Microorganisms for Sustainability 6
Series Editor: Naveen Kumar Arora

Deepak G. Panpatte
Yogeshvari K. Jhala
Rajababu V. Vyas
Harsha N. Shelat *Editors*

Microorganisms for Green Revolution

Volume 1: Microbes for Sustainable Crop Production

Springer

Microorganisms for Sustainability

Volume 6

Series editor

Naveen Kumar Arora, Environmental Microbiology, School for Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

More information about this series at http://www.springer.com/series/14379

Deepak G. Panpatte • Yogeshvari K. Jhala
Rajababu V. Vyas • Harsha N. Shelat
Editors

Microorganisms for Green Revolution

Volume 1: Microbes for Sustainable Crop Production

Springer

Editors
Deepak G. Panpatte
Department of Agricultural Microbiology,
B. A. College of Agriculture
Anand Agricultural University
Anand, Gujarat, India

Yogeshvari K. Jhala
Department of Agricultural Microbiology,
B. A. College of Agriculture
Anand Agricultural University
Anand, Gujarat, India

Rajababu V. Vyas
Department of Agricultural Microbiology,
B. A. College of Agriculture
Anand Agricultural University
Anand, Gujarat, India

Harsha N. Shelat
Department of Agricultural Microbiology,
B. A. College of Agriculture
Anand Agricultural University
Anand, Gujarat, India

ISSN 2512-1901 ISSN 2512-1898 (electronic)
Microorganisms for Sustainability
ISBN 978-981-10-6240-7 ISBN 978-981-10-6241-4 (eBook)
https://doi.org/10.1007/978-981-10-6241-4

Library of Congress Control Number: 2017958135

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

The book *Microbes for Sustainable Crop Production (Volume I)* is the need of the current era to mitigate adverse effects of chemical farming. The world's food requirement demands high agricultural productivity, but the conventional farming practices have several limitations. Shrinking farmland, rising input costs, and slow adoptions of mechanization are further depleting farmers' income. Soil quality is the "key" factor in current and ancient sustainable agricultural approaches. To improve and maintain soil health, microbial inoculants are strongly promoted the world over as a part of current strategies. In the last few decades, the world perceived steps toward maintaining diversity of microbes and their possible benefits in sustainable agricultural productivity. The advent of powerful new technologies for the production and application of microbial inoculants has accelerated the step of viable agricultural development.

The book includes a collection of literature and reviews on diverse aspects of sustainable agriculture through microbial inoculants. Attempts have been made to summarize the developments achieved till date and future prospects. It would provide an overview of innovative ideas for one and all interested in doubling the farmers' income, including academicians, researchers, students, and entrepreneurs desiring organic and sustainable agriculture using plant-microbe positive interaction phenomenon for achieving the second green revolution and to eliminate hunger from the earth. Microbial approaches can reduce stress on the environment,

agricultural ecosystem, and soil biodiversity in a sustainable manner, ultimately facilitating transformation of soil and agriculture.

Editors comprising a team of agricultural microbiologists of Anand Agricultural University have compiled the knowledge and experiences of renowned scientists across the globe in this book. I assure this book will be very useful for readers in the field of agricultural microbiology for bridging knowledge gap.

Anand Agricultural University
Anand, Gujarat, India

N.C. Patel,

Preface

Microorganisms are the first to arrive and last to leave the earth and represent the driving force of the universe due to their prime importance in functioning of all the biogeochemical cycle which creates the atmosphere of earth. In the last few decades, we have witnessed the increased production in the agriculture sector as a result of the green revolution. The concept of green revolution was undoubtedly proved to be a boon for our agriculture sector. But as we know that every coin has two sides, the introduction of high-yielding crops during the green revolution has increased our dependence on chemical-based agro-inputs as high-yielding crops are also highest-eating crops. To satisfy the crop's hunger and to maintain its health, farmers are incorporating tremendous amount of chemicals in the agroecosystem unknowingly and as a result of which our natural resources are getting spoiled and threatening the survival of humans. Irresponsible and excessive use of chemical inputs may throw devastating impacts on the water, air, and soil environments, as well as their cost cannot make economic and profitable agricultural products. In ancient time, farming was totally dependent on natural inputs, and presently, the concept of organic farming was revived due to the increased awareness of consumers for chemical-free food. The undetachable component of the modern concept of organic farming is microorganism. In many communities of the world, soil is being worshiped like a mother as it is nurturing life. Similarly, since sowing, plants are interacting with soil encompassing microorganisms which serves as a motherhood to crops by providing them nutrition and protection. The use of bio-inputs such as biofertilizers, biopesticides, and biodegraders comprising of agriculturally beneficial microorganisms keeps our biogeochemical cycles alive by acting as miniature factories inside the soil and provides continuous supply of nutrients as well as plant protection metabolites when required. There exist various groups of microbes including bacteria, cyanobacteria, actinomycetes, fungus, and endophytes. The inoculants based on either single or multiple beneficial strains of beneficial microorganisms are commercially produced and popularized among farming communities the world over. Moreover, presently, we are experiencing many natural disasters like flood, drought, and high or low temperature due to climate change which in turn has a negative impact on the agroecosystem and reduces the sustainability of agriculture. Microbes are also having the capacity to cope up with such stress conditions by virtue of their God gift that it shares with crops to nourish them under stressed conditions.

The book entitled *Microbes for Sustainable Crop Production (Volume I)* addresses the two major fields of microbial inoculants, viz., biofertilizers and biopesticides for agriculture, with the help of reputed national and international scientists working in the field of agricultural microbiology. Each chapter will emphasize on the mechanism of action and recent advances in agricultural microbiology. The outlooks of the authors are methodical and firm based on their own experiences during their carrier in the field of agricultural microbiology.

I hope this book will be extremely useful to the researchers in the field of agricultural microbiology especially those who are working on the development of microbial inoculants for sustainable agriculture as a source of valuable information.

Anand, Gujarat, India

Deepak G. Panpatte
Yogeshvari K. Jhala
Rajababu V. Vyas
Harsha N. Shelat

Contents

About the Editors

Deepak G. Panpatte is a research scholar working for the past 7 years. His research interests include agriculturally beneficial microorganisms, viz., biofertilizers, biopesticides, and biodegraders. He has done pioneering work in the development of fortified biocontrol bacterial consortium with phyto-extracts for the management of phytopathogenic nematodes and fungi. He has received six awards, three for presentation of research outcomes and three for his remarkable role in agriculture sector. His publication profile includes 13 research papers, 6 book chapters with Springer Publishing House, 1 practical manual, 26 popular articles, and 2 editorial pages.

Yogeshvari K. Jhala is an assistant professor having 10 years of teaching and research experience. Her field of interest is agriculturally beneficial microorganisms, viz., biofertilizers, biopesticides, and biodegraders. She has world over first time reported five unique strains of methanotrophic bacteria. For her outstanding research work on methanotrophic bacteria, she was honored with the All India Best Research Award and Young Faculty Award. Her publications include 17 research papers, 6 book chapters, 2 teaching manuals, 18 popular articles, and 2 editorial pages.

Rajababu V. Vyas is serving as research scientist and head of the Department of Agriculture Microbiology, Anand Agricultural University (AAU), Anand. Vyas is working on agriculturally beneficial microorganisms on isolation and characterization, the development of mass production technique, laboratory and field testing of biofertilizers for crop production, developed native microbial agents for biological control of insect pests and plant parasitic nematodes for crop protection, and PGPR for bioremediation of methane and agro-waste, to support organic farming approach, for 31 years. Vyas' publication assortment includes 112 research publications; 2 review papers; 4 books and manuals; 5 training manuals; and 8 book chapters, 2 in CAB International and Michigan State University Press, US publications. Vyas is a recipient of six prestigious awards and instrumental for technology patenting, commercialization, licensing, and services.

Harsha N. Shelat is an associate research scientist and award-winning researcher at Anand Agricultural University, Anand, India. She has a professional skill of 33 years in the field of agriculturally advantageous microbes chiefly biofertilizers, biodegraders, and microbial pesticides. Her contribution in society is the development and dissemination of Liquid Biofertilizer Technology for the benefit of the farming community. She has written books (2) and book chapters (5) and published more than 35 research papers in national as well as international journals.

Introduction

Microbes are “hidden miniature packages of nature” influencing the agroecosystem. Biotic factors of the agroecosystem mainly comprise of plants, animals, and microorganisms which are living and require air, water, and nutrients to survive and flourish, but the biological basis for plant health goes beyond survival and productivity. Soil serves as the mother for plants, and a healthy, balanced soil ecosystem provides a habitat for crops to grow without the need for interventions such as agrochemicals. Any organisms in the agroecosystem cannot flourish individually, and that’s the reason why research on the interaction of microorganisms with higher forms of life has gained great momentum in the last 10 to 15 years. Majority of the life processes of plants only become possible through interaction with microorganisms. Using these “little helpers” as a biological alternative to agrochemicals is a highly contemporary field of research. Soil microorganism functions to maintain soil quality, plant growth, yield, and plant health. Beneficial microorganisms are generally classified into three broad groups, viz., biofertilizers, biopesticides, and biodegraders, based on their ecological function during plant-microbe interactions. The microbes classified in all the three groups produce a nexus as their functions are overlapping and some of the microbes can perform all the three roles simultaneously, so broadly, agriculturally beneficial microorganisms are those which can fix atmospheric nitrogen, decompose organic wastes and residues, detoxify soil invaded with chemicals, suppress plant diseases and soilborne pathogens, enhance nutrient cycling, and

produce bioactive compounds such as vitamins, hormones, and enzymes that stimulate plant growth.

The readers will be enriched with a detailed account of all the aspects that are required for making a microbe "agriculturally beneficial." The views of the authors are thorough and authoritative based on their long research experience in the subject area. We hope that this book will be very useful for all those who are actively involved in the research on agriculturally beneficial microorganism for apprehending their benefits in sustainable agricultural productivity.

Anand, Gujarat, India Deepak G. Panpatte

Wonders of Microbes in Agriculture for Productivity and Sustainability

1

Rajababu V. Vyas, Deepak G. Panpatte, Yogeshvari K. Jhala, and Harsha N. Shelat

Abstract

During the green revolution which we have witnessed in the 1970s, we became self-dependent for food production. The major outbreak of green revolution is deterioration of physical, chemical and biological properties of soil due to excessive use of agrochemicals to maximize crop yield. Presently, sustainability and health of soil are of great concern and that's why people are looking for alternatives of agrochemicals. Organic amendments and microorganisms are now being harnessed for their efficient use as biofertilizers and biopesticides. Soil microorganisms interact with plant roots where they get nutrition from root exudates and degrading organic matter. Although beneficial microorganisms possess ability to deal with various environmental issues, their application in well-organized way to resolve environmental problems is yet to be realized. In this chapter, we will elaborate the importance of microbial technologies in agriculture for the larger benefit of the farming and scientific community.

Keywords

Sustainability • Agrochemicals • Biofertilizers • Biopesticides • Microorganisms

R.V. Vyas (✉) • D.G. Panpatte • Y.K. Jhala • H.N. Shelat
Department of Agricultural Microbiology, B. A. College of Agriculture, Anand Agricultural University, Anand 388 110, Gujarat, India
e-mail: rvvyas@aau.in

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*, Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_1

1.1 Sustainable Agriculture: A Perfect Agricultural System

The current concern of the day is basic need to feed the global human population which may reach up to 8.9 billion by 2050, with majority of increase in the developing countries of Asia and Africa (Wood 2001) and believed that more than 70% of population will be urbanized. To feed such ever-growing population, we need to produce 70% more food. In present time, per capita food availability differ largely between countries, wherein average food accessibility is about 3600 kcal/person/day in developed countries, whereas in the developing countries it may be 3000 kcal/group/day which represents instability of the global food system. Climate change also limits the availability of natural resources and thereby creates hurdle in meeting the nutritional requirements of the growing population. Green revolution has paid high ecological cost with global pollution, unfavourable climate change and loss of biodiversity too (Vance 1998). For sustainable agriculture, high productivities of plants and animals are ensured using their natural adaptive potentials, with a nominal disturbance of the environment (Noble and Ruaysoongnern 2010). It is our duty to develop more promising strategies to reach goal sustainable agricultural development that could improve the nutrition of crops as well as their protection from biotic (pathogens, pests) and abiotic (including pollution and climatic change) stresses (Yang et al. 2009). Sustainable agriculture comprises of advances in agricultural management practices and technologies which can be the solution for problems of conventional agriculture like reduced production with high cultivation cost, depletion of topsoil and increasing consumption of agrochemicals and energy resources. The availability of best quality farmland is gradually becoming the main objective for farmers which can be taken care by policy makers. World needs model agricultural system that ensures food production in sustainable manner.

1.2 Conservation of Natural Resources, Soil and Environment

Soil erosion results in soil deprivation followed by the pollution of both surface- and groundwater. Common causes of pollution include organic wastes from agriculture and processing industries, municipal wastes and anthropogenic production of greenhouse gases like methane and carbon dioxide (Parr and Hornick 1992). Present day's agricultural practices involve the use of agrochemicals that directly or indirectly causes pollution, thereby destructing our agroecosystem. Such pollution can be minimized by utilizing proper management practices, judicious use of agrochemicals and utilization of farm waste for energy production. Beneficial and effective microorganisms ensure sustainable crop production, crop protection and natural resource conservation when applied in soil, plant and environment as inoculants. Soil is considered to be the basic element for recycling of matter and relocation of energy by utilizing microorganisms. Soil microorganisms are generally considered as sink for elements and catalysts for transformation reactions. The governments of various countries are emphasizing sustaining soil to maintain life support functions

by implementing several regional or global programmes through FAO to monitor soil quality. Such programmes aim to adjudge microbiological indicators, as soil microbial community are having prime importance in decomposition and nutrient cycling, quick and strong response of microorganisms to toxicity.

1.3 Role of Soil Microorganisms in Sustainable Agricultural Production

Stability of ecosystem is largely affected by activities of micro- and macroorganisms (Schimel 2007). Farmers' can achieve higher crop yield with microorganisms which ensures higher fertilizer use efficiency in eco-friendly manner. Functional processes in soil such as nutrient cycling, decomposition of residues as well as positive or negative plant-microbe interactions are governed by soil microbes which regulate soil health and productivity (Harris 2009). Microorganisms that are helpful for overcoming difficulties linked to the use of agrochemical are now extensively promoted in agriculture. Sickness of soil due to unnecessary soil erosion, use of agrochemicals and their leaching into groundwater as well as inappropriate treatment of human and animal wastes pose serious environmental threat. Even though scientists have endeavoured to resolve such issues using conventional chemical and physical methods. Since years, soil microbiologists classified soil microorganisms as 'beneficial' or 'harmful' depending on their effect on soil quality, crop growth and yield. An important alteration is taking place globally in agricultural practices and food production. During the era of green revolution, the driving force for agriculture is to increase the yield potential and productivity of food crops, but presently the scene has been changed, and more emphasis is given to achieve more sustainable productivity by management of agricultural resources to satisfy human needs while conserving environmental quality and natural resources for future. Upgradation of agricultural sustainability needs to emphasize natural resources that depend on soil biological process.

1.4 Microbial Management of Soil Fertility

The main consideration for biological management of soil fertility is to utilize soil management practices to positively affect microbial populations and processes. Microbial populations and processes are having ameliorating effect on soil fertility to remove constraints to maintain soil productivity. Microorganisms are actively participating in biogeochemical cycles' functioning and improve availability of nutrients to the plants as well as help in degradation of organic matter. Soil structure and water holding capacity are greatly affected by burrowing and particle transport activities of soil microflora as well aggregation of soil particles by fungi and bacteria. Farmers are generally facing problem of decreasing soil fertility and that's why regulation of erosion and improvement of soil fertility are now major concerns for development of sustainable agriculture. There are many direct and indirect benefits of

implementing microbiological management of soil for sustainable agriculture production, viz. reduced input costs by improving resource use proficiency, prevention of pollution and land degradation, improved yield and crop quality as well as microbe-mediated remediation and rehabilitation of barren land into productive one.

1.5 Principles of Natural Ecosystems and Role of Beneficial and Effective Microbial Consortium

Presently in agriculture, newer concepts such as alternative agriculture, sustainable agriculture, soil quality, integrated pest management, integrated nutrient management and beneficial microorganisms are being explored by the agricultural researchers.

1.5.1 Efficient Soil Microbes

Basically, agriculture is the system wherein farmers try to incorporate certain agroecological elements and inputs to get desired crop and livestock production. So generally, farmers as well as researchers are keen to devise means of maintaining beneficial soil microorganisms as component of agroecosystem. Soil microorganisms have often been controlled advantageously when crops in various agroecological zones are grown and cultivated as crop rotations and without pesticides use. Conceptually scientists are majorly interested to improve soil quality by using potential and efficient microorganisms as soil and plant inoculants. The soil microorganisms can speed up plant growth and improve their resistance to pathogens. Microorganisms uphold growth of plants and thereby have primary effects on both soil and crop qualities. Wide arrays of benefits are possible depending on their predominance and activity of microorganisms in soil at particular time. However, as now people are moving towards organic farming, there is growing attention to get higher economic and agronomic yield of high quality at higher net returns, without the use of agrochemicals. To achieve this goal, it is necessary to choose best soil and agricultural management practices to get sustainable agriculture which can enhance diversity of efficient soil microorganisms that in turn can enhance the growth, yield and quality of agricultural produces. In specific sense, healthy living soil with better quality is base of a forthcoming sustainable agriculture.

1.6 Role of Rhizospheric Microbial Interactions in Environment and Agriculture Sustainability

Microorganisms interact with plants in the rhizosphere (Glick 1995; Barea et al. 2002). Microbial activity and diversity are always high in the rhizosphere as compared to bulk soil due to a variety of physical, chemical and biological events taking place in the rhizosphere micro-environment (Kennedy 1998). Some of the microbial

interactions can be explored as a low-cost biotechnology and form a basis for a strategy to help maintain eco-friendly practices which confirms firmness and throughput of both agricultural systems and natural ecosystems (Kennedy and Smith 1995).

1.7 Application of Beneficial and Effective Microorganisms: Fundamental Considerations

Generally, it is believed that incorporation of organic matter in soil can increase number of microorganisms in the soil as microorganisms require complex organic molecules to carry out their own metabolic activities. Heavy applications of organic materials, such as seaweed, fish meal and chitin from crushed crab shells, can increase number of antibiotic-producing microorganisms which provides foundation for formation of disease suppressive soil within sort time. The possibility of establishment of dominance by desired beneficial microorganism with organic farming will depend on the ecosystem and environmental conditions. It can take a lot of time to establish a stable relationship between higher and lower forms of plants for development of sustainable agroecosystem. If we succeed in the establishment of noteworthy population of specific microorganism, whether it will be advantageous to plants is another question that remains to be answered. So it is impossible to predict that plant beneficial microorganisms become predominant in conservation farming. If we take into consideration the useful anaerobic microorganisms, their numbers would increase significantly even under natural farming conditions. These facts altogether propose the requirement to isolate specific microorganisms from soil and evaluate their physiological and ecological potential to be introduced as mixed cultures into soil where their beneficial effects can be recognized.

1.8 Beneficial and Effective Microorganisms in Agroecosystem: A New Facet

Microorganisms are generally utilized as bioinoculant for sustainable agriculture as biofertilizers, biopesticides and biodegraders. Even though these perceptions and related approaches have significance, they also have restrictions. For example, the key limitation in using microbial inoculants is lack of reproducibility and consistent performance under field conditions. Most of the claims done by manufacturers of microbial inoculants are really exaggerated. When we apply microorganisms on the soil, then we have to focus on augmenting their synergistic effects. For the establishment of synergistic effect, we have to be cautious to apply such microbial inoculants to build up microbial population to the desired threshold level which facilitates achievement of desired positive effects on crop production or crop protection. The most trustworthy method is to apply helpful microorganism into soil as part of a mixed inoculum and in adequately amount to maximize the possibility of its adaptation. Inoculation of beneficial microorganisms can help to define the structure and

establishment of soil ecosystems. If one would apply organic matter to the soil, it ensures greater microbial diversity as they contain their own microflora.

1.9 Ecological, Agronomic and Biotechnological Impacts

Microorganisms are considered as bioinoculant for sustainable agriculture by virtue of genetic dependence of plants on symbiotic microorganisms (Seckbach 2002; Noble and Ruaysoongnern 2010). The importance of plant-microbe symbiosis for providing nutrients to the crops has been uncovered by the study of nitrogen fixation (Franche et al. 2009) and phosphate solubilization (Smith and Read 2008). Generally, microbes are utilized in sustainable agricultural practices as they would replace agrochemicals. This switch is typically partial and only occasionally may be widespread (Provorov and Tikhonovich 2003). Unfortunately there are a limited number of symbiotic associations that occur between plants and microorganisms. To cope up with this limitation, researchers have to pay attention to design strategies for cocultivation of plants and microorganisms (Rengel 2002; Provorov and Tikhonovich 2003). Generally, when we look towards non-symbiotic association occurring between nonlegumes and rhizosphere bacteria, the ecological competence and genotypic specificity of interactions between partners are poorly interrelated (Kozhemyakov et al. 2004). Consequently, development of these relations may be attained by choosing microbial strains for application to extensive range of plant genotypes. For improving the defensive symbiosis, direct and indirect eradication of plant pathogens and pests could be combined. Such combinations have been demonstrated for take-all disease in wheat (caused by *Gaeumannomyces graminis* var. *tritici*), which is suppressed by a multibacterial inoculant comprising of *Acidobacteria, Planctomycetes*, *Nitrospira*, *Chloroflexi*, *Azospirillum* and *Thermoanaerobacter* (Sanguin et al. 2009). In defensive symbiosis between plants and microorganisms, specificity towards pest genotype is significant rather than host specificity. Indirect suppression of pathogen infections may be due to microbe-derived secondary metabolites. The projections for an upcoming expansion of agricultural microbiology may include the creation of new multifaceted endo- and ecto-symbiotic groups based on comprehensive metagenomic approaches. A combination of nitrogen and phosphorous providing symbionts would seem encouraging, including the endosymbiotic rhizobia + VAM-fungi (Shtark et al. 2010) or rhizosphere nitrogen-fixer *Phyllobacterium* + phosphate solubilizer *Bacillus* (Rojas et al. 2001). The operative management of plant-microbe association is can be achieved using molecular methodologies (Kupriyanov et al. 2010).

1.10 Alternative Agricultural Management Approaches

Some of the important alternative agricultural management approaches currently being practised throughout the world for promoting biological activities in soils include the following.

1.10.1 Organic Agriculture

Organic agriculture is considered as the natural agricultural system which incorporates human, animal and crop products for sustainable agroecosystem. It also ensures holistic interactions between plants and microorganisms in the whole ecosystem. In organic approach maintenance of soil organic matter for management of soil fertility is a prime concern, wherein plant nutrients are generally provided through microbe-mediated decomposition of organic matter, the use of biofertilizers and biopesticides and development of pest-resistant varieties. Presently, soil scientists from different parts of world are concentrating on development of new crop varieties that enables efficient uptake organic nutrients from soil.

1.10.2 Biodynamic Agriculture

In biodynamic system of agriculture, specific plant and animal substances are fermented for a year or more which is then utilized to enhance compost and manure used in the farming operation. Such components can also be applied directly to soil as a spray to enhance biological activity. The philosophy behind biodynamic agriculture is that a healthy, active soil microbial population will improve plant-microbe interactions, nutrient cycling and reduce soil pathogens.

1.11 Integrated Plant Nutrient Supply (IPNS) System

The basic concept of IPNS is the promotion and maintenance of soil fertility for sustaining crop productivity through optimizing all possible resources (both renewable and non-renewable), such as organic, inorganic and biological components in an integrated manner appropriate to each farming situation in its ecological, soil and economic possibilities. The principal aim of IPNS is efficient and judicious use of all major resources of plant nutrients in an integrated manner, so as to get maximum yield without any deleterious effects on physicochemical and biological properties of soil. Major components of IPNS are FYM/compost, green manures, crop residues/recyclable wastes, synthetic fertilizers, biofertilizers, biological control agents and biopesticides.

1.12 Microbes in Management of Environmental Menace

Environmental pollution is major constrain worldwide as a large number of toxic, mutagenic and carcinogenic chemicals pose severe threats to the environment and public health. Restoration of polluted environment through microorganisms in present-day bioremediation, i.e. the use of microorganisms to remove toxic pollutants from the environment, is the most promising technology (Zafar et al. 2007; Lal et al. 2010). A wide array of site-specific microorganisms are capable of carrying out

bioremediation reactions, and many have already been used at sites previously contaminated with polycyclic aromatic hydrocarbons (PAHs), nitroaromatic compounds, chlorinated organics, etc. (Carvalho et al. 2005). In many cases, the pollutants are not entirely mineralized, and their products may gather and generate their own exclusive health hazards (Singh 2006). To find the solution of such problem, different bioremediation strategies are utilized including the use of various combinations of microorganisms. Biocatalysts have a huge amount of catabolic potential for bioremediation, but interactions of bacteria and pollutants are always complex, and appropriate remediation does not often take place. Metabolic engineering of microorganisms involve redirecting the cell's metabolism to attain a specific objective. One of the leading and best examples of this was the superbug of *Pseudomonas* sp. B13 endowed with five different catabolic pathways from three different bacteria which allow degradation of methylphenols and methylbenzoates by single organism.

1.12.1 Microbes for Management of Green House Gases (GHGs)

Greenhouse gases are major concern of the day, and sustainable agriculture allows humus formation in the soil to the tune of 0.3–1.0 tons C/ha/year. Climate change can be obtained by homeostasis of the microbial communities. Soil microorganisms are generally utilized for remediation of greenhouse gases like carbon dioxide and methane nitrous oxide. Methane emission from rice ecosystem is also realized by scientists, and remediation strategies for the same are yet to be designed (van De Woestyne et al. 1994). Most recently, methanotrophic microorganisms are utilized for rendering their services for remediation of methane emission from rice by utilizing 800–1000 kg CH_4/ha/year (Mohanty et al. 2006).

1.12.1.1 Biofuel Production by Microorganisms

Presently, we all are witnessing global energy crisis. Presently, the potential of microorganisms to produce various biofuels such as alcohols, hydrogen, biodiesel and biogas is being researched for exploration at large scale. Maintainable biofuels are crucial to guarantee a continuous, safe supply of energy for living beings as well as industries. Microorganism based biofuels can reduce our dependence on nonrenewable fuel sources. Liquid biofuels obtained from plant or microbes can be best substitutes for petroleum based fuels if cheap method for its commercial manufacturing is discovered. Researchers have found variety of alternative fuels, but none of them appears to be in the forefront.

1.13 Microorganisms for Biofuel Production

Microbial biofuels are considered to be of aid to meet world energy demands as living organisms integrate and concentrate energy in their biomass, and thereby biomass can serve as an attractive substitute for energy (Lee 2003). Photosynthetic microorganisms like cyanobacterial stores solar energy within biomass during photosynthesis are

released through biochemical transformation. Generally, solar energy is stored in the form of carbohydrate within biomass which is having low-energy content, so we need to concentrate the same for fuel production. Fermentation of biomass by microorganisms under anaerobic conditions seems to be effective and extensively utilized method for such concentration process. Renewable fuels produced by microorganisms comprise of hydrocarbon, ethanol, methane and hydrogen.

1.13.1 Photobiological Hydrogen Production

Algae like *Chlorella* are capable of producing hydrogen and oxygen through direct photolysis of water in the presence of suitable electron acceptor within the chloroplast. Here, water serves as electron donor and sunlight as the energy source to produce hydrogen that can be stored in microbial cell and utilized as energy source. Here, the whole process is renewable as the energy is consumed; the water is regenerated.

1.13.2 Conversion of Biomass Energy

Structural and storage carbohydrates in biomass having low-energy content cannot be used as fuel directly. It is necessary to concentrate the energy content further for fuel applications. The use of microorganisms to produce commercially valuable fuels depends on getting the right microorganisms which can produce the desired fuel efficiently (Tanaka et al. 1988). Anaerobic microbial fermentation is proficient and broadly used way for such conversion processes.

1.13.3 Alcohol (Ethanol) Production

Bioethanol is an important energy source for the regions having plentiful amount of plant deposits. Agricultural waste containing higher amount of starch and sugar can be converted to ethanol. A large number of microorganisms can produce ethanol, but all are not appropriate for industrial processes. Yeast strain, especially *Saccharomyces*, has been widely studied due to its high efficiency for conversion of sugars into alcohol. The yeasts commonly used in industrial alcohol production include *S. cerevisiae* (ferment glucose, fructose, maltose and maltoriose), *S. uvarum*, *S. diataticus*, etc. The efficiency range for ethanol production is 1–2 g ethanol/h/g cells. Some of the bacterial strains are also used for ethanol production due to their high-temperature tolerance but less efficient as compared to yeast cells for alcohol production (Lee 2003).

1.13.4 Methane Production

Methane can be used to generate energy in the form of mechanical, electrical and heat energy. Anaerobic degradation of waste material can produce large amount of methane. In classical method for methane production, generally, mixture of anaerobic bacteria is used, and after generation of methane, they can be retained in digester. In the process of fermentation, a large amount of organic matter is being degraded, and 90% of the substrate energy is recollected in the form of methane gas which can be easily purified. Fermentative bacteria can hydrolyse polymers such as proteins, lipids and polysaccharides which can be degraded to smaller molecules with the production to acetate and other saturated fatty acids, carbon dioxide and hydrogen gas as major end products. The second group of bacteria are obligate hydrogen-producing acetogenic bacteria that metabolize low-molecule organic acids (end products of the fermentative bacteria) to hydrogen and acetate.

1.13.5 Electricity from Biofuel Cells

In biofuel cells, the chemical energy is converted into electrical energy at ambient temperature. Biofuel cells can produce electric energy more efficiently as compared to conventional power engines without any pollution. The basic mechanism for generation of fuel cells remains same as that of combustion engine wherein two electrodes were placed in electrolyte solution separated by ion exchange membrane which allows the electrochemical equivalent of ignition to occur.

