:237–256

De Hoog GS (1972) The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. Stud Mycol 1:1–41

DeBach P (1964) Biological control of insect pests and weeds. Chapman &Hall, London

Dolci P, Guglielmo F, Secchi F, Ozino OI (2006) Persistence and efficacy of *Beauveria brongniartii* strains applied as biocontrol agents against *Melolontha melolontha* in the Valley of Aosta (northwest Italy). J Appl Microbiol 100:1063–1072. doi:10.1111/j.1365-2672.2006.02808.x

Engel P, Moran NA (2013) The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev 37:699–735. doi:10.1111/1574-6976.12025

Fan Y, Fang W, Guo S, Pei X, Zhang Y, Xiao Y, Li D, Jin K, Bidochka MJ, Pei Y (2007) Increased insect virulence in *Beauveria bassiana* strains overexpressing an engineered chitinase. Appl Environ Microbiol 73:295–302

Fang W, Leng B, Xiao Y, Jin K, Ma J, Fan Y, Feng J, Yang X, Zhang Y, Pei Y (2005) Cloning of *Beauveria bassiana* gene Bbchit1 and its application to improve fungal strain virulence. Appl Environ Microbiol 71:363–370

Ghikas DV, Kouvelis VN, Typas MA (2010) Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8S-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B. brongniartii*. BMC Microbiol 10:174

Gupta SC, Leathers TD, El-Sayed GN, Ignoffo CM (1994) Relationships among enzyme activities and virulence parameters in *Beauveria bassiana* infections of *Galleria mellonella* and *Trichoplusia ni*. J Invertebr Pathol 64:13–17

Hallsworth JE, Magan N (1994) Effect of carbohydrate type and concentration on polyols and trehalose in conidia of three entomopathogenic fungi. Microbiol-SGM 140:2705–2713

Hirsch PR, Mauchline TH, Clark IM (2010) Culture independent molecular techniques for soil microbial ecology. Soil Biol Biochem 42(6):878–887

Hirsh J, Galidevara S, Strohmeier S, Devi KU, Reineke A (2013) Effects on diversity of soil fungal community and fate of an artificially applied *Beauveria bassiana* strain assessed through pyrosequencing. Microb Ecol 66:608–620

Hsiao WF, Khachatourians GG (1997) The role of extracellular enzymes in the virulence of the entomopathogenic fungus, *Verticillium lecanii*, to oat-bird cherry aphid, *Ropalosiphum padi* (Homoptera: Aphididae). Chin J Entomol 17:227–236

Inglis GD, Johnson DL, Cheng KJ, Goettel MS (1997) Use of pathogen combinations to overcome constraints of temperature on entomopathogenic Hyphomycetes against grasshoppers. Biol Control 8:143–152

Inglis GD, Duke GM, Kawchuk LM, Goettel MS (1999) Influence of oscillating temperatures on the competitive infection and colonization of the migratory grasshopper by *Beauveria bassiana* and *Metarhizium flavoviride*. BioControl 14:111–120

Jaber LR, Enkerli J (2016) Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Sci Tech. doi:10.1080/09583157.2016.1243227

Jackson MA, Dunlap CA, Jaronski ST (2010) Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. BioControl 55:129–145. doi:10.1007/s10526-009-9240-y

Jaronski ST (2007) Soil ecology of the entomopathogenic ascomycetes: a critical examination of what we (think) we know. In: Maniana K, Ekesi S (eds) Use of entomopathogenic fungi in biological pest management. Research Sign Posts, Trivandrum, pp 91–144

Jaronski ST (2010) Ecological factors in the inundative use of fungal entomopathogens. BioControl 55:159–185. doi:10.1007/s10526-009-9248-3

Kabaluk T, Goettel M, Ericsson J, Erlandson M, Vernon B, Jaronski ST, Mackenzie K, Cosgrove L (2007) Promise versus performance: working toward the use of *Metarhizium anisopliae* as a biological control for wireworms. Bull IOBC/WPRS 30(7):69–76

Kim JJ, Xie L, Han JH, Lee SY (2014) Influence of additives on the yield and pathogenicity of conidia produced by solid state cultivation of an *Isaria javanica* isolate. Mycobiology 42(4):346–352. doi:10.5941/MYCO.2014.42.4.346

Łabanowska BH, Bednarek H (2011) Efficacy of *Beauveria brongniartii* as Melocont in the control of the European cockchafer (*Melolontha melolontha*). IOBC/wprs Bull 66:179–182

Łabanowska BH, Olszak RW (2003) The soil pests and their chemical and biological control on strawberry plantations in Poland. IOBC wprs Bull 26(2):93–99

Li DC (2006) Review of fungal chitinases. Mycopathologia 161:345–360

Loesch A, Hutwimmer S, Strasser H (2010) Carbon utilization pattern as a potential quality control criterion for virulence of *Beauveria brongniartii*. J Invertebr Pathol 104:58–65

Malusá E, Pinzari F, Canfora L (2016) Efficacy of biofertilizers: challenges to improve crop production. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity – vol. 2: functional applications. Springer, New Delhi, pp 17–40. doi:10.1007/978-81-322-2644-4_2

Mayerhofer J, Enkerli J, Zelger R, Strasser H (2015) Biological control of the European cockchafer: persistence of *Beauveria brongniartii* after long-term applications in the Euroregion Tyrol. BioControl 60:617–629

Mazid S, Rajkhowa RC, Kalita JC (2011) A review on the use of biopesticides in insect pest management. Int J Sci Adv Technol 1(7):169–178

Meszka B, Sobiczewski P,. Bryk H, Chałańska A, Ślusarski Cz, Ciesielska J, Malusà E (2014) Effect of active steam disinfection on soil microorganisms and strawberry plants health and yield. In: Proceedings of XVI international conference on organic fruit growing, Hohenheim, Germany, pp 258–259

Miller M, Palojärvi A, Rangger A, Reeslev M, Kjøller A (1998) The use of flurogenic substrates to measure fungal presence and activity in soil. Appl Environ Microbiol 64:613–617

Pereira RM, Stimac JL, Alves SB (1993) Soil antagonism affecting the dose response of workers of the red imported fire ant, *Solenopsis invicta*, to *Beauveria bassiana* conidia. J Invertebr Pathol 61:156–161

Pinzari F, Ceci A, Abu-Samra N, Canfora L, Maggi O, Persiani AM (2016) Phenotype MicroArray™ system in the study of fungal functional diversity and catabolic versatility. Res Microbiol. ISSN 0923-2508, http://dx.doi.org/10.1016/j.resmic.2016.05.008

Read AF, Taylor LH (2001) The ecology of genetically diverse infections. Science 292:1099–1102

Rodrıguez-Gomez D, Loera O, Saucedo-Castaneda G, Viniegra-Gonzalez G (2009) Substrate influence on physiology and virulence of *Beauveria bassiana* acting on larvae and adults of *Tenebrio molitor*. World J Microbiol Biotechnol 25:513–518

Sahai AS, Manocha MS (1993) Chitinases of fungi and plants: their involvement in morphogenesis and host-parasite interaction. FEMS Microbiol Rev 11:317–338

Schreiter S, Ding GC, Grosch R, Kropf S, Antweiler K, Smalla K (2014) Soil type-dependent effects of a potential biocontrol inoculant on indigenous bacterial communities in the rhizosphere of field-grown lettuce. FEMS Microbiol Ecol 90(3):718–730. doi:10.1111/1574-6941.12430

Schwarzenbach K, Enkerli J, Widmer F (2009) Effects of biological and chemical insect control agents on fungal community structures in soil microcosms. Appl Soil Ecol 42:54–62

Schwieger F, Tebbe CC (2000) Effect of field inoculation with Sinorhizobium meliloti L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*) – linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. Appl Environ Microbiol 66(8):3556–3565

Seidl V (2008) Chitinases of filamentous fungi: a large group of diverse proteins with multiple physiological functions. Fungal Biol Rev 22:36–42

Sparling GP (1997) Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Pankhurst CE, Double BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International, Wallingford, pp 97–119

Srikanth J, Santhalakshmi G (2012) Effect of media additives on the production of Beauveria brongniartii, an entomopathogenic fungus of *Holotrichia serrata*. Sugar Tech 14:284–290

St Leger RJ, Cooper RM, Charnley K (1986) Cuticle-degrading enzymes of entomopathogenic fungi: regulation of production of chitinolytic enzymes. J Gen Microbiol 132:1509–1517

St Leger RJ, Allee LL, May B, Staples RC, Roberts DW (1992) World-wide distribution of genetic variation among isolates of *Beauveria* spp. Mycol Res 96:1007–1015

Stern VM, Smith RF, van den Bosch R, Hagen KS (1959) The integration of chemical and biological control of the spotted alfalfa aphid. Integr Control Concept Hilgardia 29:81–101

Tartanus M, Łabanowska BH, Malusá E Tkaczuk C, Chałanska A (2016) Holistic approach for an effective control of white grub of European cockchafer (Melolontha melolontha) in organic strawberry plantations in Poland. Proceedings of XVII International Conference on Organic Fruit Growing, Hohenheim, Germany, pp 293–294

Tesi R, Gelsomino A, Baldi A, Lenzi A, Peruzzi A (2007) Soil disinfection with steam alone or combined with CaO in a greenhouse radish crop. Adv Hortic Sci 21:75–82

Trabelsi D, Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: a review. Biomed Res Int 863240. 10.1155/2013/863240

Triolo E, Materazzi A, Luvisi A (2004) Exothermic reactions and steam for the management of soil-borne pathogens: five years of research. Adv Hortic Sci 2:89–94

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzón A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009) Fungal entomopathogens: new insights on their ecology. Fungal Ecol 2:149–159

Vidal S (2015) Entomopathogenic fungi as endophytes:plant–endophyte–herbivore interactions and prospects for use in biological control. Curr Sci 108:1–9

Watrous J, Roach P, Alexandrov T, Heath BS, Yang JY, Kersten RD, van der Voortg M, Poglianoh K, Grossi H, Raaijmakersg JM, Moorec BS, Laskind J, Bandeirac N, Dorresteina PC (2012) Mass spectral molecular networking of living microbial colonies. PNAS 109:E1743–E1752. doi:10.1073/pnas.1203689109

Xu J, Baldwin D, Kindrachuk C, Hegedus DD (2006) Serine proteases and metalloproteases associated with pathogenesis but not host specificity in the Entomophthoralean fungus *Zoophthora radicans*. Can J Microbiol 52:550–559

Yao H, He Z, Wilson Campbell MJCD (2000) Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microb Ecol 40:223–237

Influence of Climate Change, Rhizosphere, and Cultivation on Soil Fertility Determinants

30

C.S. Sumathi and V. Rajesh Kannan

Abstract

Agriculture is the largest food-producing sector. The productivity of agriculture depends on climate change, nature of soil, and cultivation type. Arbuscular mycorrhizal fungi (AMF) are agriculturally important fungi that produce glomalin protein. The hyphae of AMF release glomalin in soil and mycorrhizal roots. The two fractions are easily extractable glomalin (EEG) and total glomalin (TG). The glomalin fractions sequester soil organic carbon and help to maintain the soil fertility. The present study analyzes the variations of soil fertility determinants according to the variations of climatic change, soil nature, and cultivation. Athani (11°31′18″ N, 77°34′49″ E), Erode district, Tamil Nadu, India, is prevalent for turmeric cultivation. Periodically every month the samples were collected from August 2006 to March 2007. The samples used were rhizosphere, turmeric root, and rhizome. The extraction methods of glomalin proteins were followed as per the methodologies adopted by Wright and Upadhyaya (Soil Sci 161:575–586, 1996). The root easily extractable glomalin (EEG-R) and soil easily extractable glomalin (EEG-S) concentrations increased in the months of October 2006 and January 2007, respectively. On February 2007, soil and root total glomalin concentrations were increased. The present study represents climatic factors are playing a significant role on soil nutrients, microbial communities, plant growth, and mycorrhizal proteins.

C.S. Sumathi (✉)
PG and Research Department of Microbiology, K. S. Rangasamy College of Arts and Science, Tiruchengode 637 215, Tamil Nadu, India
e-mail: sumathisamiappan@gmail.com

V.R. Kannan
Rhizosphere Biology Laboratory, Department of Microbiology, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_30

Keywords
Glomalin • Mycorrhizal proteins • Soil fertility • *Arbuscular mycorrhizae* • Climate change

30.1 Introduction

Soil fertility represents the sufficient accessibility of mineral nutrients dissolved in soil solution by plants. Generally, soil fertility is influenced by biotic and abiotic factors. Soil microorganisms actively participate in cycling of soil nutrients, whereas they are restricted to upper organic soil. The plant roots and the associated microorganisms penetrate deep into the soil to assess nutrients and water. Arbuscular mycorrhizal fungi (AMF), a type of mycorrhizal fungi, forms symbiotic association with most of the plant species. AMF involve in three important mechanisms such as influence on plant physiology, soil ecological interactions, and soil engineering. AMF carry out mechanisms like disease resistance, phosphate translocation, and nutrient uptake (Rillig 2004). AMF increases the soil quality by supporting the formation of soil aggregates. Thus, AMF is not only a factor but also a key determinant of soil quality. An additional point to be paid attention about AMF is the production of soil fertility proteins lining the hyphal strands and excreted into the rhizosphere.

The mycorrhizal proteins are named after the phylum *Glomeromycota* as "glomalin." Glomalin is a glycoprotein produced by hyphae of AMF with a molecular weight of >60 KDa in size and may be a hydrophobin-like protein (Wright and Upadhyaya 1996). The protein occurs as lining of mycorrhizal hyphae. The protein concentration was influencing the formation of aggregates in crop soils (Wright et al. 1999; Wright and Anderson 2000; Rillig et al. 2001a). The amino acid sequence of glomalin is highly related to stress proteins; hence, the production of protein was increased with limited hyphal growth (Rillig and Steinberg 2002; Lovelock et al. 2004). Glomalin acts as "soil glue" and makes the soil particles bind together. Mycorrhizal protein is strongly correlated with soil aggregation. Frequent disturbance of soil by tillage, cropping, and grazing reduced glomalin and soil aggregation (Nichols and Miller 2013). This property is related to climatological influence, soil type, and cropping systems. The concentrations of glomalin in soil seem to be responsive to global change factors such as elevated atmospheric CO_2 (Rillig et al. 1999). Glomalin is highly heat stable and extractable from soil by autoclaving. Recent non-autoclaving method of extraction was introduced by Driver et al. (2005). The factors influencing the concentration of glomalin include soil nutrients, temperature, season, moisture, species and diversity of AMF, and host plant metabolism (Rillig et al. 2001a, b). Gadkar and Rillig (2006) identified and characterized glomalin protein. In order to assess the role of AMF under crop cultivation conditions, the changes in the colonization and glomalin concentrations were recorded. So far mostly the concentration of glomalin was characterized in the grassland,

rain forest, floodplain soils, and river foam (Nichols and Miller 2013). A very few studies had been carried out over the distribution of glomalin in soil under various agroecosystems. In the present study, the characterization of glomalin in turmeric rhizosphere was done; the likely relationships that occur between glomalin and climatic factors, soil fertility, plant growth, and survival of rhizosphere microbial population were studied.

30.2 Materials and Methods

30.2.1 Site Description and Sample Collection

The prevalent turmeric cultivation area Athani (11°31′18″ N, 77°34′49″ E) of Erode district, Tamil Nadu, India, was selected for the study. The rhizosphere, turmeric roots, and rhizomes were used as samples and collected periodically every month from August 2006 to March 2007. The surface soil of turmeric plant was removed and dug near the rhizosphere zone. The samples collected were used to assess the seasonal patterns of turmeric plant. Rhizospheres were air-dried. The root-adhering soil particles were removed by gently washing with tap water, blot dried, and stored at 4° C for further analysis.

30.2.2 Climatic Data

Climatological data [maximum and minimum temperature, rainfall, number of rainy days, relative humidity (RH%), sunshine, and evaporation] were collected from Tamil Nadu Agricultural University and Research Station, Bhavanisagar, Tamil Nadu, India.

30.2.3 pH

Measured 10 g of air-dried soil sample and 100 ml of distilled water were added to make a suspension of 1:10 (w/v) dilution and read with a digital pH meter (Systronics-335).

30.2.4 Electrical Conductivity

Ten grams of air-dried rhizosphere was weighed, and suspension was made by adding 100 ml of distilled water and was measured with a digital electrical conductivity meter (DEC-1-USA).

30.2.5 Analysis of Soil Nutrients

The total nitrogen (N) and available phosphorus (P) were determined, respectively, by micro-kjeldahl and molybdenum blue methods (Jackson 1973). Using ammonium acetate solution (pH 7), exchangeable potassium (K) was extracted and measured with a digital flame photometer (Jackson 1973), and soil organic carbon (SOC) and soil organic matter (SOM) were estimated using rapid dichromate oxidation method (Walkey and Black 1934). The micronutrients, viz., Cu, Zn, Fe, and Mn, were also estimated as described by Lindsay and Norvell (1978).

30.2.6 Turmeric Growth and Yield

The plant height, root length, shoot and root biomass, and number of leaves present in each plant were recorded. The shoot and root dry weights were measured by oven drying at 80° C to get a constant weight.

30.2.7 Analysis of Phytochemical Status

The total chlorophyll of turmeric leaves was estimated by following Witham's method (Witham et al. 1971). The total carbohydrate and protein concentration was estimated by anthrone and Lowry's method, respectively (Hedge and Hoftreiter 1962; Lowry et al. 1951). The phenol contents of turmeric leaves were analyzed using sodium carbonate-folin phenol reagent (Malick and Singh 1980). The spectrophotometric estimation of curcumin (cur) was followed to assess the quality of turmeric (ASTA 1997).

30.2.8 AM Fungal Factors

EEG and TG of turmeric root and rhizosphere were extracted and quantified according to Bradford assay (Wright and Upadhyaya 1996). The AM fungal colonization in turmeric roots was calculated by trypan blue method (Philips and Hayman 1970).

30.2.9 Population of Rhizosphere Microorganisms

The standard serial dilution and sterile microbial plating techniques were employed for the enumeration of soil microbial population in the rhizosphere. Nutrient agar for isolation of bacteria, actinomycete isolation agar for actinomycetes, and Sabouraud agar for fungus isolation were used.

30.2.9.1 Statistical Analysis

Analysis of variance (ANOVA) was calculated for the data obtained and the means separated using Duncan's multiple range test (DMRT). Pearson's bivariate correlation analysis (SPSS version 10) was used to assess the relationships between glomalin and climatic factors, soil nature, plant growth, and rhizosphere microbial communities (Zar 1984).

30.3 Results

30.3.1 Climatic Factors

The climatic factors existed at the period of study is given in Table 30.1. The maximum temperature reached was 35.6° C during the month of March 2007 (time of harvest), and the minimum temperature was recorded in the month of January 2007 (16.9° C).

The winter season was noted to have minimum temperature levels consequently. The same time, the relative humidity (RH%) was greater starting from November 2006 to February 2007. The RH% was low during the month of August 2006. The total rainfall was high at November 2006 with 180.6 mm, and the number of rainy days was 9. Rainfall occurred from August 2006 to February 2007, except in the month March 2007 had no rainfall.

The maximum wind velocity was 6.0 Km/h. On November 2006, wind velocity had declined, and the rainfall was maximum. On January and February 2007,

Table 30.1 Meteorological factors in turmeric (*Curcuma longa*) plantations of Erode district, Tamil Nadu, India

S. no.	Month	Temperature (°C)		Relative humidity (%)	Rainfall		Wind velocity (Km/h)	Sunshine (h/day)	Evaporation (mm)
		Maximum	Minimum		Total rainfall (mm)	No. of rainy days			
1.	Aug 2006	34.8	24.0	80.1	39.9	3.0	6.0	5.9	4.6
2.	Sep 2006	33.9	23.6	85.5	81.5	7.0	3.0	5.7	3.4
3.	Oct 2006	33.1	23.3	88.3	176.3	6.0	2.2	5.6	3.2
4.	Nov 2006	31.2	21.9	95.8	180.6	9.0	0.7	5.2	2.5
5.	Dec 2006	30.6	17.4	91.6	4.0	1.0	1.0	7.3	3.4
6.	Jan 2007	31.4	16.9	91.6	5.2	1.0	1.3	8.2	4.1
7.	Feb 2007	33.2	18.8	90.3	43.4	2.0	2.0	8.4	4.2
8.	Mar 2007	35.6	20.8	88.0	0.0	0.0	2.0	7.6	5.2

brighter sunshine was recorded at 8.2 and 8.4 h/day, respectively. Water evaporation rate, brighter sunshine, nil rainfall, and maximum temperature were the factors that played role for dry conditions on March 2007. The influence of biotic and abiotic factors on turmeric growth is given in Table 30.5. The maximum temperature had significantly negative correlation with relative humidity at the level of $P < 0.05$. Rainfall had shown significantly positive correlation with the number of rainy days ($r = 0.902$, $P < 0.01$). The wind velocity and RH had significantly negative correlations with each other with the level of $P < 0.01$. Sunshine was found to have significantly negative correlations with minimum temperature, rainfall, and number of rainy days. Evaporation had significantly negative correlation with rainfall and number of rainy days.

30.3.2 Turmeric Growth and Yield

The variations among the morphological parameters like number of leaves, shoot height, root length, and biomass of shoot, root, and rhizome had increased during its growth period up to 7 months (February 2007), and growth was reduced at the harvest period of 8 months (March 2007). The maximum leaf number reached to 9 (Table 30.2). The plants measured with shoot height of about 53 cm. The plants were noted to reach the maximum heights sequentially growing until January 2007 and slightly attained pause to their growth phase thereby declined in the shoot height. Till February 2007, growth of roots occurred consequently increased and supported plant growth. The maximum shoot and root biomass were recorded during the months of December 2006 and January 2007, respectively. At harvesting stage, the rhizome biomass was estimated to extend at peak, ranging from 7.0 to 177.2 g.

The number of leaves showed significantly positive correlation with manganese ($r = 0.791$, $P < 0.05$). Plant height had negative correlation at significant levels with minimum temperature, wind velocity, total nitrogen, and exchangeable potassium and showed significant positive correlations with relative humidity and sunshine. Root length had significant negative correlation with wind velocity, soil K and plant height. Shoot biomass showed significant negative correlation with maximum temperature and water evaporation, and it was having positive correlation with relative humidity and copper. Root biomass had equally significant positive as well as negative correlation with sunshine, plant height and root length, minimum temperature, wind velocity, and total nitrogen, respectively. Rhizome biomass mostly had significant positive correlation with sunshine, zinc, and plant height but negatively correlated with exchangeable potassium content (Table 30.5).

Table 30.2 Seasonal variations in growth and yield of turmeric under agrochemical application practices

Month	No. of leaves	Shoot height (cm)	Root length (cm)	Shoot biomass (g)	Root biomass (g)	Rhizome biomass (g)
Aug 2006	$5.0^{bc} \pm 1.1$	$53.0^{a} \pm 1$	$14.1^{a} \pm 0.3$	$17.3^{b} \pm 0.4$	$7.1^{a} \pm 0.1$	$7.0^{a} \pm 0.1$
Sep 2006	$9.0^{f–i} \pm 0.5$	$60^{b} \pm 1.1$	$16.6^{b–f} \pm 0.4$	$16.6^{b–f} \pm 0.4$	$9.62^{bc} \pm 0.2$	$7.6^{a} \pm 0.1$
Oct 2006	$5.0^{bc} \pm 1.1$	$61.5^{bc} \pm 2$	$16.7^{f} \pm 0.2$	$24.89^{d} \pm 0.5$	$10.43^{cd} \pm 0.1$	$20.8^{ab} \pm 0.4$
Nov 2006	$5.0^{bc} \pm 1.1$	$92.0^{d–g} \pm 2$	$16.8^{fg} \pm 0.1$	$41.1^{i} \pm 0.8$	$11.97^{e} \pm 0.1$	$40.0^{bc} \pm 0.5$
Dec 2006	$5.0^{bc} \pm 0.5$	$97.5^{h} \pm 2.3$	$17^{g} \pm 0.2$	$36.45^{gh} \pm 0.6$	$13.85^{f} \pm 0.2$	$59^{cd} \pm 1.4$
Jan 2007	$6.0^{de} \pm 1.1$	$103.2^{i} \pm 2$	$18^{i} \pm 0.4$	$33.1^{efg} \pm 0.6$	$17.5^{hi} \pm 0.2$	$78.2^{de} \pm 1.7$
Feb 2007	$5.0^{bc} \pm 0$	$103^{i} \pm 1$	$18.1^{i} \pm 0.4$	$18.95^{b} \pm 0.2$	$14.65^{g} \pm 0.1$	$106^{f} \pm 1.5$
Mar 2007	$5.0^{bc} \pm 0.5$	$101.5^{hi} \pm 1$	$17.7^{h} \pm 0.2$	$15.35^{a} \pm 0.2$	$12.5^{e} \pm 0.1$	$177.2^{g–i} \pm 3.5$

Means followed by common letter(s) are not significantly different at 5% level according to DMRT. ± values represent standard error at 5% level of significance

30.3.3 Microbial Population in Turmeric Rhizosphere

In the rhizosphere, the bacterial population was found maximum (117×10^5 CFU/g) and 11×10^5 CFU/g as minimum. The population of actinomycetes was high in soil sample with a colony count of about 117×10^4 CFU/g. The fungal population was minimum during September 2006 and maximum during October 2006 (Table 30.3). The AMF root colonization varied from 63.6% to 85%.

The rhizosphere bacterial population had significantly positive correlation with shoot biomass ($r = 0.726$, $P < 0.05$). The actinomycete population was significant and negatively correlated the factors like relative humidity, plant height, root length, root biomass, turmeric leaf protein, TG-S, TG-R, and curcumin. It was positively correlated to minimum temperature and wind velocity (Table 30.5). AMF was found to have significantly positive correlation with sunshine ($r = 0.756$, $P < 0.05$) and negative correlation with nitrogen ($r = -0.778$, $P < 0.05$) and potassium ($r = -0.764$, $P < 0.05$).

30.3.4 Phytochemical Variations in Turmeric

From December 2006 to January 2007, the turmeric leaves were estimated to have highest levels of Chl *a* (Fig. 30.1). While nearing the harvesting stage, the Chl *a* concentration was declined. Chl *a* showed positive correlation with copper ($r = 0.792$, $P < 0.05$) and negative correlation with maximum temperature, and available phosphorus at significance level of $P < 0.05$. Chl *b* showed significant

Table 30.3 Influence of biotic and abiotic factors on microbial populations in turmeric rhizosphere

Month	Bacteria (10^{-4}/g soil)	Actinomycetes (10^{-3}/g soil)	Fungi (10^{-3}/g soil)	AMF%
Aug 2006	$11^{a} \pm 1$	$117^{fgh} \pm 3$	$18^{e} \pm 1.1$	$70^{c} \pm 1.5$
Sep 2006	$84^{def} \pm 2.5$	$69^{de} \pm 1.5$	$8.0^{a} \pm 0$	$80^{def} \pm 2$
Oct 2006	$28^{a} \pm 1.5$	$40^{c} \pm 1.1$	$25^{h} \pm 1.1$	$68^{b} \pm 1.5$
Nov 2006	$117^{gh} \pm 3$	$20^{a} \pm 0.5$	$14^{bc} \pm 0.5$	$63^{a} \pm 1.5$
Dec 2006	$86^{f} \pm 2$	$14^{a} \pm 0.5$	$16^{d} \pm 0.5$	$71^{c} \pm 1.5$
Jan 2007	$54^{bc} \pm 2$	$9^{a} \pm 0.5$	$18^{e} \pm 1.1$	$80^{def} \pm 2$
Feb 2007	$22^{a} \pm 1.1$	$4^{a} \pm 0$	$10^{a} \pm 0.5$	$85^{h} \pm 2$
Mar 2007	$52^{bc} \pm 1.5$	$28^{ab} \pm 1.1$	$21^{fg} \pm 1.1$	$82^{g} \pm 1.5$

Means followed by common letter(s) are not significantly different at 5% level according to DMRT. ± values represent standard error at 5% level of significance

positive correlation with iron at the level of 1%. Total chlorophyll content was negatively correlated to maximum temperature and available phosphorus and positively correlated to copper, shoot biomass, and Chl *a* at significant levels.

The carbohydrate contents were increased gradually from the period of August 2006 to December 2006; there occur a sudden increase in the value on March 2007 (Fig. 30.2). Carbohydrates had significant and positive correlation only to rhizome biomass ($r = 0.757$, $P < 0.05$) (Table 30.5).

There occurred a collinear increase in the phenol throughout the study period and higher during the plant's older stages (Fig. 30.3). The total phenolic compounds have negative significant correlation with minimum temperature and soil total nitrogen and positively correlated to sunshine at the significance level of 5%. The protein contents slope upward gradually till January 2007 (0.564 mg/0.1 g) and were found to turn down during the last period of 2 months (Fig. 30.4). Protein content has significantly positive correlation with relative humidity, plant height, and root biomass whereas negatively correlated to maximum temperature (Table 30.5). The quantity of curcumin increased linearly throughout the sampling period (Fig. 30.5). It was estimated that curcumin had reached its maximum level at the final rhizome harvesting time. It was having positive correlation with sunshine ($r = 0.834$, $P < 0.05$).

30.3.5 Status of Soil Fertility Determinants

EEG-S was found maximum during the months of October and November 2006 (1.47 µg/g soil). Though there was a sudden reduction of EEG-S on December 2006 and in subsequent months, moderate concentrations were maintained. During the middle of the sampling period, a zigzag phase of soil total glomalin concentrations appeared but initially with lesser concentrations of protein. On January 2007, EEG-R was estimated as peak value of 2.565 µg/g (Fig. 30.6). In general, total root glomalin initially had lowest protein concentrations and rose up on later sampling periods. The months of February and March 2007 were pronounced to have higher protein concentration (Fig. 30.7).

Table 30.4 Variations of soil edaphic and nutrient factors in turmeric rhizosphere

	Physical		Macronutrients				Micronutrients				
Month	pH	EC (dS/m)	OC%	OM%	N (1000 Kg/acre)	P (Kg/acre)	K (1000 Kg/acre)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
Aug 2006	$8.27^{a} \pm 0.04$	$0.365^{f} \pm 0.015$	$2.83^{e} \pm 0.04$	$48.89^{e} \pm 2.7$	$85.5^{f} \pm 1.5$	$5^{a} \pm 0.1$	$427.5^{gh} \pm 2.5$	$26.13^{d} \pm 0.5$	$1.43^{c} \pm 0.03$	$1.18^{c} \pm 0.08$	$1.03^{b} \pm 0.01$
Sep 2006	$8.43^{g} \pm 0.03$	$0.235^{b} \pm 0.005$	$3.36^{h} \pm 0.05$	$58.35^{h} \pm 1.0$	$81d^{e} \pm 5.5$	$5.75^{cd} \pm 0.15$	$432.5^{h} \pm 22.5$	$25.52^{b} \pm 0.4$	$2.2^{e–h} \pm 0.04$	$0.66^{a} \pm 0.04$	$1^{b} \pm 0.02$
Oct 2006	$8.41^{f} \pm 0.02$	$0.2^{a} \pm 0.05$	$2.59^{d} \pm 0.02$	$44.8^{d} \pm 3.5$	$88.5^{g} \pm 2.5$	$6^{e} \pm 0.1$	$380^{c–f} \pm 10$	$28.23^{fgh} \pm 0.6$	$1.6^{d} \pm 0.03$	$1.06^{b} \pm 0.1$	$1.06^{c} \pm 0.04$
Nov 2006	$8.39^{de} \pm 0.01$	$0.18^{a} \pm 0.01$	$3.145^{g} \pm 0.01$	$54.3^{g} \pm 2.6$	$91.5^{h} \pm 1.5$	$5.75^{cd} \pm 0.25$	$432.5^{h} \pm 5$	$25.29^{b} \pm 0.5$	$1.07^{a} \pm 0.01$	$0.53^{a} \pm 0.11$	$1.71^{df} \pm 0.03$
Dec 2006	$8.28^{a} \pm 0.06$	$0.42^{gh} \pm 0.01$	$3.07^{f} \pm 0.02$	$52.96^{f} \pm 1.3$	$70^{bc} \pm 1.0$	$5^{a} \pm 0.2$	$382.5^{c–f} \pm 17.5$	$25.85^{c} \pm 0.3$	$1.05^{a} \pm 0.02$	$0.86^{ab} \pm 0.08$	$1.7^{d–f} \pm 0.05$
Jan 2007	$8.28^{a} \pm 0.09$	$0.335^{de} \pm 0.055$	$1.653^{a} \pm 0.01$	$28.49^{a} \pm 1.0$	$59^{a} \pm 3.0$	$5.25^{b} \pm 0.25$	$227.5^{b} \pm 7.5$	$26.93^{e} \pm 0.2$	$1.15^{a} \pm 0.01$	$1.78^{ef} \pm 0.03$	$2.06^{gh} \pm 0.17$
Feb 2007	$8.45^{h} \pm 0.02$	$0.365^{f} \pm 0.005$	$2.99^{f} \pm 0.03$	$51.6^{f} \pm 3.0$	$64.5^{a} \pm 3.5$	$6.25^{f} \pm 0.12$	$185^{a} \pm 12.5$	$24.8^{a} \pm 0.3$	$1.595^{d} \pm 0.03$	$1.41^{d} \pm 0.21$	$0.62^{a} \pm 0.02$
Mar 2007	$8.32^{bc} \pm 0.02$	$0.286^{c} \pm 0.04$	$2.2^{bc} \pm 0.01$	$38^{bc} \pm 2.7$	$70.2^{c} \pm 2.1$	$6.53^{h} \pm 0.22$	$169^{a} \pm 5$	$24.5^{a} \pm 0.4$	$1.29^{b} \pm 0.02$	$2.3^{gh} \pm 0.12$	$0.68^{b} \pm 0.03$

Means followed by a common letter(s) are not significantly different at 5% level according to DMRT. ± values represent standard error at 5% level of significance

Table 30.5 Biotic and abiotic factor influence on turmeric growth

Parameters	Max temp	Mini temp	RH	RF	Rainy days	Wind	Sun shine	Evap	EC	OC	OM	N	K	Mn	Cu	Zn	EEG-S	TG-S	EEG-R	TG-R	Cur
Max temp	–																				
Min temp	0.579	–																			
RH	−0.752*	−0.568	–																		
RF	−0.170	0.580	0.267	–																	
Rainy days	−0.164	0.638	0.164	0.902**	–																
Wind	0.669	0.623	−0.952**	−0.128	−0.030	–															
Sun	−0.056	−0.831*	0.177	−0.775*	−0.853**	−0.281	–														
Evap	0.700	−0.123	−0.550	−0.751*	−0.791*	0.445	0.585	–													
EC	−0.095	−0.597	−0.226	−0.832*	−0.801*	0.187	0.677	0.501	–												
OC	−0.061	0.421	−0.070	0.385	0.569	0.133	−0.521	−0.507	−0.125	–											
OM	−0.056	0.427	−0.075	0.386	0.573	0.136	−0.525	−0.506	−0.130	1.000**	–										
N	0.128	0.852**	−0.173	0.809*	0.812*	0.304	−0.969**	−0.519	−0.659	0.533	0.535	–									
K	−0.222	0.588	−0.184	0.553	0.716*	0.288	−0.876**	−0.662	−0.315	0.628	0.630	0.795*	–								
Mn	0.497	0.557	−0.521	0.178	0.344	0.416	−0.263	0.000	−0.286	0.394	0.404	0.195	0.185	–							
Cu	−0.828*	−0.459	0.522	0.030	0.091	−0.445	−0.032	−0.486	0.047	−0.262	−0.265	−0.101	0.269	−0.575	–						
Zn	0.466	−0.356	−0.136	−0.621	−0.783*	0.015	0.702	0.861**	0.295	−0.799*	−0.799*	−0.638	−0.872**	−0.218	−0.266	–					
EEG-S	0.134	0.452	0.103	0.749*	0.465	−0.052	−0.412	−0.266	−0.733*	−0.204	−0.201	0.486	0.059	0.146	−0.151	−0.011	–				
TG-S	−0.220	−0.587	0.663	−0.139	−0.206	−0.622	0.598	0.068	0.081	−0.106	−0.111	−0.507	−0.677	−0.308	−0.053	0.302	−0.132	–			
EEG-R	−0.286	−0.583	0.304	−0.213	−0.493	−0.404	0.536	0.156	0.169	−0.798*	−0.800*	−0.544	−0.526	−0.342	0.330	0.523	0.307	0.108	–		
TG-R	−0.131	−0.657	0.582	−0.309	−0.427	−0.623	0.719*	0.221	0.233	−0.145	−0.151	−0.613	−0.778*	−0.317	−0.173	0.440	−0.199	0.939**	0.216	–	
Cur	−0.104	−0.763*	0.504	−0.526	−0.623	−0.549	0.832*	0.422	0.348	−0.452	−0.457	−0.760*	−0.871**	−0.487	0.007	0.661	−0.293	0.864**	0.359	0.919**	–

*Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). Nonsignificant results were omitted

OC organic carbon, *OM* organic matter, *N* nitrogen, *P* phosphorus, *K* potassium, *Fe* iron, *Mn* manganese, *Zn* zinc, *EEG-S* easily extractable soil glomalin, *EEG-R* easily extractable root glomalin, *TG-S* soil total glomalin, *TG-R* root total glomalin, *Cur* curcumin

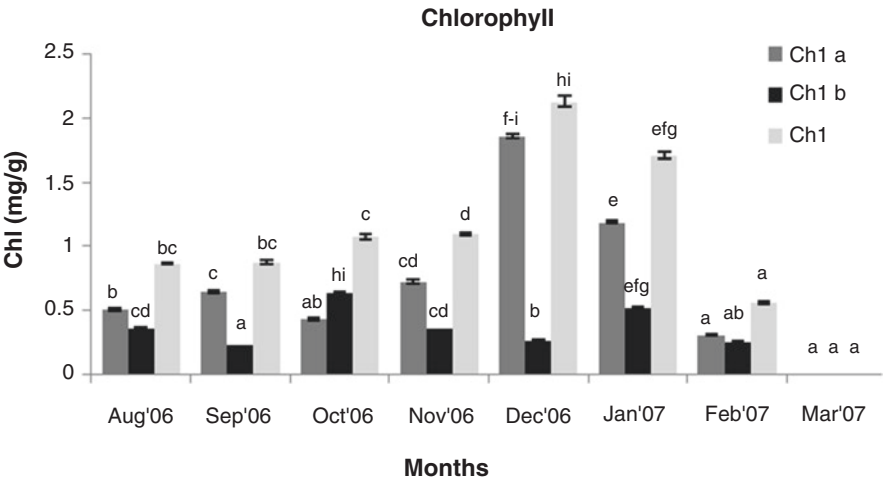


Fig. 30.1 Variations of Chlorophyll concentration in turmeric leaves. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

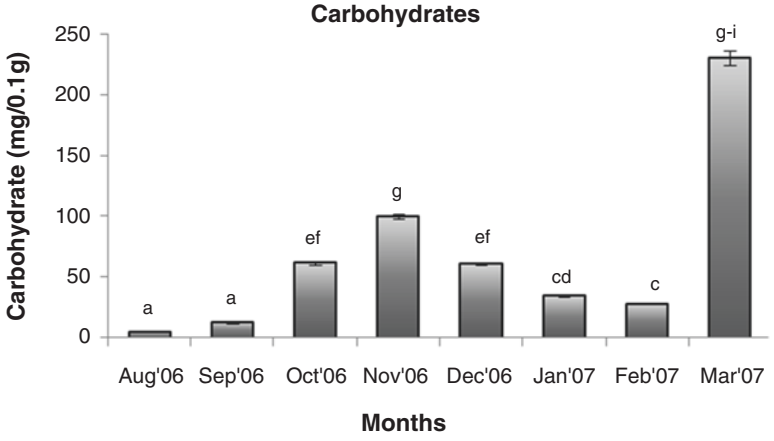


Fig. 30.2 Variations of Carbohydrate concentration in turmeric leaves. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

EEG-S had positive correlation with rainfall ($r = 0.749$, $P < 0.05$) and negative correlation with EC ($r = -0.733$, $P < 0.05$) at significant levels. Soil total glomalin (TG-S) had significantly positive correlation with plant height and root length at the level of $P < 0.01$ and $P < 0.05$, respectively. EEG-R had significant and negative correlation with OC ($r = -0.798$, $P < 0.05$) and OM ($r = -0.800$, $P < 0.05$). TG-R had significantly positive correlations with sunshine, plant height, root length, rhizome biomass, and TG-S and negative correlation with potassium at significant level.

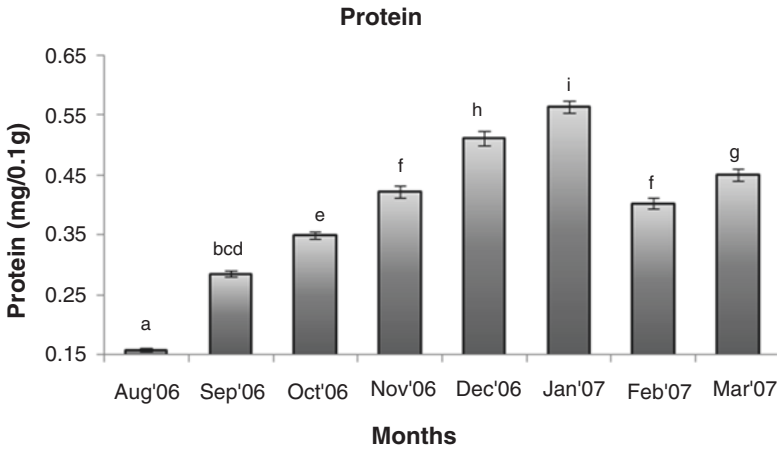


Fig. 30.3 Variations of Protein concentration in turmeric leaves. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

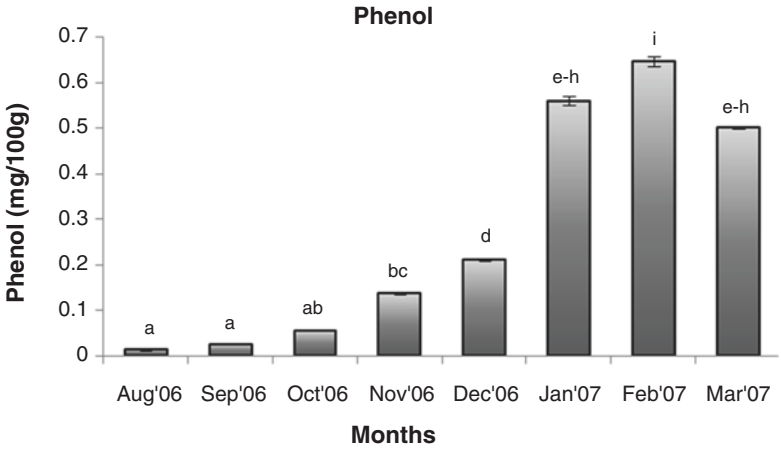


Fig. 30.4 Variations of Phenol concentration in turmeric leaves. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

30.3.6 Soil Fertility Status

The pH of the turmeric rhizosphere was alkaline in nature. The electrical conductivity was maximum in December 2006 and minimum in March 2007, and it showed significant negative correlation with both rainfall ($r = -0.832$, $P < 0.05$) and number of rainy days ($r = -0.801$, $P < 0.05$). On January 2007, the SOC content was minimum and showed maximum values on September 2006. Corresponding results

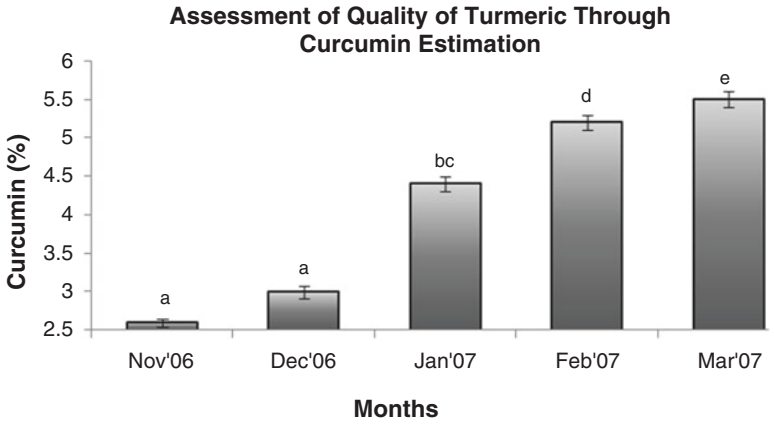


Fig. 30.5 Assessment of quality of turmeric through curcumin estimation. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

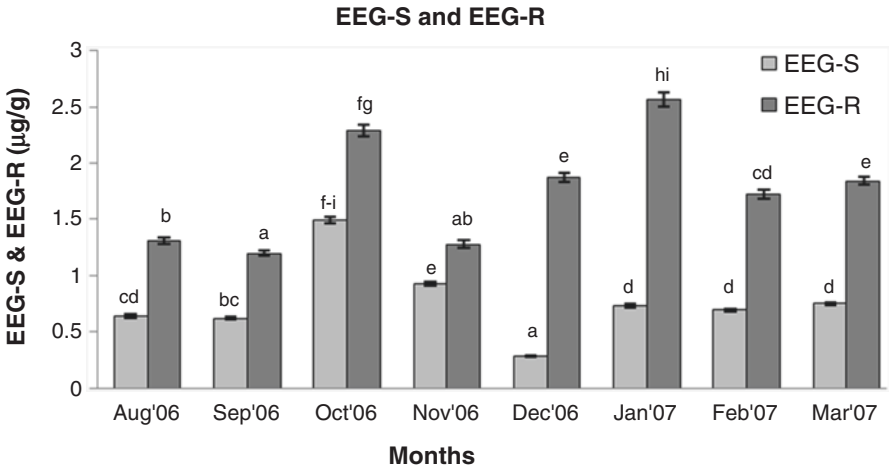


Fig. 30.6 EEG-S and EEG-R. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

were also observed in the case of organic matter (OM). Total soil nitrogen showed highest on October 2006 (91.5 × 1000 Kg/acre). The available phosphorus content was minimum and maximum during the months of August 2006 and March 2007, respectively. The exchangeable potassium was higher during the initial stages of the plant growth and reduced when the plant growth increases. The iron contents were showing reduced results during the harvest stage of the plant, i.e., February and March 2007. In this period, the iron levels were showing statistically similar values. The maximum Fe levels were estimated during October 2006 of about 28.23 ppm.