1.13.6 Generation of Electricity from Hydrogen Gas

Energy content of hydrogen is 18.7 kJ/g which seems to be fourfold greater than ethanol and twofold more than methane. Microorganisms produce hydrogen as a part of their metabolic reactions. Generation of energy in the form of hydrogen by microorganisms or components of microorganisms is still in its infancy, but there may be three possible routes for hydrogen production (Waites et al. 2001).

Biophotolysis of Water It comprises breakdown of water using sunlight as energy source and does not need any exogenous substrate. The energy so produced is generally utilized to produce reduced nicotinamide adenine dinucleotide phosphate (NADPH). In the presence of a bacterial hydrogenase and suitable electron carrier, molecular hydrogen can be produced.

Photoreduction It is a light-assisted breakdown of organic compounds performed by photosynthetic bacteria. Photoreduction process is anaerobic and hence inhibited by oxygen, dinitrogen and ammonium ions. Hydrogen production is performed by nitrogenase enzyme which reduces protons and nitrogen. Purple non-sulphur bacte-

ria, such as *Rhodospirillum* spp., carry out efficient photoreduction which photometabolizes organic acids.

1.13.7 Generation of Electricity from Methanol

Direct methanol fuel cell (DMFC) contains dilute mixture of 2% methanol in water. The methanol is converted into formate on the anode. The proton then reacts with oxygen as in a PEM cell. The metabolically active microorganisms, such as *Proteus vulgaris* and *Anabaena variabilis* immobilized in a biofuel cell, could convert energy in their substrate (glucose for the former and light for the later) into electricity (Allen and Bennetto 1993). A biofuel cell in which bacteria *Proteus vulgaris* and *Escherichia coli* were used as sulphate reduction catalysts was in operation for 5 years, demonstrating thus its long-term stability. The disadvantage of biofuel cell is that the power output is low (1 kW at 40 mA/cm^2). Thus, it is used for specific purposes, such as small medical and military apparatuses used in the field and in space missions. Biofuel cells are considered to be eco-friendly and can be used as substitutes in order to reduce greenhouse gas emission.

1.13.8 Algal Biofuels

Algae carry out photosynthesis by utilizing energy from sunlight and carbon dioxide to produce biomass comprising oil that can be transformed into biodiesel. Advantages of algal biofuel are that algae are having 100 more oil production capacity as compared to any terrestrial plant on per acre basis and algae can be grown on barren lands using non-potable water. Technology for production of algal biofuels is in its infancy due to high production cost as well as inadequate information about scale-up. Currently, cost for large-scale production of algal biofuel is 10–30 times costly as compared to other biofuels.

1.13.9 Current Research

Presently, microbiologists are exploring several avenues of to produce biofuel more competently.

These include:

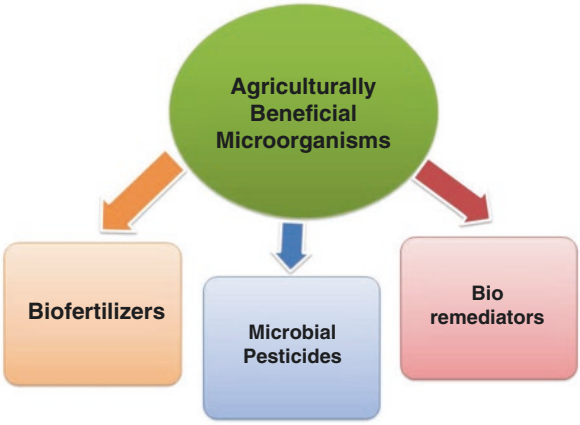
- Large-scale production of microbial cellulase which converts celluloses into fermentable sugars
- Genetic engineering of yeast cells to produce more alcohol-tolerant yeast strains to be employed for bioethanol production
- Selection and large-scale use of microbial strains that transform sugars into biobutanol as substitute to bioethanol

- Isolation and utilization algal strains yielding high oil content for biodiesel production

1.14 Landmarks of Anand Agricultural University for Microorganism-Based Sustainable Agricultural System

Research on agriculturally important beneficial microorganisms was started at GAU, now AAU, as biofertilizer research in 1979 with major thrust to identify and isolate efficient native microbial cultures which fix atmospheric nitrogen or solubilize/mobilize phosphorous and potash suitable for different agroclimatic conditions, total 60 gene sequences of beneficial bacteria were deposited at NCBI, USA. Indian patent published for biofertilizer cum biopesticide technology. Technologies of liquid biofertilizers and Bio-NPK consortium were commercialized for the ultimate users, the farming community (http://aau.in/college-menu/department/765~815).

1.14.1 Major Thrust Areas



1.14.1.1 Biofertilizers

Many native microorganisms useful as biofertilizers are identified, tested and promoted by AAU, the then GAU (Vora et al. 2008), to develop low-cost eco-friendly bio-inputs for crop production generating more than 50 recommendations in different crops for farming community of Gujarat state in last three decades.

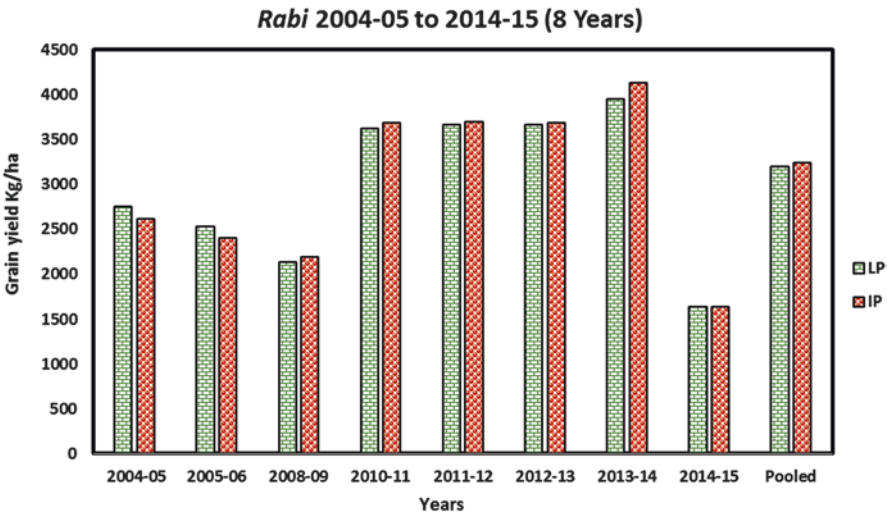
Nitrogen fixers	*Azolla pinnata*, *Rhizobium* spp., *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Acetobacter diazotrophicus*, *Derxia gummosa*
Phosphate solubilizers	*Bacillus circulans*, *Bacillus coagulans*, *Torulospora globasa*, *Pseudomonas fluorescens* (siderophore), *Thiobacillus* (SOM), *Aspergillus niger* (avirulent), *Trichoderma* sp., *Paecilomyces* sp.
Potash mobilizers	*Bacillus* spp., *Enterobacter asburiae*, Fungi: *Trichoderma*, *Aspergillus*
Zinc mobilizers	*Pseudomonas* spp., *Bacillus* sp., *Rhizobium* sp.

Liquid Biofertilizers

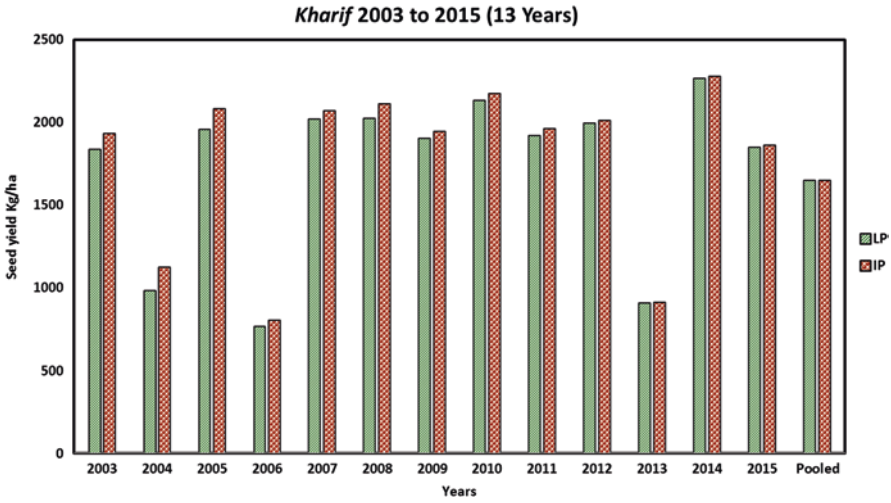
Liquid biofertilizers (LBFs) were developed and promoted: N-fixers *Azotobacter chroococcum*, *Azospirillum lipoferum* and phosphate culture (*Bacillus coagulans*) (Vyas et al. 2008). *Anubhav* liquid biofertilizer formulations as individual culture was successfully launched, and the product has a minimum cell count of 10^8/ml with shelf life above 1 year. Drip irrigation and greenhouse cultivation are suitable for field crops. Liquid biofertilizers are advantageous over marketed carrier-based products are having shelf life of 6 months. During the last decade, demonstrations in maize, wheat, mung, etc. at farmers' fields in tribal areas of Gujarat in **lab-to-land** efforts recorded saving of 25% RD of N + P with significant yield increase.

1.14.1.2 Demonstrations of Anubhav Liquid Biofertilizers in Life Sustaining Crops

1. **Wheat**



2. **Maize**



These plant growth-promoting bacteria have the capacity to produce phytohormones such as IAA and GA_3 that work as plant probiotics in the rhizosphere. The brand *Anubhav* liquid biofertilizers was sold to the end users since 2005 at an affordable price by the department and bulk supply to Govt. of Gujarat for Krushi kits. *Anubhav* liquid biofertilizers are chiefly benefiting farmers of Gujarat and nearby states of Western India like Maharashtra, Madhya Pradesh and Rajasthan. In the last 10 years, the university sale of *Anubhav* liquid biofertilizers is more than 2 lakhs litres (worth of Rs. 22.5 million; from department single window http://aau.in/college-menu/department/765~815).

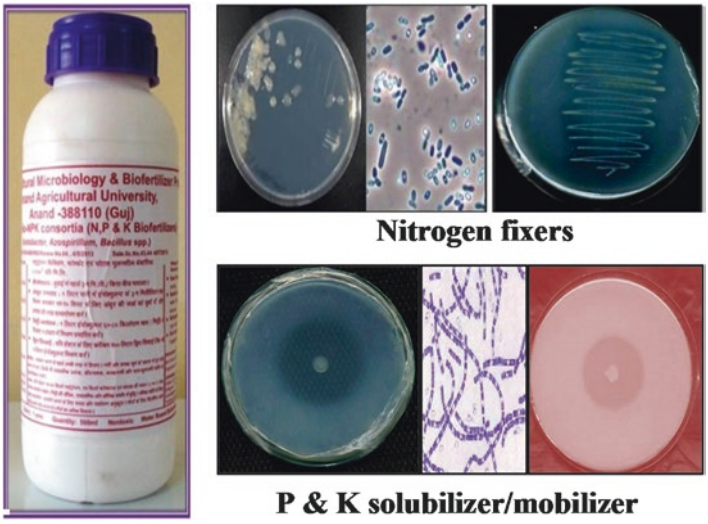
Anubhav biofertilizer products	Remarks
Azotobacter (N fixer)	Available round the year from the Department of Microbiology as retail sale but bulk supply with prior indent only
Azospirillum (N fixer)	
Phosphate culture (PSB)	
Potash culture (KMB)	
BIO NP (*Azotobacter/Azospirillum/Rhizobium* + PSB) (two cultures)	
Bio-NPK (*Azotobacter*, *Azospirillum*, PSB(2), KMB(1) (Total five cultures)	
Rhizobium (symbiotic N fixer)	Production as per indent and seasonal need for leguminous crops (pulses, oilseeds, Lucerne, etc.)

Anubhav LBFs are disseminated under lab to land, and farmer's awareness created in the last 4 years (2010, 2011, 2012 and 2014) through Krushi Mahotsav programme. Liquid biofertilizers *Azotobacter*, *Azospirillum* and phosphate culture were mass multiplied and supplied to the tune of 1 lakh bottles (500 ml) for inclusion in Krushi kit during 4 years and distributed to farmers of Gujarat (18,000 villages) http://agri.ikhedut.aau.in/1/fld/807. This herculean task was attained in incessant

collaboration of Department of Agriculture, GOG, through Gujarat Agro Industries Corporation Ltd., Ahmedabad and Gujarat State Seed Corporation Ltd., Gandhinagar. Moreover, *Rhizobium* and PSB to the tune of 45,000 litres were supplied to GOG for inclusion in RKVY and ATMA schemes for *Kharif* and *Rabi* 2011–2012.

1.14.1.3 New Products (2014–2015): Anubhav Bio-NPK Consortium with Multiple Utility as Biofertilizer cum Biopesticide

Recently, the Department of Agricultural Microbiology has developed and launched a new product 'Bio-NPK consortium' having multiple utility as biofertilizer cum biopesticide on the occasion of Rabi Krushi Mahotsav – December 11, 2014. This product contains five strains of agriculturally beneficial microorganism (two nitrogen fixers, two phosphate solubilizers and one potash mobilizer) and is the one-time solution for all the macronutrient (N, P, K) requirement of crops. Moreover, this formulation will also provide an additional benefit of protecting plant from phytopathogenic fungi and nematodes. Patent application entitled 'Technology for Native Plant Growth Promoting Bacterial (PGPB) Consortium Formulations, Useful as Biofertilizer cum Biopesticide' filed vide No 1060/DEL/2013 dtd. April 9, 2013 and published vide No.50/2014 dtd. December 12, 2014.



1.14.1.4 Beneficial Fungi as Myco-phosphate Solubilizer and Myco-potash Mobilizer

- Mycopesticides, *Paecilomyces lilacinus*-A, *Trichoderma viride*-A and *Trichoderma harzianum*-M, were found to give good P solubilization zones on PKVK agar medium. In broth, ThM showed highest P-solubilizing ability (309.33 µg/ ml). HPLC analysis showed production of pyruvic acid, formic acid, orotic acid, citric acid and butyric acid by mycopesticides. Quantitative analysis for IAA production found highest in ThM (12.60 µg/ ml).

- Mycopesticide, *Trichoderma viride*, and biodegrader fungus, *Aspergillus wentii*, are found K solubilizer on mica agar plates and confirmed as myco-potash cultures.

1.14.1.5 Protocols Ready to Be Transferred by Public-Private Partnership and Technological Consultancy Services for Microbial Inputs (Vyas et al. 2014)

- Technology of *Azolla pinnata* cultivation has been developed and domesticated following unremitting support to farmers over the last three decades.
- Liquid biofertilizers (LBFs), *Azotobacter chroococcum*, *Azospirillum lipoferum* and phosphate culture (*Bacillus coagulans*), potash-mobilizing bacteria (*Enterobacter asburiae*) and Bio-NPK consortium, etc.

Liquid biofertilizer technology cultures like nitrogen fixers and phosphate solubilizers are under **Indian patent deposits for 10 years from 2011**	Three cultures deposited at IMTECH (GOI), Chandigarh
	Access no. MTCC 5464
	(*Azotobacter chroococcum*)
	Access no. MTCC 5465
	(*Bacillus coagulans*)
	Access no. MTCC 6567
	(*Azospirillum lipoferum*)

- Developed mass production technology for fungal biopesticides based on solid substrate fermentation technique with standardization of dust/granular formulations. In vitro mass production technique for native entomopathogenic nematode and bacterial complex in liquid and solid state is also evolved.
- The Indian patent of the PGPB consortium (five bacteria) the Bio-NPK, nitrogen fixers (*Azotobacter*, *Azospirillum*) as well as phosphate solubilizers and potash mobilizer (three *Bacillus* spp.). Bio-NPK technology has been patented and published in **Indian Patent Journal No. 50/2014 dtd. 12/12/14**.
- Department of Science and Technology (DST,GOI) – Lockheed Martin (FICCI and Stanford University) India Innovation Growth Programme, IIGP 2013 Technology Commercialization and Entrepreneurship Workshop (led by Stanford Graduate School of Business, IC^2 Institute, Univ. Texas, USA). Liquid biofertilizers of AAU was listed amongst the best 30 technologies of 2013.
- https://www.youtube.com/watch?v=m8hxJqR_3UI
- International efforts for awareness of AAU technologies including liquid biofertilizer technology in Indo-US Bilateral Workshop on Technology Commercialization, July 8–13, 2012, Michigan State University (MSU), East Lansing, Michigan, USA.

1.14.1.6 Individual Liquid Biofertilizer and Bio-NPK Consortium Technologies Commercialization (Licensing)

To reach the remotest farmers, AAU has licensed the liquid biofertilizer/Bio-NPK production technologies to few companies (2011–2015) through AAU BPDU,

NAIP-1, ICAR World bank-financed project and generated revenue of Rs. 50 Lakhs. (Success story LBF*:* http://www.icar.org.in/node/5667; http://www.aau.in/business-planning-development-unitnaip-i)

1.14.1.7 Environmental Impact of LBF

From **2005 to 2015,** university sale as well as GOG distribution of liquid biofertilizers is more than **2 lakhs** litres covering thousands of hectares of land across the state, saving 25% of N&P fertilizers like Urea, DAP, SSP and also the government subsidy input on chemical fertilizers. This in turn is also useful in the reduction of environment pollution by curtailing fertilizer usage-based agroecosystem and environmental pollution. In the near future, three entrepreneurs who have received AAU LBF Technology transfer by public-private partnership will emerge as key producers having capacity up to 5 lakhs litres per annum expected production, sale and use up to 15–18 lakhs litres annually in Gujarat, and surrounding states will also help protecting environment, save subsidy of GOI, etc. benefiting to the mankind and particularly to the farming community as a low-cost agro input. India has about 157.9 million hectares of arable land, and liquid biofertilizer application can reduce demand of chemical fertilizers and save the government subsidy, which in turn is also useful in the reduction of environment pollution with improved soil health and with better productivity.

1.14.1.8 Recommendations for Farmers

Recommendations include:

Azolla pinnata (fresh) and BGA for lowland rice
Azolla pinnata (dry) for wheat, potato and tobacco (saving 30–50 kg N/ha)
Azotobacter chroococcum (ABA-1) for pearl millet, sorghum, paddy, *Amaranthus* (Rajgara), sugarcane, maize, potato, wheat, pigeon pea, tobacco, SRI rice, onion, sesame and cotton *Azospirillum lipoferum* (ASA-1) for pearl millet, finger millet, paddy, sorghum, guinea grass, maize, sesame, tobacco, tobacco and onion (saving 20–40 kg N/ha)
Acetobacter diazotrophicus (ACG-2) for sugarcane (saving 100 kg N/ha)
Rhizobium spp. RBA 5, ARS 21 for pigeon pea
Rhizobium spp. F 75, IC-76 for chickpea
Rhizobium spp. GMBS 1 for green gram (saving 30–50 kg N/ha)
Bacillus circulans (PBA 4) for cowpea
Bacillus brevis (PBA-12) for sorghum (fodder), wheat (durum), pearl millet and wheat *Bacillus coagulans* (PBA-13) for pigeon pea, wheat
Bacillus coagulans (PBA-14) for cowpea
Bacillus coagulans (PBA-16) for sorghum (dual and fodder), urad bean, sesame, pearl millet, sesame and SRI rice
Bacillus coagulans (PBA-17) for urad bean and groundnut
Torulaspora globosa (PBA-22) for pigeon pea, maize, sorghum and groundnut (saving 20–50 kg P_2O_5/ha)
Enterobacter asburiae KMBW1 for potato (25% saving of potash)

Azospirillum lipoferum (ASA-1) + *B. coagulans* (PBA-16) for chilli and brinjal nursery (25% saving of RDF)
Bio-NPK consortium for groundnut, potato and wheat (25% saving of RDF N:P:K)

1.14.1.9 Microbial Pesticide

Fungal	*Beauveria brongniartii*, *Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Trichoderma* spp.
Bacterial	*Bacillus popilliae*, *Bacillus thuringiensis* spp., *Pseudomonas fluorescens*
Others	*Rickettsia*-like organism (RLO), native entomopathogenic nematode, *Xenorhabdus* bacterial symbiont of *Steinernema* spp.

- Developed mass production technology for microbial pesticides based on solid/liquid substrate fermentation with formulations, successfully employed in field for control of insects and phyto-nematodes (Vyas et al. 2010).
- Microbial control of white grubs through bacterial pathogen, *Bacillus popilliae* (Vyas et al. 1991a), and fungus. *Beauveria brongniartii* (Vyas et al. 1991b) has been established in laboratory to field conditions and developed cheap mass production technology for microbial pesticides based on solid substrate fermentation technique (Vyas et al. 1990).
- New *Rickettsia*-like organism (RLO) and *Bacillus thuringiensis* var. *galleria* have been reported from Gujarat for the first time (Jani et al. 1993).
- The entomopathogenic fungi, viz. *Beauveria brongniartii* and *Metarhizium anisopliae* were proved useful for white grub control and simultaneously parasitic on eggs of root-knot nematodes (Vyas et al. 1990).
- In vitro mass production technique for native entomophilic nematode, *Steinernema* sp., has been developed (liquid and solid state based on *Xenorhabdus*) and standardized with formulation preparation (Vyas et al. 1999, 2006).
- Biological control of root-knot disease by nematode egg-parasitizing fungus, *Paecilomyces lilacinus*, in groundnut and cotton has been successfully demonstrated by fungus application at 25 kg/ha (spore dust/granules having 10^9 conidia/g based on rice grain substrate as carrier) (Vyas et al. 1995).
- Molecular characterization of EPN, *S. thermophilus* and three native undetermined isolates of Gujarat were carried out by RAPD-PCR, which showed two isolates in same cluster which was later on taxonomically identified as *S. riobrave* (Umarao et al. 2002; Vyas et al. 2005).
- Native insect pathogenic beneficial nematode, *Steinernema riobrave* – *Xenorhabdus* bacterial complex and exo- and endotoxins of *Xenorhabdus* spp. have been proved suppressive to root-knot nematodes, *Meloidogyne* spp. for the first time in the country (Vyas et al. 2006, 2010).
- In vitro toxicity of *Xenorhabdus* metabolites, exo- and endotoxic factors against *A. niger* showed fungi static and suppress collar rot disease on groundnut probably the first report (Vyas et al. 2005).

- Molecular characterization of EPNs, native *Xenorhabdus* and *Bacillus thuringiensis* and *Pseudomonas* isolates by RAPD/RFLP (Hinge et al. 2010).
- A great diversity of fluorescent *Pseudomonas* in the middle Gujarat is recorded (Panpatte et al. 2015a) and has been proven to be a microbial biocontrol agent for *Fusarium* wilt disease in pigeon pea.
- Moreover developed new fortified consortium formulation comprising of *P. fluorescence*, *P. putida and Providencia vermicola* fortified with phyto-extracts in the middle Gujarat is recorded and has been proven to be a microbial biocontrol agent for *Fusarium* wilt and root-knot nematode disease complex (Panpatte et al. 2015b, 2016).

1.14.1.10 Bioremediators

Bioremediators/Biodegraders	*Emericella*, *Aspergillus*, *Pseudomonas*, *Cellulomonas*, *Pleurotus*, etc. for biodegradation of different agricultural wastes and biodegradable plastics

Microbial Consortium for Degradation Agro-waste

Composting of banana pseudostem waste by consortium of cellulolytic and lignolytic isolates (*Cellulomonas*, *Pleurotus*, *Propionibacterium*, *Lactobacillus*, etc.) (Dabhi et al. 2014).

1.14.2 New Frontiers in Current Decade

1.14.2.1 Biodegradation of Plastic

Emericella nidulans, *Aspergillus wentii*, *Pseudomonas*, etc. having thermoplastic and biodegradable plastic adoring capacity, attacking different plastics and reduce degradation time (Kushwah et al. 2013).

1.14.2.2 Microorganisms for Reduction of GHG Methane

Methane is one of the potent greenhouse gases (GHG), and about 10–30% of the methane is emitted by methanogens in rice cultivation. Methylotrophic bacteria (MOB), the only biological sink to remove methane from atmosphere, are considered to be significant part for reducing the potential quantity of emitted methane which is utilized by aerobic methane-oxidizing bacteria present in rice rhizosphere. Besides their main role in methane degradation, methylotrophic bacteria have also the ability to promote plant growth through one or more mechanisms. Native methylotrophic isolates under study are *Bacillus aerius* AAU M8, *Bacillus amyloliquefaciens* AAU M14, *Bacillus subtilis* AAU M17, *Bacillus megaterium* AAU M29 and *Paenibacillus illinoisensis* AAU M 17. Studies have shown that methylotroph improves plant growth by the production of phytohormones like indole-3-acetic acid (IAA), cytokinins and enzyme, viz. 1-aminocyclopropane-1-carboxylate

(ACC) deaminase which lower down ethylene concentration in plants, and production of bio-protectants to reduce incidence of plant pathogens (Jhala et al. 2014).

1.14.2.3 Isolation and Identification of Bacterial Strain Tolerating Heavy Metals for Bioremediation of Contaminated Soil

Isolates predominantly gram-positive *Bacillus* spp. and *Micrococcus* sp. and gram-negative *Pseudomonas* sp. were detected in polluted soil samples and studied for tolerating heavy metals (Pb, Ni, Cd, Cr, Co, Fe, Zn and Cu) under laboratory conditions; selected cultures are under investigation for their bioremediation potential.

1.14.2.4 Bioplastic (PHA/PHB) from *Azotobacter*

Production of poly(3-hydroxyalkanoates) through *Azotobacter* spp. utilizing agro-waste as substrate for indigenous production of bacterial bioplastic and biopolymer is fully biodegradable by soil inhabited by polyethylene adoring bacteria *Pseudomonas* capable to enhance decomposition in vitro, dual approach for minimizing plastic wastes and hazards (Bhatt 2012; Patel 2014).

1.15 Conclusion

In conclusion, it can be narrated that agricultural soil constitutes both plant and microorganisms as important and interactive components about less than 1% on earth's surface; nevertheless, it keeps the earth living for productivity and sustainability. On the other hand, currently soil receives high amount of different toxic agrochemicals in various forms causing ill effects on beneficial soil microflora and fauna. In this nexuses it is revealed that natural allies, the wonderful and useful agriculturally beneficial microorganisms, have best potential for strategical non-chemical, green farming approach to sustain agroecosystem for crop production, crop protection and soil reclamation for healthy life on globe in a long-run tactics of mankind.

Acknowledgements Authors are grateful to Anand Agricultural University authorities, Vice Chancellor, Director of Research and Dean (Agri.) for all support like utilization of research information and accomplishments of the chapter.