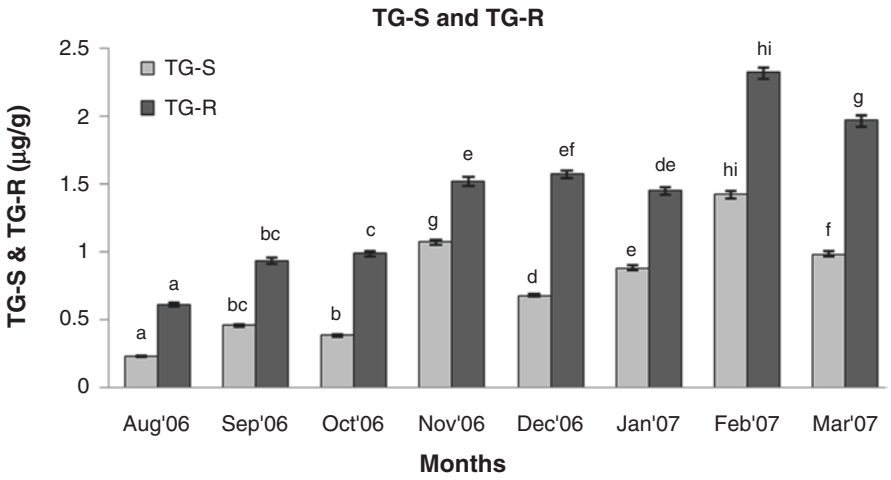


Fig. 30.7 TG-S and TG-R. Note: Means followed by common letter(s) are not significantly different at 5% level according to DMRT. *Error bars* represent standard error at 5% level of significance

In succession, the rhizosphere samples were noticed to have reduced values of manganese during middle of the study period. The maximum level of manganese was detected during the initial stages of the study period. The maximum copper values were present on rhizosphere on March 2007 measuring of 2.3 ppm. The zinc content was maximum during January 2007, but it suddenly dropped to lower extend on February 2007 (Table 30.4).

The organic carbon content of the rhizosphere had significantly positive correlation with rainfall ($r = 0.902$, $P < 0.01$) (Table 30.5). The correlation between OC and OM was highly significant and positive ($r = 0.100$, $P < 0.01$). The exchangeable potassium found to have significantly negative correlation with number of rainy days ($r = -0.876$, $P < 0.01$) and significant positive correlation with total nitrogen ($r = 0.795$, $P < 0.05$). Zinc was found to have significant negative correlation with number of rainy days, organic carbon, organic matter, and phosphorus content, but it was positively correlated to water evaporation.

30.4 Discussion

Agriculture is a highly vulnerable sector and is sensitive to climatic factors. Soil fertility is an important factor which determines the success of agricultural productivity. As soil fertility is concerned, the climatic factors have strong impact; one such example is influence of temperature on nutrient uptake (Kristoffersen et al. 2005), and cool conditions affect uptake due to reduced mineralization, less diffusion toward roots (Barber 1995), lower uptake kinetics (Bravo and Uribe 1981), and reduced root growth. From the present study, it was observed that climatic factors

have significant correlation and they do showed positive and negative significant influence on soil nutrients like nitrogen, potassium, and zinc. This indicates the role of climatic factors on the soil fertility and nutrient uptake. In addition, relative humidity, rainfall, and sunshine are the most notable climatic factors which had positive correlations with plant morphological and biochemical characters. The climatic factors play an eminent role in the growth of a plant; studies on emergence pattern, growth, development, and quality of a plant species influenced by edaphic factors are necessary for better crop production (Ghorbani et al. 1999). The correlations of the climatic factors are supported by Zhang et al. (2006), who found significant relations between the meteorological factors, viz., maximum and minimum temperature, evaporation, sunshine, and wind speed. Climatic factor also accelerates many vital processes of the plant thereby involving the metabolic activities of the plant growth and development, thus showing the significant positive correlation with the plant growth and yield.

The seasonal modifications of morphological parameters like number of leaves, shoot height, root length, and shoot, root, and rhizome biomass had increased during its growth period up to 7 months (February 2007) and suddenly reduced on March 2007. This may be the attaining of senescence phase by the plant. The growth, quality, and yield of turmeric were studied by Hossain et al. (2005), which revealed that a steady state rises in the rhizome biomass throughout the study.

Microbial community structure was greatly influenced by seasonal variation (Dunfield and Germida 2003), thereby affecting the fatty acid composition or the genetic diversity. In contrast to that, the differences in the microbial communities are not related to the growth of the plants. The seasonal fluctuations of bacterial diversity in agricultural soils were studied by Meier et al. (2008).

The influence of seasonal variation on AMF colonization and structural establishment was supported by Rajesh Kannan et al. (2006). The variation in the colonization by AMF occurred according to the growth of the plant (Rajesh Kannan et al. 2009). Several biotic and abiotic factors are responsible for such microbial population variations (Sumathi et al. 2008).

In the present study, a special context is given to soil fertility determinants, glomalin. The significant positive correlation between rainfall and EEG-S and sunshine and TG-R proves the role of climatic factors on soil fertility. Not only to the climatic factors, both the fractions interlinked within themselves and showing significant correlations. In addition to that, total glomalin also contributes the increase in the curcumin production. The increased concentration of glomalin showed the soil as fertile one, which implies that there is an increased and active life cycle of AMF.

30.5 Conclusion

The information about the seasonal dynamics of AMF hyphal product (glomalin) are very few; these findings may be helpful to understand the life cycle of AMF and its applications. From this study, the protein content of the turmeric was compared with the level of mycorrhizal proteins both in the soil and curcumin (Sumathi et al. 2008).

To our knowledge no such works had been published; this novel idea expresses the strength of mycorrhizal colonization, involvement of mycorrhizal proteins in the translocation of elements, and improvement of the turmeric quality and yield.

Acknowledgment The first author conveys the thanks to the Council of Scientific and Industrial Research (CSIR), New Delhi, for funding this project in the mode of Senior Research Fellowship (SRF).

References

ASTA'S analytical methods manual (1997) 4th edn

Barber SA (1995) Soil nutrient bioavailability: a mechanistic approach. Wiley, New York

Bravo FP, Uribe EG (1981) Temperature dependence of the concentration kinetics of absorption of phosphate and potassium in corn roots. Plant Physiol 67:815–819

Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biol Biochem 37:101–106. doi:10.1016/j.soilbio.2004.06.011

Dunfield KE, Germida JJ (2003) Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified Canola (*Brassica napus*). Appl Env Microbiol 69:7310–7318. doi:10.1128/AEM.69.12.7310-7318.2003

Gadkar V, Rillig MC (2006) The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. FEMS Microbiol Lett 263:93–101

Ghorbani R, Seel W, Leiferr C (1999) Effects of environmental factors on germination and emergence of Amaranthus retroflexus. Weed Sci 47(5):505–510

Hedge JE Hofreiter BT (1962) In: Whistler RL, Be Miller JN (eds) Carbohydrate chemistry 17. Academic, New York

Hossain MA, Ishimine Y, Akamine H et al (2005) Effects of seed rhizome size on growth and yield of turmeric (*Curcuma longa* L.) Plant Prod Sci 8:86–94

Jackson NL (1973) Soil chemical analysis. Prentice Hall, New Delhi

Kristoffersen AO, Riley H, Sogn TA (2005) Effects of P fertilizer placement and temperature on root hair formation, shoot growth and P content of barley grown on soils with varying P status. Nut Cycl Agroecosys 73:147–159. doi:10.1626/pps.8.482

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for Zn, Fe, Mn and Cu. Soil Sci Soc Am J 42:421–428

Lovelock CE, Wright SF, Nichols KA (2004) Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil. Soil Biol Biochem 36:1009–1012. doi:10.1016/j.soilbio.2004.02.010

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275

Malick CP, Singh MB (1980) Plant enzymology and histoenzymology. Kalyani Publishers, New Delhi

Meier C, Wehrli B, van der Meer JR (2008) Seasonal fluctuations of bacterial community diversity in agricultural soil and experimental validation by laboratory disturbance experiments. Microb Ecol 56:210–222. doi:10.1007/s00248-007-9337-8

Nichols KA, Millar J (2013) Glomalin and soil aggregation under six management systems in the Northern Great plains, USA. Open J Soil Sci 3:374–378. http://dx.doi.org/10.4236/ojss.2013.38043

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Brit Mycol Soc 55:158–161

Rajesh Kannan V, Dhanapal K, Joseph T et al (2006) Studies on dynamics of AM fungal association and spore density in *Elettaria cardamom* Maton. (Small cardamom). J Plant Crops 34:489–493

Rajesh Kannan V, Sumathi CS, Manian S (2009) Arbuscular mycorrhizal fungi colonization in upland rice as influenced by agrochemical application. Rice Sci 16:307–313. doi:10.1016/S1672-6308(08)60095-5

Rillig MC (2004) Arbuscular mycorrhizae, glomalin and soil aggregation. Can J Soil Sci 84:355–363. doi:10.4141/S04-003

Rillig MC, Steinberg PD (2002) Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification? Soil Biol Biochem 34:1371–1374. doi:10.1016/S0038-0717(02)00060-3

Rillig MC, Wright SF, Allen MF et al (1999) Rise in carbon dioxide changes soil structure. Nature 400:628

Rillig MC, Wright SF, Nichols KA et al (2001a) Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant Soil 233:167–177. doi:10.1023/A:1010364221169

Rillig MC, Wright SF, Kimball BA et al (2001b) Elevated carbon dioxide (FACE) and irrigation effects on water stable aggregates in an agricultural sorghum field: a possible role for arbuscular mycorrhizal fungi. Glob Chang Biol 7:333–337. doi:10.1046/j.1365-2486.2001.00404.x

Sumathi CS, Balasubramanian V, Ramesh N et al (2008) Influence of biotic and abiotic features on *Curcuma longa* L. plantation under tropical condition. Mid-East J Sci Res 3:171–178

Walkey A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic titration method. Soil Sci 34:29–38

Witham FH, Blaydes DF, Devlin RM (1971) Experiments in plant physiology. Van Nostrand, New York

Wright SF, Anderson RL (2000) Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. Biol Fert Soils 31:249–253. http://dx.doi.org/10.1007/s003740050653

Wright SF, Upadhyaya A (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci 161:575–586

Wright SF, Starr JL, Paltineanu IC (1999) Changes in aggregate stability and concentration of glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi during transition from plow- to no-till management. Soil Sci Soc Am J 63:1825–1829. http://dx.doi.org/10.2136/sssaj1999.6361825x

Zar JM (1984) Biostatistical analysis. Prentice Hall, New Jersey

Zhang JT, Wenming R, Bin L (2006) Relationships between vegetation and climate on the Loess Plateau in China. Folia Geobot 41:151–163

Bacterial Endophytes: Potential Candidates for Plant Growth Promotion

31

Pramod Kumar Sahu, Amrita Gupta, G. Lavanya, Rahul Bakade, and Dhananjaya P. Singh

Abstract

Decreasing resources and enhancing needs have made the situation imperative to boost the agricultural productivity. Agrochemical mediated improvement in productivity has reached to a phase where it has more disadvantages than benefits. Degrading soil quality, water quality, polluting food chain, etc. has driven the interest for the minimal use of these agrochemicals. The moment we think of reducing agrochemical use, one must have an alternative productivity enhancer and essentially sustainable enhancer. Microorganisms have promising roles in fulfilling this need. Microbes take part in enhancing nutrient mobilization, nutrient availability and nutrient use efficiency. This makes it potential agent for partially substituting agrochemicals. Endophytes are a class of microbes living inside the plants with a complex effect in plant growth. A lot of workers have reported significance of endophytic microbes in boosting crop production and minimizing agrochemical load. In this regard, this chapter focuses on various studies conferring the constructive role of bacterial endophytes. Endophytes have tremendous roles in stimulating plant growth, inducing systemic resistance, and alleviating abiotic stresses, nutrient use efficiency, and many more. Exploring the endophytic treasure can make sizable contribution in sustainable agriculture. Understanding, inducing, and/or inoculating these bacterial endophytes can enhance plant growth and health. This chapter also deals regarding the mode of

P.K. Sahu (✉) • A. Gupta • D.P. Singh
ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, Maunath Bhanjan, Uttar Pradesh 275103, India
e-mail: pramod15589@gmail.com

G. Lavanya
Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangaluru 560065, India

R. Bakade
ICAR-Research Complex for Eastern Region (ICAR-RCER), Patna, Bihar, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_31

entry, colonization, and isolation techniques of bacterial endophytes, which will widen the arena of understanding about bacterial endophytes.

Keywords
Bacterial endophytes • Surface sterilization • Plant growth promotion • Biocontrol • Abiotic stress alleviation

31.1 Bacterial Endophytes

Plants endorse the life in soil and are required for essential soil development. In sustaining soil microbes, the plants never live detached (Kobayashi and Palumbo 2000). It constitutes a vast niche of endophytic organisms and not a single plant species lack at least single endophytes (Strobel et al. 2004). Endophytic bacteria are a class of microorganism that dwell in internal plant tissue and do not impart any negative effects to the plant (Schulz and Boyle 2006; Backman and Sikora 2008). The term endophyte was coined by Anton de Bary in 1886 to describe "microorganisms that colonizes internal tissues of stems and leaves" (Wilson 1995). Endophytes are defined as microorganisms living in plant tissues free from disease symptoms. It colonizes inner parts of host tissues and the entire plant without damaging or eliciting robust defense responses.

As an internal colonizer, they could provide a boundary across the invading pathogens directly or by producing bioactive compounds (Thomas and Upreti 2014; Podolich et al. 2015). There are more than 200 genera and 16 bacterial phyla that are correlated with endophytic diversity such as *Actinobacteria*, *Proteobacteria*, and *Firmicutes* (Golinska et al. 2015). As the diversification of bacteria ranges from gram positive to gram negative, most of the endophytic bacterial species belong to α-, β-, and γ-proteobacteria subgroup (Kuklinsky-Sobral et al. 2004). Out of these, the γ-proteobacteria group is the most diverse and dominant.

Bacterial endophytes can be isolated from internal parts of the surface-sterilized plant tissue. It makes a way through the plant tissue from the root hair and also from the aerial part of the plant. It enters the tissue via germinating radical, secondary roots, stomata, or a result of foliar damage and uses the apoplastic route (Sattelmacher 2001; Reinhold-Hurek et al. 2007). By unique behavior with plants, they can be notable from the nonbacterial endophytes. Utmost bacterial endophytes interact with plants in a biotrophic and mutualistic association (Hallmann et al. 1997; Bacon and Hinton 1996; Kobayashi and Palumbo 2000). They are also associated with the exchange of nutrients, enzymes, functional agents, and signals (Parsek and Greenberg 2000; Hardoim et al. 2015).

Bacterial endophytes colonize above (vegetation) and beneath soil (root) host tissues establishing long-haul natural associations, without doing substantive harm to the host. They can be further perceived by predominating the growth of many fungi, bacteria, and nematodes (Bacon and Hinton 1996; Hallmann et al. 1997; Parsek and Greenberg 2000; Hardoim et al. 2015). Tight coalition between the host

plant and endophyte is intervened through the action of admixture produced by the microorganism and the host cells (Reinhold-Hurek and Hurek 2011; Brader et al. 2014). Literature documented that distinct effect of endophytic bacteria on plant health and growth endophytes benefited to the host by enhancing nutrient availability, uptake, stress tolerance, disease resistance, etc. Enhanced plant growth can be established through plant growth-promoting efficacy of endophytes, an interaction that alters internal plant hormone production, increases receptiveness of nitrogen- and phosphorus-like nutrients (Glick 2012), etc. Therefore, it is also used in the form of bioinoculants in agriculture to increase plant yield.

It produces a wide spectrum of compounds such as antibiotic, exoenzymes, siderophores, and other antimicrobial compounds which can suppress the growth of pathogens and act as a biocontrol agent (Raaijmakers and Mazzola 2012; Christina et al. 2013; Brader et al. 2014; Wang et al. 2014). It has found to be stimulating an underlying pathogen defense mechanism, called as induced systemic resistance (ISR) that provides an increased level of protection to a wide variety of pathogens (Pieterse et al. 2014).

31.2 Interaction with Plants

Bacteria can prevail as free-living organisms in soils or adhere to the surface of roots or phyllosphere and may also create the symbiotic relationship with plants (Smith and Goodman 1999). The external colonization of microbes can be apart in two categories on the basis of localization, phyllospheric microorganisms in which it gets across with the exterior part of the leaf surface of the plant and rhizospheric microorganisms which relate with roots. Low humidity and high irradiation are adapted by a phyllospheric microorganism that helps to shield plants from airborne pathogens, and nutritionally rich zone deposition containing compounds, such as amino acid, organic acid, vitamins, sugars, etc., is disguise by the roots in the rhizosphere part of the plant. Plants live in close coalition with microorganisms that fulfill significant functions in agricultural ecosystems. Sustainability of the different ecosystems has an important role in the synergy between microorganism and other biotic factor. It is thus necessary to understand the association and functions carried out by microbial communities. Plant microbe interaction plays crucial role in balancing the ecosystem. Organic and inorganic compounds that are produced by a plant create nutritionally enriched environment and favor heavy colonization of diverse microbes.

Microbial communities affect the plant physiology directly or indirectly, in a positive or negative manner, by various interactions like mutualism, commensalism, amensalism, and pathogenic consequences. In plants, commensalism or mutualism is one of the most common interaction found (Campbell 1995). An endophytic bacterium is an example of plant–microbe interaction wherein bacteria live in a less competitive environment of the host plant tissue without any extreme damage to host cells (James and Olivares 1998). The *Rhizobium*-legume symbiosis and arbuscular mycorrhizal (AM) symbiosis are established by molecular cross talk for

mutual identification of signals generated from both plants and microbial partners. Rhizospheric bacteria or fungi produced symbiotic signals, which occur in the form of lipochitooligosaccharides (LCOs). It is activated by symbiotic pathway and signals are perceived via lysine-motif receptors situated on the plasma membrane of the plant cell, and it triggers the CSP which regulates the interaction among the plant and rhizospheric microorganism.

Secondary metabolites and hydrolytic enzymes are known to be produced by endophytic bacteria. Studies on plant metabolites provided a wide opportunity for discovery of novel endophytic secondary metabolites (Brader et al. 2014). The majority of compounds produced by endophytes possess antibacterial or antifungal activity. Abundant endophytic bacteria with their metabolic signal pathway intricate the interaction in the endosphere of the plant. It creates great difficulty for model in vitro experiments. Quorum sensing is a complex interaction that occurs among the bacteria as observed in the case of the *Methylobacterium*–plant colonization by quorum sensing signals (Dourado et al. 2014). Metagenomic analysis confers the occurrence of three quorum sensing systems, autoinducer-2 system, diffusible signal factor system, and N-acyl homoserine lactone (AHL) system in endophytic microbiome of rice (Sessitsch et al. 2012). The AHL signaling was commonly reported in endophytic microbiome of *Populus deltoides* (Schaefer et al. 2013). In plant–pathogen interaction, quorum sensing was affirmed in olive (*Olea europaea* L.) disease caused by *Pseudomonas savastanoi* pv. *savastanoi*, *Pantoea agglomerans*, and *Erwinia toletana.* They all yield AHLs and impaired virulence was observed in AHL quorum sensing mutant *E. toletana*. Knot development competency by the pathogen was retaken when olive was co-inoculated with *P. savastanoi* AHL synthase mutant (Hosni et al. 2011). Plants intensely associate in AHL signaling as existence of the AHL modified the expression of a number of plant genes in addition to those convoluted in plant defense response, and sometimes plant also mimics the bacterial AHLs (Hartmann et al. 2014).

31.3 Method of Isolation

Isolation of endophytes is one of the most crucial steps in bacterial endophyte research. Bacterial endophytes are isolated from surface-sterilized plant tissue by various methods. Surface sterilization is done to ensure removal of the entire surface microflora. The method used for surface sterilization must be strong enough to remove the entire surface microflora; at the same time, it should be gentle on endophytes (i.e., surface sterilants shouldn't kill the endophytes). This may be a little difficult to achieve, because if we use a very strong surface sterilization to ensure complete removal of surface microflora, it kills some of the endophytes near the surface. On the other hand, if we use relatively diluted sterilants in order to "not kill" any endophytes near the surface, the surface microflora may not be eradicated. The culture-dependent surface sterilization methods, sometimes, may not eradicate surface organisms (Anand et al. 2006; Manter et al. 2010). Therefore, the method used for surface sterilization should be standardized according to different types of

tissues and sterilants used, so that eradication of surface flora can be achieved with minimum harm to endophyte population.

31.3.1 Surface Sterilization and Endophyte Isolation

A healthy plant tissue is washed to harvest root and shoot with the least damage. Plantlets are surface sterilized by using standard protocols, root and shoot separated, and grounded in phosphate buffer. The filtrate is plated on nutrient agar (NA)/TSA plates (strictly monitored up to a week to ensure freedom from all accidental contaminants) in appropriate dilutions. Tissue imprints of surface-sterilized roots and shoots are also plated to ensure elimination of surface microflora (Sessitsch et al. 2002). Here, care should be taken to judgment of sterilization, as the tissue imprint of a surface sterile tissue may also give microbial colony from cut edges of tissue because of sap coming out from tissue that contains some endophytes. This shouldn't be considered as contaminants. Plates after incubation at 30 °C are observed for distinct colony types for 2–5 days. All colonies found to be distinct morphotypes are streaked on NA plates to get a pure culture. The common methodology used for isolation of the bacterial endophyte (Zinniel et al. 2002; Upreti and Thomas 2015) is depicted in Fig. 31.1.

The surface sterilization for metagenomic studies in endophytes has been evaluated by Burgdorf et al. (2014) by spraying yeast cells on wheat and checking its presence by DGGE analysis after surface sterilization. They found that the physical surface sterilization methods are more consistent and effective than chemical sterilants. There are several variants of surface sterilization method according to the nature of tissue used (Table 31.1).

31.4 Mode of Entry

The endophyte gets the benefit of the close niche to host, which bypasses the complex competition for food and space with other microbes as observed in the rhizosphere. Great efforts have been taken in research to confer the mode of entry of the endophyte to the plant. Due to the absence of penetration structure, bacteria cannot enter through physical force. The pathogenic bacteria enters through stomata, lenticels, wounded parts, lateral root emerging points, and germinating radicals or by degrading cell wall-bound polysaccharides of host plant roots. It was also proposed that bacteria may enter through the epidermal junction (Sprent and De Faria 1998) or by passive plant uptake during transpiration (Quadt-Hallman et al. 1997).