References

Allen RM, Bennetto HP (1993) Microbial fuel-cells. Appl Biochem Biotechnol 39(1):27–40

Barea JM, Gryndler M, Lemanceau PH, Schuepp H, Azcon R (2002) The rhizosphere of mycorrhizal plants. In: Gianinazzi S, Schuepp H, Barea JM, Haselwandter K (eds) Mycorrhiza technology in agriculture: from genes to bioproducts. Birkhauser Verlag, Basel, pp 1–18

Bhatt MA (2012) Production of Poly (3-Hydroxyalkanoates) by Azotobacter spp. utilizing agro-waste as substrate and its biodegradability in vitro- An M. Sc. Thesis submitted to Sardar Ptael University, V. V. Nagar, Gujarat

Carvalho MF, Ferreira JR, Pacheco CC, De Marco P, Castro PML (2005) Isolation and properties of a pure bacterial strain capable of fluorobenzene degradation as sole carbon and energy source. Environ Microbiol 7:294–298

Dabhi BK, Vyas RV, Shelat HN (2014) Use of banana waste for the production of cellulolytic enzymes under solid substrate fermentation using bacterial consortium. Int J Pure Appl Sci 3(1):1–9

Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59

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

Harris J (2009) Soil microbial communities and restoration ecology: facilitators or followers? Science 325:573–574

Hinge VR, Patel BA, Vyas RV (2010) Differentiation among four Meloidogyne species from Gujarat by RAPD – PCR. Ind J Nematol 40(2):167–170

http://aau.in/college-menu/department/765~815

http://agri.ikhedut.aau.in/1/fld/807

http://www.aau.in/business-planning-development-unitnaip-i

http://www.icar.org.in/node/5667

https://www.youtube.com/watch?v=m8hxJqR_3UI

Jani JJ, Vyas RV, Yadav DN (1993) A new Rickettsia Like Organism associated with white grubs. Curr Sci 65(9):720–721

Jhala YK, Vyas RV, Shelat HN, Patel HK, Patel HK, Patel KT (2014) Isolation and characterization of methane utilizing bacteria from wetland paddy ecosystem. World J Microbiol Biotechnol 30:1845–1860

Kennedy AC (1998) The rhizosphere and spermosphere. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (eds) Principles and applications of soil microbiology. Prentice Hall, Upper Saddle River, pp 389–407

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

Kozhemyakov AP, Provorov NA, Zavalin AA, Shott PR (2004) Analysis of interactions between different barley and wheat cultivars with rhizospheric growth promoting bacteria on the variable nitrogen background. Agrokhimia 3:33–40

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

Kushwah P, Vyas R, Jhala Y, Patel H (2013) Diversity of plastic degrading microorganisms and their appraisal on biodegradable plastic. Appl Ecol Environ Res 11(3):441–449

Lal R, Pandey G, Sharma P, Kumari K, Malhotra S, Pandey R, Raina V, Kohler HPE, Holliger C, Jackson C, Oakeshott JG (2010) Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiol Mol Biol Rev 74:58–80

Lee YK (2003) Microorganisms and production of alternative energy. In: Kun LE (ed) Microbial biotechnology: principles and applications. World Scientific Publishing Co. Pte. Ltd, pp 655–670

Mohanty SR, Bodelier PLE, Floris V, Conrad R (2006) Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. Appl Environ Microbiol 72:1346–1354

Noble AD, Ruaysoongnern S (2010) The nature of sustainable agriculture. In: Dixon R, Tilston E (eds) Soil microbiology and sustainable crop production. Springer, Berlin, pp 1–25

Panpatte DG, Shelat HN, Jhala YK (2015a) Compatibility of biocontrol bacteria with Phytoextracts. J Pure Appl Microbiol 9(4):3083–3087

Panpatte DG, Shelat HN, Jhala YK, Darji VB, Parvez N, Pathak L, Khatri K (2015b) Isolation and characterization of native Pseudomonas fluorescens for biocontrol of Fusarium wilt in Greengram. Green Farming 6(1):127–132

Panpatte DG, Shelat HN, Jhala YK, Dhole AM (2016) Inhibition of multiple fungal phytopathogens by biocontrol bacteria. Nat J Life Sci 13(1):29–31

Parr JF, Hornick SB (1992) Agricultural use of organic amendments: a historical perspective. Am J Altern Agric 7:181–189

Patel KT (2014) Mass Production Technology of Azotobacter in Laboratory Fermentor on Agro-industrial Wastes with Assessment of Alginate and Poly-β-hydroxybutyrate (PHB) Production Potential- A Ph.D. thesis submitted to Anand Agricultural University, Anand, Gujarat

Provorov NA, Tikhonovich IA (2003) Genetic resources for improving nitrogen fixation in legume–rhizobia symbiosis. Genet Resour Crop Evol 50:89–99

Rengel Z (2002) Breeding for better symbiosis. Plant Soil 245:147–162

Rojas A, Holguin G, Glick BR, Bashan Y (2001) Synergism between Phyllobacterium sp. (N2-fixer) and Bacillus licheniformis (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiol Ecol 35:181–187

Sanguin H, Sarniguet A, Gazengel K, Moenne-Loccoz Y, Grundmann GL (2009) Rhizosphere bacterial communities associated with disease suppressiveness stages of take-all decline in wheat monoculture. New Phytol 184:694–707

Schimel J (2007) Soil microbiology, ecology, and biochemistry for the 21st century. In: Paul EA (ed) Soil microbiology, ecology and biochemistry, 3rd edn. Academic Press, London

Seckbach J (ed) (2002) Symbiosis: mechanisms and model systems. Kluwer Academic Publishers, Dordrecht, p 850

Shtark OY, Borisov AY, Zhukov VA, Provorov NA, Tikhonovich IA (2010) Intimate associations of beneficial soil microbes with host plants. In: Dixon R, Tiltson E (eds) Soil microbiology and sustainable crop production. Springer Science and Business Media, Berlin/Heidelberg, pp 119–196

Singh OV (2006) Proteomics and metabolomics: the molecular make-up of toxic aromatic pollutant bioremediation. Proteomics 6(20):5481–5492

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, London, pp 503–512

Tanaka K, Kashiwagi N, Ogawa T (1988) Effects of light on the electrical output of bioelectrochemical fuelcells containingAnabaena variabilis M-2: Mechanism of the post- illumination burst. J Chem Technol Biotechnol 42:235–240

Umarao GS, Vyas RV, Ganguly AK (2002) Molecular characterization of Steinernema Thermophilum Ganguly & Singh (Nematoda: Steinernematidae). Int J Nematol 12(2):215–219

van De Woestyne M, Gellens V, Anasi I, Verstraete W (1994) Anaerobic digestion and interregional recycling of organic soil supplements. In: Marchaim U, Ney G (eds) Sustainable rural environment and energy network (SREN) – biogas technology as an environmental solution to pollution, REUR technical series number 33. FAO, Rome

Vance CP (1998) Legume symbiotic nitrogen fixation: agronomic aspects. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Molecular biology of model plant-associated bacteria. Kluwer Academic Publishers, Dordrecht, pp 509–530

Vora MS, Shelat HN, Vyas RV (2008) Handbook of biofertilizers and microbial pesticides. Satish Serial Publishing House, Delhi

Vyas RV, Dn Y, Patel RJ (1990) Studies on the efficacy of Beauveria brongniartii (Sacc). Petch against white grub. Ann Biol 6(2):123–128

Vyas RV, Yadav DN, Patel RJ (1991a) Efficacy of bacillus popilliae var holotrichiae against white grub (Holotrichia Consanguinea). Indian J Agric Sci 61(1):80–81

Vyas RV, Yadav DN, Patel RJ (1991b) Mass production of a entomogenous fungi Beauveria brongniartii on solid substrates. Indian J Exp Biol 29:795–797

Vyas RV, Patel DB, Patel DJ, Patel BA (1995) Bio-efficacy of Paecilomyces lilacinus against Meloidgoyne incognita. Ind J Mycol Pl Pathol 25(1&2):131

Vyas RV, Patel NS, Patel DJ (1999) Mass production technology for Entomopathogenic nematode Steinernema sp. Ind J Nematol 29:178–181

Vyas RV, Maghodia AB, Patel BA, Patel DJ (2005) In vitro testing of Xenorhabdus metabolites against groundnut collar-rot fungus, Aspergillus niger. Int Arac News 25:34–36

Vyas RV, Maghodia AB, Patel BA, Patel DJ (2006) Isolation of native Xenorhabdus bacteria from Steinernema spp. and role of their exo and endo toxic factors for management of root-knot nematodes (Meloidogyne spp.) on tomato. Indian J Nematol 36(2):241–246

Vyas RV, Shelat HN, Vora MS (2008) Biofertilizers techniques for sustainable production of major crops for second green revolution in Gujarat – an overview. Green Farming 1:68–72

Vyas RV, Shelat HN, Jhala YK (2010) Microbial pesticides – an alternative tool to combat insect pests in GOI organic farming. Org Farming News Lett 6(4):14–17

Vyas RV, Singh B, Shelat HN and Shekh AM (2014) Present scenario & future prospects: AAU BPDU, approaches to promote agri-business by technology transfer and public-private partnerships for second green revolution. In: Handbook of Technology Transfer and Commercialization: Experiences of US and India. Michigan State University Press

Waites MJ, Morgan NL, Rockey JS, Higton G (2001) Industrial microbiology: an introduction. Blackwell Science Ltd, p 288

Wood N (2001) Nodulation by numbers: the role of ethylene in symbiotic nitrogen fixation. Trend Plant Sci 6:501–502

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Zafar S, Aqil F, Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol 98:2257–2261

Microbial Biofertilizer: A Potential Tool for Sustainable Agriculture

2

Udaya Kumar Vandana, Ankita Chopra,
Sanchita Bhattacharjee, and P.B. Mazumder

Abstract

Surplus use of chemical fertilizers in crop field to meet the increasing demand of crop production has greatly hampered the soil ecosystem and human health. An alternative environment-friendly approach to sustainable agriculture is encouraging the use of biofertilizers. Plant growth-promoting microorganisms are one such group of potent biofertilizers. Many bacteria and fungi can develop close associations with the crop plant which improves growth, immunity and overall development of the plant. Thus understanding the action of various mechanisms exhibited by these microorganisms can show us the way to formulate the microbes to be used as biofertilizers. Continuous efforts are made to develop strategies for optimizing bioformulations. This chapter gives a deep understanding of the transformation of a microbe into a fertilizer. Distinctive properties of plant growth-promoting microbes and strategies to develop and optimize the bioformulations in addition to the phenomenon of integrated management have been discussed broadly.

Keywords

Fertilizer • Plant growth-promoting microorganisms • Biofertilizers • Bioformulation

U.K. Vandana • A. Chopra • S. Bhattacharjee • P.B. Mazumder (✉)
Department of Biotechnology, Assam University, Silchar 788011, Assam, India
e-mail: uday21microbe@gmail.com; pbmazumder65@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_2

2.1 Introduction and Brief History on Microbial Biofertilizers

Agricultural practices always work with the aim of improving crop yield. For increasing productivity chemical fertilizers are being used. It leads to spoilage of the soil health through affecting its biodiversity by altering the chemical composition, microbial flora and ecosystem(s) (Wall et al. 2015). Early nineteenth-century chemical fertilizer industries started producing synthetic fertilizers and pesticides consisting of phosphorous (P), potassium (K) and nitrogen (N) to boost crop production and disease protection (Belay et al. 2002; Meng et al. 2013). Many researchers in recent years found the negative impact of chemical fertilizers and their hazardous nature on soil and human health. Farmers are being the target population for pesticide poisoning due to their direct exposure and lack of technical knowledge (Amundson et al. 2015). Current agricultural practices are quite dependent on synthetic chemical fertilizers as they directly help in increasing the required elements in soil. Studies suggest that long-term continuous application of chemicals results in soil acidification and reduced soil quality which ultimately hampers human health and creates environmental imbalance (Geisseler and Scow 2014). Hence there is an increasing need to have alternative sustainable agricultural practices and biotechnological approaches to increase crop productivity, improve soil health and conserve biodiversity. In this approach microbes play a vital role in maintaining agricultural sustainability by maintaining diversity of ecosystems and improving soil health in a safer way (McDaniel et al. 2014; Altieri 1999). Continuous interaction between the plant and its surrounding microbiome helps build some positive interactions. Depending upon the site of interaction, it is designated as phyllosphere, rhizosphere, epiphytic and endophytic bacteria (Rout 2014; Philippot et al. 2013; Lindow and Brandl 2003; Hartmann et al. 2009; Dong et al. 2003). Bacteria possessing the traits which benefit the plant in growth and disease protection are termed as plant growth-promoting bacteria (PGPB) (Mantelin and Touraine 2004; Bashan 1998; Bashan and de-Bashan 2005). Bioformulations were in agricultural practice in the history where discovery of Bassi in 1835 illustrated *Beauveria bassiana* infection in silkworm (Brownbridge et al. 2012). This discovery laid a path for identifying the role of microbes in disease protection. The discovery of Bt (*Bacillus thuringiensis*) toxin gave more strength to the idea of researchers to think more about microbes as an alternative for chemicals (Sayyed et al. 2003). Later most of the bacteria were reported for their plant growth-promoting and biocontrol activity. Many studies reported the successful application of various bioformulations in controlling the disease and improving plant growth (Glick and Bashan 1997). Beneficial microbes such as *Pseudomonas* spp. (Ahemad and Khan 2012a), *Bacillus* spp. (Canbolat et al. 2006), *Klebsiella* spp. (Ahemad and Khan 2011), *Rhizobium* (Ahemad and Khan 2009), *Azospirillum* (Rodrigues et al. 2008) and *Burkholderia* sp. (Guo et al. 2015) have been reported in different crops like rice (Mirza et al. 2006), green gram (Wani et al. 2007), wheat (Khalid et al. 2004), chickpea (Verma et al. 2014), maize (Braud et al. 2009a, b), black gram (Ganesan 2008), barley (Canbolat et al. 2006), *Brassica* (Belimov et al. 2005), soybeans (Gupta et al. 2005), sunflower (Faisal and

Hasnain 2005) and tomato (Ahemad and Kibret 2014). The commercialization of PGPR started in the late eighteenth century, and its popularity increased over the time with successful use as bioinoculants. The application of PGPB in sustainable agriculture is the need of the hour (Brockwell and Bottomley 1995; Vessey 2003). Mechanism of action of these microbial inoculants varies; researcher found that these are specific to host and region. Moreover, bacteria need to face unfavourable conditions after inoculation which make them reduce their expressive traits (Bashan 1998). Bacterial consortiums were made with multiple bacteria to combine multiple traits that benefit plant growth and combat against phytopathogens. Based on their expressive traits, numerous numbers of biofertilizers came into existence with various types of formulation. Moreover, recent development in studies on agriculture reveals that microbiome activities in soil and sustainable agriculture are interlinked to each other. This chapter will collectively focus on plant growth-promoting bacteria (PGPB) and their mechanism of action in growth promotion and role in bioformulations for sustainable development of agriculture.

2.2 Mechanisms of Action of Plant Growth-Promoting Rhizobacteria

2.2.1 Phosphate Solubilization

Phosphorus, a key nutritional element, plays an indispensable role in several plant developmental processes like macromolecular biosynthesis, photosynthesis, respiration, signal transduction and energy transfer (Khan et al. 2010). Despite the abundance of phosphorus in soil, sometimes it becomes inaccessible to plants, as they can only absorb soluble forms of phosphorus, i.e. mono- and dibasic phosphate (Jha et al. 2012). To resolve the problems related to plant phosphorus deficiency, chemically synthesized phosphate fertilizers are used. But the use of phosphate fertilizer comes with various drawbacks like release of highly volatile and poisonous hydrogen fluoride (HF) gas during manufacture (Sharma et al. 2013), heavy metal accumulation in soil and plant after application, eutrophication and hypoxia of lakes and marine estuaries (Lugtenberg et al. 2013), etc. Phosphate solubilizing microbes (PSM) provide an eco-friendly alternative to chemical fertilizers. The common mechanisms used by PSM for phosphate solubilization include (i) organic acid (acetic, malic, tartaric, gluconic, lactic, 2-ketogluconic, oxalic and succinic, citric) secretion (Patel et al. 2015) and (ii) extracellular enzyme (non-specific phosphatases, phytases, phosphatases and C-P lyases) production (Bloemberg and Lugtenberg 2001). Phosphate solubilization trait is widespread among rhizosphere microflora. Some of the efficient PSMs identified till date are *Aspergillus niger*, *Penicillium* sp., *Kluyvera cryocrescens, Pseudomonas aeruginosa, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus atrophaeus, Paenibacillus macerans,* etc.

2.2.2 Nitrogen Fixation

Nitrogen (N) is the major mineral element required by plants for growth and development but is also the most limiting available nutrient for plant growth (Valentine et al. 2010). Dinitrogen constitutes a major portion of atmospheric gas (78%). However, most organisms cannot use this form of nitrogen. Prokaryotes are involved in the task of making dinitrogen available to other eukaryotes via the ATP-dependent process of biological nitrogen fixation (BNF) where dinitrogen is reduced to ammonia (Dos Santos et al. 2012). Bioavailability of nitrogen in the form of ammonia and nitrates is limited. Modern agriculture depends largely on nitrogen fertilizers for high crop yields (Galloway et al. 2008). The drawbacks of using chemical nitrogen fertilizers are:

(i) Production of nitrogen fertilizers requires a vast amount of non-renewable fossil fuel (Erisman et al. 2007).
(ii) High emission of greenhouse gases, which constitute a key factor in climate change.
(iii) Half of the nitrogen fertilizer applied is lost to leaching, resulting in significant health and environmental problems (Olivares et al. 2013).
(iv) Increase in soil acidity due to release of hydrogen ions in fertilizer applied on soil (Arma 2016).

Therefore, replacing chemical nitrogen fixation by BNF can generate a new perspective of agricultural sustainability (Farrar et al. 2014). Legumes fix atmospheric N through symbiotic nitrogen fixation (SNF). A part of the N fixed by legumes can be transferred to neighbouring non-fixing plants by means of N-transfer (Fustec et al. 2009). N-transfer is the movement of N from one legume plant (donor) to another nonlegume plant (receiver) in a mixed stand of plant community (Høgh-Jensen and Schjoerring 2000; Pirhofer-Walzl et al. 2012). N-transfer facilitates more efficient utilization of fixed N, minimizes N losses and maintains a good level of biomass production (Thilakarathna et al. 2016). *Paenibacillus polymyxa* P2b-2R, an endophytic strain, is capable of fixing nitrogen (N) and promoting growth in a broad range of hosts including canola (*Brassica napus* L.) (Anand et al. 2013; Padda et al. 2016). Recently it was reported that inoculation of maize and wheat with nitrogen-fixing rhizobacterium *Pseudomonas protegens* Pf-5 X940 largely improved nitrogen content and biomass accumulation in both vegetative and reproductive tissues, and this beneficial effect was positively associated with high nitrogen fixation rates in roots (Fox et al. 2016).

2.2.3 Phytohormone Production

Phytohormones produced by plant-associated microflora can stimulate plant growth and development by modulating endogenous plant hormone levels (Gray 2004) (Van Loon 2007). The most important microbial plant growth regulators reported

till date include auxins such as indole-3-acetic acid, cytokinins and gibberellins (GAs). Eighty percent of rhizospheric microbes isolated from various crops are reported to produce auxin as secondary metabolites (Ahemad and Khan 2011). Plant-associated rhizobacteria can synthesize auxin in either L-tryptophan-dependent or L-tryptophan-independent pathways. Three tryptophan-dependent routes for auxin synthesis are known in rhizobacteria which are (i) indole-3-pyruvic acid (IPyA) pathway found in *Rhizobium, Bradyrhizobium* and *Azospirillum*; (ii) indole-3-acetamide (IAM) pathway used by some pathogenic bacteria like *Agrobacterium tumefaciens, Pseudomonas syringae, Pantoea agglomerans,* etc.; and (iii) tryptamine pathway found in *Bacillus licheniformis* and *Bacillus megaterium*. Rhizobacterial IAA has been identified as a key effector molecule in plant-microbe interaction causing either phytostimulation or pathogenesis (Ahemad and Khan 2012b; Mahanty et al. 2016). Besides IAA, there are reports of microbial phytostimulation by cytokinin production. *Bacillus megaterium* has been reported to enhance the growth of *Arabidopsis thaliana* and *Proteus vulgaris* seedlings via cytokinin synthesis (Castro et al. 2008). Bacteria belonging to diverse genera such as *Pseudomonas, Azospirillum, Bacillus, Proteus, Klebsiella, Xanthomonas, Pseudomonas,* etc. are well-characterized cytokinin producers. Apart from that gibberellin (GA) production has been detected in both bacteria and fungi. Though the exact role of bacterial GA is not known yet, GA-producing bacteria are still used for enhancing seed germination rate (Goswami et al. 2016).

2.2.4 Insecticidal Protein Production

Insect pests cause a major crop loss. Reduction of 39% yield and loss amounting US$ 500 million annually is caused by the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in corn (*Zea mays*) cultivation in Brazil. Native strains of entomopathogenic nematodes active against *S. frugiperda* represent a promising alternative to the intensive use of chemical insecticides to control fall armyworm population in corn plantations. Conventional control methods are ineffective especially when pest attacks the below-ground plant parts. Protecting plants with microbial agents such as PGPR is an ecologically friendly approach (Péchy-Tarr et al. 2013). Insecticidal toxins so far have been exploited mainly in two bacterial groups *Bacillus thuringiensis (Bt) and Photorhabdus/Xenorhabdus* species. *B. thuringiensis* is a gram-negative rod-shaped bacterium which produces a diverse range of insecticidal protein such as crystal (Cry) and cytolytic (Cyt) toxins (Roh et al. 2007). *Photorhabdus/Xenorhabdus* species are gram-negative bacteria producing insecticidal toxins (Tc) and live in symbiotic relationship with entomopathogenic nematodes (ffrench-Constant et al. 2007). Two related strains of *P. fluorescens* CHA0 and Pf-5 exhibit both antifungal activity and insecticidal activity. Their insecticidal activity depends greatly on a large protein production termed as the Fit toxin (Péchy-Tarr et al. 2013) which also contributes to oral insecticidal activity (Ruffner et al. 2013). *Yersinia entomophaga* MH96 secretes Yen-Tc protein toxin complex which when ingested by sensitive insects causes its death within 72 h of infection (Busby

et al. 2012). Insecticidal toxin (Tc) formed by three-component (TcA-, TcB- and TcC-like proteins) complexes were found effective for symbiosis and insecticidal activity (ffrench-Constant et al. 2007). Symbiotic bacterial interactions with nematodes is one of the viable alternative for chemicals as their interaction leads bacteria to produce factors that can control/kill the insect host and facilitate the growth of nematodes. Bacterial ureases have been studied extensively for their role in insecticidal activity (Salvadori et al. 2012).

2.2.5 Antibiotic Production

Indirect mechanism of plant growth promotion by bacteria involves antibiotic production as well which have inhibitory effects on pathogenic organisms in the rhizosphere (Glick 1995) (Ahmad et al. 2008). Antibiotics constitute a wide and heterogeneous group of low molecular weight chemical organic compounds that are produced by a wide variety of microorganisms (Raaijmakers et al. 2002). The basis of antibiosis relies on the secretion of molecules which can reduce or kill the growth of target pathogen (Glick et al. 2007). Some antibiotic compounds are diffusible such as phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin and cyclic lipopeptides, and some are volatile like hydrogen cyanide (HCN) (Haas and Défago 2005). Mostly the *Pseudomonas* genus in comparison to other bacterial species has the ability to produce antibiotics (Santoyo et al. 2012). Pyoluteorin (Plt), phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin (Prn), hydrogen cyanide (HCN) and pyoluteorin (Plt) and protein-type (bacteriocins) are some types of antimicrobial compounds synthesized by *Pseudomonas* (Haas and Keel 2003). 2,4-DAPG is the most efficient antibiotic in the control of plant pathogens and can be produced by various strains of *Pseudomonas* (Nakkeeran et al. 2006). This antibiotic has antifungal, antibacterial and antihelmintic properties (Loper and Gross 2007; Velusamy et al. 2006; Cronin et al. 1997). Thomashow and Weller (1988) demonstrated the first experimental proof that a *Pseudomonas* antibiotic can suppress plant disease in an ecosystem. *Pseudomonas fluorescens 2–79* strain (isolated from the rhizosphere of wheat) synthesized phenazine antibiotic phenazine-1-carboxylic acid (PCA) which could suppress take-all disease caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt) on wheat. *Pseudomonas* PCA-negative mutants are partially devoid of their ability to inhibit the fungus in vitro and to suppress take-all disease in vivo.

Recently, *Pseudomonas* and *Bacillus* species are known to have a new class of biocontrol agent called lipopeptide (LP) bio-surfactants which possess positive effect on competitive interactions with organisms such as bacteria, fungi, nematodes and plants (De Bruijn et al. 2007; Raaijmakers et al. 2010). *Bacillus* LPs were mostly studied as antagonists, but they also facilitate root colonization (Bais et al. 2004). Khabbaz et al. (2015) reported that *Pseudomonas fluorescens* Pf 9A-14, *Pseudomonas* sp. Psp. 8D-45 and *Bacillus subtilis* Bs 8B-1 showed broad-spectrum antagonistic activity and provided suppression of *Pythium* damping-off and root rot of cucumber. *Pseudomonas* strains contained genes for biosynthesis of antibiotics,

viz. PCA, 2, 4-diacetylphloroglucinol, pyrrolnitrin and pyoluteorin, whilst *B. subtilis* Bs 8B-1 contained antibiotic lipopeptides such as fengycin, bacillomycin, bacilysin, surfactin and iturin A. These antagonistic bacteria have also shown a significant increase in fresh weights of both cucumber and radish plants. The antagonistic activity of the three bacterial strains and the growth inhibition of *Phytophthora capsici* and *Rhizoctonia solani* might have been due to the production of different types of antibiotics.

2.2.6 Siderophore Production

Along with antibiotics, siderophores also function in root disease suppression (Martínez-Viveros et al. 2010). The term "siderophores" is derived from the Greek word meaning "iron carriers". They are relatively low molecular weight, ferric ion-specific chelating agents produced and utilized by bacteria and fungi growing under low iron stress (Neilands 1995). The primary function of these compounds is to scavenge the ferric iron [Fe (III)] from different terrestrial and aquatic habitats and thereby make it available for microbial and plant cells for their cellular growth and metabolism (Ahmed and Holmström 2014). The importance of iron (Fe) in the growth of almost all living organisms is because it acts as a catalyst in enzymatic processes, oxygen metabolism, electron transfer and DNA and RNA syntheses (Aguado-Santacruz et al. 2012). Acquirement of Fe through siderophore production displays the competitive fitness of plant growth-promoting bacteria to colonize plant roots (Barton and Abadia 2006) thereby outcompeting the pathogenic microorganisms in the rhizosphere (Siddiqui 2006). The primary role of siderophore is to sequester iron, but it also forms complexes with other essential elements, viz. Mo, Mn, Co and Ni, in the environment and make them available for microbial cells (Bellenger et al. 2008) (Braud et al. 2009a, b). pH influences Fe(III)-siderophore complex formation. Fe has to compete against free proton for siderophore binding sites and also against metals such as divalent cations (Cd2+, Cu2+, Ni2+, Pb2+ and Zn2+) (Albrecht-Gary and Crumbliss 1998), trivalent cations (Mn3+, Co3+ and Al3+) and actinides (Th4+,U4+ and Pu4+) (Weber 2005).

2.2.7 Hydrogen Cyanide Production

Many rhizobacteria are capable of producing a volatile compound known as HCN which plays a role in biocontrol of certain plant pathogens (Martínez-Viveros et al. 2010) (Gupta et al. 2015). HCN genes are widely distributed among many *Pseudomonas* strains producing antibiotic 2,4-DAPG (Haas and Défago 2005). The *hcnAB* genes are shown to be particular in detecting HCN-producing pseudomonas among the bulk isolates (Svercel et al. 2007). In addition with the established hypothesis of biocontrol by HCN-producing strains, another new hypothesis evolved where it is stated that HCN is involved in geochemical processes and regulation of nutrient availability. HCN is also involved in metal sequestration (Wongfun

et al. 2013), and this sequestration leads to increased availability of phosphate (Rijavec and Lapanje 2016).

2.2.8 Bacterial Volatile Compounds

Many PGPRs have been reported to secrete volatile compounds known as bacterial volatile compounds (BVCs) which trigger plant growth and immunity (Chung et al. 2016). For a rhizobacteria to contribute in plant's growth promotion, it is studied that there must be a close association between the microbe and the root, but volatile compound-producing rhizobacteria does not require any established physical contact to trigger growth response (Ortíz-Castro et al. 2009). BVC are low molecular weight compounds (<300 Da) secreted by bacteria (Chung et al. 2016). Bacterial volatiles include inorganic compounds such as ammonia, H2S, HCN and NO, and therefore these volatiles are referred to as BVCs rather than volatile organic compounds (VOCs) (Audrain et al. 2015). BVCs such as 2,3-butanediol and acetoin accelerate plant growth and induce systemic resistance (Ryu et al. 2003). Bacteria-emitting BVC was reported to colonize the maize tissue both underground and aboveground and secrete BVC which strikes the plant's physiology, growth and defence (D'Alessandro et al. 2014). *Bacillus* sp. B55 secretes sulphur-containing BVC-dimethyl disulphide (DMDS) which increased sulphur content in *Nicotiana attenuata* and also enhanced the plant growth (Meldau et al. 2013).

2.2.9 Rhizoremediation

Microbes have the potential to detoxify various soil contaminants (petroleum hydrocarbons (PHCs), pesticides halogenated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), heavy metals, etc.) through diverse mechanisms like bioexclusion, biosorption, bioleaching and bioaccumulation. Degradation of contaminants occurs in the rhizosphere by combined action of microbial products and plant root exudates. Bioremediation of non-biodegradable heavy metals has been reported to be done by different plant beneficial rhizobacteria like *Achromobacter xylosoxidans, Azotobacter chroococcum, Ochrobactrum* sp.*, Bacillus subtilis, Bacillus megaterium, Bradyrhizobium, Pseudomonas sp., Mesorhizobium, Brevibacillus* sp.*, Kluyvera ascorbata, Pseudomonas putida, Ralstonia metallidurans, Rhizobium, Sinorhizobium* sp.*, Pseudomonas aeruginosa, Variovorax paradoxus, Psychrobacter* sp.*, Xanthomonas* sp., etc. (Mahanty et al. 2016). Microbes can do either biotransformation or biodegradation to detoxify the pesticides. For microbial biodegradation, enzyme systems involved are hydrolases, esterases and the mixed function oxidases (MFO) in the first metabolic stage and the glutathione S transferases (GST) system in the second phase (Ortiz-Hernández et al. 2013). It has been reported that *Azospirillum, Enterobacter, Azotobacter, Bacillus, Klebsiella, Gordonia, Paenibacillus, Serratia, Pseudomonas*, etc. can reduce pesticide toxicity in soil (Shaheen and Sundari 2013).

2.2.10 Induced Systemic Resistance

Defence responses in plants can be activated via two mechanisms. One is induced systemic resistance (ISR) triggered by nonpathogenic PGPR, and the other is systemic acquired resistance (SAR) triggered by a pathogenic agent (Pieterse et al. 2009). SAR leads to activation of pathogenesis- related (*PR*) genes and involves salicylic acid (SA) as signalling molecule (Durrant and Dong 2004). ISR is SA independent but requires signalling pathway of jasmonic acid (JA) followed by ethylene signalling (van Loon et al. 1998). Yet both ISR and SAR require nonpathogenesis-related protein (NPR1), a key regulatory protein. ISR prepares the plant to encounter pathogen by priming for enhanced defence. During pathogen or insect attack, the defence response is accelerated leading to faster and enhanced resistance (Conrath et al. 2006). SAR and ISR pathways have been reported to exert additive effect on *A. thaliana* against a broad range of pathogens (van Wees et al. 2000). The enhanced defence response due to the additive effect was supported by molecular studies which revealed an increased expression of pepper defence genes *CaTin1*, *CaPR1* and *CaPR4* after application of combined treatment of *Bacillus pumilus* INR7 with a chemical inducer, benzothiadiazole (BTH), in the field and subsequent suppression against bacterial spot disease caused by *Xanthomonas axonopodis* pv. vesicatoria in pepper (Yi et al. 2013).

2.2.11 Induced Systemic Tolerance

Induction of microbe-driven abiotic stress tolerance in plant is referred to as "induced systemic tolerance (IST)" (Yang et al. 2009). Molecular mechanism of plant-microbe crosstalk associated with IST is largely unknown. Beneficial microbes can enhance survivability of stress-affected plants by diverse mechanisms. One of the most important mechanisms is the modulation of hormonal status in host plant. In response to stress stimuli (salinity, drought, metal toxicity, etc.), retardation in plant growth and development is due to the increase in stress ethylene level. Some plant growth-promoting bacteria produce ACC deaminase enzyme which cleaves ACC, the precursor of ethylene to ammonia and α-ketobutyrate (KB), thereby lowering ethylene level and promoting plant growth under stress. The level of ACC deaminase activity differs among bacterial genera under various environmental conditions (Singh and Jha 2016). Experimental evidence suggests that bacteria showing ACC deaminase activity approximately >20 nmol α-ketobutyrate (KB) mg-1 h-1 are sufficient to reduce the growth inhibitory effects of stressors (Penrose and Glick 2003). Volatile emission is another important microbial trait involved in plant growth stimulation under stress (Ryu et al. 2003). For instance, VOC produced by *Bacillus subtilis* confers salt tolerance in *Arabidopsis thaliana* by modulating the expression of high-affinity Na+ transporter HKT1 in a tissue-specific manner (Zhang et al. 2008).