The colonization process of bacteria *Enterobacter asburiae* JM22 and *Pseudomonas fluorescens* 89B-61 was demonstrated by applying bacteria on seeds and leaves (Quadt-Hallmann et al. 1997). The tracing of bacterial endophyte *Enterobacter asburiae* JM22 was done by immunological technique using immune gold labeling coupled with transmission electron microscopy. Surface-sterilized seeds of cotton were treated with bacterial cells, dried, and planted. After 2 weeks

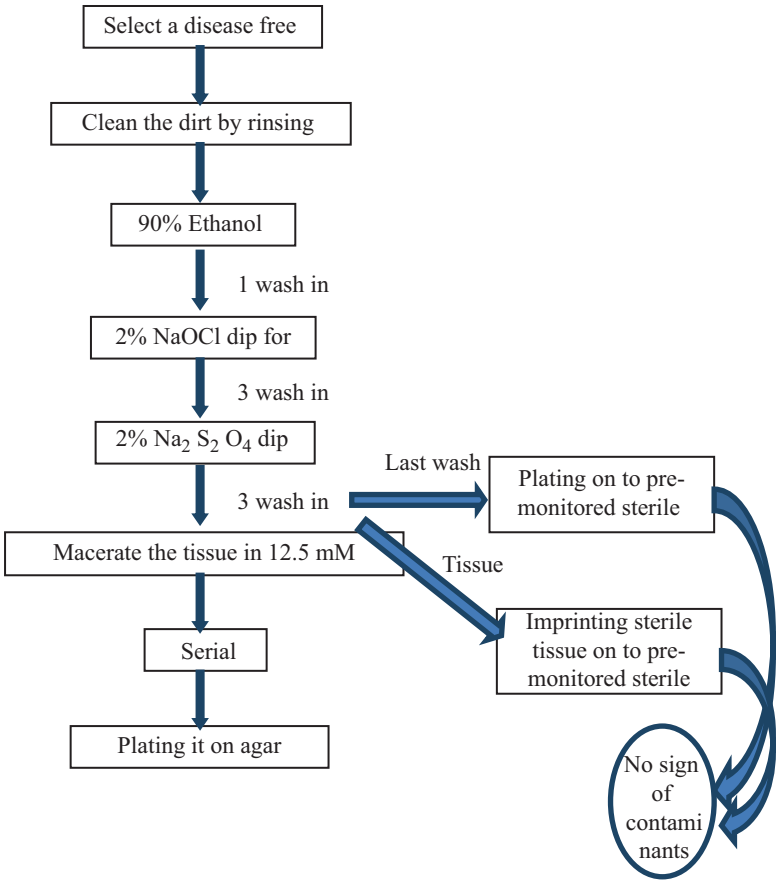


Fig. 31.1 General method for bacterial endophyte isolation

of planting, different plant parts were sampled, surface sterilized, and subjected to tracking by various methods. It was found that JM22 had the capacity to penetrate the root for its entry. There was indirect indication of production of cellulose by JM22, similar to plant pathogenic bacteria (Quadt-Hallmann et al. 1997). Also, entry through wounds was observed.

Apart from seed-transmitted endophytes which are already present in plants, the root is the major source of entry. However, there are other possible sites where endophytes in fruits can enter, namely, through flowers (Sharrock et al. 1991) and natural openings of leaves (stomata or stem lenticels; Kluepfel 1993), via the mealy bug in sugarcane (Ashbolt and Inkerman 1990), etc.

Entry of *Burkholderia* sp. strain PsJN in *Vitis vinifera* L. cv. Chardonnay was determined under gnotobiotic conditions. The visualization of the mode of entry and colonization was done by tagging with *gfp* (PsJN::*gfp*2x) or *gusA* (PsJN::*gusA*11) gene. Secretion of cell wall-degrading endoglucanase and endopolygalacturonase

Table 31.1 Different surface sterilization methods for endophyte isolation

S. No	Plant tissue	Sterilants used	Duration	Post sterilization	References
1.	Stem of wild rice	70% ethanol	1 min shaking	Transferred to media	Elbeltagy et al. (2001)
2.	Stem of rice	0.01% Tween- 20	30 min	Maceration in 0.8% saline and transferred to media	Elbeltagy et al. (2001)
3.	Tomato roots and hypocotyl	90% ethanol (washed in autoclaved distilled water)	Quick dip	Macerated in PBS, dilute, inoculate to media	Zinniel et al. (2002)
		2% NaOCl (washed thrice in autoclaved distilled water)	1–3 min		
		2% $Na_2S_2O_3$ (washed thrice in autoclaved distilled water)	10 min (to remove chloramines residues)		
4.	Any plant material	70% ethanol, sometimes flamed, air-dried in laminar airflow	Thoroughly treated with alcohol	Outer tissues removed with sterile scalpel, inner tissues carefully excised and placed on water agar plates	Strobel and Daisy (2003)
5.	Soybean roots and stem soaked in distilled water and drained	70% ethanol	30 sec	Macerated, diluted, and inoculated to media	Hung and Annapurna (2004)
		0.1% HgCl2	3 min for roots and nodule; 5 min for stem		
		Washed ten times in distilled water			
6.	Tomato roots washed in tap water to remove dirt	70% ethanol	5 min	Roots divided into small fragments and placed onto S media (for endophytic actinomycetes isolation)	Cao et al. (2004)
		0.9% NaOCl (washed thrice in distilled water)	20 min		
		10% $NaHCO_3$	10 min (to retard growth of fungi)		

(continued)

Table 31.1 (continued)

S. No	Plant tissue	Sterilants used	Duration	Post sterilization	References
7.	Rye grass and alfalfa roots	Roots washed in deionized water	–	Final rinse was plated in 1/10th TSA, same water was used for PCR using eubacterial primers to ensure sterility	Phillips et al. (2008)
		Shaken in rotary shaker for 0.5 h			
		–95% ethanol			
		–5.25% NaOCl			
		Five rinses in sterile distilled water			
8.	Stem pieces	70% ethanol and flamed	–	Aseptically placed on LB agar plates	Bhore et al. (2010)
9.	Leaf (cleaned in 5% Teepol washed)	70% ethanol	30 s	Leaves were cut into pieces of 1 cm^2 size using sterile scalpel, placed on sterile media	Bhore et al. (2010)
		5% NaOCl	3 min		
		100% ethanol (washed in autoclaved distilled water)	10 s		
10.	Ginseng leaves	Tap water wash	10 min	Dried with sterile filter paper, macerated in PBS with sterile quartz, and plated on PDA	Gao et al. (2015)
		5% NaOCl	5 min		
		Sterile water rinse	4 times		
		70% ethanol	30 s		
11.	Turmeric rhizomes	70% ethanol	3 min	Rhizomes were dried, sliced, and placed onto agar media	Kumar et al. (2016)
		0.5% NaOCl	3 min		
		70% ethanol (washed thrice in distilled water)	30 s		
12.	Rambutan fruit	70% ethanol	10 min	1 g tissue mixed with 1 ml 0.85% NaCl, inoculate into sugar agar plate	Suhandono et al. (2016)
		2.5% NaOCl	10 min		
		70% ethanol (rinse three times in sterile deionized water)	10 min		

was observed as the mode of entry (Compant et al. 2005). After entry into the plant tissue, it remains localized inside the plant and spread systemically by vascular bundle or apoplastic movement (Quadt-Hallman et al. 1997). Sharma et al. (2005) studied the colonization pattern of one endophyte isolate in wheat by tagging it with *gusA*/*gfp* genes. They found that cracks developed near lateral root emergence are major sites from where the endophyte enters and spreads to intercellular spaces as well as vascular bundles. A similar mode of endophyte entry was also observed in rice roots (Hallmann et al. 2001).

31.5 Role of Bacterial Endophytes in Plants

Endophytic bacteria have a significant role in supporting plant growth. They differ from biocontrol strains and involve in the plant growth promotion by improved nutrient cycling and minerals such as nitrogen, phosphorus, and other nutrients.

Endophytic bacteria promote plant growth through several mechanisms, which include nitrogen fixation (Elbeltagy et al. 2001), phosphate solubilization (Wakelin et al. 2004), production of indole acetic acid (Lee et al. 2004), and siderophore production (Costa and Loper 1994). They are also known to supply vitamins to the plants (Pirttila et al. 2004) and exert plant growth. They increase availability of nutrients in the rhizosphere, influence root growth and morphology, and promote other beneficial plant–microbe symbioses. Endophytic bacteria reside in specific plant tissues and develop a close relationship with the plant, by exchanging nutrients, enzymes, siderophores, and biosurfactants and also stimulating plant signals (Hardoim et al. 2015).

Several endophytes positively affect plant growth promotion by phenomena like stomatal regulation, osmotic adjustment, altered root morphology, enhanced nutrient uptake, and alteration of metabolism (Compant et al. 2005).

31.5.1 Role in Plant Growth Promotion

Endophytic bacteria provide a large array of beneficial effects to their host plant. It promotes plant growth by producing plant growth-enhancing substances such as indole acetic acid (IAA, Naveed et al. 2015) and cytokinins (CK, Garcia de Salamone et al. 2001) and improving nutrient absorption, including nitrogen fixation (Mirza et al. 2001). Besides growth enhancement, endophytic bacteria also benefit the host plant by enhancing adaptation for abiotic or biotic stress via phytohormone signaling. The endophytic bacteria get advantage of being close to the host and protected from the harsh external environment (Sturz et al. 2000). The endophytic bacterial population density varies with the bacterial species, host genotypes, developmental stage of host, inoculum density, and environmental conditions (Pillay and Nowak 1997; Tan et al. 2003).

Endophyte *Burkholderia phytofirmans* PsJN is reported to enhance cold tolerance by altering metabolism of carbohydrates and photosynthetic activity in

grapevine (Ait et al. 2006; Fernandez et al. 2012). The bacterium promotes the host plant to acclimatize to cold condition, lowering the cell damage by accumulation of metabolites such as starch, proline, and phenolic compounds and increasing photosynthetic activity. The same was observed in the wheat grown under reduced irrigation conditions. The presence of endophytic bacteria balances the metabolism and reduces the effect of drought stress in wheat (Naveed et al. 2014). In the rice plant, it was observed to improve the salinity stress tolerance by the accumulation of glycine betaine-like compounds like *Pseudomonas pseudoalcaligenes.*

ABA is a crucial phytohormone for growth and development, which increases under stress condition. Endophytic *Azospirillum* spp. are reported to accumulate the abscisic acid (ABA) in mitigating water stress tolerance in maize. Plant growth-promoting hormones IAA and gibberellins further enhance the effect (Cohen et al. 2009). ABA majorly affects water balance and osmotic stress tolerance in plants (Tuteja 2007).

The major sources of nitrogen for agricultural soil are from mineral fertilizer and biological nitrogen fixation. A number of endophytic diazotrophic bacteria have already been reported to colonize the interior roots of maize, rice, and grasses (Barraquio et al. 1997) and are believed to be capable of contributing nitrogen nutrition in sugarcane (Boddey et al. 1995), rice (Yanni et al. 1997), and wheat (Webster et al. 1998). N2-fixing bacteria constitute only a small proportion in the total endophytic pool of the plant (Barraquio et al. 1997; Martínez et al. 2003).

Reiter et al. (2003) identified the nitrogen-fixing bacteria in nitrogen-poor soils from sweet potato by PCR amplification of nitrogenase (*nifH*) gene. In this analysis, the sequences to those from rhizobia, including *Sinorhizobium* sp. strain NGR234, *Sinorhizobium meliloti*, *Rhizobium etli*, and also *Klebsiella* spp. and *Paenibacillus odorifer*, are detected (Reiter et al. 2003). In legume nodules, also endophytic bacteria are found. Along with *R. leguminosarum* bv. *trifolii*, which is the normal clover symbiont, few other species of rhizobia were also found in red clover nodules, including *Rhizobium* (*Agrobacterium*) *rhizogene* (Sturz et al. 1997). Generally, other endophytic bacteria are unable to form nodules.

Ethylene, a gaseous hormone, exhibits a diverse range of biological effects in plants, even at low concentrations (Abeles et al. 2012). It is involved in germination, differentiation, root and shoot primordial formation, branching and elongation of roots, lateral bud development, flowering, flower senescence, fruit ripening, abscission, anthocyanin production, synthesis of volatile organic compounds (VOCs) for aroma formation in fruits, hydrolysis of storage products, leaf senescence, and abscission (Glick 2014). The enzyme ACC deaminase degrades 1-aminocyclopropane-1-aarboxylate (ACC) which is an immediate precursor of ethylene in plants which is produced during normal plant development and when the plant is exposed to various environmental stresses (Abeles et al. 2012). The bacterium is acting as a sink for ACC after it cleaved into α-ketobutyrate and ammonia, thereby lowering plant ACC levels, which starts its conversion into ethylene. The presence of ACC deaminase-producing bacteria protects the plant from ill effects of inhibiting the level of ethylene during abiotic stress.

Production of ACC deaminase was reported by the endophytic diazotrophic *Achromobacter xylosoxidans* AUM54 isolated from *Catharanthus roseus* grown in saline soil (Karthikeyan et al. 2012). It was showed that endophytic bacteria might play an important role in higher salinity tolerance of the halophyte plant *Limonium sinense* which was naturally associated with ACC deaminase-producing putative endophytes (Qin et al. 2014). Thirteen isolates possessing ACC deaminase activity were obtained that belonged to genera *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Serratia*, *Klebsiella*, *Microbacterium*, *Isoptericola*, and *Streptomyces*. Out of them, four ACC deaminase-producing strains were shown to stimulate growth of the host plants. In a study with tomato plants pretreated with ACC deaminase-producing *Pseudomonas fluorescens* YsS6 and *P. migulae* 8R6, grown under 165 and 185 mM, NaCl levels showed enhanced biomass and flowering than the treatment with ACC deaminase-deficient mutants of the bacteria (Ali et al. 2014).

The potential of bacterial endophytes in fixing dinitrogen and promoting plant growth has renewed the interest in such interactions. Non-rhizobial nitrogen fixers like *Azoarcus* spp., *Herbaspirillum* spp., *Gluconacetobacter diazotrophicus*, etc. have been isolated from the endosphere of kallar grass, rice, and sugarcane, respectively (Cocking 2003).

31.5.2 Role of Bacterial Endophytes in Biocontrol

The roles of plant-associated microorganisms have been extensively studied in inducing suppressiveness of soilborne diseases. Mostly, these microorganisms are root-associated bacteria, and rhizobacteria are a major group of them. Rhizobacteria have the capacity to colonize the developing root system in the presence of competing soil microflora; some rhizobacteria are able to enter in the root and establish its association with the host plant internally, i.e., endophytic phase. The association of rhizobacteria with the host plant is considered as a sign of healthy plant and also contributes in general soil suppressiveness.

The disease can be controlled by applying either the specific introduction of biological control agent or various agronomic and cultural practices to enhance the suppressiveness by altering the rhizosphere microorganism. The application of suitable PGPR is reviewed, to control some specific soilborne fungal pathogen (Kloepper 1993). Few of the soilborne pathogens like *Fusarium oxysporum*, *Pythium* spp., *Phytophthora* spp., *Aphanomyces* spp., *Sclerotium rolfsii*, *Gaeumannomyces graminis*, *Rhizoctonia solani*, *Verticillium* spp., and *Thielaviopsis basicola* are found to be negatively affected by PGPR. The approach to introducing a specific PGPR is to manipulate the indigenous bacterial communities of the rhizosphere, as biological control agents, in a manner that can help in enhancing soil suppressiveness for nematodes and few other soilborne plant pathogens. The work on nematode control strategies demonstrates that it is possible to achieve at least limited induced soil suppressiveness by modification in microbial community structure and function and by several cultural practices like inclusion of antagonistic plants, organic amendments, applications of biorational nematicides, etc.

31.5.2.1 Endophyte Bacteria Induced Systemic Resistance

The endophyte is not only effective for plant growth but it is also related to PGPR-mediated biological control of several soilborne plant pathogens with the strategy called induced systemic resistance (ISR) (Kloepper and Ryu 2006). Since 1991, from the first report of induction of resistance in cucumber by endophyte bacterium *Pseudomonas fluorescens* strain G8-4 against anthracnose disease (Wei et al. 1996), there have been intensive reports of ISR by endophytes. Microbe-associated molecular patterns (MAMPs) play a key role here in mimicking the pathogen for induction of host resistance and provide resistance against diverse groups of plant pathogens. Cucumber endophyte *Bacillus pumilus* strain INR7, isolated from a surviving plant from heavily infested cucumber field with *Erwinia tracheiphila* wilt, is shown to reduce severity of wilt disease in further field trials (Zehnder et al. 2001). The ISR induced by long-run application of INR7 also reduced a number of cucumber beetles which are vectors for *Erwinia tracheiphila.*

ISR against viruses by endophytic bacteria was reported by Zehnder et al. (2000) in *Bacillus pumilus* strain SE34, *Bacillus subtilis* strain IN937b, and *Bacillus amyloliquefaciens* strain IN937a against CMV in tomato. Similarly, Murphy et al. (2003) reported a reduction of CMV in tomato using different strains of endophyte bacterium *B. subtilis*. Co-elicitation of ISR and plant growth was reported by Zhang et al. (2004) by seed application followed by soil drench of endophytic bacteria *B. pumilus* strain SE34 and *S. marcescens* strain 90-166. Biohardening with endophytic bacteria was found to induce systemic resistance in banana against bunchy top virus. Few strains of endophytic bacteria are found to enhance defense-related enzymes and PR proteins with 60% disease control. Apart from disease control, morphological and physiological traits also improved (Harish et al. 2008).

Wheat endophytic actinobacteria capable of suppressing many fungal pathogens were studied for induction of host resistance in *Arabidopsis*. Induction of systemic acquired resistance (SAR) for fungal pathogen *Fusarium oxysporum* and ISR against bacterial pathogen *Erwinia carotovora* mediated by JA/ET pathways was observed by application of endophytic actinobacteria (Conn et al. 2008). There have been many such reports where endophytes have been successfully demonstrated to induce systemic resistance against an array of plant pathogens.

31.5.2.2 Endophytic Bacteria in the Control of Pest Insects in Agriculture

Many studies have also revealed the use of endophytic bacteria for the control of insect pest. *Pseudomonas maltophilia* is reported to cause alternation in larval growth and reduction in the emergence of adult *Helicoverpa zea* of *Plutella xylostella* (Bong et.al.1991). The reduction in viability has been reported to be 80–50% by isolate EN 4 of *Kluyvera ascorbata* and EN5 of *Alcaligenes piechaudii*. The strains of *Bacillus thuringiensis* S 1905 and S 2122 are shown to cause 100% of mortality in caterpillar in the third instar of *Plutella xylostella* after 48 h of bioassay exposure, whereas S 2124 showed 98.33% of mortality after 96 h (Praca 2012). About 100% of mortality of *Plutella xylostella* was observed in the second instar with the application of *B. thuringinesis*. Macedo et al. (2012) reported that the few

strains of *B. thuringiensis* which are toxic to *Diatraea saccharalis* (Lepidoptera: Crambidae) cause more than 75% of mortality.

Melatti et al. (2010) found that the strains S 29, S 40, S 616, and S1576 of *Bacillus aizawai* and S1168 of *Bacillus kurstaki* were toxic to the cotton aphid (*Aphis gossypii*) and showed a mortality of more than 50%. Out of these strains, S 29 and S 1168 were the most effective and showed 76% and 73% of mortality against *A. gossypii*, respectively. In the case of *Spodoptera frugiperda*, among 58 subspecies of *Bacillus thuringiensis*, only *Bacillus thuringiensis morrisoni* is proven to cause 80% of mortality in caterpillar of *Spodoptera frugiperda* (Polanczyk et al. 2003). Along with this, five isolates of *Bacillus thuringiensis* were the best obtained among 24 isolates which showed 31.6–100% of mortality. In the pathogenicity study of *Spodoptera frugiperda* and *Sphenophorus levis*, the isolates of *Bacillus thuringiensis* IB 17.3 and IB 8.2 are found to be the most efficient against caterpillar of *Spodoptera frugiperda*, and isolate IB 26.2 is efficient in the control of larvae of *Sphenophorus levis* and showed 75% of mortality.

31.5.3 Role in Alleviating Abiotic Stress

Productivity of agricultural crops as well as the microbial activity in soil affected by abiotic stress plant and soil microorganism is significantly affected by extreme conditions such as extended drought, excessive rain flooding, high temperatures, frost, and low temperatures, Microorganisms could play a crucial role in enhancing crop tolerance in stress-prone areas. Endophytic bacteria are associated with plant roots and alleviate the ill effects derived from abiotic stresses (such as drought, low temperature, salinity, metal toxicity, high temperatures, etc.) on plants through the assembly of exopolysaccharides and biofilm formation.

When plants are exposed to stress conditions, bacterial endophytes affect plant cells by particular systems like induction of osmoprotectants and heat shock proteins. As compared to rhizospheric counterparts, endophytes are proven better against abiotic stresses (Hallmann et al. 1997; Ryan et al. 2008; Turner et al. 2013). In plants, many rhizosphere microorganisms trigger defense systems and activate a systemic feedback as the root zone bacteria activate signaling pathways. This results in induction of resistance (ISR) in the host. *Bacillus* sp. has been shown to induce ISR as most of the nonpathogenic bacteria are well studied for beneficial effect under abiotic stress situation (Chakraborty et al. 2006).

Rhizosphere microbes can elicit physical or chemical changes relevant to plant resistance in the form of ISR and tolerance to abiotic stress. PGPR induced difference in plants. IST depicts to elevate resistance to abiotic stress (Yang et al. 2009). Signal transduction in plant defense responses can interconnect with other plant stress responses by metabolic pathway. The genes involved in plant responses toward biotic and abiotic stresses can be correlated (Dimkpa et al. 2009).

Bacterial existence in the plant endorses acclimation to chilling temperature, minor cell damage, higher photosynthetic activity, and aggregation of cold-stress-related metabolites such as starch, proline, and phenolic compounds. In wheat

plants grown under reduced irrigation condition, bacteria are found to alleviate the effects of drought stress and maintain metabolic balance (Naveed et al. 2014). *Pseudomonas pseudoalcaligenes* was exhibited to induce accumulation of higher concentrations of glycine betaine-like compounds leading to enhanced salinity stress tolerance in rice (Jha et al. 2011).

Water stress alleviation was reported in maize by abscisic acid (ABA) accumulating endophytic *Azospirillum* spp. Further, the effect was also seen in IAA and gibberellin accumulation (Cohen et al. 2009). Under stress condition, ABA level increases and regulates plant growth.

Plant-synthesized ethylene causes secondary effects of biotic and abiotic stress (Bleecker and Kende 2000); at the same time, it is also involved in vital physiological processes such as seed germination, plant growth, fruit ripening, senescence, and pathogen resistance (Abeles et al. 2012). ACC-deaminase-producing strains lower the effect of stress by degrading ACC, the precursor of ethylene (Grichko and Glick 2001; Mayak et al. 2004).

31.5.4 Other Benefits from Bacterial Endophytes

Bacterial endophytes have many other potential uses in agriculture including phytoremediation, heavy metal tolerance to plant, enhancing nutrient use efficiency, etc.; some of the important roles are listed in Table 31.2. In nature plants are grown with a number of microorganisms; some are harmful directly or indirectly, and to reduce the effect of these, nature also recruited some endophytes. The cost of remediation can strongly decrease by using some biological techniques, and for that bioremediation is the only alternative which is economical. Combined action of plants and associated microbes forms the basis of phytoremediation.