Table 2.1 List of some beneficial plant growth-promoting traits

Trait	Role	Microbe	Reference
Phosphate solubilization	1. Organic acid production	*Bacillus licheniformis*; *B. amyloliquefaciens*; *Penicillium sp.*	Chen et al. (2006) and Wakelin et al. (2004)
	2.Phytase production	*Bacillus mucilaginosus; Aspergillus niger*	Li et al. (2007) and Vassilev et al. (2007)
	3. Phosphatase production	*Burkholderia cepacia, Serratia marcescens*	Ryu et al. (2005) and Unno et al. (2005)
Nitrogen fixation	1. Symbiotic	*Rhizobium phaseoli; Vesicular-arbuscular mycorrhizal fungi*	Shah et al. (2010)
	2. Non-symbiotic	*Gluconacetobacter diazotrophicus*	Bhattacharyya and Jha (2012)
Phytohormone production	1. IAA production	*Bacillus licheniformis; Phoma glomerata* and *Penicillium* sp.	Goswami et al. (2016) and Waqas et al. (2012)
	2. Cytokinin production	*Bacillus megaterium*	Castro et al. (2008)
	3. Gibberellin production	*Acetobacter diazotrophicus, Phoma glomerata* and *Penicillium* sp.	Basti et al. (1998) and Waqas et al. (2012)
Biocontrol	1. Extracellular enzyme production		
	(a) Chitinase	*Enterobacter agglomerans*	Nielsen and Sörensen (1999)
	(b) Glucanase	*Bacillus cepacia*	Compant et al. (2005)
	2. Antibiotic production	*Pseudomonas fluorescens; Trichoderma koningii*	Thomashow and Weller (1988) and Xiao-Yan et al. (2006)
	3. Siderophore production	*Pseudomonas aeruginosa*	Braud et al. (2009a, b)
	4. HCN production	*Pseudomonas chlororaphis*	Nandi et al. (2015)
Potassium solubilization	Production and excretion of organic acid and inorganic acid	*Bacillus mucilaginosus*	Ullman et al. (1996)
Induced systemic tolerance	1. ACC deaminase production	*Achromobacter piechaudii; Trichoderma asperellum; Penicillium citrinum*	Mayak et al. (2004), Viterbo et al. (2010) and Jia et al. (2000)
	2. Exopolysaccharide production	*Oceanobacillus profundus*	Qurashi and Sabri (2011)
	3. VOC production	*Bacillus amyloliquefaciens*	Choi et al. (2014)

2.3 Strategies for Development and Optimization of Bioformulations

2.3.1 Large-Scale Production of Strains

For mass production of inoculants, the viable cells of the strains have to prove efficient enough in maintaining their genetic stability, exerting the desired effect on target crops and their survival under adverse conditions. Preparation of microbial inoculum is considered to be key factor in maintaining viability of the inoculant on seed (Moënne-Loccoz et al. 1999). The production of microbial inoculants starts with preparation of broth culture to reach high population density of bacterial cells. The main factors during inoculum preparation include (i) the specified growth media; (ii) optimal growth conditions such as pH, temperature, O2, etc.; (iii) purity of the media; and (iv) cost (Herrmann and Lesueur 2013). The microbial cultures are then inoculated on different types of carrier which serves as the delivery vehicle of live biofertilizers from the factory to the field (Bashan et al. 2014). Acclimatization of inoculants in the carrier material for several days prior to application to seed can improve the inoculums' efficacy (O'Callaghan 2016).

2.3.2 Formulation

Jones and Burges (1998) regarded formulation as vital factor in bioinoculant development. Roles of formulation are to (i) stabilize the microbe, (ii) help in the delivery of the microbe to the target zone, (iii) protect the microbe during seed storage and (iv) enhance the functionality of the microbe in situ after planting. Over the years scientists have been trying to improve the survival of pre-inoculated seeds, and so various formulation efforts are being targeted. Microbial formulations are divided into conventional type and advanced type. Conventional type includes (1) **solid formulation** (peat, granules, powders, etc.), but microbial shelf life is less in it due to desiccation and (2) **liquid formulation**, based on broth cultures, but they lack carrier protection and quickly lose viability on the seed. Advanced type involves the most promising technique for constructing carriers of microorganisms called (1) **microencapsulation** formulation which has been proven to be advantageous over conventional types (John et al. 2011). Biofilms have been proposed as possible bioformulation for both bacteria and fungi (Seneviratne et al. 2008). Recently it was reported that *Trichoderma atroviride* spores can be formulated by an adhesive, xanthan gum, provided optimal storage conditions are maintained and thus can be effectively delivered on to seeds (Swaminathan et al. 2016).

2.3.3 Storage and Transport

Formulation is important during storage and transport of the biofertilizers (Malusá et al. 2012). Thus endurance of bioinoculant is necessary during its storage period

and also after its application onto the soil where it has to compete with other native microbes for space and nutrient (Bashan et al. 1995). The carriers and optimum conditions required to maintain the bioinoculants differs as it depends on the strains used. PGPR continued to multiply and maintain their metabolic activity when peat was used as carrier (Rice et al. 2000). The sludge-based carrier could maintain rhizobia population at neutral pH and water holding capacity even after 130 days of storage at 25 ° C (Ben Rebah et al. 2002). Encapsulation of microbial cells offers longer viability when stored at 4 ° C. Moreover encapsulated bacteria could be stored at 4 °C or room temperature for up to 6 months with static population size (Rouissi et al. 2010). Long-term storage of bioinoculants results in cell sedimentation. Vandergheynst et al. (2007) used hydrophobic silica nanoparticles for thickening the oil phase which greatly cut down cell sedimentation thereby improving cell viability during storage. The reason behind is the dispersed water retaining the oil which prevented cells from desiccation. More insights into overcoming the problem of cell sedimentation using nanomaterials will be beneficial for further long-term storage of biofertilizers.

2.3.4 Inoculation in the Field

Introduction of the biofertilizers into the field depends on various factors including concentration of the inoculums, mode of biofertilizer application, competition of inoculants with the native niche for survival and user-friendliness of the bioinoculant (Dey et al. 2012). Farmers need to have proper knowledge about how microbes perform in soil prior to their inoculation in the fields (Date 2001). The lower quantity of inoculants having high cell concentration (10^4–10^6) shows similar efficiency as the higher quantity of inoculants with lesser cell concentration does (Schulz et al. 2008). Mode of biofertilizer application is mainly done by four ways: (a) inoculation of seeds with powder formulation, (b) water-suspended peat sprayed onto furrow during seed sowing, (c) soil inoculation with peat granules and (d) liquid formulations (Bashan 1998). Biofilm-based application of microbial consortium was proved to be advantageous for fixing N_2 in the soybean over the conventional practise of rhizobia inoculation (Jayasinghearachchi and Seneviratne 2004). Since the microbial population in the soil could get diluted along with time, repeated application of bioinoculum during the growing season is required to escalate the effect of microbial application (Bashan et al. 1995) (Malusá et al. 2012). Agrichemicals are often used as seed dressing. Thus compatibility of seed inoculants with those agrichemicals such as pesticides is the most important because pesticides have been reported to alter the structure and function of the bioinoculum (Fox et al. 2007; O'Callaghan 2016).

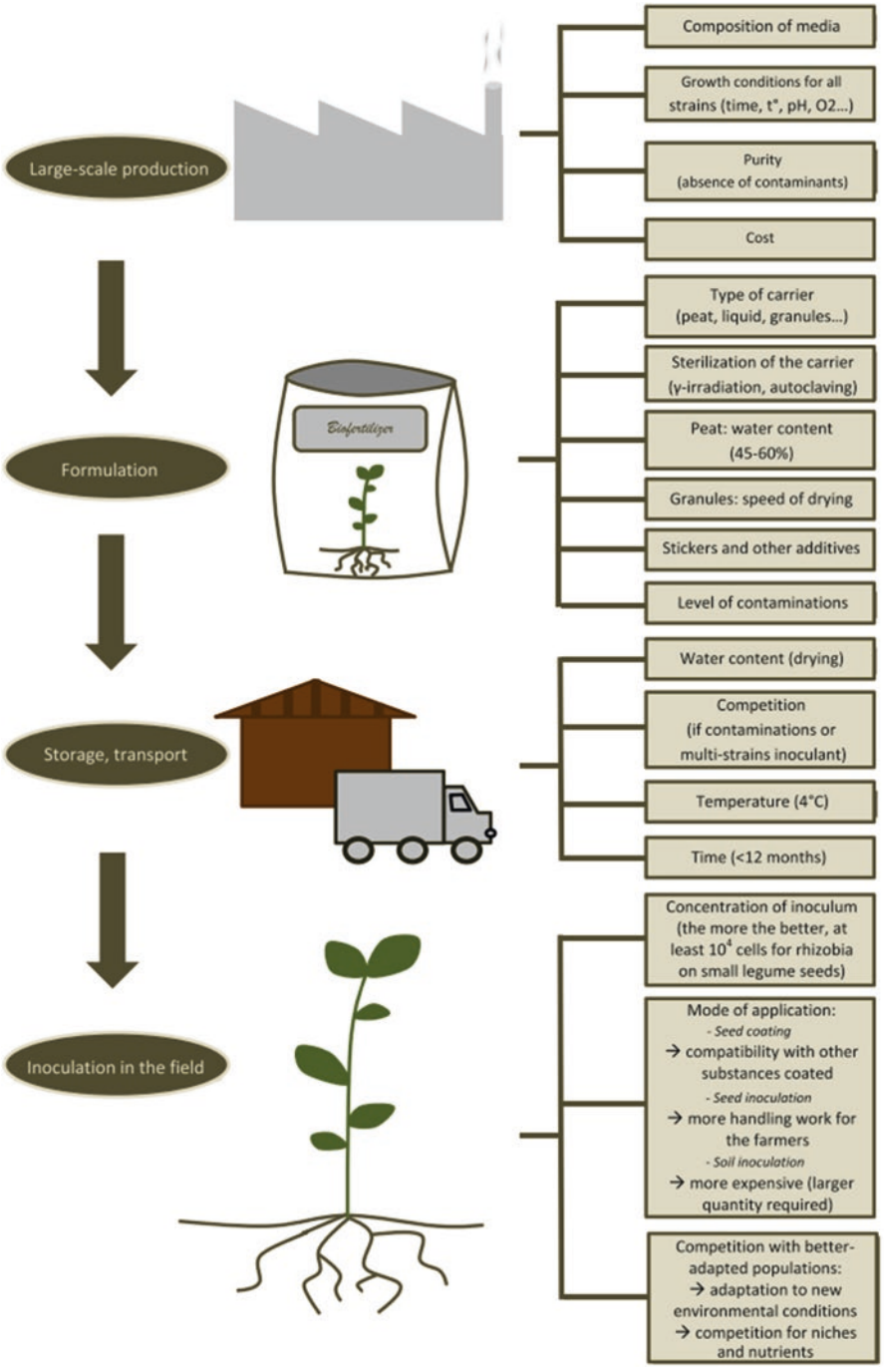


Fig. 2.1 Steps involved in inoculum preparation to inoculation in field (Herrmann and Lesueur 2013)

2.3.5 Integrated Management

Crop production is at stake due to increase in incidences of pests, namely, animal pests (insects, nematodes, mites, etc.), plant pathogen (bacteria, protozoa, fungi, virus) and weeds. Crop protection is being developed for prevention and control of pests (Oerke 2006). Integrated crop protection management can be broadly classified into four types: (i) integrated soil fertility management (ISFM) (ii) integrated pest management (IPM), (iii) integrated weed management (IWM) and (iv) integrated nutrient management (INM). However on a broader perspective, it is seen that all the four kinds are interrelated.

Soil infertility is the considered to be greatest obstacle for increasing crop yield in developing nations worldwide. For farmers to get benefited by the application of modernized tools in farming, soil fertility has to be restored (Khosro and Yousef 2012). Physical, chemical and biological properties of soil also influence the crop plant's ability to resist or tolerate insect pests. A fertile soil possesses high organic matter and beneficial organisms which fight infection and provide nutritional balance to the plant. Imbalanced nutrition in soil can reduce pest resistance (Altieri and Nicholls 2003) (Magdoff and van Es 2000). The soil microbes can thus be involved in integrated pest management programmes (Gadhave et al. 2016). The techniques used by farmers for pest management are also applicable for soil fertility management and vice versa (Altieri and Nicholls 2003).

Pests contribute to huge amount of crop loss (Oerke 2006). FAO regards IPM as a pillar of both sustainable intensification of crop production and pesticide risk reduction (http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/ipm/en/) since it promotes biological activity in soil, minimizing the use of pesticides by incorporating alternative methods to control pests (Hobbs et al. 2008).

Among pests, weeds are also considered as major biotic constrain to food production (Rigby and Cáceres 2001). Integrated weed management system follows cultural practices, viz. crop rotation, irrigation, sowing, intercropping, etc., to reduce weed emergence (Barberi 2002). Biofertilizer like *Azolla* forms a thick mat of thallus on standing water surface in the lowland rice farming system preventing light to penetrate the weed seeds resulting in weed suppression (Kathiresan 2002). Insect pests are also welcomed by many weed species and so indirectly IWM also exerts positive influence on IPM (Kathiresan 2007).

INM aims to subside the harmful impact of chemical fertilizers containing elements like N, P, K, etc. (Adesemoye and Kloepper 2009) by development of microbial inoculants consisting of nitrogen fixing, phosphorus dissolving and potassium mobilizing organisms (Sangeetha and Suseela Bhai 2016). Adesemoye et al. (2008) showed that plant N content was increased after inoculation with PGPR which might have resulted from increased fertilizer N utilization efficiency in an INM system. Co-inoculation of wheat plant with *Azospirillum* and P-solubilizing bacteria increased N and P uptake by the plant (El-Komy 2005).

A deep insight into understanding the interaction among microbe-fertilizer-plant can help in developing new strategies for integrated management. This will focus on improving the agricultural practices by lowering the adverse effects exerted on the

environment due to the use of conventional agriculture practices (Geisseler and Scow 2014). Microbial fertilizers are promising than the conventional chemical fertilizers since they do not possess threat to the ecosystem in the long run.

2.3.6 Commercialization

Key for extensive commercialization of bioinoculants demands coordination between the research and industrial sector and insightfulness of the farmers (Glick 2012). Steps involved in successful commercialization are as follows:

1. Expressive biological functional traits of bacteria should be well determined.
2. Indigenous varieties should be engineered for appropriate environmental conditions.
3. Better evaluated understanding on rhizosphere, phyllosphere, endophytic microbes interactions and their beneficial and harmful effects.
4. Inter- and intramicrobial communication studies on healthy biodiversity (plant-fungi, bacteria-fungi, bacteria-insects, bacteria-bacteria) for welfare of the plant.
5. Farmer friendly methods of application development.

2.4 Mechanism of Biofertilizer Action on Plant

Depending upon the mechanism of action, present-day microbial biofertilizers can be broadly divided into two categories, viz. nutrient uptake stimulators and biopesticides. The fate of the designed bioformulation and its performance under field condition largely depends upon the properties of microbe(s) by which it is made of. Various microbes can promote plant growth either directly or indirectly by diverse mechanisms. Depending upon the microbial functional trait, bioformulations are classified into three major groups: nitrogen fixers, phosphate solubilizers and plant growth-promoting microbes (PGPM).

Nitrogen-fixing microorganisms convert atmospheric dinitrogen into plant-usable form as ammonia by an ATP-driven process called biological nitrogen fixation (BNF) (Gothwal et al. 2008). Biological nitrogen fixers can be free-living, associative or symbiotic in nature (Mazid and Khan 2014). As specific nitrogen fixers can only colonize certain plant groups, so depending upon that, the specific bioformulation for a plant is recommended. For example, bioformulations containing symbiotic nitrogen fixer, *Rhizobium* is appropriate for leguminous plants. Similarly, *Azospirillum,* a free-living nitrogen fixer, is particularly applied to C4 plants, because it is dependent on the salt of organic acids like malic and aspartic acid for nitrogen fixation (Mazid and Khan 2014).

The key enzyme complex required for biological nitrogen fixation is nitrogenase encoded by the nif gene cluster (Goswami et al. 2016). Nitrogenase complex is made up of two components, viz. dinitrogenase reductase (iron protein) and dinitrogenase (molybdenum – iron protein). Dinitrogenase component is responsible for

fixing nitrogen by using the electrons provided by dinitrogenase reductase (Mahanty et al. 2016).

In case of legume-*Rhizobia* (*Rhizobium*/*Bradyrhizobium*/*Sinorhizobium*/*Azorhizobium*/*Mesorhizobium*) association, (iso)flavonoids present in plant root exudate act as stimuli for the activation of nodulation genes (nod, nol, noe) of compatible rhizobia and subsequent production of nodulation factor (lipochitin oligosaccharides) to initiate root curling followed by nodulation of leguminous plant (Ibáñez et al. 2015). Research studies reported that plant ethylene level increases upon *Rhizobium* infection in order to prevent subsequent rhizobial infection and promote nodulation (Abeles et al. 1992; Mahanty et al. 2016). It has been found that some rhizobial strains increase nodule number by producing a phytotoxin called rhizobitoxine which inhibits ACC synthase enzyme in legumes and thereby lowers plant ethylene level (Vijayan et al. 2013).

Another important group of microbial biofertilizers called phosphate solubilizing microbes can solubilize bound phosphorus from organic or inorganic complexes and make it available for plant uptake. Low molecular weight inorganic acids (such as gluconic and citric acids) produced by soil bacteria possess carboxyl and hydroxyl groups which can chelate the cations (calcium, aluminium, iron) bound to insoluble phosphatic compounds accompanying the release of plant-usable soluble phosphorus. *Rhizobium leguminosarum*, *Rhizobium meliloti* and *Bacillus firmus* have been reported to produce 2-ketogluconic acid for mineral phosphate solubilization (Abd-Alla 1994; Sridevi and Mallaiah 2009). Microbes can also mineralize complex structured organic phosphorus (tricalcium phosphate, rock phosphate, aluminium phosphate, etc.) by secreting a range of enzymes like non-specific phosphatases which catalyse the hydrolysis of phosphoric esters and convert organic phosphorus to inorganic form, phosphatases and C-P lyases that break C-P bonds in organophosphonates and phytases for phosphorus release from phytic acid (Goswami et al. 2016). It has been found that some microbes can perform both solubilization and mineralization activity (Pereira and Castro 2014) proving them extremely efficient biofertilizing agent.

Besides nitrogen fixation and phosphate solubilization, other prominent microbial traits involved in plant growth enhancement include phytohormone production, siderophore production, antibiotic production, HCN production and ACC deaminase production. Phytohormone-producing bacteria are ubiquitous in plant rhizosphere and serve as a potent candidate for biofertilizer formulation due to its ability of regulating plant growth by modulating endogenous hormonal level in plants. *Agrobacterium tumefaciens*, *Bacillus megaterium, Pseudomonas syringae*, *Pantoea agglomerans*, *Rhizobium*, *Bradyrhizobium*, *Erwinia herbicola,* etc. are reported to enhance plant growth by IAA production (Goswami et al. 2016).

Bacterial siderophore production is involved in improving plant iron nutrition. Iron predominantly exists in soil as Fe^{3+} which easily forms insoluble oxides and hydroxides inaccessible for assimilation in both plant and bacteria. Siderophore, a low molecular weight compound (usually <1 KDa), produced by bacteria and fungi under iron-limiting condition binds with Fe^{3+} ion and reduces it to Fe^{2+} molecule.

Release of Fe^{2+} molecules in rhizosphere by microbes benefits plants in terms of iron utilization (Mahanty et al. 2016).

Occurrence of ACC deaminase production trait in bacteria is directly linked to induced systemic tolerance (IST). Under stressful condition, plant ethylene level increases. 1-aminocyclopropane-1-carboxylic acid (ACC) is the precursor of ethylene. ACC deaminase produced by bacteria cleaves ACC into α-ketobutyrate and ammonia, thereby reducing plant ethylene level, so that plant can grow well under unfavourable condition. Scientific studies suggested that ACC deaminase-producing bacterial strains like *Achromobacter piechaudii* ARV8, *Pseudomonas fluorescens*YsS6, *Pseudomonas migulae* 8R6, etc. can reduce adverse effect of different stress conditions (drought, salinity, flooding, temperature, heavy metal toxicity, etc.) on plant growth and yield (Ali et al. 2012; Glick 2014; Mayak et al. 2004; Goswami et al. 2016). *Pseudomonas putida* Rs-198 confer salt tolerance in cotton by decreasing Na^+ absorption and increasing the rate of uptake of other divalent cations like K^+, Mg^{2+} and Ca^{2+}(Yao et al. 2010).

Another promising strategy of microbial plant growth promotion is the biocontrol. Biocontrol can be achieved by beneficial microbes by production of various anti-phytopathogenic metabolites, viz. HCN, 2,4 diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), pyoluteorin (Plt), pyrrolnitrin (Prn), oomycin A, viscosinamide, butyrolactones, kanosamine, zwittermicin A, aerugine, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antitumor antibiotics FR901463, cepafungins and antibiotic karalicin (Bhattacharyya and Jha 2012). It was reported that soil inoculation with *Pseudomonas fluorescens* and arbuscular mycorrhizal fungi can prevent root rot disease in *Phaseolus vulgaris* L (Neeraj and Singh 2011; Bhardwaj et al. 2014). Mycorrhiza produces bioactive compounds called Myc factors which are perceived by host roots for activation of symbiosis (SYM) pathway (Bhardwaj et al. 2014).

It was observed that some biofertilizers like *R. leguminosarum*, *Rhizobium sp*. IRBG 74 and Bradyrhizobium sp. IRBG 271 can increase net photosynthetic rate of plants (Mahanty et al. 2016). PGPR Strains like *Achromobacter xylosoxidans*, *Azotobacter chroococcum*, *Bacillus subtilis*, *Bradyrhizobium*, *Pseudomonas* sp., *Brevibacillus* sp., *Kluyvera ascorbata*, Mesorhizobium, etc. were reported to possess bioremediation potential (Shinwari et al. 2015; Mahanty et al. 2016). Biofertilizers with bioremediation potential may play pivotal role in restoring fertility of contaminated unfertile soil.

2.5 Commercially Available Bioformulations: Success and Drawback

In the present era marked by global warming and food scarcity, biofertilizers have arisen as a promising substitute to hazardous agrochemicals. Problems arising due to the use of various chemical fertilizers in modern agricultural practices are innumerable and increasing day by day. It has been reported that chemical fertilizers cause mineral imbalance in plant body resulting in the reduction of valuable

nutrients in food. For example, excess of potassium treatment in plant can decrease ascorbic acid and carotene in foods. Moreover, methemoglobinemia may arise due to consumption of vegetables grown in NO_3 rich soil (Mazid and Khan 2014). On the contrary, biofertilizers can perform all functions of agrochemicals like soil enrichment, plant growth stimulation, yield enhancement, etc. without causing any deleterious effect to the ecosystem.

Rhizobium, belonging to the family *Rhizobiaceae*, is a potent biofertilizer able to fix atmospheric nitrogen by forming symbiotic relation with legumes (lentil, pea, black gram, soybean, ground nut, etc.) and certain nonlegumes (*Parasponia*) (Saikia et al. 2007; Mazid and Khan 2014). Some crop-specific inoculants of *Rhizobium* include *Rhizobium japonicum* for soybean, *R. trifolii* for berseem, *R. lupini* for chickpea, *R. phaseoli* for green gram and *R. Meliloti* for lucerne. Though rhizobium is a very good substitute of nitrogen fertilizers, its application is limited by crop specificity and variable response under field condition. Another important nitrogen-fixing biofertilizer, *Azotobacter,* can fix nitrogen non-symbiotically. Problem associated with *Azotobacter* application is that it requires a large amount of organic C and Mo for stimulating nitrogenase enzyme activity during N fixation (Khan et al. 2011; Mazid et al. 2011). For optimizing biofertilizer activity, we should first know the constraints. Major constrains related to application of biofertilizer in agricultural system include (Table 2.2):

- Limited resource generation
- Problems in quality control
- Problems with inoculation techniques
- Compatibility with host genotype
- Standardization of proper dosage
- Occurrence of mutation in microbial strain throughout the bioformulation development
- Lack of assurance about the biofertilizer activity under various climatic conditions
- Impact of season change on biofertilizer activity
- Influence of native soil microflora
- Wrong inoculation techniques
- Unavailability of suitable carrier resource
- Lack of awareness among farmers
- Market level constraints
- Inadequate experienced staff

2.6 Conclusion and Future Perspective

Major constraint for biofertilizers is that their effect in field and lab conditions varies. Commercialization of biofertilizers is lacking a regulatory body. Policy making authorities should make guidelines in preparation of biofertilizer and its activity to be accepted globally. Farmer-friendly approaches with novel techniques of

Table 2.2 List of some commercially available microbial biofertilizers

Commercial bioformulation	Microbial ingredient(s)	Benefits	Reference
BiotaMax	*Bacillus subtilis, B. megaterium, B. licheniformis, B. pumilus, B. laterosporus, Paenibacillus polymyxa, Trichoderma harzianum, T. viride, T. polysporum, T. koningii*	Increases root mass – stronger, healthier root systems	http://www.biotamax.com
		Process nutrients more efficiently	
		Degrade organic material	
		Produces plant growth hormones	
		May result in a decreased need for traditional fertilizers	
		Reduced root oxidation	
JumpStart®	*Penicillium bilaiae*	Increased root development	http://www.novozymes.com/en/solutions/agriculture/bioag-in-australia
		Improved nitrogen fixation in legume crops	
		Improved stress tolerance	
		Improved seed quality	
		Earlier, more even maturity	
		Savings on costs, handling, transportation, storage and time requirements compared to more phosphate fertilizer	
		Lower environmental impact	
		Higher yield	
Custom B5™	*Bacillus subtilis, B. laterosporus, B. licheniformis, B. megaterium, B. pumilus*	Enhance soil productivity	http://www.biotamax.com
Ovalis Rhizofertil	*Pseudomonas putida* I-4163	Improve soil quality by mineral amendment and stimulate plant growth	https://www.agriculture-xprt.com/products/ovalis-rhizofertil-biofertilizers-518517

(continued)

Table 2.2 (continued)

Commercial bioformulation	Microbial ingredient(s)	Benefits	Reference
Biofox	*Fusarium oxysporum*	Effective against *Fusarium moniliforme*	www.biofox.com
AgBio	*Streptomyces griseoviridis* strain K61	Prevent *Fusarium* spp., *Alternaria brassicicola*, *Phomopsis* spp., *Botrytis* spp., *Pythium* spp. and *Phytophthora* spp. thatcause seed, root, stem rot and wilt disease of ornamental and vegetable crops	http://www.agbio-inc.com
EcoGuard	*Bacillus licheniformis* SB3086	Effective for prevention of fungal diseases like dollar spot and anthracnose	https://www.harrells.com/uploads/products/labels/ecogua.pdf
PONCHO/ VOTiVO™ mix	*Bacillus firmus* mixed with clothianidin	Provide plant protection against insects and nematode	http://fs1.agrian.com/pdfs/Poncho_VOTiVO_Labelnewa.pdf
Custom N_2	*Paenibacillus polymyxa*	Improve plant's nitrogen nutrition	http://www.biotamax.com
Bioshield™	*Serratia entomophila*	Effective against soil-dwelling grass grub larvae	Jackson (2017)

application methods need to be developed. Although biofertilizers are employed in agriculture practices, they couldn't make huge impact like chemical fertilizers due to lack of educated farmers and repugnance of biofertilizers due to their incompatibility with new soils. Government of individual countries over the globe should encourage organic farming by offering special incentives. Above all successful biofertilizer usage will come into existence where limitations are reduced to an extent that it can compete with the market of chemical industries.