The degradation of copious nitro-aromatic compounds like 2,4,6-trinitrotoluene has been reported by the endophytic bacteria *Methylobacterium* of hybrid poplar trees (Van Aken et al. 2004). The genetically constructed endophytes *Burkholderia cepacia* and *Herbaspirillum seropedicae* were proven by increasing the ability of accumulation of nickel and tolerance to the inoculated plants of yellow lupine (Lodewyckx et al. 2001). Similarly, Barac et al. (2004) demonstrated the use of engineered bacterial endophytes, *Burkholderia cepacia* G4, for increasing tolerance to toluene (aromatic hydrocarbons) and also for reducing transpiration of the same to the atmosphere. Toluene is among the four components of BTEX contamination (benzene, toluene, ethylbenzene, and xylene isomers); therefore, it increases phytoremediation by reducing toxicity.

Degradation of organochlorine herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) by bacterial endophyte *Pseudomonas* was demonstrated in an experiment in which the treated plant did not accumulated 2,4-D in plant, and there were no signs of phytotoxicity (Germaine et al. 2006). The untreated plant showed accumulation of herbicide and sign of phytotoxicity like leaf abscission, callus development, and reduction in biomass. During this experiment more numbers of other rhizosphere bacterial populations were observed to enhance degradation of 2,4-D.

Table 31.2 List of some other beneficial activities of bacterial endophytes

S. No.	Beneficial effects of endophytes	Plant	References
1.	Useful for biotization step in micropropagation	Micropropagated plants	Nowak (1998)
2.	Endophyte with genetically constructed nickel resistance system (*Burkholderia cepacia* L.S.2.4 and *Herbaspirillum seropedicae*) enhances plant tolerance to nickel	*Lupinus luteus* L	Lodewyckx et al. (2001)
3.	Genes for nitro-aromatic compound degradation is more prevalent than rhizosphere microflora	–	Siciliano et al. (2001)
4.	Genetically engineered *Burkholderia cepacia* G4 increased plant tolerance to toluene	Yellow lupine	Barac et al. (2004)
5.	Degradation of 2,4,6-trinitrotoluene by phytosymbiotic strain *Methylobacterium*	Hybrid poplar tree (*Populus deltoids* x *P. nigra*)	Von Aken et al. 2004
6.	Toluene degradation	Poplar	Taghavi et al. (2005)
7.	Degradation of herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) by *Pseudomonas* sp. for reducing phytotoxicity	Pea (*Pisum sativum*)	Germaine et al. (2006)
8.	Lead accumulation by heavy metal-resistant endophytic bacteria and providing tolerance to plant	Rape (*Brassica napus*) roots	Sheng et al. (2008)
9.	PAH degradation by *Brevundimonas* and *Pseudomonas* spp.	Prairie plants	Phillips et al. (2008)
10.	Antitumor activity of endophytic *Streptomycetes*	Pharmaceutical plants	Li et al. (2008)
11.	Remediation of contaminated soils and groundwater	–	Weyens et al. (2009)
12.	ABA and GA produced by endophyte *Azospirillum lipoferum* for drought stress alleviation	Maize	Cohen et al. (2009)
13.	Endophytic bacteria (*Elsholtzia splendens* and *Commelina communis*) providing copper tolerance in copper mine wasteland	Rapeseed	Sun et al. (2010)
14.	Improving shelf life of agricultural produces	–	Bhore et al. (2010) and Ali et al. (2012)
15.	*Herbaspirillum seropedicae* produce amphiphilic lipopeptides serobactin A, B, and C; act as siderophores	Colonizing crops of grass family	Rosconi et al. (2013)

The benefits of using endophytes for xenobiotic remediation are its ability to manipulate more genetically easily than plants (Newman and Reynolds 2005). The bacteria required genetic engineering of a xenobiotic degradation pathway to improve xenobiotic remediation. Also, the gene expression studies of contaminant catabolic genes can reveal efficiency of the remediation process. The uptake of any toxic xenobiotics and its degradation in plant is another significant advantage of employing endophytic pollutant degraders, so that the effect of phytotoxicity gets reduced and eliminates toxic effect on herbivores of the nearby area.

The horizontal gene transfer is a general phenomenon in bacteria, and the endophyte niche became a hot spot for that. The natural transfer of degradative plasmid, pTOM-Bu61, was reported to a large number of different other endophytic bacteria in plants (Taghavi et al. 2005). The horizontal gene transfer also supported efficient degradation of toluene in poplar plants. The application of natural endophytic bacteria having the capacity to degrade contaminants is the best approach for phytoremediation.

31.6 Future Prospects in Utilizing Endophytes for Plant Growth

The enormous potential of endophytes can be harnessed in a much efficient way by precisely understanding the ecology and behavior inside the plant. Research on plant-specific mode of entry and survival during off-season may help in designing crop-specific strategy for endophyte application/augmentation. Studying one endophyte in isolation may give a different picture than reality. Knowing its interaction with other microflora inside the plant is much more important when field performance is concerned. Understanding of their numbers and mode of action should be deciphered with respect to yet-to-culture organisms and their possible impacts on plant physiology.

A lot is yet to be known in endophytes. Real-time measurement of entry, movement, and activity in plant like antagonism to harmful and beneficial microflora, nutrient acquisition, nutrient use efficiency, induction of host resistance, crop-specific production of primary and secondary metabolites, dependency of plants on endophytes or dependency of endophytes on plants, etc. will give new insight of endophyte ecology. Upcoming research on these all aspects may add to the potential of bacterial endophytes in various aspects of plant growth promotion.

Acknowledgments The ICAR-National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM) is gratefully acknowledged for its continuous support and guidance.

References

Abeles FB, Morgan PW, Saltveit ME Jr (2012) Ethylene in plant biology, 2nd edn. Academic, San Diego

Ait BE, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72(11):7246–7252

Ali S, Charles TC, Glick BR (2012) Delay of flower senescence by bacterial endophytes expressing ACC deaminase. J Appl Microbiol 113:1139–1144

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167

Anand R, Paul L, Chanway C (2006) Research on endophytic bacteria: recent advances with forest trees. In: Schulz B, Boyle C, Sieber T (eds) Microbial root endophytes, vol 9. Springer-Verlag, Berlin, pp 89–103

Ashbolt NJ, Inkerman PA (1990) Acetic acid bacterial biota of the pink sugar cane mealybug *Saccharococcus sacchari* and its environment. Appl Environ Microbiol 56:707–712

Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. Biol Control 46:1–3

Bacon CW, Hinton NS (1996) Symptomless endophytic colonization of maize by *Fusarium moniliforme*. Can J Bot 74:1195–1202

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Barraquio WL, Revilla L, Ladha JK (1997) Isolation of endophytic diazotrophic bacteria from wetland rice. Plant Soil 194:15–24

Bhore SJ, Ravichantar N, Loh CY (2010) Screening of endophytic bacteria isolated from leaves of Sambung Nyawa [*Gynura procumbens* (Lour.) Merr.] for cytokinin-like compounds. Bioinform 5:191–197

Bleecker AB, Kende H (2000) Ethylene: a gaseous molecule in plants. Annu Rev Cell Dev Bio 16:1–18

Boddey RM, de Oliveira OC, Urquiaga S, Reis VM, Olivares FL, Baldani VLD, Döbereiner J (1995) Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. Plant Soil 174:195–209

Bong CFJ, Sikorowski PP (1991) Effects of cytoplasmic polyhedrosis virus and bacterial contamination on growth and development of the corn earworm, Helicoverpa zea. J Invert Pathol 57(3):406–412

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37

Burgdorf RJ, Laing MD, Morris CD, Jamal-Ally SF (2014) A procedure to evaluate the efficiency of surface sterilization methods in culture-independent fungal endophyte studies. Braz J Microbiol 45(3):977–983

Campbell N (1995) In: Brady EB (ed) Prokaryotes and the origins of metabolic diversity, 5th edn. The Benjamin/Cummings Publishing Company, Reedwood City, pp 502–519

Cao L, Qiu Z, You J, Tan H, Zhou S (2004) Isolation and characterization of endophytic *Streptomyces* strains from surface-sterilized tomato (*Lycopersicon esculentum*) roots. Lett Appl Microbiol 39:425–430

Chakraborty U, Chakraborty B, Basnet M (2006) Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. J Basic Microbiol 46:186–195

Christina A, Christapher V, Bhore SJ (2013) Endophytic bacteria as a source of novel antibiotics: an overview. Pharmacogn Rev 7(13):11–16

Cocking EC (2003) Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant Soil 252(1):169–175

Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. Botany 87:455–462

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Barka EA (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71(4):1685–1693

Conn VM, Walker AR, Franco CMM (2008) Endophytic Ac*tino*bacteria induces defense pathways in *Arabidopsis thaliana*. MPMI 21:208–218

Costa JM, Loper JE (1994) Characterization of siderophore production by the biological control agent *Enterobacter cloacae*. MPMI 7(4):440–448

Dimkpa C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stresses conditions. Plant Cell Environ 32:1682–1694

Dourado MN, Bogas AC, Pomini AM, Andreote FD, Quecine C, Marsaioli AJ, Araujo WL (2014) *Methylobacterium*-plant interaction genes regulated by plant exudate and quorum sensing molecules. Braz J Microbiol 44(4):1331–1339

Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, Isawa T, Mitsui H, Minamisawa K (2001) Endophytic colonization and in planta nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. Appl Environ Microbiol 67(11):5285–5293

Fernandez O, Theocharis A, Bordiec S, Feil R, Jacquens L, Clement C, Fontaine F, Barka EA (2012) *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. MPMI 25(4):496–504

Gao Y, Liu Q, Zang P, Li X, Ji Q, He ZY, Yang H, Zhao X, Zhang L (2015) An endophytic bacterium isolated from *Panax ginseng* C.A. Meyer enhances growth, reduces morbidity, and stimulates ginsenoside biosynthesis. Phytochem Lett 11:132–138

Garcia de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47(5):404–411

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2, 4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:1–15

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39

Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M (2015) Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie Van Leeuwenhoek 108:267–289. doi:10.1007/s10482-015-0502-7

Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase containing plant growth promoting bacteria. Plant Physiol Biochem 39:11–17

Hallmann J, Quadt Hallmann A, Mahaffe WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hallmann J, Quadt-Hallmann A, Miller WG, Sikora RA, Lindow SE (2001) Endophytic colonization of plants by the biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. Phytopathology 91(4):415–422

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R (2008) Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. Appl Soil Ecol 39(2):187–200

Hartmann A, Rothballer M, Hense BA, Schroder P (2014) Bacterial quorum sensing compound is important modulators of microbe-plant interactions. Front Plant Sci 5:131

Hosni T, Moretti C, Devescovi G, Suarez-Moreno ZR, Fatmi MB, Guarnaccia C, Pongor S, Onofri A, Buonaurio R, Venturi V (2011) Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease. ISME J 5(12):1857–1870

Hung PQ, Annapurna K (2004) Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.) Omonrice 12:92–101

James EK, Olivares FL (1998) Infection and colonization of sugarcane and other graminaceous plants by endophytic diazotrophs. Crit Rev Plant Sci 17:77–119

Jha Y, Subramanian RB, Patel S (2011) Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiol Plant 33:797–802

Karthikeyan B, Joe MM, Islam R, Sa T (2012) ACC deaminase containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus* through reduced ethylene levels and induction of antioxidative defense systems. Symbiosis 56(2):77–86

Kloepper JW, Ryu CM (2006) Bacterial endophytes as elicitors of induced systemic resistance. In: Schulz et al (eds) Microbial root endophytes, vol 9. Springer-Verlag, Berlin, pp 33–52

Kloepper JW, Tuzun S, Liu L, Wei G (1993) Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. Pest management: biologically based technologies. American Chemical Society Books, Washington, DC, pp 156–165

Kluepfel DA (1993) The behavior and tracking of bacteria in the rhizosphere. Annu Rev Phytopathol 31:441–472

Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon CW, White JF (eds) Microbial endophytes. Marcel Dekker, New York, pp 199–233

Kuklinsky-Sobral K, Araujo WL, Mendonca C, Geran LC, Piskala A, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD (2016) Isolation and characterization of bacterial endophytes of Curcuma longa L. 3 Biotech 6(1):1–8

Lee S, Flores-Encarnacion M, Contreras-Zentella M, Garcia-Flores L, Escamilla JE, Kennedy C (2004) Indole-3-acetic acid biosynthesis is deficient in Gluconacetobacter diazotrophicus strains with mutations in cytochrome c biogenesis genes. J Bacteriol 186(16):5384–5391

Li J, Zhao G, Chen H, Wang H, Qin S, Zhu W, Xu L, Jiang C, Li W (2008) Antitumour and antimicrobial activities of endophytic *Streptomycetes* from pharmaceutical plants in rainforest. Lett Appl Microbiol 47:574–580

Lodewyckx C, Taghavi S, Mergeay M, Vangronsveld J, Clijsters H, van der Lelie D (2001) The effect of recombinant heavy metal resistant endophytic bacteria in heavy metal uptake by their host plant. Int J Phytoremediation 3:173–187

Macedo CL, Martins ES, Macedo LLP, Santos AC, Praça LB, Góis LAB, Monnerat RG (2012) Selection and characterization of *Bacillus thuringiensis* strains effective against *Diatraea saccharalis* (Lepidoptera: Crambidae). Pesq Agrop Braz 47(12):1759–1765

Manter D, Delgado J, Holm D, Stong R (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microbial Ecol 60:157–166

Martínez L, Caballero J, Orozco J, Martínez-Romero E (2003) Diazotrophic bacteria associated with banana (*Musa* spp.) Plant Soil 257:35–47

Mayak S, Triosh T, Glick BR (2004) Plant growth promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530

Melatti VM, Praça LB, Martins ES, Sujii E, Berry C, Monnerat RG (2010) Selection of *Bacillus thuringiensis* strains toxic against cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). Bio Assay 5(2):1–4

Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA (2001) Isolation partial characterization and the effect of plant growth-promoting bacteria (PGPB) on micro propagated sugarcane in vitro. Plant Soil 237(1):47–54

Murphy JF, Reddy MS, Ryu C-M, Kloepper JW, Li R (2003) Rhizobacteria-mediated growth promotion of tomato leads to protection against *Cucumber mosaic virus*. Phytopathology 93:1301–1307

Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A (2014) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73:121–131

Naveed M, Qureshi MA, Zahir ZA, Hussain MB, Sessitsch A, Mitter B (2015) L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. Ann Microbiol 65:1391–1389

Newman L, Reynolds C (2005) Bacteria and phyto-remediation: new uses for endophytic bacteria in plants. Trends Biotechnol 23:6–8

Nowak J (1998) Benefits of in vitro "biotization" of plant tissue cultures with microbial inoculants. In Vitro Cell Dev Biol-Plant 34(2):122–130

Praca LB, Gomes ACMM, Cabral G, Martins ES, Sujii EH, Monnerat RG (2012) Endophytic colonization by Brazilian strains of Bacillus thuringiensis on cabbage seedlings grown in vitro. Bt Res 3(1)

Parsek MR, Greenberg EP (2000) Acyl homoserine lactone quorum sensing in Gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. Proc Natl Acad Sci U S A 97(16):8789–8793

Phillips LA, Germida JJ, Farrell RE, Greer CW (2008) Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. Soil Biol Biochem 40(12):3054–3064

Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA (2014) Induced systemic resistance by beneficial microbes. Ann Rev Phytopathol 52:347–375

Pillay VK, Nowak J (1997) Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can J Microbiol 43:354–361

Pirttila AM, Joensuu P, Pospiech H, Jalonen J, Hohtola A (2004) Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures. Physiol Plant 121(2):305–312

Podolich O, Ardanov P, Zaets I, Pirttilä AM, Kozyrovska N (2015) Reviving of the endophytic bacterial community as a putative mechanism of plant resistance. Plant Soil 388(1–2):367–377

Polanczyk RA, Silva RFP, Fiuza LM (2003) Screening of *Bacillus thuringiensis* isolates to *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae). Arq Inst Biol 70(1):69–72

Qin S, Zhang YJ, Yuan B, Xu PY, Xing K, Wang J, Jiang JH (2014) Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte *Limonium sinense* (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. Plant Soil 374:753–766

Quadt-Hallmann A, Kloepper JW, Benhamou N (1997) Bacterial endophytes in cotton: mechanisms of entering the plant. Can J Microbiol 43(6):577–582

Raaijmakers JM, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Ann Rev Phytopathol 50:403–424

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14(4):435–443

Reinhold-Hurek B, Krause A, Leyser B, Miche L, Hurek T (2007) The rice apoplast as a habitat for endophytic N2-fixing bacteria. In: Sattelmacher B, Horst WJ (eds) The apoplast of higher plants compartment of storage, transport and reactions. Springer, Berlin, pp 427–443

Reiter B, Bürgmann H, Burg K, Sessitsch A (2003) Endophytic *nifH* gene diversity in African sweet potato. Can J Microbiol 49:549–555

Rosconi F, Davyt D, Martinez V, Martinez M, Abin-Carriquiry JA, Zane H, Butler A, de Souza EM, Fabiano E (2013) Identification and structural characterization of serobactins, a suite of lipopeptide siderophores produced by the grass endophyte *Herbaspirillum seropedicae*. Environ Microbiol 15:916–927

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278(1):1–9

Sattelmacher B (2001) The apoplast and its significance for plant mineral nutrition. New Phytol 149:167–192

Schaefer AL, Lappala CR, Morlen RP, Pelletier DA, Lu TY, Lankford PK, Harwood CS, Greenberg EP (2013) LuxR- and luxI-type quorum-sensing circuits are prevalent in members of the *Populus deltoides* microbiome. Appl Environ Microbiol 79(18):5745–5752

Schulz B, Boyle C (2006) In: BJE S, CJC B, Sieber TN (eds) What are endophytes? Microbial root endophytes. Springer-Verlag, Berlin, pp 1–13

Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002) Cultivation- independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and *Actinomycetes*-specific PCR of 16S rRNA genes. FEMS Microbiol Ecol 39:23–32

Sessitsch A, Hardoim P, Doring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T, Sarkar A, Bodrossy L, van Overbeek L, Brar D, van Elsas JD, Reinhold-Hurek B (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. MPMI 25(1):28–36

Sharma PK, Sarita S, Prell J (2005) Isolation and characterization of an endophytic bacterium related to *Rhizobium/Agrobacterium* from wheat (*Triticum aestivum* L.) roots. Curr Sci 89(4):608–610

Sharrock KR, Parkes SL, Jack HK, Rees-George J, Hawthorne BT (1991) Involvement of bacterial endophytes in storage rots of buttercup squash (*Cucurbita maxima* D. hybrid 'Delica'). NZJ Crop Hortic Sci 19:157–165

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156(3):1164–1170

Siciliano S, Fortin N, Himoc N et al (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl Environ Microbiol 67:2469–2475

Smith KP, Goodman RM (1999) Host variation for interactions with beneficial plant-associated microbes. Ann Rev Phytopathol 37:473–491

Sprent JI, De Faria SM (1998) Mechanisms of infection of plants by nitrogen fixing organisms. Plant Soil 110(2):157–165

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fertil Soils 25:13–19

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30

Suhandono S, Kusumawardhani MK, Aditiawati P (2016) Isolation and molecular identification of endophytic bacteria from Rambutan fruits (*Nephelium lappaceum* L.) cultivar Binjai. HAYATI J Biosci 23(1):39–44

Sun LN, Zhang YF, He LY, Chen ZJ, Wang QY, Qian M, Sheng XF (2010) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour Technol 101(2):501–509

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. Appl Environ Microbiol 71:8500–8505

Tan Z, Hurek T, Reinhold-Hurek B (2003) Effect of N-fertilization, plant genotype and environmental conditions on *nifH* gene pools in roots of rice. Environ Microbiol 5:1009–1015

Thomas P, Upreti R. (n.d.) Testing of bacterial endophytes from non-host sources as potential antagonistic agents against tomato wilt pathogen *Ralstonia solanacearum*. Adv Microbiol 4:656–666. doi: 10.4236/aim.2014.410071

Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14:209

Tuteja N (2007) Abscisic acid and abiotic stress signaling. Plant Signal Behav 2(3):135–138

Upreti R, Thomas P (2015) Root associated bacterial endophytes from *Ralstonia solanacearum* resistant and susceptible tomato cultivars and their pathogen antagonistic effects. Front Microbiol 6:255. doi:10.3389/fmicb.2015.00255

Van Aken B, Peres C, Doty S, Yoon J, Schnoor J (2004) *Methylobacterium* populi sp. nov., a novel aerobic, pink pigmented, facultatively methylotrophic, methane-ultilising bacterium isolated from poplar trees (*Populus deltoides x nigra* DN34). Evol Microbiol 54:1191–1196

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol Fert Soils 40(1):36–43

Wang M, Xing Y, Wang JXY, Wang G (2014) The role of the chi1 gene from the endophytic bacteria *Serratia proteamaculans* 336x in the biological control of wheat take all. Can J Microbiol 60(8):533–540

Webster G, Jain V, Davey MR, Gough C, Vasse J, Denarie J, Cocking EC (1998) The flavonoid naringenin stimulates the intercellular colonization of wheat roots by *Azorhizobium caulinodans*. Plant Cell Environ 21:373–383

Wei L, Kloepper JW, Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathology 86:221–224

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254

Wilson D (1995) Endophyte: the evolution of a term, and clarification of its use and definition. Oikos 73:274–276

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yanni YG, Rizk RY, Corich V et al (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of potential to promote rice growth. Plant Soil 194:99–114

Zehnder GW, Yao C, Murphy JF, Sikora EJ, Kloepper JW (2000) Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. Biol Control 45:127–137

Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW (2001) Application of rhizobacteria for induced resistance. Eur J Plant Pathol 107:39–50

Zhang S, Reddy MS, Kloepper JW (2004) Tobacco growth enhancement and blue mold disease protection by rhizobacteria: relationship between plant growth promotion and systemic disease protection by PGPR strain 90–166. Plant Soil 262:277–288

Zinniel DK, Lambrecht P, Harris NB (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68:2198–2208. doi:10.1128/AEM.68.5.2198- 2208.2002

Microbial Community Composition and Functions Through Metagenomics

32

Vivek Kumar, Anjali Singh, Madhu Bala Tyagi, and Ashok Kumar

Abstract

Metagenomic approaches have provided a better understanding of microbial diversity and function across the terrestrial biome. Initial studies on soil metagenomics involved construction of libraries and sequencing of cloned genes to know the product encoded, but now a days direct sequence-based information plays an important role in functional profiling of environmental DNA. The rich information obtained from soil metagenome provides new insight into the taxonomic and functional diversity of soil microorganism. Some of the techniques of molecular biology research such as clone-based gene sequence analysis, molecular fingerprinting, next-generation sequencing, and many others have proved very useful in analyzing unknown environmental DNA sample and opened a flux gate of exciting research finding. Additionally, development of new environmental DNA isolation method as well as improved cloning systems has accelerated the pace of research. More importantly, metagenomic tools have resulted in discovery of several novel genes coding for protease, lipase, amylase, alcohol oxidoreductase, antibiotic resistance, etc., from ecological niches including meadows, crop fields, and others. With metagenomic approaches, new dimension in the characterization of complex microbial community has been attained. Surely, metagenomic approaches can be used to build a predictive understanding of how microbial diversity and function vary across terrestrial biome.