References

Abd-Alla MH (1994) Solubilization of rock phosphates by Rhizobium and *Bradyrhizobium*. Folia Microbiol Off J Inst Microbiol Acad Sci Czech Repub 39(1):53–56

Abeles FB, Morgan PW, Saltveit ME (1992) Ethylene in plant biology. In: *Ethylene in Plant Biology*. Elsevier Science, San Diego, pp 264–296

Adesemoye AO, Kloepper JW (2009) Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85(1):1–12

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(10):876–886
Aguado-Santacruz G et al (2012) Impact of the microbial siderophores and phytosiderophores on the iron assimilation by plants: a synthesis. Rev Fitotec Mex 35(1):9–21
Ahemad M, Khan MS (2009) Toxicity assessment of herbicides quizalafop-p-ethyl and clodinafop towards rhizobium pea symbiosis. Bull Environ Contam Toxicol 82(6):761–766
Ahemad M, Khan MS (2011) Effects of insecticides on plant-growth-promoting activities of phosphate solubilizing rhizobacterium Klebsiella sp. strain PS19. Pesticide Biochem Physiol 100(1):51–56
Ahemad M, Khan MS (2012a) 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, Khan MS (2012b) Effects of pesticides on plant growth promoting traits of Mesorhizobium strain MRC4. J Saudi Soc Agril Sci 11(1):63–71
Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud University – Sci 26(1):1–20
Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163(2):173–181
Ahmed E, Holmström SJM (2014) Siderophores in environmental research: roles and applications. Microbial Biotechnol 7(3):196–208
Albrecht-Gary AM & Crumbliss AL (1998) Coordination chemistry of siderophores: thermodynamics and kinetics of iron chelation and release. In *Metal Ions In :*Biological Systems. pp. 239–327
Ali S, Charles TC, Glick BR (2012) Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. J Appl Microbiol 113(5):1139–1144
Altieri MA (1999) The ecological role of biodiversity in agroecosystems. Agric Ecosyst Environ 74(1–3):19–31
Altieri MA, Nicholls CI (2003) Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems. Soil Tillage Res 72(2):203–211
Amundson R et al (2015) Soil and human security in the 21st century. Science 348(6235):1261071–1–1261071–6
Anand R, Grayston S, Chanway C (2013) N2-Fixation and seedling growth promotion of Lodgepole pine by endophytic Paenibacillus polymyxa. Microb Ecol 66(2):369–374
Arma MJ (2016) Effect of biological fertilizer on the growth and nodules formation to soya bean (Glicine max (l .) merrill) in ultisol under net house conditions. J Exp Biol Agric Sci 4(6):617–624
Audrain B et al (2015) Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev 39(2):222–233
Bais HP, Fall R, Vivanco JM (2004) Biocontrol of Bacillus Subtilis against infection of Arabidopsis roots by Pseudomonas syringae is facilitated by biofilm formation and Surfactin production. Plant Physiol 134:307–319
Barberi P (2002) Weed management in organic agriculture: are we addressing the right issues? Weed Res 42(3):177–193
Barton LL, Abadia J (2006) *Iron nutrition in plants and Rhizospheric microorganisms*. Springer, Dordrecht
Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16(4):729–770
Bashan Y, de-Bashan LE (2005) Bacteria/plant growth-promotion. Enc Soils Environ 1:103–115
Bashan Y et al (1995) Survival of Azospirillum brasilense in the bulk soil and rhizosphere of 23 soil types. Appl Environ Microbiol 61(5):1938–1945
Bashan Y et al (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378(1–2):1–33
Basti F et al. (1998). Production of indole-3-acetic acid and gibberellins A 1 and A 3 by Acetobacter diazotrophicus and Herbaspirillum seropedicae in chemically-defined culture media, pp.7–11.

Belay A, Claassens AS, Wehner FC (2002) Effect of direct nitrogen and potassium and residual phosphorus fertilizers on soil chemical properties, microbial components and maize yield under long-term crop rotation. Biol Fertil Soils 35(6):420–427

Belimov AA et al (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.) Soil Biol Biochem 37(2):241–250

Bellenger JP et al (2008) Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. Nat Geosci 1(4):243–246

Ben Rebah F, Tyagi RD, Prévost D (2002) Wastewater sludge as a substrate for growth and carrier for rhizobia: the effect of storage conditions on survival of Sinorhizobium meliloti. Bioresour Technol 83(2):145–151

Bhardwaj D et al (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13(1):1–10

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(4):1327–1350

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

Braud A, Hoegy F et al (2009a) New insights into the metal specificity of the Pseudomonas aeruginosa pyoverdine-iron uptake pathway. Environ Microbiol 11(5):1079–1091

Braud A, Jézéquel K et al (2009b) Enhanced phytoextraction of an agricultural Cr- and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere 74(2):280–286

Brockwell J, Bottomley PJ (1995) Recent advances in inoculant technology and prospects for the future. Soil Biol Biochem 27(4–5):683–697

Brownbridge M et al (2012) Persistence of Beauveria bassiana (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. Biol Control 61(3):194–200

Busby JN et al (2012) Structural analysis of Chi1 chitinase from Yen-Tc: the multisubunit insecticidal ABC toxin complex of Yersinia entomophaga. J Mol Biol 415(2):359–371

Canbolat MY et al (2006) Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fertil Soils 42(4):350–357

Castro RO, Cantero EV, Bucio JL (2008) Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signal Behav 3(4):263–265

Chen YP et al (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Choi S et al (2014) Genome Sequence of Bacillus amyloliquefaciens GB03, an Active Ingredient of the First Commercial Biological Control Product. Genome Announc 2(5):2–3

Chung JH, Song GC, Ryu CM (2016) Sweet scents from good bacteria: case studies on bacterial volatile compounds for plant growth and immunity. Plant Mol Biol 90(6):677–687

Compant S 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(9):4951–4959

Conrath U et al (2006) Priming: getting ready for battle. Mol Plant-Microbe Interact 19(10):1062–1071

Cronin D et al (1997) Role of 2, 4-Diacetylphloroglucinol in the Interactions of the Biocontrol Pseudomonad Strain F113 with the Potato Cyst Nematode Globodera rostochiensis. Applied Environ Microbiol 63(4):1357–1361

D'Alessandro M et al (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant Cell Environ 37(4):813–826

Date RA (2001) Advances in inoculant technology: a brief review. Aust J Exp Agric 41(3):321–325

De Bruijn I et al (2007) Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in Pseudomonas species. Mol Microbiol 63(2):417–428

Dey R, Pal KK, Tilak KVBR (2012) Influence of soil and plant types on diversity of rhizobacteria. Proc Natl Acad Sci India Sect B - Biol Sci 82(3):341–352

Dong Y et al (2003) Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of Medicago sativa and Medicago truncatula. Appl Environ Microbiol 69(3):1783–1790
Dos Santos PC et al (2012) Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics 13(1):162
Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209
El-Komy HMA (2005) Coimmobilization of Azospirillum lipoferum and Bacillus megaterium for successful phosphorus and nitrogen nutrition of wheat plants. Food Technol Biotechnol 43(1):19–27
Erisman JW et al (2007) Reduced nitrogen in ecology and the environment. Environ Pollut 150(1):140–149
Faisal M, Hasnain S (2005) Bacterial Cr(VI) reduction concurrently improves sunflower (Helianthus annuus L.) growth. Biotechnol Lett 27(13):943–947
Farrar K, Bryant D, Cope-Selby N (2014) Understanding and engineering beneficial plant-microbe interactions: plant growth promotion in energy crops. Plant Biotechnol J 12(9):1193–1206
ffrench-Constant RH, Dowling A, Waterfield NR (2007) Insecticidal toxins from Photorhabdus bacteria and their potential use in agriculture. Toxicon 49(4):436–451
Fox JE et al (2007) Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants. Proc Natl Acad Sci U S A 104(24):10282–10287
Fox AR et al (2016) Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen-fixing bacterium Pseudomonas protegens Pf-5 X940. Environ Microbiol 18(10):3522–3534
Fustec J et al (2009) Nitrogen rhizodeposition of legumes. In: Sustainable Agriculture, pp 869–881
Gadhave KR, Hourston JE, Gange AC (2016) Developing soil microbial inoculants for Pest management: can one have too much of a good thing? J Chem Ecol 42(4):348–356
Galloway JN et al (2008) Transformation of the nitrogen cycle. Science 320:889–892
Ganesan V (2008) Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. Curr Microbiol 56(4):403–407
Geisseler D, Scow KM (2014) Long-term effects of mineral fertilizers on soil microorganisms – a review. Soil Biol Biochem 75:54–63
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.
Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39
Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15(2):353–378
Glick BR et al. (2007). Promotion of plant growth by ACC deaminase-producing soil bacteria. In: New Perspectives and Approaches in Plant Growth-Promoting Rhizobacteria Research. pp 329–339
Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of Plant Growth Promoting Rhizobacteria (PGPR). Cogent Food Agric 2(1):1–19
Gothwal RK et al (2008) Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. Appl Ecol Environ Res 6(2):101–109
Gray WM (2004) Hormonal regulation of plant growth and development. PLoS Biol 2(9)
Guo JK et al (2015) Burkholderia metalliresistens sp. nov., a multiple metal-resistant and phosphate-solubilising species isolated from heavy metal-polluted soil in Southeast China. Anton Leeuw Int J Gen Mol Microbiol 107(6):1591–1598
Gupta A et al (2005) In situ characterization of mercury-resistant growth-promoting fluorescent pseudomonads. Microbiol Res 160(4):385–388
Gupta G et al (2015) Microbial & Biochemical Technology Plant Growth Promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7(2):96–102

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3(4):307–319
Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing pseudomonas spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41:117–153
Hartmann A et al (2009) Plant-driven selection of microbes. Plant Soil 321(1–2):235–257
Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97(20):8859–8873
Hobbs PR, Sayre K, Gupta R (2008) The role of conservation agriculture in sustainable agriculture. Philos Trans R Soc Lond Ser B Biol Sci 363(1491):543–555
Høgh-Jensen H, Schjoerring JK (2000) Below-ground nitrogen transfer between different grassland species: direct quantification by 15N leaf feeding compared with indirect dilution of soil 15N. Plant Soil 227(1–2):171–183
Ibáñez F et al (2015) Sequence and expression analysis of putative Arachis hypogaea (peanut) Nod factor perception proteins. J Plant Res 128(4):709–718
Jackson, T.A., Boucias, D.G. & Thaler, J.O., 2001. Pathobiology of amber disease, caused by Serratia Spp., in the New Zealand grass grub, Costelytra zealandica. Journal of invertebrate pathology, 78(4), pp.232–243.
Jayasinghearachchi HS, Seneviratne G (2004) A bradyrhizobial-Penicillium spp. biofilm with nitrogenase activity improves N_2 fixing symbiosis of soybean. Biol Fertil Soils 40(6):432–434
Jha CK, Patel B, Saraf M (2012) Stimulation of the growth of Jatropha curcas by the plant growth promoting bacterium Enterobacter cancerogenus MSA2. World J Microbiol Biotechnol 28(3):891–899
Jia YJ et al (2000) 1-aminocyclopropane-1-carboxylate (ACC) deaminase induced by ACC synthesized and accumulated in Penicillium citrinum intracellular spaces. Biosci Biotechnol Biochem 64(2):299–305
John RP et al (2011) Bio-encapsulation of microbial cells for targeted agricultural delivery. Crit Rev Biotechnol 31:211–226
Jones K, Burges HD (1998) Technology of formulation and application. In: Burges HD (ed) Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. Kluwer Academic Publishers, Dordrecht, pp 7–27
Kathiresan R (2002) Weed management in rice–black gram cropping system. Indian J Weed Sci 34:220–226
Kathiresan R (2007) Integration of elements of a farming system for sustainable weed and pest management in the tropics. Crop Prot 26(3):424–429
Khabbaz SE et al (2015) Characterisation of antagonistic Bacillus and Pseudomonas strains for biocontrol potential and suppression of damping-off and root rot diseases. Ann Appl Biol 166(3):456–471
Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96(3):473–480
Khan MS et al (2010) Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch Agron Soil Sci 56(1):73–98
Khan TA, Mazid M, Mohammad F (2011) A review of ascorbic acid potentialities against oxidative stress induced in plants. J Agrobiol J Agrobiol 28(282):97–111
Khosro M, Yousef S (2012) Bacterial biofertilizers for sustainable crop Production : a review. J Agric Biol Sci 7(5):307–316
Li X et al (2007) Growth promoting effect of a transgenic Bacillus mucilaginosus on tobacco planting. Appl Microbiol Biotechnol 74(5):1120–1125
Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Appl Environ Microbiol 69(4):1875–1883
Loper JE, Gross H (2007) Genomic analysis of antifungal metabolite production by Pseudomonas fluorescens Pf-5. Eur J Plant Pathol 119(3):265–278
Lugtenberg B, Malfanova N, Kamilova F, Berg G (2013) Plant growth promotion by microbes: Endophytic bacteria with plant growth promoting and biocontrol abilities. Blackwell Publishing, Hoboken, pp 575–586

Magdoff F, van Es H (2000) Building soils for better crops, 2nd edn. Sustainable Agriculture Network, Beltsville
Mahanty T et al (2016) Biofertilizers: a potential approach for sustainable agriculture development. Environ Sci Pollut Res:1–21
Malusá E, Sas-Paszt L, Ciesielska J (2012) Technologies for Beneficial Microorganisms Inocula Used as biofertilizers. Sci World J:1–12
Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55(394):27–34
Martínez-Viveros O et al (2010) Mechanisms and practical considerations involved in plant growth promotion by Rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319
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
Mazid M, Khan TA (2014) Future of bio-fertilizers in Indian agriculture: an overview. Int J Agric Food Res 3(3):10–23
Mazid M et al (2011) Significance of Sulphur nutrition against metal induced oxidative stress in plants significance of Sulphur nutrition against metal induced oxidative stress in plants. J Stress Physiol Biochem 7(3):165–184
McDaniel MD, Tiemann LK, Grandy AS (2014) Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecol Appl 24(3):560–570
Meldau DG et al (2013) Dimethyl disulfide produced by the naturally associated bacterium bacillus sp B55 promotes Nicotiana attenuata growth by enhancing sulfur nutrition. Plant Cell 25(7):2731–2747
Meng H et al (2013) Soil pH dynamics and nitrogen transformations under long-term chemical fertilization in four typical chinese croplands. J Integr Agric 12(11):2092–2102
Mirza MS et al (2006) Molecular characterization and PCR detection of a nitrogen-fixing Pseudomonas strain promoting rice growth. Biol Fertil Soils 43(2):163–170
Moënne-Loccoz Y et al (1999) Effect of inoculum preparation and formulation on survival and biocontrol efficacy of Pseudomonas fluorescens F113. J Appl Microbiol 86(1):108–116
Nakkeeran S, Fernando WGD, Siddiqui ZA (2006) Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. In: Siddiqui ZA (ed) PGPR: Biocontrol and biofertilization. Springer, Dordrecht, pp 257–296
Nandi M et al (2015) Pyrrolnitrin and hydrogen cyanide production by Pseudomonas chlororaphis strain PA23 exhibits Nematicidal and repellent activity against Caenorhabditis elegans. PLoS One 10:1–19
Neeraj S, Singh K (2011) Organic amendments to soil inoculated arbuscular mycorrhizal fungi and Pseudomonas fluorescens treatments reduce the development of root-rot disease and enhance the yield of Phaseolus vulgaris L. Eur J Soil Biol 47(5):288–295
Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270(45):26723–26726
Nielsen MN, Sörensen J (1999) Chitinolytic activity of Pseudomonas fluorescens isolates from barley and sugar beet rhizosphere. FEMS Microbiol Ecol 30(3):217–227
O'Callaghan M (2016) Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl Microbiol Biotechnol 100(13):5729–5746
Oerke E-C (2006) Crop losses to pests. J Agric Sci 144(1):31
Olivares J, Bedmar EJ, Sanjuán J (2013) Biological nitrogen fixation in the context of global change. Mol Plant-Microbe Interact 26(5):486–494
Ortíz-Castro R et al (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4(8):701–712
Ortiz-Hernández ML et al (2013) Mechanisms and strategies for pesticide biodegradation: opportunity for waste, soils and water cleaning. Rev Int de Contaminacion Ambiental 29:85–104
Padda KP, Puri A, Chanway CP (2016) Plant growth promotion and nitrogen fixation in canola (*Brassica napus*) by an endophytic strain of *Paenibacillus polymyxa* and its GFP-tagged derivative in a long-term study. Botany 94(12):1209–1217

Patel K, Goswami D, Dhandhukia P & Thakker J (2015) Bacterial metabolites in sustainable agroecosystem
Péchy-Tarr M et al (2013) Control and host-dependent activation of insect toxin expression in a root-associated biocontrol pseudomonad. Environ Microbiol 15(3):736–750
Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant 118(1):10–15
Pereira SIA, Castro PML (2014) Phosphate-solubilizing rhizobacteria enhance Zea mays growth in agricultural P-deficient soils. Ecol Eng 73:526–535
Philippot L et al (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799
Pieterse CMJ et al (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5(5):308–316
Pirhofer-Walzl K et al (2012) Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. Plant Soil 350(1–2):71–84
Qurashi AW, Sabri AN (2011) Osmoadaptation and plant growth promotion by salt tolerant bacteria under salt stress. Afr J Microbiol Res 5(21):3546–3554
Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Anton Leeuw Int J Gen Mol Microbiol 81(1–4):537–547
Raaijmakers JM et al (2010) Natural functions of lipopeptides from Bacillus and Pseudomonas: more than surfactants and antibiotics. FEMS Microbiol Rev 34(6):1037–1062
Rice W et al (2000) Rhizobial inoculant formulations and soil pH influence field pea nodulation and nitrogen fixation. Can J Soil Sci 80(3):395–400
Rigby D, Cáceres D (2001) Organic farming and the sustainability of agricultural systems. Agric Syst 68(1):21–40
Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785
Rodrigues E et al (2008) Azospirillum amazonense inoculation: effects on growth, yield and N-2 fixation of rice (Oryza sativa L.) Plant Soil 302(1–2):249–261
Roh JY et al (2007) Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. J Microbiol Biotechnol 17:547–559
Weber RWS (2005) Mycorrhizas: anatomy and cell biology. Mycologist 19(3):133
Rouissi T et al (2010) Centrifugal recovery of rhizobial cells from fermented starch industry wastewater & development of stable formulation. Ind Biotechnol 6(1):41–49. Available at: http://www.liebertonline.com/doi/abs/10.1089/ind.2010.6.041.
Rout ME (2014) The plant microbiome. Adv Bot Res 69:279–309
Ruffner B et al (2013) Oral insecticidal activity of plant-associated pseudomonads. Environ Microbiol 15(3):751–763
Ryu C-M et al (2003) Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci U S A 100(8):4927–4932
Ryu C et al (2005) Study of mechanisms for plant growth promotion elicited by rhizobacteria in Arabidopsis Thaliana. Plant and Soil 268(1):285–292
Saikia SP et al (2007) Biological nitrogen fixation with non-legumes: an achievable target or a dogma? Curr Sci 92:317–322
Salvadori JDM et al (2012) Characterization of entomopathogenic nematodes and symbiotic bacteria active against Spodoptera frugiperda (Lepidoptera: Noctuidae) and contribution of bacterial urease to the insecticidal effect. Biol Control 63(3):253–263. Available at: https://doi.org/10.1016/j.biocontrol.2012.08.002.
Sangeetha KP, Suseela Bhai R (2016) Integrated plant nutrient system – with special emphasis on mineral nutrition and biofertilizers for black pepper and cardamom – a review. Crit Rev Microbiol 42(3):439–453
Santoyo G, Orozco-Mosqueda MDC, Govindappa M (2012) Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of Bacillus and Pseudomonas : a review. Biocontrol Sci Tech 22(8):855–872

Sayyed AH, Cerda H, Wright DJ (2003) Could Bt transgenic crops have nutritionally favourable effects on resistant insects? Ecol Lett 6(3):167–169
Schulz TJ et al (2008) Soybean seed inoculant and fungicidal seed treatment effects on soybean. Crop Sci 48:1975–1983
Seneviratne G et al (2008) Fungal-bacterial biofilms: their development for novel biotechnological applications. World J Microbiol Biotechnol 24(6):739–743
Shah MK, Naveed M, Akhter MJ (2010) Substrate-dependent Auxin production by Rhizobium phaseoli improves the growth and yield of Vigna radiata L. under salt stress conditions. J Microbiol Biotechnol 20:1288–1294
Shaheen S, Sundari SK (2013) Exploring the applicability of PGPR to remediate residual organophosphate and carbamate pesticides used in agriculture fields. Int J Agric Food Sci Technol 4(10):947–954
Sharma SB et al (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2(1):587
Shinwari ZK et al (2015) Biological activities of commonly used medicinal plants from ghazi brotha, attock district. Pak J Bot 47(1):113–120
Siddiqui ZA (2006) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui ZA (ed) PGPR: Biocontrol and biofertilization. Springer, Dordrecht, pp 111–142
Singh RP, Jha PN (2016) The multifarious PGPR Serratia marcescens CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (Triticum aestivum L.) PLoS One 11(6):e0155026
Sridevi M, Mallaiah KV (2009) Phosphate solubilization by Rhizobium strains. Ind J Microbiol 49(1):98–102
Svercel M, Duffy B, Défago G (2007) PCR amplification of hydrogen cyanide biosynthetic locus hcnAB in pseudomonas spp. J Microbiol Methods 70(1):209–213
Swaminathan J et al (2016) Formulations for delivering Trichoderma atroviridae spores as seed coatings, effects of temperature and relative humidity on storage stability. J Appl Microbiol 120(2):425–431
Thilakarathna MS et al (2016) Nitrogen fixation and transfer of red clover genotypes under legume grass forage based production systems. Nutr Cycl Agroecosyst 106(2):233–247
Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from Pseudomonas Fluorescens in biological control of Gaeumannomyces graminis var. tritici. J Bacteriol 170(8):3499–3508
Ullman WJ et al (1996) CHEMICAL laboratory evidence for microbially mediated silicate mineral dissolution in nature. Chem Geol 132:11–17
Unno Y et al (2005) Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of Lupin analysed by phytate utilization ability. Environ Microbiol 7:396–404
Valentine AJ, Benedito VA, Kang Y (2010) Legume nitrogen fixation and soil abiotic stress: from physiology to genomics and beyond. In: Foyer C, Zhao M (eds) Nitrogen metabolism in plants in the post-genomic era. Wiley-Blackwell, Oxford, pp 207–248
Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119(3):243–254
van Loon LC, Bakker PA, Pieterse CM (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483
van Wees SC et al (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(15):8711–8716
Vandergheynst J et al (2007) Water-in-oil emulsions that improve the storage and delivery of the biolarvacide Lagenidium giganteum. BioControl 52(2):207–229
Vassilev N et al (2007) Simultaneous phytase production and rock phosphate solubilization by Aspergillus niger grown on dry olive wastes. Ind Crop Prod 26(3):332–336
Velusamy P et al (2006) Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol. Can J Microbiol 52(1):56–65

Verma JP et al (2014) Evaluation of plant growth promoting activities of microbial strains and their effect on growth and yield of chickpea (Cicer arietinum L.) in India. Soil Biol Biochem 70:33–37

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

Vijayan R, Palaniappan P, Tongmin SA, Elavarasi PMN 2013. Rhizobitoxine enhances nodulation by inhibiting ethylene synthesis of Bradyrhizobium elkanii from Lespedeza species: validation by homology modeling and molecular docking study. World J Pharm Pharm Sci, 2, pp.4079–4094

Viterbo A et al (2010) Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. FEMS Microbiol Lett 305(1):42–48

Wakelin SA et al (2004) Phosphate solubilization by Penicillium spp. closely associated with wheat roots. Biol Fertil Soils 40(1):36–43

Wall DH, Nielsen UN, Six J (2015) Soil biodiversity and human health. Nature 28:69–76

Wani PA, Khan MS, Zaidi A (2007) Effect of metal tolerant plant growth promoting Bradyrhizobium sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70(1):36–45

Waqas M et al (2012) Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules 17(9):10754–10773

Wongfun N et al (2013) Weathering of granite from the Damma Glacier area: the contribution of cyanogenic bacteria. Geomicrobiol J 31:93–100

Xiao-Yan S et al (2006) Broad-spectrum antimicrobial activity and high stability of Trichokonins from Trichoderma koningii SMF2 against plant pathogens. FEMS Microbiol Lett 260(1):119–125

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4

Yao L et al (2010) Growth promotion and protection against salt stress by Pseudomonas putida Rs-198 on cotton. Eur J Soil Biol 46(1):49–54

Yi H-S, Yang JW, Ryu C-M (2013) ISR meets SAR outside: additive action of the endophyte Bacillus pumilus INR7 and the chemical inducer, benzothiadiazole, on induced resistance against bacterial spot in field-grown pepper. Front Plant Sci 4:122

Zhang H et al (2008) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mol Plant-Microbe Interact 21(6):737–744

Potentials of Microbial Inoculants in Soil Productivity: An Outlook on African Legumes

3

Bukola Rhoda Aremu, Elizabeth Temitope Alori, Raphael Funso Kutu, and Olubukola Oluranti Babalola

Abstract

Nutrient availability is one of the major limiting factors affecting legume production in Africa. With the limited arable land resources, meeting the dietary requirement of the ever-increasing world population becomes a serious challenge. The most frequently deficient nutrient on crop fields is nitrogen (N). Inconvenient increase in prices of chemical nitrogen fertilizers together with the environmental problems associated with their excessive use calls for alternative low-cost and ecologically friendly soil-plant fertilization technologies. Soil microorganisms play significant roles in nutrient mineralization and supply to plant hence promoting plant growth. Soil microbes suppress soilborne plant diseases and destroy environmentally hazardous compounds in soil. Microbial inoculants are agricultural amendments that use microorganisms such as rhizobia and endophytes to promote legume growth. These microbes form symbiotic relationships with the target leguminous plant, and both parts benefit. The structure and function of the plant microbiome are major determinants of plant health and productivity. Microbial inoculants are the potential tools for sustainable agriculture.

Keywords

Microbiome • Nitrogen fixation • Soil fertility • Soil health • Soil quality

B.R. Aremu • R.F. Kutu • O.O. Babalola (✉)
Food Security and Safety Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa
e-mail: olubukola.babalola@nwu.ac.za

E.T. Alori
Department of Crop and Soil Sciences, Landmark University, P.M.B, 1001 Omu-Aran, Nigeria

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*, Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_3

3.1 Introduction

The over-reliance on conventional agricultural systems, which depend highly on non-sustainable energy inputs and intensive use of herbicides, fertilizers and pesticides, does not hold the answer to obtaining higher yields from food crops grown in Africa. In order to meet the ever-rising demand for sustainable land use, increased feed and biofuel production as alternative to nonrenewable fossil fuels, there has been an expanding demand to improve the soil quality through the production and utilization of nitrogen-rich composts. The residual effect of these non-sustainable practices of may have an adverse effect on the community as a whole. Legumes are fit and often used for the building up of a friendly and advantageous association with soil microorganisms known as rhizobia that create pull knobs for lessening of climatic dinitrogen to effectively assimilable structures for use by the host plants. An extraordinary level of organism group's specificity exists in rhizobia-legume associations, emerging from a signal interchange in the two partners. The root nodule initiation, on the other hand, requires a set of vastly coordinated events at the root epidermal and cortical cells; there has been an expanding reliance on concentrated agribusiness. These unsustainable practices may prompt to the decay of soil quality and require the generation of nitrogen manures to the detriment of nonrenewable fossil powers. Besides the growing cost of improvement, the excessive usage of manures is in addition responsible for the damage to various organic frameworks (Hawkesford 2011). Hence, the use of microbial inoculants has proved to be a promising technology to obtain an increase food production and a sustainable agricultural system. Soil microorganisms are capable of enhancing plant growth and protect soils from disease and abiotic stresses (Glick 2012). Microorganisms establish associations with plants and promote plant growth by means of several beneficial characteristics such as nutrient availability from genetic processes of biological N fixation (BNF) and phosphate solubilization and stress alleviation through 1-aminocyclopropane-1-carboxylate deaminase expression modulation and the production of phytohormones and siderophores, among several others (Alori 2016).

The introduction of beneficial microbes to soil and plant (inoculation) is less aggressive and causes less damage to the environment compared to chemical fertilization. Microbial inoculation technology is therefore a sustainable agronomic practice that reduces production costs. There are increasing applications of symbiotic or free-living N-fixing bacteria in sustainable agricultural systems (Koki and Takayoshi 2013). The application of inoculants is seen as being very attractive since it would substantially reduce the use of chemical fertilizers and pesticides, and there are now an increasing number of inoculants being commercialized for various crops (Babalola 2010; Babalola and Glick 2012a, b; Berg 2009). Microbial inoculants comprise three major groups of soil microbes which are symbiotic nitrogen-fixing rhizobia, plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF).

Rhizobia species are well investigated because of their symbiotic relationship with leguminous plants and their agronomical application as inoculants in the cultivation of economic crops (Ajilogba and Babalola 2013; Ajilogba et al. 2013; Torres et al. 2012). The soybean-*Bradyrhizobium* association is a good example of the

efficiency of BNF, while *B. elkanii* and *B. japonicum* are species that are commonly used to inoculate this leguminous plant. In this system, the BNF is so efficient that attempts to increase grain yields by adding nitrogenous fertilizers are not successful in plants that had been effectively inoculated with the recommended *Bradyrhizobium* strains (Souza et al. 2015).

Legumes have served man as source of food, feed, fuel wood and fertility from the very early times, hence, described as 'soil improvers' (Ajilogba and Babalola 2013; GRDC 2013). The unique ability of legumes to fix atmospheric N through symbiotic association with root-nodule bacteria had been used to improve the yields of legumes in sub-Saharan Africa (Abaidoo et al. 2013). Moreover, most of the soils used for legume production in Africa are poor in nutrient status, especially total N, organic carbon and available phosphorus and, therefore, relatively unproductive (Laditi et al. 2012; Machido et al. 2011). Leaching, denitrification, volatilization, nutrient mining and depletion by crop and crop residue removal for alternative uses have all contributed to the further worsening of the low fertility situation (Yakubu et al. 2010). Hence, the replenishment of depleted soil nutrients, especially N, depends largely on the addition of inorganic fertilizers, which rank first among the external inputs that are required to maximize agriculture outputs, but in turn contributes substantially to environmental pollution (Alori et al. 2012). On the other hand, most farmers cannot afford inorganic fertilizers due to their high cost and non-availability on time in the region (Yakubu et al. 2010) resulting in low to suboptimal use (Kutu and Asiwe 2010; Kutu and Diko 2011) that neither mitigate the nutrient mining problem nor guarantee soil fertility restoration for optimum crop growth and productivity. Consequently, this has led to a renewed farmers' interest on BNF, which provides a continuous in situ supply of N for plant growth, adds organic matter to the soil and is economically viable (Yakubu et al. 2010). Most importantly, inoculation of legume crop is recommended when the field has not been cropped with the host plant for the past 3–5 years or when it has never been planted to the host (Yakubu et al. 2010). Moreover, inoculation of legume can increase rhizobia populations in fields where environmental conditions for the bacteria's long-term survival are not favourable. For instance, the rhizobia population of a field with pH below 6.0, periodically flooded conditions or extremely sandy soils can be greatly improved by microbial inoculation for maximum legume production (Machido et al. 2011).

The success of a legume grain crop is dependent on its capacity to form effective nitrogen-fixing symbioses with root-nodule bacteria. Many soils, however, do not have adequate amount of native rhizobia in terms of number, quality or effectiveness to enhance BNF. These situations call for the provision of external source of rhizobia through inoculation that to enable effective legume nodulation and N_2-fixation. Three of such situations were identified that legumes generally need inoculation: (1) where compatible rhizobia are absent, (2) where the population of compatible rhizobia is small and (3) where the indigenous rhizobia are ineffective or less effective in N_2-fixation with the intended legume than selected inoculant strains (Vanlauwe and Giller 2006).

Ronner et al. (2016) discussed the history of rhizobia inoculants used for grain legume improvement in Nigeria. However, information on the exploration of the

potential of microbial inoculants in the production of African legume is limited. This review therefore detailed the legume microbiome in soil. It provides an overview of the interaction of endophytic microbes with legumes, legume microbial inoculants for organic farming, legume microbial inoculants for soil fertility and legume microbial inoculants for soil health improvement. Legumes commonly planted in African were well expatiated.

3.2 Legumes Microbiome in Soil

The rhizosphere is the area of soil encompassing the root which is influenced by it. The importance of the rhizosphere emerges from the discharge of natural material from the root and the consequent impact of expanded microbial action on nutrient cycling and plant development. In the rhizosphere, the amounts and the classes of substrates are not quite the same as those in the mass soil, and this prompts to colonization by various populaces of microbes including bacteria, fungi, protozoa and nematodes. Other physicochemical elements which can be distinctive in this area are acidity, humidity and nutrient status, electrical conductivity and redox potential. The relationship among organisms and roots can be helpful (water take-up, soil stability, growth advancement, N_2-fixation, biocontrol, antibiosis, beneficial interaction), detrimental (disease, phytotoxicity) or unbiased (nutrient flux, free catalyst discharge, connection, allelopathy, rivalry)—these impacts frequently rely on soil conditions and in this manner should be considered as factors (Chaparro et al. 2012). Relationships that are helpful to farming integrate mycorrhizae, legume nodulation and formation of antimicrobial complexes that restrain the development of pathogens. Clearly, balancing the effect of the beneficial elements of the rhizosphere will assist in manipulation of the rhizosphere.

3.3 Rhizobia

Rhizobia are free-living facultative saprophytic organism dwelling in the root knobs of the most legumes. They exist in the rhizoplane, rhizosphere as well as in the soil apart from the rhizosphere in small quantity. Rhizobia are more prevalent in the rhizosphere of the legumes as a result of the plant root exudate. Diversity of the host legumes is significantly found in connection with various gene pools of indigenous rhizobia. The formation of nodules by rhizobia in relation to legumes is highly specific. These rhizobia are described by their capacity to deliver hypertrophies (swellings) or knobs on the stems or roots of most however not all legumes (Mus et al. 2016). Not all the legumes form nodules and those that form only do so with specific rhizobia. On the other hand, some of the rhizobia are promiscuous, having the capability of nodulating more than one legumes (Ampomah et al. 2008). These rhizobia are unique among the soil microbes due to the N-fixing capability whenever in mutualistic relationship with compatible legumes. The physiological versatility of these rhizobia enables their adaptation to the complex and competitive soil

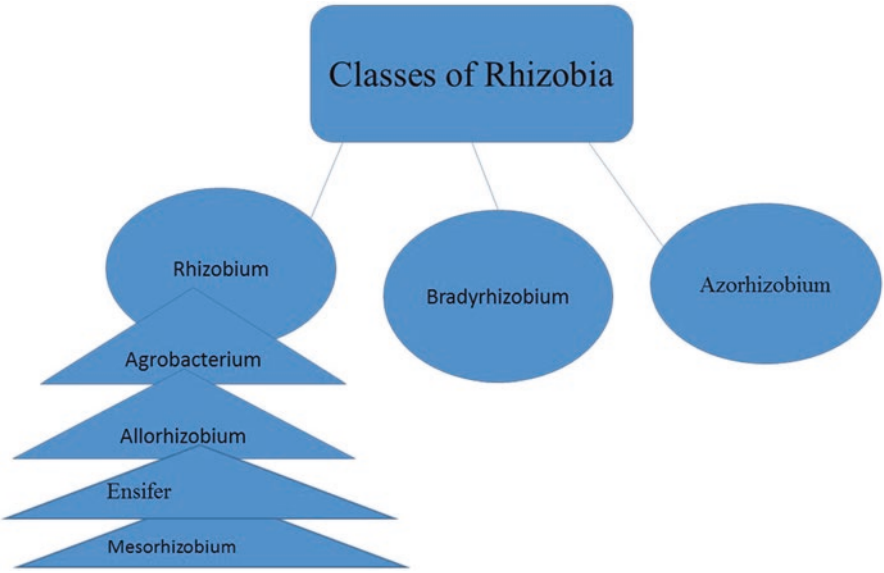


Fig. 3.1 Classes of rhizobia

environment. Rhizobia convert atmospheric dinitrogen (N_2) into absorbable ammonia for the improvement of plant growth and productivity. The process is eco-friendly and without any exogenous release to the soil.

The rhizobia nodulation ability and N-fixing ability with a wide range of legumes also enhance their persistence in the soil. The taxonomy of these rhizobia is in the state of flux (Shamseldin et al. 2017). Presently, there are three groups as illustrated in Fig. 3.1. The difficulty in the classification of rhizobia is due to the nodulation genes borne on the plasmid or found on the chromosomal symbiotic islands that move between the soil microorganisms, which weaken the infection based on taxonomic analysis (Shamseldin et al. 2017).

There are two major genera of rhizobia, which include the *Rhizobium* species (spp.) that are associated with legumes and the *Bradyrhizobium* species which are in the other hand associated with soybean and cowpea. When grown on a special growth medium called yeast-mannitol agar (YMA), *Rhizobium* spp. produce an acid growth reaction, while the *Bradyrhizobium* spp. produce alkaline reaction. When rhizobia live on organic material in the soil, without legume partner, they are called saprophytes. Many factors like environmental and soil conditions (soil moisture, pH and temperature), cropping history and vegetation affect the number of rhizobia in the soil. Rhizobia bacteria require the availability of molybdenum (Mo), a soil element for effective nitrogen fixation. Although Mo is abundantly present in soils, its availability is greatly influenced by soil pH and considered most adequate at pH values of between 6.5 and 7.0.

Rhizobia are reported to influence crop growth, yield, and nutrient uptake by different mechanisms (Dudeja and Giri 2014). They fix N, help in promoting

free-living N-fixing bacteria, increase the supply of other nutrients such as phosphorus (P) and iron (Fe), produce plant hormones, enhance other beneficial bacteria or fungi, control bacterial and fungal diseases and help in controlling insect pests (Dudeja and Giri 2014). This symbiosis can therefore help reduce the requirements for the addition of nitrogenous fertilizer during the growth of leguminous crops. Inoculation with rhizobia induces the proliferation of plant growth-promoting microorganisms (PGPMs) like *Bacillus*, *Rahnella*, *Pseudomonas*, *Mesorhizobium*, *Streptomyces*, *Sinorhizobium* and *Azospirillum*, among others. Inoculation with *rhizobia* also causes a perturbation of the microbial community. Legumes include some of the most important commercial crops under cultivation, such as soybean (*Glycine max*), pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris* L.).

3.4 Legume-Nodulating Bacteria

Apart from rhizobia, there are other bacteria that possess ability to nodulate leguminous plants. Presently, these belong to three main groups belonging to *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* (Shamseldin et al. 2017). The family *Rhizobiaceae*, *Phyllobacteriaceae*, *Bradyrhizobiaceae*, *Hyphomicrobiaceae* and *Brucellaceae* belong to the largest class (*Alphaproteobacteria*), while the *Betaproteobacteria* formed the second of the only one family, the *Burkholderiales*, which contains only two genera (Fig. 3.2). There are 98 legume-nodulating bacteria that are attributed to 18 main genera with 238

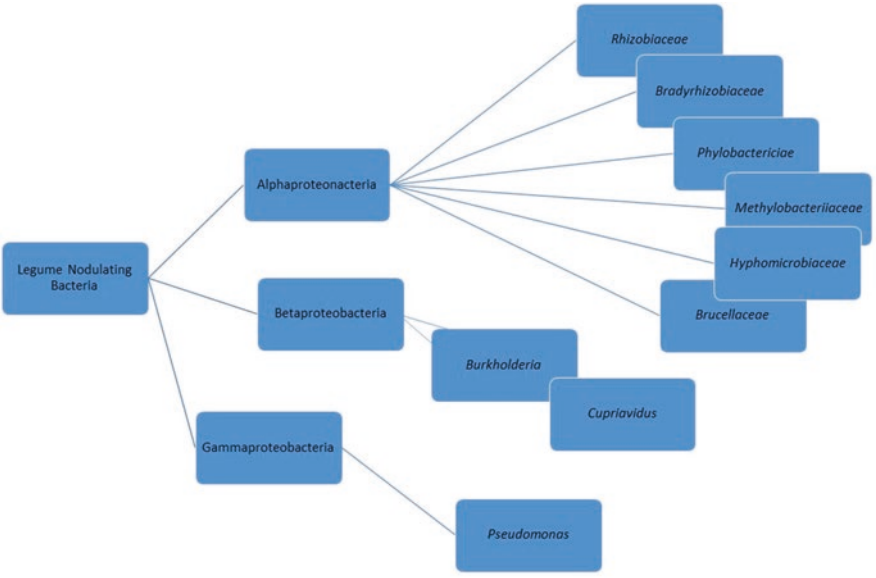


Fig. 3.2 Legume nodulating bacteria

species, out of which *Rhizobium* and *Bradyrhizobium* are the two largest genera (Berrada and Fikri-Benbrahim 2014).

3.5 Interactions of Endophytic Microbes with Legumes

Endophytic microbes are microbes that colonize the inside of plant tissues (legume and nonlegume) without causing any harm to the host plant. Endophytes have been reported in about 300, 000 species of plants (Dudeja and Giri 2014) and have potential use in sustainable agriculture. Endophytic microbes play major role in agricultural environment and produce many natural products that could be used in agriculture, industry and medicine (Ruby and Raghunath 2011). Endophytic microbes may be more important than rhizospheric microbes in promoting plant growth because they escape competition with rhizosphere microorganisms and achieve close contact with the plant tissues. Colonization of host plant by endophytes depends on seasonal changes, soil hydric stress and plant defence response among others (Dudeja and Giri 2014). About 200 genera of culturable and nonculturable bacteria belonging to 16 phyla have been reported as endophytes that include *Acidobacteria*, *Cyanobacteria*, *Firmicutes*, *Nitrospira*, *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Fusobacteria*, *Proteobacteria*, *Spirochaetes*, *Planctomycetes*, *Verrucomicrobia*, *Chlorobi*, *Gemmatimonadetes*, *Aquificae* and *Chloroflexi* (Sessitsch et al. 2012). However, the genera *Gluconobacter*, *Pseudomonas*, *Stenotrophomonas*, *Serratia*, *Bacillus*, *Enterobacter* and *Burkholderia* belong to the phylum *Proteobacteria*, while *Actinobacteria* and *Firmicutes* are the most predominant and studied endophytes (Babalola 2010; Kumar et al. 2013; Ryan et al. 2008; Taghavi et al. 2009; Taghavi et al. 2010; Weilharter et al. 2011). The nodules in the roots of legumes particularly pea (*Pisum sativum*), lucerne (*Medicago sativa*) and chickpea (*Cicer arietinum*) and nonlegumes such as oat (*Avena sativa*), rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*), maize (*Zea may*), carrot (*Daucus carota* L.), banana, coffee, citrus plant and wheat (*Triticum aestivum*) contain a verse load of endophytic bacteria (Dudeja and Giri 2014; Saini et al. 2015). Dudeja et al. (2012) and Dudeja and Giri (2014) reported the isolation of endophytic bacteria from the nodules and roots of many legumes, pea, cowpea, alfalfa, chickpea, *Conzattia*, mung bean, fenugreek, *Acacia*, *Kennedia*, soybean, *Psoralea*, *Mimosa*, *Oxytropis*, clover, *Scorpiurus*, *Vicia*, *Sesbania*, *Lotus*, *Hedysarum*, *Ornithopus*, bean, *Onobrychis*, *L. tetragonolobus*, *Leucaena*, peanut, *Argyrolobium*, *Melilotus* and *Medicago*. Similarly, endophytic bacteria were isolated from the nodules of *Sophora alopecuroides* (Zhao et al. 2013). The array of endophytic bacteria that have been reportedly isolated from legume tissues include *Inquilinus*, *Rhodopseudomonas*, *Paracoccus*, *Ornithinicoccus*, *Serratia*, *Pedobacter*, *Bacillus*, *Starkeya*, *Staphylococcus*, nose *Mycobacterium*, *Brevibacillus*, *Lysinibacillus*, *Pseudomonas*, *Nocardia*, *Sphingomonas*, *Dyella*, *Phyllobacterium*, *Aerococcus*, *Ochrobactrum*, *Agromyces*, *Stenotrophomonas*, *Methylobacterium*, *Actinobacteria*, *Paenibacillus* and *Streptomyces* among others. A single host plant may comprise several genera and

species of endophytes (Dudeja and Giri 2014). Wang et al. (2013) and Palaniappan et al. (2010) isolated 72 and 39 endophytic bacteria from *Arachis hypogea* and *Lespedeza* sp., respectively.

Endophytes improve plant growth attributes with respect to increased biomass, germination rates, hydraulic activity, nitrogen content, root and shoot length, chlorophyll content, yield tolerance to biotic (pest and pathogen) and abiotic (such as salinity, acidity, flood and drought) stresses and protein content (Khan et al. 2017; Sánchez-Romera et al. 2016). The impact of endophytes on the host plant can be through direct biochemical activities like BNF, phosphate solubilization, phytohormone production and inhibition of ethylene biosynthesis in response to abiotic and biotic stresses (induce systemic tolerance) or indirect such as inducing resistance to pathogen (Bhattacharyya and Jha 2012). More also, endophytic bacteria produce secondary metabolites that affect the plant directly or indirectly. These metabolites include ammonia, organic acids and enzymes like pectinases and celluloses (Dudeja and Giri 2014). Listed in Table 3.1 are examples of some legume microbial inoculants and their beneficial properties.

3.6 Legume Microbial Inoculants for Organic Farming

Certain microbial inoculants such as nitrogen-fixing rhizobia and mycorrhizae can improve soil nutrients and reduce disease infestation on legume plant under organic farming (Bhardwaj et al. 2014). When using purchased commercial inoculant in organic production of grains legume, forage legume or cover crops, it is important to avoid inoculants produced from genetically modified organisms, recombinant DNA technology, sewage sludge or ionizing radiation (Mapelli et al. 2012). The reason for using microbial inoculants in organic farming is as a result of the fact that most rhizobia species are organotrophs, that is, they get the derived energy from organic matter (Mendes et al. 2013). There is insufficient accessibility and availability of degradable organic compounds in many soils, while carbon accessibility is the most widely known constraining component for the growth of soil bacteria (Rousk and Bååth 2007). The nutritive cations of the soil minerals are released through the activities of these bacteria for their own sustenance as well as for plant nourishment. The mineral weathering microorganisms have been identified from different environments, especially from rhizosphere and ectomycorrhizosphere (Collignon et al. 2011), and often can add to the growth of plant in nutrient-poor soils (Leveau et al. 2010; Mapelli et al. 2012).

3.7 Legume Microbial Inoculants for Nitrogen Fixation

In soils with low mineral N content, nitrogen-fixing microorganisms provide ammonium into the legume biomass that allows for faster growth. The symbiosis is initiated through the legume root infection by the rhizobia and the formation of root nodules where biological N fixation occurs through the action of a bacterial enzyme,

Table 3.1 Examples of some legume microbial inoculants and their beneficiary properties

Legume crop	Microbial inoculants	Beneficial properties	References
Soybean	*Rhizobium* sp., *Bradyrhizobium* sp., *Trichoderma harzianum*	Production of growth hormones and biocontrol	N'cho et al. (2015)
Pea	*Rahnella* sp.	P-solubilization, production of 1-aminocyclopropane-1-carboxylate (Bilia et al. 2014) deaminase	Vyas et al. (2010)
Cowpea	*Bradyrhizobium* spp.	Production of growth-stimulating hormones	Morel et al. (2012)
Chickpea	Arbuscular Mycorrhiza	Protective response under restrictive condition	Farzaneh et al. (2009)
Cowpea	*Scutellospora reticulata* and *Glomus pansihalos*	Bio-remediation	Alori and Fawole (2012)
Pea	*Pseudomonas* sp.	Production of growth-stimulating hormones	Germaine et al. (2009)
Faba bean	*Rhizobium leguminosarum* bv. viciae and arbuscular mycorrhizal fungi	Protective response under restrictive environmental condition	Abd-Alla et al. (2014)
Chickpea	*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Mesorhizobium*	Production of growth	Saini et al. (2015)
Pigeon pea	*Bacillus* sp. and *Rhizobium* spp.	Production of growth	Rajendran et al. (2008)
Chickpea	*Pseudomonas* sp. and *Mesorhizobium* sp.	Production of indole acetic acid (IAA)	Malik and Sindhu (2011)
Pea, Lentin	*Bacillus thuringeinsis*-KR1 and *Rhizobium leguminosarum*	Growth and nodulation	Mishra et al. (2009)
Pigeon pea	Rhizobacteria and *Rhizobium*	Nodulation and nitrogen fixation	Tilak et al. (2006)
Common bean	*Rhizobium* and *Pseudomonas*	Growth and yield	Sáncheza et al. (2014)
Lentil	*Rhizobium leguminosarum*	Growth nodulation and yield	Muhammad et al. (2012)
Common bean	*Rhizobium-Azospirillum*	N fixation	Remans et al. (2008)

called 'nitrogenase' (Masson-Boivin et al. 2009). Inoculating the legume plant with efficient nitrogen-fixing microorganisms improves its potential to biologically fix atmospheric N. These kinds of microbial inoculants, also known as soil inoculants, are agricultural amendments that use microorganisms known as rhizobia to promote legume growth. These bacteria form symbiotic relationships with the target leguminous plant, and both parts benefit. Inoculation of legumes with microbial inoculants results in a tremendous increase in number and mass of nodules, nitrogenase activity, leghaemoglobin content of nodule and dry mass of root and shoot (Abd-Alla et al. 2014). Nitrogen fixation is very efficient in satisfying the high N requirements

of legumes because the conversion of gaseous N_2 to NH_3 takes place inside the plant. All of the fixed N is readily available and in the form required for combination with carbohydrates to produce the amino acids used for the manufacture of protein. Furthermore, since N fixation in the root nodules is directly dependent on the translocation of carbohydrates from the leaves, the rate of fixation is fully 'synchronized' with the rate of plant growth (Zhang et al. 2014).

In addition, to reinstate N availability of poor soil, it depends solely on the number of successful strains of the *Rhizobium* close to the rhizosphere to accelerate N fixation. Every legume requires a particular type of *Rhizobium* for effective nodulations. Although numerous legumes might be modulated by diverse strains of rhizobia, growth and N availability could only be possible by specific strains rhizobia for specific legumes (Mahdi et al. 2010). It is consequently critical to coordinate microsymbionts wisely for most extreme N fixation. A strain of rhizobia that nodulates and fixes a lot of N in one legume variety may likewise do in relationship to certain other leguminous species. This must, however, be confirmed by testing. Leguminous plants that show this propensity to react comparatively to specific strains of rhizobia are considered 'effectiveness' classes. Hence the amount of N fixed varies according to the legume species and variety. More also, alkalinity significantly inhibited nodulation and N fixation in legumes inoculated with microbial inoculant (Abd-Alla et al. 2014). The potential for N fixation is directly related to rhizobia survival, the extent of effective nodulation and plant growth factors. Any adverse soil condition or environmental stress that affects plant growth is likely to slow down the N fixation process. Nitrogen fixation is also affected by the level of available N in the soil. High soil N levels reduce N fixation because legumes will preferentially use most of the available soil N before they begin to fix atmospheric N. Nodule formation will be progressively inhibited as soil nitrate-N levels rise above about 39.2 kg ha^{-1}, and little fixation will occur with soil nitrate-N levels above 61.6 kg ha^{-1} (Abd-Alla et al. 2014).

3.8 Legume Microbial Inoculants for Soil Fertility and Increased Crop Productivity

The sustainable productivity of an agroecosystem largely depends on the ability of the soil to supply essential nutrients to the growing plants (Abd-Alla et al. 2014). Recently, there is an emerging demand to decrease the dependence on chemical fertilizers that has become a major input in crop production worldwide and ultimately increase the sustainability of agriculture. Today, only 30–50% of applied N fertilizer and 10–45% of P fertilizer are taken up by crops (Singh et al. 2016). Hence there is a need to explore alternative sources which are environment friendly and cost effective. Microbial inoculant, an alternative source of N and P fertilizer, in legume symbiosis, is a promising technology (Youseif et al. 2017). The symbiosis between legumes and rhizobia is one of the important ecological mutualisms. Legumes vary in their potential to improve soil fertility. In this wises legumes could be ranked as follows: green manure crops > forage crops > low harvest index grain

legumes > high harvest index grain legumes (Abd-Alla et al. 2014). Hence, legumes microbial inoculation has become a significant practice in the development of sustainable soil management system.

Members from the rhizosphere microbiome can altogether impact on the nutrient status of plants. Commonly known are the nitrogen-fixing rhizobia and the mycorrhizal fungi that enhance P take-up (Miransari 2011). The significance of symbionts such as mycorrhizal fungi for the translocation of nutrients and minerals from soil to the plant (Adeleke et al. 2012; Gianinazzi et al. 2010; Wallander et al. 2013), the production of stable soil aggregates and the destruction of soil borne plant pathogens (Pozo and Azcón-Aguilar 2007) is well reported (Salvioli and Bonfante 2013). Apart from *Rhizobium* and *Bradyrhizobium*, other N-fixing living bacteria like *Cyanobacteria*, *Proteobacteria*, *Actinobacteria,* and *Bacilli* have been recognized in the rhizosphere (Gaby and Buckley 2011). For instance, exploration of the cowpea rhizosphere showed a high genetic difference of mutualistic rhizobia species in western Amazon (Guimarães et al. 2012). Results of glasshouse trials and 16S rRNA gene sequencing revealed that *Bradyrhizobium*, *Rhizobium*, *Burkholderia* and *Achromobacter* species are highly effective for nodulation of cowpea (Guimarães et al. 2012). Notwithstanding the broad research on N fixation by rhizobia, the exchange of nitrogen is related to the amounts of comparable legume. The legume-specific beneficial interaction to other agriculturally critical plant species has not been revealed. Geurts et al. (2012) revealed that understanding the central contrast between the apparently comparative cell reactions incited by *Rhizobium* and mycorrhizal organisms will be important to accomplish this 'old dream'. Rhizosphere microorganisms can likewise encourage the take-up of particular trace elements, like iron which is plentiful in soil under acidic to basic conditions just like Cu, Mn and Zn. and exists fundamentally in the insoluble ferric oxide form that is not accessible for the growth of microorganism. Owing to the shortage of accessible iron in numerous microbial environments and higher concentration of toxic free iron, bacteria utilize diverse mechanisms in order to manage the intracellular iron concentration through the production of siderophores (Hider and Kong 2010). On the host side, plants react to iron limitation by either expanding the dissolvability of inorganic iron in the rhizosphere or by discharging phytosiderophores that are in this manner transported once more into the root tissue by a particular take-up system (Walker and Connolly 2008).

3.9 Legume Microbial Inoculants for Soil Health Improvement

A healthy soil is one that adequately performs its functions, which are important to humans, such as providing a medium for plant growth biological activity, regulating the partitioning of water flow and storage in the environment and serving as an environmental buffer in the formation and destruction of environmentally hazardous compounds. The ability of legumes and associated microbes to degrade pollutants permits plants to grow as natural vegetation at contaminated sites. Legume

microbial inoculants degrade pollutant compounds, aid rhizoremediation, solubilize P, fix atmospheric N and secrete siderophores (Aziz et al. 2016). One of the ways to promote soil health is to inoculate legumes with rhizobial bacteria that form symbiotic relationship with the host plant. Seeds could also be inoculated with treatments comprising of beneficial microorganisms that protect seedlings from soil borne diseases (Trabelsi and Mhamdi 2013).

The microbial inoculants in the rhizosphere give the forefront resistance to plant roots against assault by soil borne pathogens. Different individuals from the rhizosphere microbiome can alienate soil borne pathogens before and during primary infection and secondary spread on and in root tissue. The major means of wiping off plant pathogen by the rhizosphere microorganisms are antibiosis (Raaijmakers and Mazzola 2012), parasitism and rivalry for trace element, nutrients and microsites (Druzhinina et al. 2011), impedance with majority detecting influencing harmfulness and induced systemic resistance (Berendsen et al. 2012; Pieterse et al. 2012). Most, if not all, rhizobacteria produce metabolites that restrain the growth of contending microorganisms. Likewise, rhizosphere fungi are productive makers of antitoxin metabolites (Brakhage and Schroeckh 2011). Among the metabolites produced by the rhizosphere microorganisms are volatile organic compounds (VOCs) that balance up the growth of plant as well as control the dialogues among microbes and plants (Bailly and Weisskopf 2012; Effmert et al. 2012). In spite of the fact that VOCs are smaller than the aggregate number of metabolites released by fungi and bacteria, they are unique in the establishment of crosstalk with the rhizosphere and in soil biological systems. VOCs are little particles (<300 Da) with high vapour weights ready to diffuse through the water-and gas-filled pores in soil (Insam and Seewald 2010). Different bacterial species including *Stenotrophomonas maltophilia*, *Serratia plymuthica*, *Pseudomonas trivialis*, *P. fluorescens*, *B. subtilis* and *Burkholderia cepacia* release VOCs that hinder mycelial development of parasitic plant pathogens (Effmert et al. 2012). These VOCs are controlled by the root exudates. Late work demonstrated that the range of volatiles discharged by rhizobacteria can be impacted by the accessible pool of root exudates. For instance, volatiles formed in soil corrected with simulated root exudates without amino acids had solid antibacterial impacts yet mellow antifungal impacts, though volatiles delivered from root exudates supplemented with amino acids had solid antifungal impacts. At last, VOCs can likewise induce systemic resistance in plants and advance plant development (Bailly and Weisskopf 2012). Members from the rhizosphere microbiome can likewise influence the plant resistant system (Berendsen et al. 2012; Pieterse et al. 2012). The systemic resistance reaction prompted in plants by valuable rhizobacteria is by and large, managed by the phytohormones jasmonic corrosive (JA) and ethylene (ET) (Zamioudis and Pieterse 2012). Nonetheless, some bacterial strains do not instigate systemic resistance by means of the JA/ET pathway however through the salicylic corrosive (SA) pathway (Pieterse et al. 2012).

3.10 Legume Microbial Inoculants for Soil Quality Enhancement/Maintenance

A range of soil factors are known to build nutrient accessibility and plant production. The most significant might be the entities including the soil microbiome of the rhizosphere, which is the soil encompassing the underlying root of plants where complex relations transpire between the roots, soil, and microorganisms. Root exudates serve as substrates and signalling molecules for microbes making an unpredictable and joined relationship among plants and the microbiome. The larger group of soil microorganisms mainly the endophytes, symbionts, pathogens and plant growth-promoting rhizobacteria have greater impact on the soil microbiome. Each microbe teams up with the general soil microbiome to impact on plant well-being and crop efficiency. Carvalhais et al. (2013) and Panke-Buisse et al. (2015) revealed in their extensive studies that plants can shape the soil microbiome through the root exudates discharge. The molecular communication changes according to the plant improvement level, closeness to neighbouring species, management methods, and many other factors (Chaparro et al. 2012).

3.11 African Legume Crops

Fabaceae or Leguminosae belong to legume commonly referred to as Fabaceae, which is one of the biggest and most financially key plant families. Legumes are the third-biggest group of angiosperms, comprising ca. 730 genera and ca. 19,400 species (Velázquez et al. 2010). In contrast with Asteraceae (23,000 spp.), Orchidaceae (22,000 spp.), and other substantial plant families, Fabaceae are target group of worldwide plant diversity for various reasons. Fabaceae incorporates numerous valuable plants, for example, crops, legumes, timber, ornamentals and therapeutic plants (Saslis-Lagoudakis et al. 2011). Habitat difference of Fabaceae is amazingly high; legumes arise from the tropics to cold zones, from the seashore to high-altitude habitats and in rain timberlands, mangroves, peat-overwhelm woodlands, occasional backwoods, savannahs and deserts.

Furthermore, Fabaceae demonstrate high differing qualities in three fundamental tropical vegetation sorts including the tropical rain timberlands, dry backwoods and woody savannahs (Särkinen et al. 2011; Simon et al. 2011). However, the other families mentioned above have similar diversity, if by any means, in only one of these vegetation categories. Plants of Fabaceae also harbour numerous explicit herbivorous creepy crawlies and sustain specific food webs. Many legume species are in mutual relationship with knob-shaping microscopic organisms with N fixation capacity and all things considered bolster imperative environment capacities (Sprent et al. 2009). There is a significant collection of confirmation from morphological and subatomic phylogenetic reviews to bolster the Fabaceae as a monophyletic family (Bruneau et al. 2008). It customarily has been partitioned into three subfamilies Caesalpinioideae, Mimosoideae and Papilionoideae, on the premise of morphological contrasts, especially in botanical characters (Peix et al. 2015).