V. Kumar • A. Kumar (✉)
School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi 221 005, India
e-mail: kasokbt@rediffmail.com

A. Singh
School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi 221 005, India

M.B. Tyagi
Botany Department, MMV, Banaras Hindu University, Varanasi 221 005, India

D.P. Singh et al. (eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives*, DOI 10.1007/978-981-10-5813-4_32

Keywords
Microbial community • Metagenomics • Function • Plant-microbe interaction

32.1 Introduction

Among all natural environments, soil is the most challenging environment for microbiologists. A diverse assemblage of microbes belonging to bacteria, archaea, fungi, algae, and protozoa constitutes the soil microbial community and plays an important role in biogeochemical cycling of essential elements, maintenance of plant health, and regulation of soil fertility. The soil microbes are also important for industry as they are important sources of new natural products. Soil microbial diversity is greatly affected by spatial heterogeneity and complex physical, chemical, and biological properties of the soil environment. Root exudates, physico-chemical changes, crop rotation, and plant growth stages have major influence on native microbial community (Buee et al. 2009). Every plant has the capacity to be colonized by more than thousand microbes. One gram of soil is known to have thousands to millions of different bacterial, archaeal, and eukaryotic members whose taxonomic diversity is accompanied by physiological and functional diversity (Delmont et al. 2011). Several indices of microbial diversity measures exist in natural environments. Notable among them are gene, phylogenetic, functional, protein, and metabolic diversity. Until recently, microbial diversity in the environment was estimated employing culture-dependent approaches which were based on analysis done on microbes cultured in artificial growth media under laboratory conditions. Based on the size of population estimated by serial dilution plating and microscopy, only about 1% of soil's microorganisms have been cultured till date (Staley and Konopka 1985). Lately, it has been realized that culture-dependent techniques do not accurately describe the microbial communities present in natural environment (Handelsman 2004). During the last three decades, the application of culture-independent approaches has allowed more accurate estimation of the microbial community. Pace et al. (1986) were the first to suggest the use of culture-independent methods to study microbial community. The approach was based on cloning of DNA directly from environmental samples followed by sequencing and functional analysis of the cloned fragment. Subsequently, the word "metagenomics" was coined by Handelsman et al. (1998) which refers to the study of collective genomes in an environmental community or genomic analysis of a population of microorganisms. Several synonyms for this word such as community genomics, environmental genomics, and population genomics also exist. The term metagenome has been used to represent the collective DNA of the entire indigenous microorganisms present in a community. A metagenomic library from a mixture of organisms was enriched on dried straw in the laboratory, and clones expressing cellulolytic activity were observed (Pang et al. 2009). Stein et al. (1996) identified a clone of 40 kb insert derived from a marine picoplankton assemblage that contained a *16S rRNA* gene of archaea. Burgmann et al. (2004) took lead in initiating metagenomic approach in the analysis of free-living diazotrophs in soil. They were able to show diversity in *nifH*

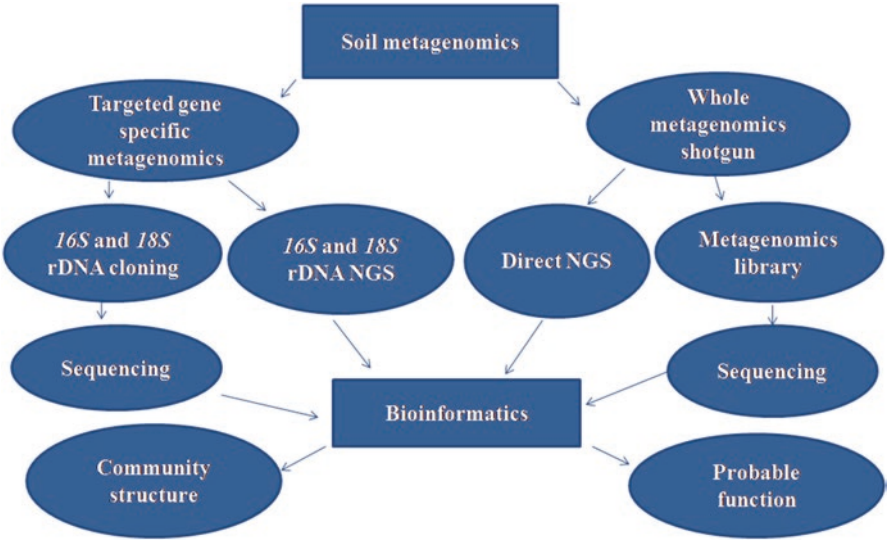


Fig. 32.1 Metadata generation and analysis

gene in soil bacteria. Choudhury et al. (2009) reported diversity of *16S rRNA* and *nifH* genes derived from rhizosphere soil and roots of an endemic drought-tolerant grass, *Lasiurus sindicus*. A detailed account of works done in the area of metagenomics specifically of plant growth-promoting rhizobacteria (PGPR) is available in the review article of Leveau (2007) and Nelson (2013). Sorensen et al. (2009) have lucidly described the molecular tools in rhizosphere microbiology from single cell to whole community analysis. Besides works on rhizosphere metagenome, several researchers have studied soil-derived metagenomic library and reported new genes/enzymes (Yun et al. 2004). However, till date, most of the metagenomic approaches have focused studies on bacterial community although analysis of archaean and fungal communities is equally important (Fierer et al. 2012). Furthermore, compared to other habitats, only a few soil environments have been studied so far because construction of libraries with DNA extracted from soil has faced difficulties in extraction and purification of DNA (Daniel 2005). Henceforth, cataloguing of microbial diversity in soil is still very difficult and challenging.

32.2 Metagenomic Approaches for Community Composition and Functional Analysis

Molecular techniques are very useful in studying structural and functional diversity of total microbial community. To this effect, detailed analysis of *rRNA* gene mostly shows existence of three domains of life, namely, bacteria, archaea, and eukarya (Sait et al. 2002). Several types of molecular techniques including *in situ* and *ex situ* hybridization, cloning of desired gene in a suitable vector and high-throughput sequencing technology, etc., are now used in the analysis of total microbial community structure (Fig. 32.1).

32.2.1 *In Situ* and *Ex Situ* Hybridization Techniques

In situ hybridization is used for detection of live and dead cells in their native habitats. For the study of microbial community, universal 16S and 18S fluorescent (Cy3-labeled) oligonucleotide probes are used. Cy3 is a photostable carbocyanine dye with good bright signals and high quantum yield (Mujumdar et al. 1989). Additionally, specific hybridization probes for tracing microorganisms and confirming their presence are used. These include Eub338 (bacteria), Euk516 (eukarya), arch915 (archaea), Alflb (α-subdivision of proteobacteria), Bet42a (β-subdivision of proteobacteria), Gam42a (γ-subdivision of proteobacteria), SRB385 (δ-subdivision of proteobacteria), HGC69a (Gram-positive bacteria with high G+C content), and CF319a (*Cytophaga-Flavobacterium* cluster of the CFB phylum) (Amann et al. 1990a, b; Stahl and Amann 1991; Manz et al. 1992; Roller et al. 1994). A nucleic acid intercalating dye, 4, 6-diamidino-2′-phenylindole (DAPI), is used for fluorescence staining, and DNA microarrays are used for *in vitro* total population analysis of the soil sample. Herein, metagenomic PCR products are hybridized with known molecular probes attached to microarrays (Gentry et al. 2006). As such signal intensity found after hybridization is presumed to be directly proportional to the abundance of organism. Several researchers have applied the above approach for the analysis of microbial diversity as well as population size. Employing the above approaches, a *16S rRNA* gene-based microarray comprising 30,000 probes to detect several cultured microbial species has been used (DeSantis et al. 2007). Similarly, PhyloChip technology has been employed for the analysis of soil microbial community profiling of mine site (Rastogi et al. 2009). However, one of the major limitations of applying hybridization technique in studying metagenome samples is the lack of probes as the available probes have been designed only for known sequences which are not suitable for the study of novel organisms. Besides chip technology, confocal laser scanning microscopy is also used in the identification of positive clones from total microbial community. Functional gene arrays (FGAs) are also used for the detection of specific metabolic group of microbes (He et al. 2007). This technique is not only beneficial for microbial community analysis but is also useful for metabolically active functional gene community analysis. GeoChip contains 2400 different functional probes for detecting ammonia oxidation, methane oxidation, nitrogen fixation, etc. (He et al. 2007). Yergeau et al. (2009) used FGA for the study of carbon and nitrogen biogeochemical cycle of Antarctica soil microbial community.

32.2.2 Clone-Based Gene Sequence Analysis

Since amplification of all the templates occurs during PCR of metagenomic DNA samples, it is very difficult to reveal the source and identity of the template. However, within a microbial community of prokaryotic and eukaryotic taxa of soil, *16S* and *18S rRNA* genes are conserved for respective domains. Additionally, certain other genes such as RNA polymerase beta unit (*rpoB*), gyrase beta subunit (*gyrB*),

recombinaseA (*recA*), and heat shock protein (*hsp60*) also have conserved functions (Ghebremedhin et al. 2008). Analysis of *16S* and *18S rRNA* gene is helpful in the detection of novel organisms that exist in nature and whose detection is not possible by traditional methods. As such these organisms are novel for researcher but not for their habitat. To overcome these difficulties, shotgun cloning strategy is now used wherein metagenomic PCR products are ligated in vector and transferred in surrogate. Each host cell receives individual gene of interest, whose sequencing is performed (DeSantis et al. 2007). Data obtained from sequencing is confirmed by matching with database of different types of public domain, viz., NCBI, GenBank, Ribosomal Database Project (RDP), Greengenes, etc. Sequencing of metadata confirms the microbial population size including their prokaryotic and/or eukaryotic origin. Based on reference sequences, their phylum, class, order, family, genus, and species are determined. Phylogenetic analysis of clones is also done to determine correlation between and among samples for α and β diversity.

32.2.3 Molecular Fingerprinting

Generally, fingerprinting of diverse microbial community is done by using 16S and 18S rDNA clone libraries. Soil microbial fingerprints are generated by direct analysis of PCR products of metagenomic DNA samples and are mainly based on restriction fragment length polymorphism. A number of molecular biology strategies, namely, denaturing gradient gel electrophoresis (DGGE)/temperature gradient gel electrophoresis (TGGE), single-strand conformational polymorphism (SSCP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), length heterogeneity polymerase chain reaction (LH-PCR), etc., are more commonly used for fingerprinting, but all these approaches are not always fruitful for metagenomic analysis. Recent advancements in genetic fingerprinting techniques have allowed simultaneous analysis of multiple samples in microbial habitats as well as comparative studies of microbes in mixed samples. Importantly, recent developments in bioinformatics tools have greatly helped the analysis of data obtained by community fingerprinting.

32.2.4 Next-Generation Sequencing

For several years, progress in the area of metagenomic study of microbial communities lagged behind mainly due to the high cost of conventional sequencing methods, but emergence of new technology specifically the next-generation sequencing (NGS) has provided cheaper option. In fact progressive advancement in techniques of next-generation sequencing and advanced microscopy has changed the scenario of microbial community structure and function (Zwolinski 2007; Jansson et al. 2012; Cardinale 2014; Mendes and Raaijmakers 2015). NGS is considered as a blessing for

metagenomics because it provides high number of metadata in a short time and is cost-effective. This technology has many high-throughput sequencing platforms, i.e., Illumina (HiSeq & MiSeq), Roche/454, GS FLX, SOLiD 5500 series, Life/APG, HeliScope, and Ion Torrent/Ion Proton platforms. Employing NGS, several bacteria and archaea have been characterized using high-throughput sequence of *16S rRNA* gene. Sequences of other genes such as *nifH* (for nitrogen fixation) and *amoA* (for ammonia monooxygenase) have been frequently used for functional characterization. The procedure in brief consists extraction and purification of total DNA and amplification of targeted genes with conserved primer having short oligonucleotide tags as well as sequence adapters. Employing bioinformatics tools search for operational taxonomy unit (OTU) for phylogeny and sequence assembly for annotations purpose as well as prediction of function of genes/proteins is made.

32.2.5 *In Silico* Tools for Metadata Analysis

As such biostatistical metadata analysis of individual soil habitat is done in terms of abundant and rare population. Earlier metagenomic shotgun sequencing data were used to identify microbes present in a microbial community along with their proportions, but existing taxonomic profiling methods are inefficient for increasingly large data sets. Segata et al. (2012) reported an approach that uses clade-specific marker genes to unambiguously assign reads to microbial clades more accurately and much faster. Herein, metagenomic data profile (taxonomic clades) of abundant microbial population is analyzed by phylogenetic analysis tool such as BLAST v2.2.22 and MetaPha1An (Altschul et al. 1990; Segata et al. 2012). Clade-specific marker gene is used for MetaPha1An database, and sequence similarity search of query is followed by non-default parameters. MetaPhlAn is used to estimate higher score of species. Significant micro-biomass co-occurrence and coexistence interactions are studied by Cytoscape plugin CoNet1.0b2 (http://pssweb05.psb.urgent.be/conet/). Kruskal-Wallis test is used for the analysis of diversity among different samples for alpha and beta diversity, and analysis of different microbial communities is done by Bray-Curtis measurement (Goodrich et al. 2014). R (http://www.r-project.org) function verdict package vegan is helpful for comparing multivariate pairwise taxonomic abundances, and 2D stress value is estimated by permutation-based multivariate analysis of variance. Nonmetric multidimensional scaling (NMDS) is used for watching dispersion of community structure on the basis of Bray-Curtis similarity distance matrix. Linear discriminate analysis (LDA) is used as biomarker (Segata et al. 2011). An automated analysis platform MG-RAST server has been used for quantitative analysis of microbial populations based on sequences (Meyer et al. 2008). Publicly available website (http://vamps.mbl.edu/.projectAB_SAND_Bb6) is used for visualization and analysis of microbial populations structure (VAMPS). Comparative studies of abundance of species are done by web server METASTAT (White et al. 2009). Moran's I test is used for autocorrelation of residual of the alpha diversity by APE R package. Hellinger-transformed taxonomic is used for data transformation, where p values play important role and

corrected for multiple comparisons using the Benjamini-Hochberg method. HUMAnN v0.98 and MEGAHIT v0.3.3 are commonly used for functional analysis of metadata (Abubucker et al. 2012). MEHAHIT v0.3.3 is used for assembling of forward and reverse metadata for determining contig length distribution. Prodigal v2.60 is helpful for assembled metadata prediction (Hyatt et al. 2012). Annotation of gene is done by DIAMOND v0.7.9 with BLASTp command (Buchfink et al. 2015). Protein functional database KEGG Orthology v54 is useful for initial mapping of protein and functional characterization and determination of activity (Edger 2010). Functional gene distribution in metagenomic sample is predicted by COG categories and SEED classes by Kruskal-Wallis test (Table 32.1).

32.2.6 Functional Analysis

Functional metagenomic approaches are dominant over genomic approach because they deal with multiple microbial populations at a time. In this approach, mostly two types of strategies are used: (a) obtaining clone-based information of soil DNA, and (b) obtaining direct sequence-based information. Soil DNA is directly cloned in vectors like pCC1FOS, pCC2FOS, Fosmid pFL12, pUC18, pUCNHD, pGEXC1066, and pGEXC2066 for library preparation (Fig. 32.2). More recently, pCC1FOS and pCC2FOS vectors are used for cloning of large metagenomic DNA. Other vectors used for cloning have limitations mainly due to insert size. Sequence information obtained from library of direct sequence and/or cloned sequence is used for functional annotation of new gene. Together with this, the known bias of prepared library from the soil sample is considered. As such revealing the function of extracted DNA of mixed microbial population is very difficult as it contains a number of unknown organisms with undefined genes and their function. To overcome the problems, functional analysis is done by preparing physical library followed by functional screening**.** First requirement is the selection of a good vector for metagenomic library preparation according to desired function. Preparation of metagenomic soil-based library is same as the cloning of genomic DNA of individual microorganism. Soil DNA is fragmented by mechanical shearing, and restriction digestion is done for obtaining the desired size of fragment. Desired fragment of DNA is ligated in vector followed by transformation and recombinant selection. Selected recombinants are further used for sequencing by primer walking or NGS methods. Sequences obtained are used for annotation of functional gene using bioinformatics tools. Several factors such as type of microbial community present in soil, collection and storage of soil, and quality of extracted DNA influence the functional analysis of soil metagenome (Daniel 2005).

Table 32.1 Server and software used for metadata analysis

No.	System	Server/software	Links
1	Assembly and annotation	Meta-Velvet	metavelvet.dna.bio.keio.ac.jp
		META-IDBA	i.cs.hku.hk/~alse/hkubrg/projects/metaidba/
		IDBA-UD	i.cs.hku.hk/~alse/hkubrg/projects/idba_ud/
		Genovo	cs.stanford.edu/group/genovo/
		HMMer3	hmmer.janelia.org
		BLAST	blast.ncbi.nlm.nih.gov
		RAPSearch2	omics.informatics.indiana.edu/mg/RAPSearch2
		RAST	rast.nmpdr.org/
		MEGAHIT	github.com/voutcn/megahit
		DIAMOND	omictools.com/diamond-tool
2	Taxonomic analysis	RDP classifier	rdp.cme.msu.edu
		NBC	nbc.ece.drexel.edu
		CARMA3	webcarma.cebitec.uni-bielefeld.de
		MEGAN	ab.inf.uni-tuebingen.de/software/megan
		SOrt-ITEMS	metagenomics.atc.tcs.com/binning/SOrt-ITEMS
		HUMAnN	uttenhower.sph.harvard.edu/human
3	Functional analysis	FragGeneScan	omics.informatics.indiana.edu/FragGeneScan
		MGA	whale.bio.titech.ac.jp/metagene
		GeneMark	exon.gatech.edu/metagenome

4	Pathway information	Model SEED	Seedviewer.theseed.org/seedviewer.cgi?page=ModelView
		KGGE	kegg/pathway.html?sess
5	Metabolic profile/metagenome functional analysis	GSEA	broadinstitute.org/gsea/
		ShotgunFunctionali	shotgun.math.chalmers.se/
		zeR	cbcb.umd.edu/~boliu/metapath/
		MetaPath	kiwi.cs.dal.ca/Software/STAMP
		STAMP	ebi.ac.uk/metagenomics/
		EBI	ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml
		metagenomics	cbcb.umd.edu/software/glimmer-mg
		RPSBlast	ncbi.nlm.nih.gov/COG/
		Glimmer-MG	
		COG	
5	Statistical analysis	R software	cran.r-project.org
		Ade4 package	cran.rproject.org/web/packages/ade4/index.html

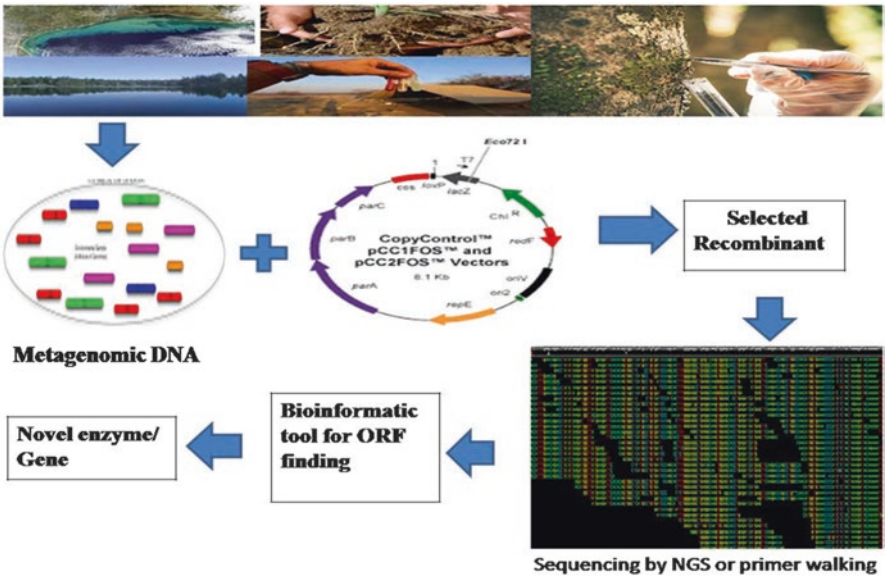


Fig. 32.2 Strategy adopted for the search of novel enzyme/gene from metagenomics

32.3 Soil Microbial Community Structure

Soil microbiota includes microorganisms present on the soil particle surface and those entrapped in soil pores. These include members from bacteria, archaea, algae, and fungal groups. As compared to eukaryotes, prokaryotes are more abundant in soil microbial community. The soil microbial community helps in increasing the fertility of agricultural soil by using their enormous genetic pool that promotes plant growth and increases nutrient availability to plants through biogeochemical cycling of essential elements. They also play important role in degradation of complex organic compounds present in soil including the xenobiotics (Hacquard et al. 2015). Soil microbes secrete different types of compounds such as polysaccharides, gums, and glycoproteins which adhere to minerals and improve soil structure (Arwidsson et al. 2010). It is very crucial for plant to survive and establish themselves against toxic compounds like polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) (Musilova et al. 2016). Richness and evenness of microbial diversity are very important for agriculture soil health and productivity (Tardy et al. 2015). Nitrogen, the most important nutrient required for plant growth and abundantly present in the form of N_2 gas in the atmosphere is made available to plants through nitrogen-fixing organisms present in soil. In general rhizospheric microbial community is known to play a beneficial role in plant growth promotion by influencing metabolism of nitrogen, iron, phosphorus, and potassium (Mendes et al. 2014). Several studies have shown that alterations in environmental conditions are important drivers for causing changes in soil microbial diversity (Yin et al. 2015; Johnston et al. 2016). Predicting the response of dryland ecosystems to a changing

environment is a significant challenge because multiple perturbations, such as land-use changes and regional climate shifts, occur simultaneously. Increased herbivores, recreation, and energy/mining activities also alter surface soil conditions in dry lands, with significant ecological impacts (Belnap 2002). Global warming is on increase with modernization of world, and an average of 0.25 °C increase in earth's temperature occurs per decade (Aydemir et al. 2014). Global anthropogenic changes are known to have also negative effect on soil microbial community composition and function. In a comparative study of OTUs from different habitats like tundra, temperate grassland, and agricultural land, several taxonomical differences were observed. Microbial community from tundra soil was more complex (92%) than those from temperate habitat (80%) (Johnston et al. 2016). The structure of bacterial, archaeal, and fungal community also shows variations during dry and rainy seasons. It has been reported that the members from Planctomycetes, Thermoprotei, and Glomeromycota decreased, whereas Proteobacteria and Ascomycota became more abundant during dry season (de Castro et al., 2016). Interestingly, microbial communities from metal-contaminated sites showed higher number of members from Firmicutes, Chloroflexi, and Crenarchaeota in comparison to Proteobacteria and Actinobacteria (Yin et al. 2015). It has been reported that Brazilian soils support more complex microbial communities than others, with an unexplored genetic diversity (Araujo et al. 2012). Siles et al. (2016) analyzed soil microbial community at four sites of Alpine forest across an altitudinal gradient (545–2000 m above sea level) and found no shift in abundance or diversity of archaeal community, but there was dominance of Thaumarchaeota. However, they reported changes in abundance and diversity of bacterial and fungal community from lower to higher altitudes. The bacterial community was mainly represented by Proteobacteria, Acidobacteria, and Bacteroidetes, whereas the fungal members belonged to Ascomycota, Basidiomycota, and Zygomycota. Sites at the lowest altitudes had the highest bacterial richness, and diversity was found correlated to environmental and edaphic factors specially soil pH. An increase in relative size of fungal community along the gradient was also observed and ascribed to soil pH and C/N ratio (Siles et al. 2016). Yang et al. (2014) employed a microarray-based metagenomic tool to study the response of microbes to climatic change at four sites/elevation of mountainous grassland and found cold-shock genes more abundant at higher elevation. Similarly, metagenomic analysis of some potential nitrogen-fixing bacteria in arable soils at different formation processes revealed significant differences in bacterial community structure (Wolińska et al. 2017). Their study showed dominance of β-Proteobacteria among the representative of potential N_2 fixers belonging to genus *Burkholderia*. For the benefit of readers, a brief detail of community structure of different groups is presented below under separate heading.

32.3.1 Bacteria

Till date majority of soil bacterial communities are reported as uncultured or unknown bacteria because their references are not available in any database.

Henceforth, metagenomics has given new insights in determining the structure and the role of native bacterial communities in increasing the fertility of agricultural soils. Most cultured bacteria from soil have been reported from phyla Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria. Among noncultured bacteria maximum percentage (20%) is reported from Acidobacteria (Riesenfeld et al. 2004). Certain bacteria like species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia* have been isolated from rhizosphere of various agricultural crops (Kloepper et al. 1989; Glick 1995; Ladha and Reddy 2000; Franche et al. 2009; Venieraki et al. 2011). Among these most of the species are involved in plant growth-promoting (PGP) activity including fixation of atmospheric N_2, solubilization of insoluble phosphate, production of phytohormones (indole acetic acid and gibberellic acid), and antagonistic compounds which are effective in the plant disease control (Miyamoto et al. 2004; Richardson et al. 2009).

A few researchers have analyzed bacterial community structure from contaminated or uncropped soil (Le et al. 2016; Souza et al. 2016). Community profiling of long-term oil-contaminated soil revealed the abundance of members from Chromobacterium and Proteobacteria (Patel et al. 2016). In another study, microbial community from crude oil-contaminated soil showed about 40% lesser fungal population than those of bacterial population (Le et al. 2016). Likewise uncropped and soil cropped by maize/soyabean for 23 years showed significant variations in microbial community composition both in terms of taxonomical and functional attributes. Cropped soil showed abundance of members from Proteobacteria and Ascomycota and a decrease in abundance of Planctomycetes, Thermoprotei, and Glomeromycota (Souza et al. 2016). Uncropped soil community revealed bacteria like *Rhizobium*, *Azospirillum*, *Xanthomonas*, *Pseudomonas*, *Acidobacterium*, etc., and fungal members from Ascomycota (Souza et al. 2016). A study conducted on bacterial community from the southwestern highland soil of Saudi Arabia which is highly susceptible to environmental changes and known as hotspot for biodiversity revealed a total of 33 different phyla dominated by Proteobacteria, Actinobacteria, and Acidobacteria (Yasir et al. 2015). In an interesting study conducted on microbiomes and metagenomes of forest biochar, it was noted that microbial community of biochar had lower prokaryotic diversity and higher eukaryotic diversity compared to surrounding soil (Noyce et al. 2016). Detailed analysis of biochar bacterial community showed lower abundance of prokaryotic microorganisms including Acidobacteria, Planctomycetes, and β-Proteobacteria, and eukaryotic biochar community was represented by protists (11%).

Fertilizers and climate are crucial for plant growth; when their ratio varies, bacterial community also changes. For example, winter wheat (*Triticum aestivum*) and summer maize (*Zea mays*) showed significant differences in proportion of bacterial groups, i.e., Acidobacteria, Chloroflexi, Actinobacteria, Proteobacteria, Bacteroidetes, etc. (Xun et al. 2016). Bacterial community structure also showed appreciable differences not only with the types of soil but also with cultivars therein. Li et al. (2016) reported that γ-Proteobacteria, α-Proteobacteria, and Actinobacteria were the main phyla from tea orchard soil. On the other hand, species of *Pseudomonas*,

Stenotrophomonas, *Rahnella*, *Agrobacterium*, *Serratia*, *Bacillus*, *Lysinibacillus*, *Flavobacterium*, and *Chryseobacterium* were identified from the maize rhizosphere (Vigliotta et al. 2016). Analysis of microbial community from notoginseng (*Panax notoginseng*), a herbal medicinal plant, showed positive correlation with bacterial community like Burkholderiales, Syntrophobacteraceae, *Myrmecridium*, and *Phaeosphaeria* for colonization to diseased plants (Dong et al. 2016).

32.3.2 Archaea

Four decades ago, a new domain of living world called archaea was recognized through *16S rRNA* analysis. These bacteria are able to survive in extreme environmental conditions (Rousk et al. 2010). Archaea constitute up to 20–30% of the total prokaryotes in pelagic marine environments but constitute only 0–10% of the total prokaryotes in soil community (Bomberg and Timonen 2009). Soil archaeal research has focused mainly on a subgroup of archaea involved in ammonia oxidation and designated as ammonium-oxidizing archaea (AOA) (Leininger et al. 2006). The presence of archaeal *amoA* gene (Francis et al. 2005) in AOA plays an important role in the global cycling of nitrogen (Konneke et al. 2005). One of the most important factors in determining composition of microbial communities is soil pH. Relative abundance and diversity of archaea of soils collected from biomes of North and South America were found to be greatly affected by soil pH; a shift of pH from 4 to 8 resulted in two-fold increase in diversity (Bengtson et al. 2012).

32.3.3 Cyanobacteria

Cyanobacteria are photosynthetic prokaryotic group of organisms and regarded as eco-friendly alternative for chemical fertilizer. They directly enhance plant growth through N_2 fixation and production of various plant growth-promoting substances such as auxin, gibberellins, and cytokinins. They indirectly promote plant growth through secretion of extracellular polysaccharides which help in soil aggregation and water retention and increase the nitrogen status of soil. Wang et al. (2013) applied metagenomic approach to study the nitrogen fixation potential of terrestrial bacterial/cyanobacterial community from soil sample. They used targeted metagenomics selecting the gene for nitrogenase reductase (*nifH*) and obtained 1.1 million *nifH* 454 amplicon sequences from 222 soil samples collected from four different states in the USA. On the basis of FrameBot (a tool for frameshift correction and nearest neighbor classification), they reported that *nifH* sequences showed the dominance of the phylum Proteobacteria followed by Cyanobacteria in Alaska and Utah sites. On the other hand, cyanobacterial nearest matches were much less common in Hawaii and Florida sites. The majority of the reads showed similarity to *Anabaena* sequences and the corresponding free-living species, but a small percentage (0.05% of the total Alaska reads) were associated with *Nostoc*, a genus containing free-living as well as lichen- and moss-associated members. An Utah site, with arid

conditions showed the presence of Utah "indicator" *Nostoc punctiforme*, a desiccation-resistant form whose filaments are known to be important in holding together desert soil crusts. In addition, many *Nostoc* strains were found to be important in nitrogen-fixing desert soil crusts. Another recent study showed the comparative metagenome analysis of cyanobacteria dominated hypolithic communities in hot desert (Namibia) and cold hyperarid deserts from Antarctica (Le et al. 2016). The most abundant hypolith metagenomes showed the presence of Actinobacteria, Proteobacteria, Cyanobacteria, and Bacteroidetes with Cyanobacteria dominating in Antarctic hypoliths. Interestingly, Cyanobacteria contributed a statistically lower percentage of Namib desert sequences (10%) as compared to Antarctic sequences (~13%). Members of the Oscillatoriales, Chroococcales, and Nostocaceae lineages were the dominant Cyanobacteria in both metagenomes. Nostocales, the member from the family Nostocaceae, were more abundant in the Namib desert (19%) than in the Antarctic desert (7%). Within the Oscillatoriophycideae, Chroococcales were more abundant in the Antarctic metagenome (47%) than in the Namib desert (18%). Dorokhova et al. (2015) studied soils of an administrative district of Moscow and found that the algal-cyanobacterial communities of undisturbed soddy-podzolic soils were predominant by the high species diversity of yellow-green algae and relatively low proportion of non-heterocystous Cyanobacteria. In most of the studied soils in Moscow, diatom dominated in the algal-cyanobacterial communities whereas the industrial zone was characterized by the least species diversity of algae, especially yellow-green algae, and had the highest proportion of Cyanobacteria in the algal-cyanobacterial communities.

32.3.4 Fungi

Fungi grow abundantly in soil and have the capacity of decomposition and accumulation of organic matter which later becomes available to the plant. They also provide protection to plants against several plant pathogens. Based on sequencing, it has been reported that members from phyla Ascomycota (Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Leomycetes) and Basidiomycota (Agaricomycetes) are abundantly present, while fungal genera from Zygomycota, Microsporidia, and Glomeromycota are rare in soil. Antarctic soil crust has approximately just doubled the population of fungi present in dry soil (Bates et al. 2011; Jung et al. 2011). Poplar growing at a metal-contaminated phytoremediation sites showed colonization of roots by arbuscular, mycorrhizal endophytic fungi. Members belonging to Helotiales and *Serendipita vermifera* were found to be highly tolerant to heavy metals such as Cd, Zn, Pb, and Cu and possessed ability to degrade complex carbohydrates like xylose and cellulose. They could possibly enter the root cells by partially degrading the cell wall (Foulon et al. 2016). Saprotrophic fungi work like chelating agent and play an important role in the removal of metals from contaminated soil (Arwidsson et al. 2010). *Tetracladium maxilliforme* are dominant fungal endophytes in root samples (Foulon et al. 2016). Higher death rates of

notoginseng, a herbal medicinal plant, during continuous cropping have been correlated to significant decrease in soil fungal community over successive years of cropping (Dong et al. 2016). Due to limitation of space, it is not possible to provide all the information related to fungal metagenomics; readers may find excellent recent reviews dealing with this topic.

32.4 Outcomes of Metagenomics

New bio-products are useful in pharmacy and agro- and food industries**.** During the last decade, approximately 70,000 natural products derived from microorganisms have been identified; among them, about 50% show bioactivity (Berdy 2012). Most of the industrially useful biomolecules are recovered from environmental samples and can be used commercially for maintaining crop health. Now a days, demand of biologically synthesized molecules is more than chemically synthesized, because most of the bio-products are eco-friendly. Metagenomics has tremendous potential to change the world of microbial biotechnology by discovering cheap and most suitable products for mankind. Acylases, phosphatases, proteases, oxidoreductases, glycosyl hydrolases, lipases/esterases, etc., are the abundant products obtained from metagenomic library. In a comparative study on the occurrence of glycosyl hydrolase-related genes in microbial community, it was noted that this type of genes comprise 0.05–6% of bacterial genome (Countinho et al. 2003), 1.7% of archaeal genome (Werner et al. 2014), and 1.5% of fungal genome (Islam et al. 2012). Most approaches used in metagenomic study proved fruitful for the recovery of novel compounds/natural products from environmental samples. Details of enzymes/genes recovered from soil metagenome are presented in Table 32.2.

32.4.1 Novel Genes/Enzymes

Functional analysis of metadata from soil community has revealed the presence of several novel genes and enzymes in the native community. Metagenomic screening of Korean soil sample library showed an Fe (II)-dependent, nonheme oxygenase, and a novel 4-hydroxyphenylpyruvate dioxygenase (HPPD) that converts 4-hydroxyphenylpyruvate to homogentisate (Lee et al. 2008). This was indeed a breakthrough as plastoquinone and tocopherol are produced by HPPD-dependent anabolic pathway in plants. Furthermore, HPPD plays an important role both for plants and animals. Plant HPPD targets several β-triketones which are used as herbicides including sulcotrione and mesotrione and animal drug for treating hereditary hypertyrosinemia. HPPD also inhibits carotenoid biosynthesis in plants. Similarly, two novel lipase-encoding genes *pwtsB* (301 amino acid) and *pwtsC* (323 amino acid) have been screened from metagenomic library. These are highly active at 20 °C (pH 8.0) and 40 °C (pH 7.0) and utilize p-nitrophenyl palmitate (p-NPP) as the substrate. *pwtsB* is a cold-adapted lipase, while *pwtsC* is a thermostable lipase acting on long-chain p-nitrophenyl esters (Wei et al. 2009). One of the frequently

Table 32.2 Important enzymes/genes obtained from soil metagenome

S. No	Enzyme/gene	Sources	Roles/functions	References
1	Alpha-xylosidase	Forest soil	Glycoside hydrolase activity towards 4-Nitrophenyl α- D-xylopyranoside	Matsuzawa et al. (2016)
2	Amylolytic (*amyM*) gene	Ground soil	Hydrolysis of soluble starch and cyclodextrins	Yun et al. (2004)
3	β-Glucanase	Paddy soil	Exoglucanase and transglycosylation activity	Zhou et al. (2016)
4	Nitrous oxide reductase	Sandy soil	Reduction of N_2O to N_2	Orellana et al. (2014)
5	Phytase gene	Agricultural field soil	Histidine acid phosphatase family phytase	Tan et al. (2014)
6	Chitobiosidase	Agricultural field soil	Salt tolerance	Cretoiu et al. (2015)
7	GH16 family	Sugarcane field soil	Carbohydrate metabolism	Alvarez et al. (2015)
8	D-Amino acid oxidase (*DAAO*)	Agricultural soil	D-amino acid oxidases	Ou et al. (2015)
9	Oxygenase genes	Artificially polluted soil	Indigo-forming activity	Nagayama et al. (2015)
10	Styrene monooxygenase	Loam soil	Catalyzes the actual monooxygenation reaction, and a flavin reductase (StyB), which reduces FAD to $FADH_2$	van Hellemond et al. (2007)
11	Lipolytic enzymes	Forest top soil	Tributyrin hydrolysis activity	Lee et al. (2004)
12	Lipase gene *LipHim1*	Normal soil sample	Lipase activity	Pindi et al. (2014)
13	Endoxylanase family GH10	Sugarcane soil	Xylanase activity	Alvarez et al. (2013)
14	Endoglucanase	Rice straw compost	Cellulolytic activity	Pang et al. (2009)
15	Alkaline protease	Saline habitat	Acts as a serine protease	Purohit and Singh (2013)
16	Esterase MH lip	Antarctic soil	Haloperoxidases and proteases activity	Berlemont et al. (2013)
17	Trehalose synthase gene	Saline-alkali soil	Shows 4.1-fold higher catalytic efficiency (Kcat/km) for maltose than trehalose	Jiang et al. (2013)
18	Xylanase gene (*Mxyl*)	Compost soil	Thermostable at 80 °C	Verma et al. (2013)
19	Chitinases (*chiA*)	Agricultural soil	Chitobiosidase activity	Cretoiu et al. (2012)
20	Xylanases and cellulase	Grassland soil	High activity over a wide range of temperatures and pH	Nacke et al. (2012)

(continued)

Table 32.2 (continued)

S. No	Enzyme/gene	Sources	Roles/functions	References
21	Esterase	Antarctic desert soil	Extremely alkaliphilic and cold active	Hu et al. (2012)
22	Endo-β-1,4-glucanase gene	Red soil	Thermal stability, halotolerance activity	Liu et al. (2011)
23	EstD2	Plant rhizosphere soil	Esterases and lipolytic activity	Lee et al. (2010)
24	β-Galactosidase	5–10 cm depth soil	Hydrolyzes lactose to glucose and galactose	Wang et al. (2010)
25	Multicopper oxidase	Mangrove soil	Laccases as biocatalysts	Ye et al. (2010)
26	Family VIII alkaline esterase	Compost soil	Tolerant to methanol	Kim et al. (2010)
27	Endoglucanase	Compost soil	Activity against carboxymethyl cellulose	Pang et al. (2009)
28	Lipases	Normal soil sample	High specificity for *p*-nitrophenyl palmitate	Wei et al. (2009)
29	Esterase	Vegetable soil	Pyrethroid hydrolyzing	Li et al. (2008)
30	Poly (DL-lactic acid) depolymerases	Compost soil	Biodegradable aliphatic polyesters	Mayumi et al. (2008)

used herbicides in agriculture is phenoxy-alkanoic acid (PAA). Soil microbes are responsible for the degradation of PAA into α-ketoglutarate through an Fe^{2+}-dependent dioxygenases encoded by *tfdA*-like gene. Putative degradation of phenoxy-alkanoic acid, by *tfdA*-like gene, encoding herbicide-degrading dioxygenases has been demonstrated in soil samples (Zaprasis et al. 2010). Polyhydroxyalkanaote synthase-encoding gene *phaC* occupies a central position among bacterial enzymes; it catalyzes the polymerization of hydroxyacyl-CoA molecule. A novel polyhydroxyalkanaote (PHA) synthase-encoding gene derived from a soil metagenomic library was found to be useful in the engineering of more efficient systems for the industrial production of bioplastics (Schallmey et al. 2011). Tao et al. (2011) reported that alluvial metagenomes contain a novel esterase gene (*estDL30*) with 1524 nucleotides and produce a 507 amino acid peptide similar to B-carboxylesterases. A phylogenetic study of gene *estDL30* showed similarity to family VII lipase and esterase of *Bacillus subtilis*, *Streptomyces coelicolor*, and *Arthrobacter oxydans*. Soil metagenomic DNA library has also been used for identification of a novel tryptophan dimerization biosynthetic gene cluster, which works as indolotryptoline antiproliferative agent (Chang and Brady 2013). In another interesting study, saline-alkali soil metagenomic library showed a new novel gene trehalose synthase (*TreS*) encoding a 552 amino acid protein (molecular weight 63.3 kDa). This enzyme functions like glycosyl hydrolase family 13 enzyme catalysis (Jiang et al. 2013). Compost soil microbial habitat with hot environment contained microbes with novel thermostable and alkalistable xylansase enzyme encoded by *mxyl* gene (Verma et al. 2013). This novel enzyme showed highest catalytic activity towards p-nitrophenyl butyrate. Functional screening of mangrove soil metagenome library revealed a novel endoglucanase gene *mgce144* encoding a

648-long polypeptide with a molecular mass of 70.8 kDa and catalytic domain activity like glycosyl hydrolase 44 (Mai et al. 2014). A novel bacterial chitinase (*Chi18H8*) with antifungal role against several agricultural crops was identified from uncultured bacterial community (Hjort et al. 2014). A study conducted to reveal the metabolic potential of two soil-derived lignocellulolytic microbial consortia, denoted as RWS and TWS (bred on wheat straw), showed the plant polysaccharide-degrading capability of microbial consortia (Jimenez et al. 2015). A study on the functional screening of soil metagenomic library confirmed the presence of genes conferring tolerance against lignocellulose-derived inhibitors (Wang et al. 2015). Before the release of fermentable sugar, lignocellulosic raw materials were treated with thermostable lignocellulose that makes harsh conditions and generates numerous small inhibitor molecules responsible for hindering microbial growth and fermentation (Forsberg et al. 2016). Sequence-based screening of plasmid library of contaminated agricultural soil showed the presence of a novel *daoE* gene, closely related to D-amino acid oxidase (DAAO) gene. The maximum activity of the recombinant protein occurred at temperature 37 °C and pH 8.0 with a Km value of 2.96 mM, Vmax of 0.018 mM/min, kcat of 10.9/min, and kcat/Km value of 1.16×10^4/mol/min (Ou et al. 2015). It is known that methanesulfonate (MSA), a catabolic oxidative compound produced during sulfur cycle, is used by bacteria as carbon and energy source, and a specific enzyme methanesulfonate monooxygenase (MSAMO) is used for the first catalytic step of MSA oxidation. To this effect, novel genes *msmA* and *msmE* have been reported from marine habitat which is involved in the synthesis of large (alpha) subunit of the MSAMO enzyme (Henriques and De Marco 2015). A novel gene coding for 343 amino acid polypeptide residue and functioning like putative lipolytic enzyme related to the hormone-sensitive lipase family has been characterized from Permafrost sample. The amino acid sequence of this polypeptide showed maximum similarity with the properties reported for the uncharacterized protein from *Sphingomonas* species (Petrovskaya et al. 2016).

32.4.2 Other Novel Biomolecules

For the first time, Gillespie et al. (2002) introduced antibiotics turbomycin A and B from a metagenomic library of soil microbial community DNA. Subsequently, nine genes conferring high level of resistance to tetracycline due to production of enzyme tetracycline destructases, were identified by soil functional metagenome screening (Forsberg et al. 2015). The environmental "resistome" is the collection of genes that directly or indirectly influence antibiotic resistance. It deals with the composition of local resistome which is involved in gene transfer due to human activity such as agriculture (Perry and Wright 2013). Notably, metagenomics plays a crucial role in studying horizontal gene transfer of antibiotic resistance genes from the environment to the clinic. A number of bacteria under abnormal growth conditions synthesize natural polyester polyhydroxyalkanoates (PHAs). Metagenomics is also fruitful in exploring knowledge about the production of a variety of PHA polymer and copolymer mixtures (Cheng and Charles 2016). Ring-hydroxylating dioxygenases

(RHDs) play a crucial role in the biodegradation of a range of aromatic hydrocarbons found on polluted sites, including those containing polycyclic aromatic hydrocarbons (PAHs) (Chemerys et al. 2014). PCR amplicon sequences of ketosynthase gene have been used to explore the structural and biosynthetic diversities of pentangular polyphenols (PP) (Kang and Brady 2014). Polyketide, a secondary metabolite having antibiotic or/and anticancer activity has been isolated from soil Acidobacteria which also produce a few other secondary metabolites (Parsley et al. 2011). Metagenome-derived short-chain dehydrogenase/reductase has a role in the attenuation of *P. aeruginosa* (PAO1) which results in reduced pyocyanin production, decreased motility, and poor biofilm formation (Bijtenhoorn et al. 2011).

32.5 Challenges for Soil Metagenomics

Isolation of pure metagenomic DNA from the soil sample is problematic because several types of impurities coprecipitate with metagenomic DNA. Humic acid, the main impurity that coprecipitates with metagenomic DNA, chelates Mg^{2+} ions during PCR and also affects the activity of *Taq* DNA polymerase (Tsai and Olson 1992). The presence of minute quantities of humic compound hinders all the molecular approaches used in metagenome study and affects the activity of enzyme. Cloning of metagenomic DNA is a crucial step, and the choice of a suitable cloning vector and suitable surrogate is very critical. Construction of metagenomic library using cosmid or fosmid vector is more appropriate as they have large insert size and have high cloning efficiency. The length of extracted DNA must be sufficient for lambda packaging because self-ligated and false colonies are frequently detected (Lam and Charles 2015). Each step of metagenomic library construction is vital and requires high level of molecular biological expertise. Metagenomics also requires the knowledge of bioinformatics tools which are required for annotation and statistical analysis applicable for probability and correlation.