On the premise of molecular phylogenetic reviews, Mimosoideae and Papilionoideae have both been settled as monophyletic, settled inside a paraphyletic Caesalpinioideae. The paraphyletic subfamily Caesalpinioideae involves a various array of 'caesalpinioid' legumes that for the most part separated right on time in the historical backdrop of the family and need distinguishing floral attributes used to gathering genera into the other two families. The caesalpinioid tribe Cercideae is proposed to be one of the most primitive separating ancestries in the family (Bruneau et al. 2008). A clade including numerous other genera of Caesalpinioideae is sister to the subfamily Mimosoideae, and a clade involving these two gatherings is sister to the subfamily Papilionoideae. In the subfamily Papilionoideae, a few noteworthy gatherings have been recognized in light of molecular phylogenies (Legume Phylogeny Working Group 2013). The dalbergioid clade is a vast gathering of 45 genera and ca. 1270 species that incorporates the shelled nut (*Arachis hypogaea* L.). The genistoid clade comprises the genus *Lupinus* L. and additionally other various genera. The millettioid group involves the unequivocally sustained millettioid and phaseoloid clades including numerous vital crop species, for example, the cultivated soybean (*Glycine max*) and common bean (*Phaseolus vulgaris* L.). Hologalegina (a casual name) is the leading significant clades of Papilionoideae, divided into two main clades, namely, the robinioids (*Robinia L.* spp., e.g. dark grasshopper) and *Sesbania scop*. spp. The division is of importance due to stem-nodulation in a few species and the repeat-loss clade that is set apart by the loss of one duplicate of the vast (roughly 25 kb) inverted repeat normally found in the chloroplast genome of angiosperms. The herbaceous genera of Papilionoideae subfamily include natural plants, for example, *Pisum* L. (pea), *Vicia* L. (vetch, broad bean), *Cicer arietinum* L. (chickpea), *Medicago* L. (hay) and *Trifolium* L. (clovers). The biggest papilionoid subgroup in number of genera is the phaseoloid/millettioid assemble, which, as Hologalegina, incorporates various trained taxa, for example, *Glycine* L. (soybean), *Phaseolus* L. (basic bean), *Vigna savi* (cowpea, mung bean), *Cajanus cajan* (L.) Millsp. (pigeon pea) and *Psophocarpus* Neck. ex DC. (Winged bean). Connections in the gathering are perplexing and incorporate components of a few tribes. For instance, the nearest relatives of glycine, the soybean family, still stay obscure with a few hopefuls proposed by different atomic reviews including the pantropical variety *Teramnus* P. Browne, *Amphicarpaea*, the tribe *Psoraleeae* (Stefanović et al. 2009) or a mix thereof (Legume Phylogeny Working Group 2013).

Could the high CO_2 levels imply that N would get to be distinctly constraining for plant development, accordingly supporting advancement of N fixation? Positively, this period denoted the starting point of two main group of nodulating legumes, the genistoids and dalbergioids, and in addition group of caesalpinioids that comprises nodulating genera. In the event that nodulated legumes advanced under states of high CO_2, then it may be normal that they would be supported by current ascents in climatic groupings of this gas. Legumes abundance has main impacts on the rate of carbon and nitrogen in biological communities. Legume crops that are indigenous to Africa range from large rain forest tree to small annual herb (Sprent et al. 2009). Those genera whose major centre of diversity is Africa will be discuss in turn.

3.11.1 *Vigna*

Vigna is a genus that belongs to the popular tribe called Phaseoleae, and it is made of about 100 species some of which include *V. radiata* (L.) R. Wilczek, (also known as mung bean), *V. mungo* Hepper, *V. heterophylla*, *Vigna marina* (Burm.) Merr., *V. luteola*, *V. subterranean*, *V. vexillata* and *V. unguiculata.* Some of the species are annual, while some other ones are perennial. They are all herbaceous with some climbing (Sprent et al. 2009). *Vigna* are valued for their tuber or seeds. They are used for human food, medicine, soil improvement and for animal feed. *V. subterranean* for instance is used for breeding salinity tolerance into other crops. *Vigna* are reported to nodulate freely, using mainly the slow-growing bradyrhizobia.

The most prevalent farming system in Africa is the small-scale characterized by mixed crop-livestock farming. In this system, legumes are incorporated into both the crop and the livestock component. Through the process of BNF, legumes have the ability to increase soil fertility and protein levels in herbage. Legumes can form tripartite symbiotic relationship with nodule-inducing rhizobia and arbuscular mycorrhizal fungi. The rhizobial symbiont is responsible for atmospheric nitrogen fixation, while the association with arbuscular mycorrhizal fungi improves the ability of the plant to take up P and other nutrients (Marcel et al. 2008).

3.11.2 Cowpea

Cowpea (*Vigna unguiculata*) is one of various species of the popularly grown genus *Vigna*. Cowpea is one of the most important food and animal feed crop commonly cultivated in the semiarid tropics covering Africa, Europe, Asia and the United States. It originates from Africa and is one of the most productive heat adapted legume used agronomically. Grain ranges from 392 to 3024 kg/ha^{-1}. Use metric unit and report in kg/ha or t ha^{-1} and provide reference to support the yield statistics.

Cowpea is valued as a nutritional supplement to cereals because of complementary protein types. It is cultivated by multiple millions of smallholders in Africa. In fact, it is estimated that 200 million children, women and men live off the plant consuming the seeds daily whenever available (National Research Council 2006). Widely appreciated by the poor, cowpea seed is rich in protein, oil and digestible carbohydrate (Adeyemi et al. 2012; El-Jasser 2011; Sebetha et al. 2010).

3.11.3 Soybean

Soybean (*Glycine* max) is an annual summer legume used as human food, livestock feed and for several industrial purposes (Ali 2010). Soybean is cultivated majorly for its oil extraction (Morel et al. 2012).

3.12 Northern Africa

The major food legume in North Africa is faba bean followed by chickpea. Others include groundnuts/peanuts, lentil soybean and pea.

3.13 West Africa

Nitrogen-fixing plants have contributed to the improvement of soil fertility in West Africa. Herbaceous and woody legumes such as X, Y and Y (examples) commonly contribute 40–70 kg N ha^{-1}, which represents about 30% of the total N applied as residues (Sanginga 2003). Soybean was first cultivated in Africa in the early twentieth century and was introduced to Nigeria in 1904. Soybeans are being used to develop sustainable cropping systems in the moist savannah. The N_2 fixed by soybeans and their residual N benefits to subsequent cereal crops in the savannah zone of Southern Guinea have been estimated to vary between 38 and 126 kg N ha^{-1} (Bala 2011).

Planted forage legumes were introduced into West Africa in 1950. In the course of intensifying mixed crop-livestock systems, the dual-purpose varieties of annual (food-feed) legumes (mainly cowpea and groundnut) have gained popularity, especially in areas where farmers have good market access and pressure on land is high (Blummel et al. 2016).

Nitrogen depletion from West African soils poses serious threats to food production. There is however the need to increase food production to meet the basic food requirement of the teaming population. The use of inorganic N fertilizers though increases food production, is however not sustainable because of their indirect negative impact on soil in the form of soil degradation in the long run. Degradation in the long run is due to 'inaccurate use' such as 'application of excessive amount and the use of the wrong fertilizer type', which are collectively described as 'fertilizer abuse'. The health harzard fertilizer abuse pose to man and animal through underground water pollution is a concern (Alori et al. 2017)

The use of imported microbial inoculants was initiated in West Africa in the 1970s. At first, there was poor response to the inoculants, because of incompatibility in the new environment. Several studies on the use of these inoculants were conducted on soybean (*Glycine max* (L.) Merrill) and cowpea (*Vigna unguiculata* (L.) Walp.) but rarely on Bambara groundnut (*Vigna subterranea* (L.) Verdc.) and groundnut or peanut (*Arachis hypogaea* L.) that are naturally more adapted and promiscuous (Svubure et al. 2010). Despite the fact that inoculation activities was initiated in sub-Saharan Africa since the 1950s, and mainly used on soybean and forage legumes, the adoption of inoculation on a commercial scale has not been widely adopted, except in a few countries. Bala et al. (2011) reported the use of inoculation in most parts of East (Kenya, Uganda and Tanzania) and Southern Africa (Republic of South Africa and Zimbabwe) where their agricultural sector is dominated by commercial farms. Regular use of microbial inoculant by farmers in West Africa is still very rare. The use of microbial inoculant and inoculant

technology in West Africa is limited to research farms (Bala 2011) with scanty record at farmers' level due to the absence of or very limited large-scale soybean production and an intensive livestock industry (Bala et al. 2011).

3.14 Future Prospects and Recommendations

It is imperative for future research to identify how the exploration of legume microbial inoculants may be optimized. In addition, advances in molecular biology that will broaden knowledge base on the processes and functionality of the diverse microbiome within the rhizosphere are needed to promote widespread adoption of legume microbial inoculant in Africa agriculture will be a welcome idea. Isolation, characterization and selection of effective strains to develop local inoculant for each legume under diverse climatic and soil conditions will improve legume cropping systems in Africa.

3.15 Conclusion

The roots of legume and nonlegume plant harbour diverse microorganisms that are able to establish mutual relationship with plant root. Microbial inoculants comprising rhizobia and endophytes are potential resources that should be maximized to enhance the production of African legumes at minimal cost.

Acknowledgements The authors would like to thank the National Research Foundation, South Africa and FSSNA for funds (grant numbers UID81192, UID104015, UID98460) that have supported research in their laboratories.

References

Abaidoo R, Buahen S, Turner A, Dianda M (2013) Bridging the grain legume gap through agronomy. IITA R4D review. Issue 9. January, 2013. Available at; http://r4dreview.org/2013/01/bridging-the-grain-legume-yield-gap-through-agronomy/. Accessed on 24/9/2013.

Abd-Alla MH, El-Enany A-WE, Nafady NA, Khalaf DM, Morsy FM (2014) 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 169:49–58. doi:http://dx.doi.org/10.1016/j.micres.2013.07.007

Adeleke R, Cloete T, Bertrand A, Khasa D (2012) Iron ore weathering potentials of ectomycorrhizal plants. Mycorrhiza 22:535–544. https://doi.org/10.1007/s00572-012-0431-5

Adeyemi S, Lewu F, Adebola P, Bradley G, Okoh A (2012) Protein content variation in cowpea genotypes (*Vigna unguiculata* L. Walp.) grown in the Eastern Cape province of South Africa as affected by mineralised goat manure. Afr J Agric Res 7:4943–4947. https://doi.org/10.5897/AJAR11.1680

Ajilogba CF, Babalola OO (2013) Integrated management strategies for tomato Fusarium wilt. Biocontrol Sci 18:117–127. https://doi.org/10.4265/bio.18.117

Ajilogba CF, Babalola OO, Ahmad F (2013) Antagonistic effects of *Bacillus* species in biocontrol of tomato Fusarium wilt. Stud Ethno-Med 7:205–216

Ali N (2010) Soybean processing and utilization. In: Singh G (ed) The soybean: botany, production and uses. CAB International, Wallingford, pp 345–374

Alori ET (2016) Phytoremediation using microbial communities II. In: Ansari AA et al (eds) Phytoremediation: management of environmental contaminants. Springer, Cham, pp 183–190

Alori E., Fawole O. (2012) Phytoremediation of soils contaminated with aluminium and manganese by two arbuscular mycorrhizal fungi. J Agric Sci 4:246–252. doi:http://dx.doi.org/10.5539/jas.v4n8p246

Alori E, Fawole O, Afolayan A (2012) Characterization of arbuscular mycorrhizal spores isolated from Southern Guinea savanna of Nigeria. J Agric Sci 4:13–19. doi:http://dx.doi.org/10.5539/jas.v4n7p13

Alori ET, Dare MO, Babalola OO (2017) Microbial inoculants for soil quality and plant health. Sustain Agric Rev 22:281–307. https://doi.org/10.1007/978-3-319-48006-0_9

Ampomah OY, Ofori-Ayeh E, Solheim B, Svenning MM (2008) Host range, symbiotic effectiveness and nodulation competitiveness of some indigenous cowpea bradyrhizobia isolates from the transitional savanna zone of Ghana. Afr J Biotechnol 7:988–996

Aziz A, Ahiabor B, Opoku A, Abaidoo R (2016) Contributions of rhizobium inoculants and phosphorus fertilizer to biological nitrogen fixation, growth and grain yield of three soybean varieties on a fluvic luvisol. Am J Exp Agric 10:1–11. https://doi.org/10.9734/AJEA/2016/20072

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570. https://doi.org/10.1007/s10529-010-0347-0

Babalola OO, Glick BR (2012a) Indigenous African agriculture and plant associated microbes: current practice and future transgenic prospects. Sci Res Essays 7:2431–2439. https://doi.org/10.5897/SRE11.1714

Babalola OO, Glick BR (2012b) The use of microbial inoculants in African agriculture: current practice and future prospects. J Food Agric Environ 10:540–549

Bailly A., Weisskopf L. (2012) The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges. Plant Signal Behav 7:79–85. doi:http://dx.doi.org/10.4161/psb.7.1.18418

Bala A. (2011) Emerging challenges in cross-border movement of inoculants in sub-Saharan Africa. N_2 Africa project (putting nitrogen fixation to work for smallholder farmers in Africa). Podcaster 8, August, 2011.

Bala A, Karanja N, Murwira M, Lwimbi L, Abaidoo R, Giller K (2011) Production and use of Rhizobial inoculants in Africa, N2Africa

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. doi:http://dx.doi.org/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. doi:https://doi.org/10.1007/s00253-009-2092-7

Berrada H, Fikri-Benbrahim K (2014) Taxonomy of the rhizobia: current perspectives. British Microbiol Res J 4:616–639

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13:66–76. doi:https://doi.org/10.1186/1475-2859-13-66

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emer-gence in agriculture. World J Microbiol Biotechnol 28:1327–1350. doi:https://doi.org/10.1007/s11274-011-0979-9

Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC (2014) Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evid Based Complement Alternat Med 2014:1–14. doi:http://dx.doi.org/10.1155/2014/651593

Blummel M, Wamatu J, Rischkowsky B, Moyo S (2016) Opportunities and limitations of multi-dimensional crop improvement in grain legumes to support increased productivity in mixed crop livestock systems, presented at the international conference on pulses for health, nutrition and sustainable agriculture in drylands, Marrakesh, Morocco, 18–20 April 2016, ILRI, Nairobi

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites–strategies to activate silent gene clusters. Fungal Genet Biol 48:15–22. doi:http://dx.doi.org/10.1016/j.fgb.2010.04.004

Bruneau A, Mercure M, Lewis GP, Herendeen PS (2008) Phylogenetic patterns and diversification in the caesalpinioid legumes this paper is one of a selection of papers published in the special issue on systematics research. Botany 86:697–718. https://doi.org/10.1139/B09-065.

Carvalhais LC, Dennis PG, Badri DV, Tyson GW, Vivanco JM, Schenk PM (2013) Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. PLoS One 8:e56457

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499. https://doi.org/10.1007/s00374-012-0691-4

Collignon C, Uroz S, Turpault M, Frey-Klett P (2011) Seasons differently impact the structure of mineral weathering bacterial communities in beech and spruce stands. Soil Biol Biochem 43:2012–2022. doi:http://dx.doi.org/10.1016/j.soilbio.2011.05.008

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP (2011) *Trichoderma*: the genomics of opportunistic success. Nat Rev Microbiol 9:749–759. https://doi.org/10.1038/nrmicro2637

Dudeja SS, Giri R (2014) Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non legumes. Afr J Microbiol Res 8:1562–1572. https://doi.org/10.5897/AJMR2013.6541

Dudeja SS, Giri R, Suneja-Madan P, Kothe E (2012) Interaction of endophytic microbes with legumes. J Basic Microbiol 52:248–260. https://doi.org/10.1002/jobm.201100063

Effmert U, Kalderás J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703. https://doi.org/10.1007/s10886-012-0135-5

El-Jasser AS (2011) Chemical and biological properties of local cowpea seed protein grown in Gizan region. World Acad Sci Eng Technol 5:466–472

Farzaneh M, Wichmann S, Vierheilig H, Kaul HP (2009) The effects of arbuscular mycorrhiza and nitrogen nutrition on growth of chickpea and barley. Pflanzenbauwissenschaften 13:15–22

Gaby JC, Buckley DH (2011) A global census of nitrogenase diversity. Environ Microbiol 13:1790–1799. https://doi.org/10.1111/j.1462-2920.2011.02488.x

Germaine KJ., Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte- mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296:226–234. doi:https://doi.org/10.1111/j.1574-6968.2009.01637.x

Geurts R, Lillo A, Bisseling T (2012) Exploiting an ancient signalling machinery to enjoy a nitrogen fixing symbiosis. Curr Opin Plant Biol 15:438–443. doi:http://dx.doi.org/10.1016/j.pbi.2012.04.004

Gianinazzi S, Gollotte A, Binet M-N, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530. https://doi.org/10.1007/s00572-010-0333-3

Glick B (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:1–15. doi:http://dx.doi.org/10.6064/2012/963401

GRDC (2013) (Grains Research and Development Corporation), Australia. Rhizobial inoculants fact sheet. www.coretext.com.au http://www.grdc.com.au/~/media/B943F697AF9A406ABBA20E136FDB7DC4.ashx

Guimarães AA, Jaramillo PMD, Nóbrega RSA, Florentino LA, Silva KB, de Souza Moreira FM (2012) Genetic and symbiotic diversity of nitrogen-fixing bacteria isolated from agricultural soils in the western Amazon by using cowpea as the trap plant. Appl Environ Microbiol 78:6726–6733. https://doi.org/10.1128/AEM.01303-12

Hawkesford MJ (2011) An overview of nutrient use efficiency and strategies for crop improvement. In: Hawkesford MJ, Barraclough P (eds) The molecular and physiological basis of nutrient use efficiency in crops. Wiley-Blackwell, Oxford, pp 3–19

Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27:637–657. https://doi.org/10.1039/B906679A

Insam H, Seewald MS (2010) Volatile organic compounds (VOCs) in soils. Biol Fertil Soils 46:199–213. https://doi.org/10.1007/s00374-010-0442-3

Khan AL, Waqas M, Asaf S, Kamran M, Shahzad R, Bilal S, Khan MA, Kang S-M, Kim Y-H, Yun B-W, Al-Rawahi A, Al-Harrasi A, Lee I-J (2017) Plant growth-promoting endophyte *Sphingomonas* sp. LK11 alleviates salinity stress in *Solanum pimpinellifolium*. Environ Exp Bot 133:58–69. doi:http://doi.org/10.1016/j.envexpbot.2016.09.009

Koki T, Takayoshi W (2013) Recent trends in microbial inoculants in agriculture. Microbes Environ 28:403–404. https://doi.org/10.1264/jsme2.ME2804rh

Kumar V, Pathak DV, Dudeja SS, Saini R, Giri R, Narula S, Anand RC (2013) Legume nodules endophytes more diverse than endophytes from roots of legumes or non legumes in soils of India. J Microbiol Biotechnol Res 3:83–92

Kutu F, Asiwe J (2010) Assessment of maize and dry bean productivity under different intercrop systems and fertilization regimes. Afr J Agric Res 5:1627–1631. https://doi.org/10.5897/AJAR09.147.

Kutu FR, Diko ML (2011) Mineralogical considerations in soil fertility management on selected farmlands in Limpopo and northwest provinces, South Africa. In: Ekosse GIE et al (eds) An innovative perspective on the role of clays and clay minerals and Geophagia on economic development. Book of conference proceeding of the first international conference of clays and clay minerals in Africa and second international conference on Geophagia in Southern Africa, pp 124–130. http://www.saweb.co.za/claymineralsafrica.co.za/html/ebook2.html

Laditi MA, Nwoke OC, Jemo M, Abaidoo RC, Ogunjobi AA (2012) Evaluation of microbial inoculants as biofertilizers for the improvement of growth and yield of soybean and maize crops in savanna soils. Afr J Agric Res 7:405–413. https://doi.org/10.5897/AJAR11.904

Legume Phylogeny Working Group (2013) Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. Taxon 62:217–248. doi:https://doi.org/10.12705/622.8

Leveau JH, Uroz S, De Boer W (2010) The bacterial genus *Collimonas:* mycophagy, weathering and other adaptive solutions to life in oligotrophic soil environments. Environ Microbiol 12:281–292. https://doi.org/10.1111/j.1462-2920.2009.02010.x

Machido DA, Olufajo OO, Yakubu SE, Yusufu S (2011) Enhancing the contribution of the legumes to the N fertility of soils of the semi-arid zone of Nigeria. Afr J Biotechnol 10:1848–1853. https://doi.org/10.5897/AJB10.338

Mahdi SS, Hassan G, Samoon S, Rather H, Dar SA, Zehra B (2010) Bio-fertilizers in organic agriculture. J Phytology 2:42–54

Malik D, Sindhu S (2011) Production of indole acetic acid by *Pseudomonas* sp.: effect of coinoculation with *Mesorhizobium* sp. *Cicer* on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiol Mol Biol Plants 17:25–32. https://doi.org/10.1007/s12298-010-0041-7

Mapelli F., Marasco R., Balloi A., Rolli E., Cappitelli F., Daffonchio D., Borin S. (2012) Mineral–microbe interactions: biotechnological potential of bioweathering. J Biotechnol 157:473–481. doi:http://dx.doi.org/10.1016/j.jbiotec.2011.11.013

Marcel GA, Heijden VD, Bardgett RD, Straalen NMV (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

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. https://doi.org/10.1016/j.tim.2009.07.004

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:https://doi.org/10.1111/1574-6976.12028

Miransari M (2011) Interactions between arbuscular mycorrhizal fungi and soil bacteria. Appl Microbiol Biotechnol 89:917–930. https://doi.org/10.1007/s00253-010-3004-6

Mishra P, Mishra S, Selvakumar G (2009) Coinoculation of *Bacillus thuringeinsis*-KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea (*Pisum sativum*

L.) and lentin (*Lens culinaris* L.) World J Microbiol Biotechnol 25:753–761. https://doi.org/10.1007/s11274-009-9963-z

Morel MA, Braña V, Castro-Sowinski S (2012) Legume crops, importance and use of bacterial inoculation to increase production, crop plant. InTech, Europe

Muhammad AI, Muhammad K, Muhammad SS, Maqshoof A, Nawaf S, Naeem A (2012) Integrated use of *Rhizobium leguminosarum*, plant growth promoting Rhizobacteria and enriched compost for improving growth, nodulation and yield of lentil (*Lens culinaris* Medik.) Chilean J Agric Res 72:104–110

Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, Paramasivan P, Ryu M-H, Oldroyd GED, Poole PS, Udvardi MK, Voigt CA, Ané J-M, Peters JW (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl Environ Microbiol 82:3698–3710. https://doi.org/10.1128/AEM.01055-16

N'cho CO, Lesueur D, Yusuf AA (2015) Combined microbial inoculation as a promising approach to enhance promiscuous soybean nodulation and nitrogen content in Sudan Savanna. Int J Sustain Agric Res 2:86–97. 10.18488/journal.70/2015.2.3/70.3.86.97

National Research Council (2006) Lost crops of Africa. Volume II: Vegetables The National Academies Press, Washington, DC

Palaniappan P, Chauhan PS, Saravanan VS, Anandham R, Sa T (2010) Isolation and characterization of plant growth promoting endophytic bacteria isolates from root nodules of *Lespedeza* sp. Biol Fertil Soils 46:807–816. https://doi.org/10.1007/s00374-010-0485-5

Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J 9:980–989

Peix A., Ramírez-Bahena M.H., Velázquez E., Bedmar E.J. (2015) Bacterial associations with legumes. Crit Rev Plant Sci 34:17–42. doi:http://dx.doi.org/10.1080/07352689.2014.897899

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. https://doi.org/10.1146/annurev-cellbio-092910-154055

Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398. doi:http://dx.doi.org/10.1016/j.pbi.2007.05.004

Raaijmakers JM, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu Rev Phytopathol 50:403–424. https://doi.org/10.1146/annurev-phyto-081211-172908

Rajendran G., Sing F., Desai A.J., Archana G. (2008) Enhanced growth and nodulation of pigeon pea by coinoculation of *Bacillus* strains with *Rhizobium* spp. Bioresour Technol 99:4544–4550. doi:http://dx.doi.org/10.1016/j.biortech.2007.06.057

Remans R, Ramaekers L, Schelkens S, Hernandez G, Garcia A, Reyes J, Mendez N, Toscano V, Mulling M, Galvez L, Vanderleyden J (2008) Effect of *Rhizobium-Azospirillum* coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in Cuba. Plant Soil 312:25–37. https://doi.org/10.1007/s11104-008-9606-4

Ronner E, Franke A, Vanlauwe B, Dianda M, Edeh E, Ukem B, Bala A, Van Heerwaarden J, Giller KE (2016) Understanding variability in soybean yield and response to P-fertilizer and rhizobium inoculants on farmers' fields in Northern Nigeria. Field Crop Res 186:133–145. doi:http://dx.doi.org/10.1016/j.fcr.2015.10.023

Rousk J., Bååth E. (2007) Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiol Ecol 62:258–267. doi:https://doi.org/10.1111/j.1574-6941.2007.00398.x

Ruby J, Raghunath M (2011) A review: bacteria endophytes and their bioprospecting. J Pharm Research 4:795–799

Ryan RP, Germaine K, Francis A, Ryan DJ (2008) Bacteria endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. doi:https://doi.org/10.1111/j.1574-6968.2007.00918.x

Saini R, Kumar V, Dudeja SS, Pathak DV (2015) Beneficial effects of inoculation of endophytic bacterial isolates from roots and nodules in chickpea. Int J Curr Microbiol App Sci 4:207–221

Salvioli A, Bonfante P (2013) Systems biology and "omics" tools: a cooperation for next-generation mycorrhizal studies. Plant Sci 203–204:107–114. doi:http://dx.doi.org/10.1016/j.plantsci.2013.01.001

Sáncheza A.C., Gutiérrezc R.T., Santanab R.C., Urrutiab A.R., Fauvarta M., Michielsa J., Vanderleydena J. (2014) Effects of co-inoculation of native *Rhizobium* and *Pseudomonas* strains on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. Eur J Soil Biol 62:105–112. doi:http://dx.doi.org/10.1016/j.ejsobi.2014.03.004

Sánchez-Romera B, Ruiz-Lozano JM, Zamarreño ÁM, García-Mina JM, Aroca R (2016) Arbuscular mycorrhizal symbiosis and methyl jasmonate avoid the inhibition of root hydraulic conductivity caused by drought. Mycorrhiza 26:111–122

Sanginga N (2003) Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. Plant Soil 252:25–39. https://doi.org/10.1023/a:1024192604607

Särkinen TE, Marcelo-Peña JL, Yomona AD, Simon MF, Pennington RT, Hughes CE (2011) Underestimated endemic species diversity in the dry inter-Andean valley of the Río Marañón, Northern Peru: an example from *Mimosa* (Leguminosae, Mimosoideae). Taxon 60:139–150. doi:http://www.jstor.org/stable/41059828

Saslis-Lagoudakis CH, Klitgaard BB, Forest F, Francis L, Savolainen V, Williamson EM, Hawkins JA (2011) The use of phylogeny to interpret cross-cultural patterns in plant use and guide medicinal plant discovery: an example from *Pterocarpus* (Leguminosae). PLoS One 6:e22275. doi:http://dx.doi.org/10.1371/journal.pone.0022275

Sebetha E, Ayodele V, Kutu F, Mariga I (2010) Yields and protein content of two cowpea varieties grown under different production practices in Limpopo Province, South Africa. Afr J Biotechnol 9:628–634

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Interact 25:28–36

Shamseldin A, Abdelkhalek A, Sadowsky MJ (2017) Recent changes to the classification of symbiotic, nitrogen-fixing, legume-associating bacteria: a review. Symbiosis 71:91–109. https://doi.org/10.1007/s13199-016-0462-3

Simon MF, Grether R, de Queiroz LP, Särkinen TE, Dutra VF, Hughes CE (2011) The evolutionary history of *Mimosa* (Leguminosae): toward a phylogeny of the sensitive plants. Am J Bot 98:1201–1221. doi:http://dx.doi.org/10.1590/S1415-475738420150053

Singh DP, Singh HB, Prabha R (2016) Microbial inoculants in sustainable agricultural productivity: vol. 1: research perspectives. Springer, New Delhi

Souza RD, Ambrosini A, Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38:401–419. https://doi.org/10.1590/S1415-475738420150053

Sprent J.I., Odee D.W., Dakora F.S. (2009) African legumes: a vital but under-utilized resource. J Exp Bot 61:1257–1265. doi:https://doi.org/10.1093/jxb/erp342

Stefanović S, Pfeil BE, Palmer JD, Doyle JJ (2009) Relationships among phaseoloid legumes based on sequences from eight chloroplast regions. Syst Bot 34:115–128. doi:http://dx.doi.org/10.1600/036364409787602221

Svubure O, Mpepereki S, Makonese F (2010) Sustainability of maize-based cropping systems in rural areas of Zimbabwe: an assessment of the residual soil fertility effects of grain legumes on maize (*Zea mays* [L.]) under field conditions. Int J Eng Sci Technol 2:141–148. doi:http://dx.doi.org/10.4314/ijest.v2i7.63755

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 trees. Appl Environ Microbiol 75:748–757. https://doi.org/10.1128/AEM.02239-08

Taghavi s., van der Lelie D., Hoffman A., Zhang Y.B., Walla M.D., Vangronsveld J., Newman L., Monchy S. (2010) Genome sequence of the plant growth-promoting endophyte bacterium *Enterobacter* sp. 638. PLoS Genet 6:e1000943. doi:http://dx.doi.org/10.1371/journal.pgen.1000943

Tilak KVBR, Ranganayaki N, Manoharachari C (2006) Synergistic effects of plant-growth promoting *rhizobacteria* and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). Eur J Soil Sci 57:67–71. https://doi.org/10.1111/j.1365-2389.2006.00771.x

Torres AR, Kaschuk G, Saridakis GP, Hungria M (2012) Genetic variability in *Bradyrhizobium japonicum* strains nodulating soybean *Glycine max* (L.) Merrill. World J Microbiol Biotechnol 28:1831–1835. https://doi.org/10.1007/s11274-011-0964-3

Trabelsi D., Mhamdi R. (2013) Microbial inoculants and their impact on soil microbial communities: a review. BioMed Res Int 2013:1–11. doi:http://dx.doi.org/10.1155/2013/863240

Vanlauwe B, Giller KE (2006) Popular myths around soil fertility management in sub-Saharan Africa. Agric Ecosyst Environ 116:34–46. doi:http://dx.doi.org/10.1016/j.agee.2006.03.016

Velázquez E, R Silva L, Peix Á (2010) Legumes: a healthy and ecological source of flavonoids. Curr Nutr Food Sci 6:109–144. doi:https://doi.org/10.2174/157340110791233247

Vyas P, Robin J, Sharma KC, Rahi P, Gulati A, Gulati A (2010) Cold-adapted and rhizosphere competent strain of *Rahnella* sp. with broad-spectrum plant growth-promotion potential. J Microbiol Biotechnol 20:1724–1734. https://doi.org/10.4014/jmb.1007.07030

Walker EL, Connolly EL (2008) Time to pump iron: iron-deficiency-signaling mechanisms of higher plants. Curr Opin Plant Biol 11:530–535. doi:http://dx.doi.org/10.1016/j.pbi.2008.06.013

Wallander H, Ekblad A, Godbold D, Johnson D, Bahr A, Baldrian P, Björk R, Kieliszewska-Rokicka B, Kjøller R, Kraigher H (2013) Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils–a review. Soil Biol Biochem 57:1034–1047. doi:http://dx.doi.org/10.1016/j.soilbio.2012.08.027

Wang K, Yan PS, Ding QL, Wu QX, Wang ZB, Peng J (2013) Diversity of culturable root associated/endophytic bacteria and their chinolytic and aflatoxin inhibition activity of peanut plant in china. World J Microbiol Biotechnol 29:1–10. https://doi.org/10.1007/s11274-012-1135-x.