Acknowledgments VK is grateful to the Indian Council of Agricultural Research (ICAR), New Delhi, for the award of Senior Research Fellowship in a research project. Research in the area of PGPR is partly supported by a research grant sanctioned to AK by the Indian Council of Agricultural Research, Government of India, New Delhi (NBAIM/ AMAAS/2014-17/PF/4).

References

Abubucker S, Segata N, Goll J (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. PLoS Comput Biol 8:e1002358. doi:10.1371/journal.pcbi.1002358

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Alvarez TM, Goldbeck R, CRd S et al (2013) Development and biotechnological application of a novel endoxylanase family GH10 identified from sugarcane soil metagenome. PLoS One 8:e70014. doi:10.1371/journal.pone.0070014

Alvarez TM, Liberato MV, Cairo JPLF et al (2015) A novel member of GH16 family derived from sugarcane soil metagenome. Appl Biochem Biotechnol 177:304–317

Amann RI, Binder BJ, Olsen RJ et al (1990a) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56:1919–1925

Amann RI, Krumholz L, Stahl DA (1990b) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J Bacteriol 172:762–770

Araujo JF, de Castro AP, Costa MMC et al (2012) Characterization of soil bacterial assemblies in Brazilian Savanna-like vegetation reveals Acidobacteria dominance. Microb Ecol 64:760–770

Arwidsson Z, Elgh-Dalgrenb K, von Kronhelm T et al (2010) Remediation of heavy metal contaminated soil washing residues with amino polycarboxylic acids. J Hazard Mater 173:697–704

Aydemir U, Candolfi C, Ormeci A et al (2014) High temperature thermoelectric properties of the type-I clathrate Ba_8 Ni_x Ge_{46}-x-y-Oy square(y). J Phys Condens Matter 26:485801. doi:10.1088/0953-8984/26/48/485801

Bates ST, Berg-Lyons D, Caporaso JG et al (2011) Examining the global distribution of dominant archaeal populations in soil. ISME J 5:908–917

Belnap J (2002) Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biol Fertil Soils 35:128–135

Bengtson P, Sterngren AE, Rousk J (2012) Archaeal abundance across a pH gradient in an arable soil and its relationship to bacterial and fungal growth rates. Appl Environ Microbiol 78:5906–5911

Berdy J (2012) Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot (Tokyo) 65:385–395

Berlemont R, Jacquin O, Delsaute M et al (2013) Novel cold-adapted esterase MHlip from an Antarctic soil metagenome. Biology 2:177–188

Bijtenhoorn P, Mayerhofer H, Muller-Dieckmann J et al (2011) A novel metagenomic short-chain dehydrogenase/reductase attenuates *Pseudomonas aeruginosa* biofilm formation and virulence on *Caenorhabditis elegans*. PLoS One 6:e26278. doi:10.1371/journal.pone.0026278

Bomberg M, Timonen S (2009) Effect of tree species and mycorrhizal colonization on the archaeal population of boreal forest rhizospheres. Appl Environ Microbiol 75:308–315

Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60

Buee M, Reich M, Murat C et al (2009) 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184:449–456

Burgmann H, Widmer F, Sigler WV et al (2004) New molecular screening tools for analysis of free-living diazotrophs in soil. Appl Environ Microbiol 70:240–247

Cardinale M (2014) Scanning a microhabitat: plant-microbe interactions revealed by confocal laser microscopy. Front Microbiol 5:94. doi:10.3389/fmicb.2014.00094

Chang FY, Brady SF (2013) Discovery of indolotryptoline antiproliferative agents by homology-guided metagenomic screening. Proc Natl Acad Sci U S A 110:2478–2483

Chemerys A, Pelletier E, Cruaud C et al (2014) Characterization of novel polycyclic aromatic hydrocarbon dioxygenases from the bacterial metagenomic DNA of a contaminated soil. Appl Environ Microbiol 80:6591–6600

Cheng J, Charles TC (2016) Novel polyhydroxyalkanoate copolymers produced in *Pseudomonas putida* by metagenomic polyhydroxyalkanoate synthases. Appl Microbiol Biotechnol 100:7611–7627

Choudhury SP, Schmid M, Hartmann A et al (2009) Diversity of *16S-rRNA* and *nifH* genes derived from rhizosphere soil and roots of an endemic drought tolerant grass, *Lasiurus sindicus*. Eur J Soil Biol 45:114–122

Cretoiu MS, Kielak AM, Al-Soud WA et al (2012) Mining of unexplored habitats for novel chitinases-chiA as a helper gene proxy in metagenomics. Appl Microbiol Biotechnol 94:1347–1358

Cretoiu MS, Berini F, Kielak AM et al (2015) A novel salt-tolerant chitobiosidase discovered by genetic screening of a metagenomic library derived from chitin-amended agricultural soil. Appl Microbiol Biotechnol 99:8199–8215
Countinho PM, Stam M, Blanc E et al (2003) Why are there so many carbohydrate-active enzyme-related genes in plants? Trends Plant Sci 8:563–565
Daniel R (2005) The metagenomics of soil. Nat Rev Microbiol 3:470–478
de Castro AP, Sartori da Silva MRS, Quirino BF et al (2016) Microbial diversity in Cerrado biome (Neotropical Savanna) soils. PLoS One 11:e0148785. doi:10.1371/journal.pone.0148785
Delmont TO, Malandain C, Prestat E et al (2011) Metagenomic mining for microbiologists. ISME J 5:1837–1843
DeSantis TZ, Brodie EL, Moberg JP et al (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 53:371–383
Dong L, Xu J, Feng G et al (2016) Soil bacterial and fungal community dynamics in relation to *Panax notoginseng* death rate in a continuous cropping system. Sci Rep 6:31802. doi:10.1038/srep31802
Dorokhova MF, Kosheleva NE, Terskaya EV (2015) Algae and cyanobacteria in soils of Moscow. AJPS 6:2461–2471
Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461
Fierer N, Leff JW, Adams BJ et al (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci U S A 109:21390–21395
Forsberg KJ, Patel S, Wencewicz TA et al (2015) The tetracycline destructases: a novel family of tetracycline-inactivating enzymes. Chem Biol 22:888–897
Forsberg KJ, Patel S, Witt E et al (2016) Identification of genes conferring tolerance to lignocellulose-derived inhibitors by functional selections in soil metagenomes. Appl Environ Microbiol 82:528–537
Foulon J, Zappelini C, Durand A et al (2016) Impact of poplar-based phytomanagement on soil properties and microbial communities in a metalcontaminated site. FEMS Microbiol Ecol 92:fiw163. doi:10.1093/femsec/fiw163
Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59
Francis CA, Roberts KJ, Beman JM et al (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci U S A 102:14683–14688
Gentry TJ, Wickham GS, Schadt CW et al (2006) Microarray applications in microbial ecology research. Microb Ecol 52:159–175
Ghebremedhin B, Layer F, Konig W et al (2008) Genetic classification and distinguishing of *Staphylococcus* species based on different partial gap, *16S rRNA*, *hsp60*, *rpoB, sodA*, and *tuf* gene sequences. J Clin Microbiol 46:1019–1025
Gillespie DE, Brady SF, Bettermann AD et al (2002) Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA. Appl Environ Microbiol 68:4301–4306
Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–114
Goodrich JK, Rienzi SCD, Poole AC et al (2014) Conducting a microbiome study. Cell 158:250–262
Hacquard S, Garrido-Oter R, Gonzalez A et al (2015) Microbiota and host nutrition across plant and animal kingdoms. Cell Host Microbe 17:603–616
Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685
Handelsman J, Rondon MR, Brady SF et al (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5(R):245–249
He Z, Gentry TJ, Schadt CW et al (2007) GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME J 1:67–77

Henriques AC, De Marco P (2015) Methanesulfonate (MSA) catabolic genes from mrine and estuarine bacteria. PLoS One 10:e0125735. doi:10.1371/journal.pone.0125735
Hjort K, Presti I, Elvang A et al (2014) Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics. Appl Microbiol Biotechnol 98:2819–2828
Hu XP, Heath C, Taylor MP et al (2012) A novel, extremely alkaliphilic and cold-active esterase from Antarctic desert soil. Extremophiles 16:79–86
Hyatt D, LoCascio PF, Hauser LJ et al (2012) Gene and translation initiation site prediction in metagenomic sequences. Bioinformatics 28:2223–2230
Islam MS, Haque MS, Islam MM et al (2012) Tools to kill: genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics 13:493. doi:10.1186/1471-2164-13-493
Jansson JK, Neufeld JD, Moran MA et al (2012) Omics for understanding microbial functional dynamics. Environ Microbiol 14:1–3
Jiang L, Lin M, Zhang Y et al (2013) Identification and characterization of a novel trehalose synthase gene derived from saline-alkali soil metagenomes. PLoS One 8:e77437. doi:10.1371/journal.pone.0077437
Jimenez DJ, Chaves-Moreno D, van Elsas JD (2015) Unveiling the metabolic potential of two soil-derived microbial consortia selected on wheat straw. Sci Rep 5:13845. doi:10.1038/13845
Johnston ER, Rodriguez RL, Luo C et al (2016) Metagenomics reveals pervasive bacterial populations and reduced community diversity across the Alaska tundra ecosystem. Front Microbiol 7:579. doi:10.3389/fmicb.2016.00579
Jung J, Yeom J, Kim J et al (2011) Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. Res Microbiol 162:1018–1026
Kang HS, Brady SF (2014) Mining soil metagenomes to better understand the evolution of natural product structural diversity: pentangular polyphenols as a case study. J Am Chem Soc 136:18111–18119
Kim YH, Kwon EJ, Kim SK et al (2010) Molecular cloning and characterization of a novel family VIII alkaline esterase from a compost metagenomic library. Biochem Biophys Res Commun 393:45–49
Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–44
Konneke M, Bernhard AE, de la Torre JR et al (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546
Ladha JK, Reddy PM (eds) (2000) The quest for nitrogen fixation in rice. IRRI, Los Banos
Lam KN, Charles TC (2015) Strong spurious transcription likely contributes to DNA insert bias in typical metagenomic clone libraries. Microbiome 3:22. doi:10.1186/s40168/015/0086/5
Le PT, Makhalanyane TP, Guerrero LD et al (2016) Comparative metagenomic analysis reveals mechanisms for stress response in hypoliths from extreme hyperarid deserts. Genome Biol Evol 8:2737–2747
Lee CM, Yeo YS, Lee JH et al (2008) Identification of a novel 4-hydroxyphenylpyruvate dioxygenase from the soil metagenome. Biochem Biophys Res Commun 370:322–326
Lee MH, Hong KS, Malhotra S et al (2010) A new esterase EstD2 isolated from plant rhizosphere soil metagenome. Appl Microbiol Biotechnol 88:1125–1134
Lee SW, Won K, Lim HK et al (2004) Screening for novel lipolytic enzymes from uncultured soil microorganisms. Appl Microbiol Biotechnol 65:720–726
Leininger S, Urich T, Schloter M et al (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809
Leveau JHJ (2007) The magic and menace of metagenomics-prospects for the study of plant growth promoting rhizobacteria. Eur J Plant Pathol 119:279–300
Li G, Wang K, Liu YH (2008) Molecular cloning and characterization of a novel pyrethroid-hydrolyzing esterase originating from the metagenome. Microb Cell Factories 7:38. doi:10.1186/1475-2859-7-38

Li YC, Li Z, Li ZW et al (2016) Variations of rhizosphere bacterial communities in tea (*Camellia sinensis* L.) continuous cropping soil by highthroughput pyrosequencing approach. J Appl Microbiol 121:787–799

Liu J, Wd L, Xl Z et al (2011) Cloning and functional characterization of a novel endo-β -1, 4-glucanase gene from a soil-derived metagenomic library. Appl Microbiol Biotechnol 89:1083–1092

Mai Z, Su H, Yang J et al (2014) Cloning and characterization of a novel GH44 family endoglucanase from mangrove soil metagenomic library. Biotechnol Lett 36:1701–1709

Manz W, Amann R, Ludwig W et al (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: problems and solutions. Syst Appl Microbiol 15:593–600

Matsuzawa T, Kimura N, Suenaga H et al (2016) Screening, identification, and characterization of α-xylosidase from a soil metagenome. J Biosci Bioeng 122:393–399

Mayumi D, Akutsu-Shigeno Y, Uchiyama H et al (2008) Identification and characterization of novel poly (DL-lactic acid) depolymerases from metagenome. Appl Microbiol Biotechnol 79:743–750

Mendes LW, Kuramae EE, Navarrete AA et al (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 8:1577–1587

Mendes R, Raaijmakers JM (2015) Cross-kingdom similarities in microbiome functions. ISME J 9:1905–1907

Meyer F, Paarmann D, D'Souza M (2008) The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinf 9:386. doi:10.1186/1471-2105-9-386

Miyamoto T, Kawahara M, Minamisawa K (2004) Novel endophytic nitrogen-fixing clostridia from the grass *Miscanthus sinensis* as revealed by terminal restriction fragment length polymorphism analysis. Appl Environ Microbiol 70:6580–6586

Mujumdar RB, Ernst LA, Mujumdar SR et al (1989) Cyanine dye labeling reagents containing isothiocyanate groups. Cytometry 10:11–19

Musilova L, Ridl J, Polivkova M et al (2016) Effects of secondary plant metabolites on microbial populations: changes in community structure and metabolic activity in contaminated environments. Int J Mol Sci 17:1205. doi:10.3390/ijms17081205

Nacke H, Engelhaupt M, Brady S et al (2012) Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes. Biotechnol Lett 34:663–675

Nagayama H, Sugawara T, Endo R et al (2015) Isolation of oxygenase genes for indigo-forming activity from an artificially polluted soil metagenome by functional screening using *Pseudomonas putida* strains as hosts. Appl Microbiol Biotechnol 99:4453–4470

Nelson KE (2013) Microbiomes. Microb Ecol 65:916–919

Noyce GL, Winsborough C, Fulthorpe R et al (2016) The microbiomes and metagenomes of forest biochars. Sci Rep 6:26425. doi:10.1038/srep26425

Orellana LH, Rodriguez-R LM, Higgins S et al (2014) Detecting nitrous oxide reductase (*nosZ*) genes in soil metagenomes: method development and implications for the nitrogen cycle. MBio 5:e01193–e01114

Ou Q, Liu Y, Deng J et al (2015) A novel D-amino acid oxidase from a contaminated agricultural soil metagenome and its characterization. Antonie Van Leeuwenhoek 107:1615–1623

Pace NR, Stahl DA, Lane DJ et al (eds) (1986) Analyzing natural microbial populations by rRNA sequences. ASM News 51:4–12

Pang H, Zhang P, Duan CJ et al (2009) Identification of cellulase genes from the metagenomes of compost soils and functional characterization of one novel endoglucanase. Curr Microbiol 58:404–408

Parsley LC, Linneman J, Goode AM et al (2011) Polyketide synthase pathways identified from a metagenomic library are derived from soil Acidobacteria. FEMS Microbiol Ecol 78:176–187

Patel V, Sharma A, Lal R et al (2016) Response and resilience of soil microbial communities inhabiting in edible oil stress/contamination from industrial estates. BMC Microbiol 16:50. doi:10.1186/s12866-016-0669-8

Perry JA, Wright GD (2013) The antibiotic resistance "mobilome": searching for the link between environment and clinic. Front Microbiol 4:138. doi:10.3389/00138

Petrovskaya LE, Novototskaya-Vlasova KA, Spirina EV et al (2016) Expression and characterization of a new esterase with GCSAG motif from a permafrost metagenomic library. FEMS Microbiol Ecol 92:fiw046. doi:10.1093/femsec/fiw046

Pindi PK, Srinath RR, Pavankumar TL et al (2014) Isolation and characterization of novel lipase gene *LipHim1* from the DNA isolated from soil samples. J Microbiol 52:384–388

Purohit MK, Singh SP (2013) A metagenomic alkaline protease from saline habitat: cloning, overexpression and functional attributes. Int J Biol Macromol 53:138–143

Rastogi G, Stetler LD, Peyton BM et al (2009) Molecular analysis of prokaryotic diversity in the deep subsurface of the former Homestake gold mine, South Dakota, USA. J Microbiol 47:371–384

Richardson AE, Barea JM, McNeill AM et al (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339

Riesenfeld CS, Schloss PD, Handelsman J (2004) Metagenomics: genomic analysis of microbial communities. Annu Rev Genet 38:525–552

Roller C, Wagner M, Amann R et al (1994) *In situ* probing of gram-positive bacteria with a high DNA G+C content using 23S rRNA-targeted oligonucleotides. Microbiology 140:2849–2858

Rousk J, Baath E, Brookes PC et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351

Sait M, Hugenholtz P, Janssen PH (2002) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. Environ Microbiol 4:654–666

Schallmey M, Ly A, Wang C et al (2011) Harvesting of novel polyhydroxyalkanaote (PHA) synthase encoding genes from a soil metagenome library using phenotypic screening. FEMS Microbiol Lett 321:150–156

Segata N, Izard J, Waldron L et al (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60. doi:10.1186/gb-2011-12-6- r60

Segata N, Waldron L, Ballarini A et al (2012) Metagenomic microbial community profiling using unique clade-specific marker genes. Nat Methods 9:811–814

Siles JA, Cajthaml T, Minerbi S et al (2016) Effect of altitude and season on microbial activity, abundance and community structure in alpine forest soils. FEMS Microbiol Ecol 92. doi:10.1093/femsec/fiw008

Sorensen J, Nicolaisen MH, Ron E et al (2009) Molecular tools in rhizosphere microbiology-from single-cell to whole-community analysis. Plant Soil 321:483–512

Souza RC, Mendes IC, Reis-Junior FB et al (2016) Shifts in taxonomic and functional microbial diversity with agriculture: how fragile is the Brazilian cerrado? BMC Microbiol 16:42. doi:10.1186/s12866-016-0657-z

Stahl DA, Amann RI (1991) Development and application of nucleic acid probes. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 205–248

Staley JT, Konopka A (1985) Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol 39:321–346

Stein JL, Marsh TL, Wu KY et al (1996) Characterization of uncultivated prokaryotes: isolation and analysis of a 40-kilobase pair genome fragment front a planktonic marine archaeon. J Bacteriol 178:591–599

Tao W, Lee MH, Yoon MY et al (2011) Characterization of two metagenome-derived esterases that reactivate chloramphenicol by counteracting chloramphenicol acetyltransferase. J Microbiol Biotechnol 21:1203–1210

Tan H, Mooij MJ, Barret M et al (2014) Identification of novel phytase genes from an agricultural soil-derived metagenome. J Microbiol Biotechnol 24:113–118

Tardy V, Chabbi A, Charrier X et al (2015) Land use history shifts *in situ* fungal and bacterial successions following wheat straw input into the soil. PLoS One 10:e0130672. doi:10.1371/journal.pone.0130672

Tsai YL, Olson BH (1992) Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Appl Environ Microbiol 58:2292–2295

van Hellemond EW, Janssen DB, Fraaije MW (2007) Discovery of a novel styrene monooxygenase originating from the metagenome. Appl Environ Microbiol 73:5832–5839

Venieraki A, Dimou M, Pergalis P et al (2011) The genetic diversity of culturable nitrogen-fixing bacteria in the rhizosphere of wheat. Microb Ecol 61:277–285

Verma D, Kawarabayasi Y, Miyazaki K et al (2013) Cloning, expression and characteristics of a novel alkalistable and thermostable xylanase encoding gene (*Mxyl*) retrieved from compost-soil metagenome. PLoS One 8:e52459. doi:10.1371/0052459

Vigliotta G, Matrella S, Cicatelli A et al (2016) Effects of heavy metals and chelants on phytoremediation capacity and on rhizobacterial communities of maize. J Environ Manag 179:93–102

Wang K, Li G, Yu SQ et al (2010) A novel metagenome-derived β -galactosidase: gene cloning, overexpression, purification and characterization. Appl Microbiol Biotechnol 88:155–165

Wang Q, Quensen JF, Fish JA et al (2013) Ecological patterns of *nifH* genes in four terrestrial climatic zones explored with targeted metagenomics using Framebot, a new informatics tool. mBio 4(5):e00592–13. doi:10.1128/mBio.00592-13

Wang Q, Fish JA, Gilman M et al (2015) Xander: employing a novel method for efficient gene-targeted metagenomic assembly. Microbiome 3:32. doi:10.1186/s40168-015-0093-6

Wei P, Bai L, Song W et al (2009) Characterization of two soil metagenome-derived lipases with high specificity for p-nitrophenyl palmitate. Arch Microbiol 191:233–240

Werner J, Ferrer M, Michel G et al (2014) *Halorhabdus tiamatea*: proteogenomics and glycosidase activity measurements identify the first cultivated euryarchaeon from a deep-sea anoxic brine lake as potential polysaccharide degrader. Environ Microbiol 16:2525–2537

White JR, Nagarajan N, Pop M (2009) Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol 5:e1000352. doi.org/10.1371/journal.pcbi.1000352

Wolińska A, Kuźniar A, Zielenkiewicz U et al (2017) Metagenomic analysis of some potential nitrogen-fixing bacteria in arable soils at different formation processes. Microb Ecol 73:162–176

Xun W, Xu Z, Li W et al (2016) Long-term organic-inorganic fertilization ensures great soil productivity and bacterial diversity after natural-to-agricultural ecosystem conversion. J Microbiol 54:611–617

Yang Y, Gao Y, Wang S et al (2014) The microbial gene diversity along an elevation gradient of the Tibetan grassland. ISME J 8:430–440

Yasir M, Azhar EI, Khan I et al (2015) Composition of soil microbiome along elevation gradients in southwestern highlands of Saudi Arabia. BMC Microbiol 15:65. doi:10.1186/s12866-015-0398-4

Ye M, Li G, Liang WQ et al (2010) Molecular cloning and characterization of a novel metagenome-derived multicopper oxidase with alkaline laccase activity and highly soluble expression. Appl Microbiol Biotechnol 87:1023–1031

Yergeau E, Schoondermark-Stolk SA, Brodie EL et al (2009) Environmental microarray analyses of Antarctic soil microbial communities. ISME J 3:340–351

Yin H, Niu J, Ren Y et al (2015) An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. Sci Rep:5. doi:10.1038/srep14266

Yun J, Kang S, Park S, Yoon H, Kim MJ et al (2004) Characterization of a novel amylolytic enzyme encoded by a gene from a soil-derived metagenomic library. Appl Environ Microbiol 70:7229–7235

Zaprasis A, Liu YJ, Liu SJ et al (2010) Abundance of novel and diverse *tfdA*-like genes, encoding putative phenoxyalkanoic acid herbicide-degrading dioxygenases, in soil. Appl Environ Microbiol 76:119–128

Zhou Y, Wang X, Wei W et al (2016) A novel efficient β-glucanase from a paddy soil microbial metagenome with versatile activities. Biotechnol Biofuels 9:36. doi:10.1186/s13068-016-0449-6

Zwolinski MD (2007) DNA sequencing: strategies for soil microbiology. Soil Sci Soc Am J 71:592–600