Weilharter A, Mitter B, Shin MV, Chain PSG, Nowak J, Sessitsch A (2011) Complete genome sequence of the plant growth-promoting endophyte *Burkholderia phytofirmans* strain PsJN. J Bacteriol 193:3383–3384. https://doi.org/10.1128/JB.05055-11

Yakubu H, Kwari JD, Ngala AL (2010) N_2 fixation by grain legume varieties as affected by rhizobia inoculation in the sandy loam soil of Sudano-Sahelian zone of North Eastern Nigeria. Niger J Basic Appl Sci 18:229–236. doi:http://dx.doi.org/10.4314/njbas.v18i2.64325.

Youseif SH, Abd El-Megeed FH, Saleh SA (2017) Improvement of Faba bean yield using *Rhizobium*/*Agrobacterium* inoculant in low-fertility sandy soil. Agronomy 7:2–12. https://doi.org/10.3390/agronomy7010002

Zamioudis C, Pieterse CM (2012) Modulation of host immunity by beneficial microbes. Mol Plant-Microbe Interact 25:139–150. doi:http://dx.doi.org/10.1094/MPMI-06-11-0179

Zhang Z, Liao H, Lucas WJ (2014) Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. J Integr Plant Biol 56:192–220

Zhao LF, Xu YJ, Ma ZQ, Deng ZS (2013) Colonization and plant growth promoting characterization of endophytic *Pseudomonas chlororaphis* strain Zong1 isolated from *Sophora alopecuroides* root nodules Brazil J Microbiol 44:629–637. doi:http://dx.doi.org/10.1590/S1517-83822013000200043

Endophytic Microorganisms: Promising Candidate as Biofertilizer

4

Manish Kumar, Raghvendra Saxena,
and Rajesh Singh Tomar

Abstract

The microbial community inhabits plants on the surface as well as inside the plant tissues as epiphytes and endophytes, respectively. The endophytic microbial community is not recovered as epiphytic microbial communities, but both are playing a very important role in plant growth promotion and as a unique biofertilizer for agricultural fields. These microbial communities are associated with several plant growth-promoting attributes and therefore enhance plant growth and agricultural yields. The endophytic bacterial and fungal communities are isolated from different plant parts by taking plant tissues during isolation processes. Stem tissues, leaf tissues and mostly roots are taken for the recovery and isolation of endophytic microorganisms. Endophytic microorganisms are very unique in their functionality and abundance. High GC-containing bacterial communities (actinomycetes), low GC-containing bacteria (firmicutes) and methylotrophic bacterial and fungal communities are generally present as endophytes in the plant tissues. The current compilation will emphasize the role of the above-said microbial communities as biofertilizers in agricultural fields as well as their abundance. The antiquity about different microbial endophytes will provide an insight to elaborate their effect, promotion and sustainability to agriculture.

Keywords

Bacterial communities • Biofertilizers • Endophytic • Epiphytic

M. Kumar (✉) • R. Saxena • R.S. Tomar (✉)
Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior, India
e-mail: mkumar@gwa.amity.edu; rsaxena@gwa.amity.edu; rstomar@amity.edu

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_4

4.1 Introduction

Because of negative and harsh impact of chemical fertilizers in soil ecosystem, microbial endophytes are used as biofertilizers in the past decades. The use of endophytes increased the crop yield and production and provided an opportunity to make agriculture sustainable (Li et al. 2016). In recent years, it has been documented that endophytic microbes have the ability to colonize interior plant tissues and finally make a strong and fruitful symbiotic association with the host plant. This association results in the enhancement and improvement in plant growth along with development of strong stress tolerance ability (Mayak et al. 2004; Saravanakumar and Samiyappan 2007). The range of these endophytic microbes is very widely associated with plants such as methylotrophs, actinomycetes, firmicutes and fungi. They are associated as epiphytic and endophytic microbial communities both. The endophytic microbial community is committed to the higher biomass production of staple crops in various types of agricultural lands. Some lands are infertile, some of them are fertile, and some are in between them.

To enhance crop yield, it is very common nowadays to incorporate growth-promoting microorganisms with low-dose chemical fertilizers or microbial consortia as bioinoculant. Biofertilizers are improving soil fertility by increasing nutrient uptake of plants and also maintaining the soil microbial floral dynamics to make them healthier. Biofertilizers are making plants resilient against adverse environment and pests. In the current context, endophytic microbial entities are discussed on how they are making better crop yields. From the past investigations, it was documented that *Rhizobium* and *Pseudomonas* have PGP (plant growth-promoting) attributes and make available nutrients to plants by metabolic modifications, solubilization of phosphates, iron chelation, atmospheric nitrogen fixation and many more attributes.

4.2 Endophytic Bacterial Communities and Their Role as Biofertilizers

In an earlier investigation, endophytic bacteria were isolated from elephant grass and were identified by molecular characterization. Bacteria such as *Sphingomonas*, *Bacillus*, *Pantoea* and *Enterobacter* sp. were identified as endophytes after sequence analysis and phylogenetic relationship were analysed afterwards. In the study, representative isolates were selected to observe their plant growth-promoting ability as well as their evolutionary relationship based on sequences. Four representative isolates of endophytic bacteria have the ability to colonize plant root faster. Moreover, plant growth-promoting attributes such as IAA production, siderophore production, ammonia production, phosphate solubilization, nitrogen fixation and ACC deaminase activity were observed in four representative endophytic bacteria. The endophytic bacteria were able to colonize host plant roots and induce an increase in shoot, root length, plant fresh weight and plant dry weight in hybrid *Pennisetum* compared to controls having no inoculation both in saline and normal condition.

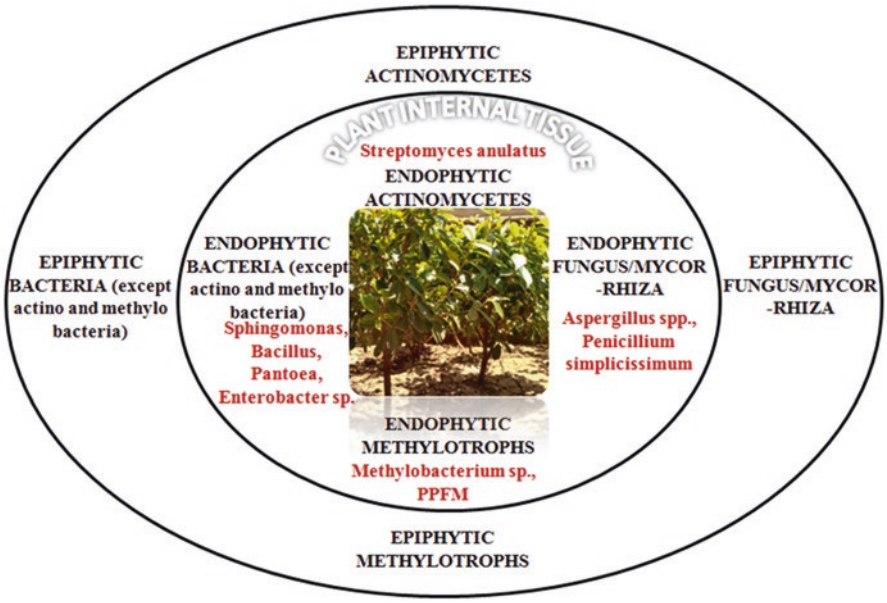


Fig. 4.1 Different types of endophytic microorganisms associated with plants involved in plant growth promotion and acting as bioinoculant and biofertilizer

Salt condition was kept up to 200 mM NaCl. Therefore, these PGP attributes make endophytic bacteria adorable and favourable for plant growth and higher yields in agricultural field (Fig. 4.1), barren land, saline soil and infertile regions. Taking this peculiarity of endophytic community, they obviously can be utilized as a better biofertilizer in the form of bioinoculant to the agricultural fields. Plant growth-promoting endophytic bacteria stimulate and enhance plant growth through various mechanisms. They promote phytohormone production, nutrient uptake, salt tolerance and biocontrol activity by reducing phytopathogens (Sturz et al. 2000; Mei et al. 2014; Bibi et al. 2012).

During the life cycle of a plant, growth promotion and development by endophytic bacteria are triggered at various times through different mechanisms (Glick 2003). The preparation of bioinoculant by taking these endophytic bacteria as biofertilizers can be applied to the field in the form of endophytic bacterial consortia. The use of mixed beneficial endophytes to the agricultural lands improves the soil quality and ultimately facilitates the plant growth.

Apart from other PGP attributes, endophytic bacteria possess ACC deaminase activity and therefore have the ability to reduce ethylene level inside the plant root as compared to ACC deaminase activity reported earlier other than endophytes. In stressed environment such as saline soil condition, plant growth-promoting endophytes are converting ACC (ethylene precursor) to ammonia and ∝-ketobutyrate using ACC deaminase enzyme (Jha and Kumar 2009; Jha et al. 2012; Glick 1995; Alexander and Zuberer 1991; Burd et al. 2000; Nabti et al. 2010) and facilitating

plant growth in adverse environment. Endophytic bacterial community is reported for high ACC deaminase activity (about 225.2–1106.6 nmol ∝-KB/h/mg) as compared to non-endophytic bacterial ACC deaminase activity (about 20 nmol ∝-KB/h/mg) (Glick 2003). However, application of plant growth-promoting endophytic bacteria in a field as biofertilizer requires attention to optimize the beneficial effects inside the host plants.

The association of biological nitrogen-fixing bacterial community with paddy roots does not mean their contribution to plants. Biological nitrogen-fixing bacteria like *Rhizobium leguminosarum* are required to be diazotrophic and endophytic in nature. In this particular endophytic association, bacteria reside inside nodules and convert nitrogen to ammonia. This type of symbiotic association protects plants by metabolic flux between plant and microbes.

4.3 Endophytic Methylotrophic Bacteria as Biofertilizer

The internal plant tissues are shelter for various microorganisms, and methylotrophs are one of them as important subpopulation of a bacterial group that can grow by utilizing reduced carbon substrates (Kumar et al. 2015, 2016). They are considered to be beneficial and non-pathogenic for plants (Pirttila et al. 2005; Meena et al. 2012). In a soybean seedling study, endophytic methylotrophic bacteria were observed to be seedling growth enhancer along with increase in root biomass (Holland and Polacco 1992). Several endophytic methylotrophs were reported earlier enhancing directly or indirectly plant growth, viz. *Methylobacterium* sp., *Methylovorus mays*, *Methylobacterium mesophilicum*, *Methylobacterium extorquens* and methanotrophs (Dourado et al. 2012; Ferreira et al. 2008; Raghoebarsing et al. 2005). Among different species of methylotrophs, pink-pigmented facultative methylotrophs (PPFM) are abundant as endophytes (Pirttila et al. 2005). Methylotrophs are reported to make agriculture sustainable (Kumar et al. 2016), and they are utilized as a source of biofertilizer also (Keerthi et al. 2015; Rekadwad 2014) to the agricultural fields. They are abundant in leaf phyllosphere and are associated as both epiphytic and endophytic bacteria. Their ability for iron acquisition and phosphate acquisition in rhizosphere makes them a potent source of biofertilizer, and farmers are applying to the fields as bioinoculants or bioformulations. Ubiquitous and cosmopolitan member of genus *Methylobacterium* sp. is found as epiphytic and endophytic bacterial community, and this PPFM (pink-pigmented facultative methylotroph) community is reported with biotechnological and agronomic potential (Daurado et al. 2015). In recent finding, PPFM and *Pseudomonas* sp. were mixed with biofertilizer, and enhanced plant growth promotion was observed at field level along with positive microbial dynamics in soil.

In earlier investigation, endophytic *Methylobacterium* sp. NPFM-SB3 was isolated from stem nodules of *Sesbania rostrata* that can form a symbiotic association with rice plant. A number of diazotrophs are reported as rice plant endophytes and sugarcane plant endophytes (Gyaneshwar et al. 2000; James et al. 2000; Baldani et al. 2000). Selection of endophytic bacteria was also based on other plant habitats

such as phyllosphere which showed better compatibility in rhizosphere region (Kishore et al. 2005). *Methylobacterium* sp. is the best example of this type of endophyte selection that is utilized as foliar spray, bioinoculant and co-inoculants with low-dose chemical fertilizers.

In potato production, methylobacterium fertilizer was used, and priming capacity of *Methylobacterium* sp. IMBG290 was also observed. Plant priming with beneficial bacterial strains induces host plant to save energy and to minimize duration for growth and development. Priming of plants by non-pathogenic bacteria allows the host to save energy and to reduce time needed for the development of defence reaction during a pathogen attack (Ardanov 2013).

4.3.1 Nitrogen-Fixing Endophytic Bacteria as Biofertilizers

Plant growth was observed to be enhanced after application of various types of bioinoculants in sterilized soil as compared to non-sterilized soil. Some of the plant growth-promoting endophytic and epiphytic microbes showed synergistic effect on increment in growth of plants (Garima and Nath 2015). Since atmospheric nitrogen cannot be reduced by plants, exterior fixed nitrogen is required by the plants for their growth and development. Therefore, these nitrogen-fixing bacterial communities can be utilized as a biofertilizer in fields. Generally farmers are applying nitrogen-containing chemical fertilizers to the field to fulfil nitrogen requirement of plants. However, during manufacturing of chemical nitrogen fertilizers, greenhouse gases are released that is very harmful for the environment. Manufacturing also leads to leaching of nitrates that is hazardous for soil ecosystem along with risk of contamination in underground and surface water. Soil fertility and agricultural sustainability are affected by continuous application of chemical fertilizers in agricultural fields. Therefore, the alternative strategy of the use of bioinoculants or biofertilizers is required for healthier crops, soil and yields. In this context, biological nitrogen fixation is triggered by endophytic bacterium *Rhizobium* sp. that colonizes and inhabits the internal compartment of plant tissue with little or no harm to the host plant. The fixation process is catalysed by the enzyme nitrogenase; therefore, this endophytic group of microorganisms can be taken as efficient biofertilizer. Internally located nitrogen-fixing bacteria have lesser competition as compared to epiphytes, and therefore fixed nitrogen is provided to plants directly. From different plants and plant parts, endophytic nitrogen-fixing bacteria were isolated and identified that contribute about 48% of nitrogen which ultimately increase plant growth. The current omics research provided information about these endophytes like *Gluconacetobacter diazotrophicus*, *Serratia marcescens* and *Azoarcus* sp., and therefore suitable bioformulations/biofertilizers can be prepared to use in agricultural fields (Gupta et al. 2012).

4.3.2 Endophytic Fungus as Biofertilizers

In the current scenario of agricultural system, it is now realized that indiscriminate and extensive application of chemical fertilizers leads to the decline of crop productivity and soil fertility. Realizing the facts, scientific community has shown much concern in eco-friendly alternatives. Biofertilizers in this direction offer eco-friendly and cost-effective solution over chemical fertilizers in order to sustain agriculture with improved crop productivity, maintain soil health and minimize environmental pollutions (Pal et al. 2015). Biofertilizers are a class of organisms which include bacteria, algae, fungi, etc. which are capable of fixing atmospheric nitrogen. They are able to mobilize nutrients through various processes like solubilization and producing plant growth-promoting agent in the soil. Biofertilizers augment nutrient availability and uptake in plants (Mishra et al. 2015; Chen 2006). Endophytic fungi live within the roots of many plant species without adversely affecting the plants. They are highly diverse in nature in their geographical area with wide diversity in extreme environments (Fisher PJ et al. 1995; Arnold and Lutzoni 2007).

Recently, the study was conducted on 30 tomato root-grown fungal endophytes isolated from central Himalayan region of India to assess their plant growth promotion ability. The study suggested that all isolated endophytes showed PGP properties; however, some of endophytes, i.e. *Fusarium fusarioides* and *Trichoderma pseudokoningii*, showed maximum growth-promoting properties; therefore, their formulation could be a potential biofertilizers in sustainable agriculture (Chadha et al. 2015). In another study, it was demonstrated that application of endophytic fungus (*Porostereum spadiceum* AGH786) on soybean [*Glycine max* (L.) Merril] seedlings had shown growth-promoting abilities under different levels of salt (NaCl) stress because of the fungal-mediated modulation of endogenous phytohormones and isoflavones. Therefore, adequate formulation of fungus offers substantial mitigation of salt stress and is suitable for agriculture in salty soil (Hamayun et al. 2017).

4.4 Conclusion

The current compilation revealed that different types of beneficial endophytic microbes are being utilized as biofertilizers at field level to enhance soil fertility and better crop production and yield. Plant growth-promoting microbial inoculants including bacteria, fungi and arbuscular mycorrhiza are used in organic farming as biofertilizers, which keep soil ecosystem nutrient rich and healthier. The above facts taking into account have shown a concern in an environment-friendly manner. Environmental balance is disturbed now due to continuous application of chemical fertilizers to the agricultural fields. Apart from this, soil health and fertility are affected, leading to diminished quality of soil in the region. Therefore, the excessive use of chemical fertilizer along with hazardous ecological impact is now in the process of replacement by naturally occurring beneficial nonhazardous microbial bioinoculants or biofertilizers. Moreover, organic farming is on the way of using

endophytic bacterial, fungal, mycorrhizal and other beneficial microbial communities as biofertilizers to enhance soil quality and fertility.

Acknowledgements Manish Kumar, Raghvendra Saxena and Rajesh Singh Tomar wish to express their sincere gratitude to Dr. Ashok Kumar Chauhan, President, RBEF parent organization of Amity University Madhya Pradesh (AUMP), for providing necessary facilities as well as valuable support.

References

Alexander DB, Zuberer DA (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil Soils 12:39–45

Ardanov P (2013) Priming capacities of endophytic *Methylobacterium* sp. on potato (*Solanum tuberosum* l.) Acta universitatis ouluensis: A scientiae rerum naturalium 613, ISBN 978–952–62-0194-8

Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical trees biodiversity hot spots? Ecology 88:541–549

Baldani JJ, Oliveira ALM, Guimmaraes SL, Baldani VLD, Reis FB, Silva LG, Reis VM, Teixeira KRS, Dobereiner J (2000) Biological nitrogen fixation in non-leguminous plants: the role of endophytic diazotrophs. In: Pedrosa FO, Hangria M, Yates G, Newton WE (eds) Nitrogen fixation: from molecules to crop productivity. Kluwer, Dordrecht, pp 397–400

Bibi F, Yasir M, Song GC, Lee SY, Chung YR (2012) Diversity and characterization of endophytic bacteria associated with tidal flat plants and their antagonistic effects on oomycetous plant pathogens. Plant Pathol J 28:20–31

Burd GI, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Chadha N, Prasad R, Varma A (2015) Plant promoting activities of fungal endophytes associated with tomato roots from central Himalaya, India and their interaction with Piriformospora indica. Int J Pharm Bio Sci 6(1):343–333

Chen J-H (2006) The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. 16–20. Land Development Department, Bangkok 10900 Thailand: 1–11

Dourado MN, Ferreira A, Araújo WL, Azevedo JL, Lacava PT (2012) The diversity of endophytic methylotrophic bacteria in an oil-contaminated and an oil-free mangrove ecosystem and their tolerance to heavy metals. Biotechnol Res Int 2012(8):1–8. 759865

Dourado MN, Neves AAC, Santos DS, Araújo WL (2015) Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic *Methylobacterium* spp. BioMed Research Int. https://doi.org/10.1155/2015/909016

Ferreira A, Quecine MC, Lacava PT et al (2008) Diversity of endophytic bacteria from eucalyptus species seed and colonization of seedlings by *Pantoea agglomerans*. FEMS Microbiol Lett 287:8–14

Fisher PJ, Petrini LE, Sutton BC, Petrini O (1995) A study of fungal endophytes in leaves, stem and roots of Gynoxis oleifolia Muchler (Compositae) from Ecuador. Nova Hedwigia 60(4):589–594

Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–114

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Gupta G, Nath JP (2015) Screening of potential PGPR candidates as future biofertilizers-a strategic approach from lab to field. Res J Biotechnol 10(11):48–62

Garima Gupta, Jitendra Panwar, Mohd Sayeed Akhtar, and Prabhat N. Jha (2012) Endophytic Nitrogen-Fixing Bacteria as Biofertilizer. E. Lichtfouse (ed) Sustainable agriculture reviews, 11:183, DOI https://doi.org/10.1007/978–94–007-5449-2_8, © Springer Science+Business Media Dordrecht

Gyaneshwar P, Reddy PM, Ladha JK (2000) Nutrient amendments influence endophytic colonization of rice by Serratia Marcescens IRBG500 and Herbaspirillum Z67. J Microbiol Biotechnol 10:694–699

Hamayun M, Hussain A, Khan SA, Kim H-Y, Khan AL, Waqas M, Irshad M, Iqbal A, Rehman G, Jan S, Lee I-J (2017) Gibberellins producing endophytic fungus Porostereum spadiceum AGH786 rescues growth of salt affected soybean. Front Microbiol 8:686. https://doi.org/10.3389/fmicb.2017.00686

Holland MA, Polacco JC (1992) Urease-null and hydrogenase- null phenotypes of a phylloplane bacterium reveal attered nickel metabolism in two soybean mutants. Plant Physiol 98:942–948

James EK, Gyaneshwar P, Barraquio WL, Mathan N, Ladha JK (2000) Endophytic diazotrophs associated with rice. In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in Rice. IRRI, Philippines, pp 119–140

Jha P, Kumar A (2009) Characterization of novel plant growth promoting endophytic bacterium Achromobacter xylosoxidans from wheat plant. Microb Ecol 58:179–188

Jha B, Gontia I, Hartmann A (2012) The roots of the halophyte Salicornia Brachiata are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. Plant Soil 356:265–277

Keerthi MM, Babu R, Joseph M, Amutha R (2015) Optimizing plant geometry and nutrient management for grain yield and economics in irrigated greengram. Am J Plant Sci 6:1144–1150

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 40:260–268

Kumar M, Srivastava AK, Pandey AK (2015) Biocontrol activity of some potent Methylotrophs isolated from Bhitarkanika mangrove sediment. Int J Curr Res Biosci Plant Biol 2:101–106

Kumar M, Tomar RS, Harshad L, Diby P (2016) Methylotrophic bacteria in sustainable agriculture. World J Microbiol Biotechnol 32:120

Li X, Geng X, Xie R, Fu L, Jiang J, Gao L, Sun J (2016) The endophytic bacteria isolated from elephant grass (Pennisetum Purpureum Schumach) promote plant growth and enhance salt tolerance of hybrid Pennisetum. Biotechnol Biofuels 9:190

Mayak S, Tirosh T, Glick BR (2004) Plant growth promoting bacteria that confer resistance in tomato and pepper to salt stress. Plant Physiol Biochem 167:650–656

Meena KK, Kumar M, Kalyuzhnaya MG, Yandigeri MS, Singh DP, Saxena AK, Arora DK (2012) Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormone. Antonie Van Leeuwenhoek 101:777–786

Mei C, Lara-Chavez A, Lowman S, Flinn B (2014) The use of endophytes and mycorrhizae in switchgrass biomass production. In: Luo H, Wu Y (eds) Compendium of bioenergy plants: switchgrass. CRC, Boca Raton, pp 67–108

Mishra S, Singh A, Keswani C, Saxena A, Sarma BK, Singh HB (2015) Harnessing plant-microbe interactions for enhanced protection against phytopathogens. In: Plant microbes symbiosis: applied facets. Springer, New Delhi, pp 111–125

Nabti E, Sahnoune M, Ghoul M, Fischer D, Hofmann A, Rothballer M, Schmid M, Hartmann A (2010) Restoration of growth of durum wheat (Triticum Durum Var. waha) under saline conditions due to inoculation with the rhizosphere bacterium Azospirillum brasilense NH and extracts of the marine alga Ulva Lactuca. J Plant Growth Regul 29:6–22

Pal S, Singh HB, Farooqui A, Rakshit A (2015) Fungal biofertilizers in Indian agriculture: perception, demand and promotion. J Eco-friendly Agric 10(2):101–113

Pirttila AM, Pospiech H, Laukkanen H et al (2005) Seasonal variation in location and population structure of endophytes in bus of scots pine. Tree Physiol 25:289–297

Raghoebarsing AA, Smolders AJP, Schmid MC, Rijpstra WIC, Wolters-Arts M, Derksen J, Jetten MSM, Schouten S, Damste JSS, Lamers LPM et al (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153–1156

Rekadwad BN (2014) Growth promotion of crop plants by *Methylobacterium organophilum*: efficient bio-inoculant and biofertilizer isolated from mud. Res Biotechnol 5:1–6

Saravanakumar D, Samiyappan R (2007) ACC deaminase from Pseudomonas Fluorescens mediated saline resistance in groundnut (Arachis Hypogea) plants. J Appl Microbiol 102:1283–1297

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

Azotobacter: A Potential Biofertilizer and Bioinoculants for Sustainable Agriculture

5

G. Chennappa, M.K. Naik, Y.S. Amaresh, H. Nagaraja, and M.Y. Sreenivasa

Abstract

Plant growth-promoting rhizobacteria (PGPR) are best known bacterial species among all other microorganisms that have more influence on physiological and structural properties of soil. PGPR helps to replace chemical fertilizer for the sustainable agriculture production by fixing the atmospheric nitrogen and producing growth-promoting substances. Among PGPR group, *Azotobacter* are ubiquitous, aerobic, free-living, and N_2-fixing bacteria commonly living in rhizosphere soil. Being the major group of soilborne bacteria, *Azotobacter* plays different beneficial roles by producing different types of vitamins, amino acids, plant growth hormones, antifungal substances, hydrogen cyanide, and siderophores. The growth-promoting substances such as indole acetic acid, gibberellic acid, arginine, etc., produced by species of *Azotobacter* have direct influence on shoot length, root length, and seed germination of several agricultural crops (soil rhizosphere). Some of the species of *Azotobacter*, viz., *A. vinelandii*, *A. chroococcum*, *A. salinestris*, *A. tropicalis*, and *A. nigricans*, are able to produce antimicrobial compounds which inhibit the growth of plant pathogens, viz., *Fusarium*, *Aspergillus*, *Alternaria*, *Curvularia*, and *Rhizoctonia* species, which can cause

G. Chennappa (✉)
Deptartment of Studies in Microbiology, Manasagangothri University of Mysore, Mysore, Karnataka, India

Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur
e-mail: chinnagurikar@gmail.com

M.K. Naik • Y.S. Amaresh
Deptartment of Plant Pathology, College of Agriculture, UAS, Raichur, Karnataka, India

H. Nagaraja • M.Y. Sreenivasa
Deptartment of Studies in Microbiology, Manasagangothri University of Mysore, Mysore, Karnataka, India
e-mail: sreenivasamy@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*, Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_5

major plant diseases and economic losses. *Azotobacter* species are efficient in fixation of highest amount of nitrogen (29.21 μg NmL^{-1} day^{-1}), production of indole acetic acid (24.50 μgmL^{-1}) and gibberellic acid (15.2 μg 25 mL^{-1}), and phosphate-solubilizing activity (13.4 mm). Species of *Pseudomonas*, *Bacillus*, and *Azotobacter* can grow and survive at extreme environmental conditions, viz., higher salt concentration, high pH environments, and even at higher temperature. *Azotobacter* is found tolerant to a higher NaCl concentration (6–8%), to maximum temperature (45 °C), and also to varied pH ranges (8–9). *A. salinestris* (GVT-1) culture filtrate has increased the paddy seed vigor index or growth and seed germination rate. *Azotobacter* species have maintained maximum levels of viable population at different temperatures in different formulations. *Azotobacter* species can grow and survive for periods in talc- and lignite-based formulations. In view of these properties, *Azotobacter* isolates can be used for sustainable agriculture as biofertilizer and bioinoculants.

Keywords
PGPR • Azotobacter • Indole acetic acid • Biofertilizer

5.1 Introduction

Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Soil microorganisms play an important role in soil processes that determine plant productivity. Bacteria living in the soil, rhizosphere and rhizoplane, and on plant tissues are called free living as they do not depend on others for their survival. Some bacteria support plant growth indirectly by the production of antagonistic substances or by inducing resistance against common plant pathogens occurring in the vicinity of roots (Tilak et al. 2005). The organic compounds released by bacteria play an important role in the uptake of mineral nutrient. The hormones produced by the rhizosphere bacteria have direct effects on growth and development of plants. The population density status of PGPR depends on the fertility of soil and human activities (Marianna et al. 2005).

Cultivation, production, and consumption of agriculture produce have been increased from the last two decades with the increasing population to sustain food supply within the available land (Chennappa et al. 2013). Asian countries which produce high agriculture productions include China, Korea, India, Pakistan, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, the Philippines, and Japan (FAO 2010). To improve the agriculture production, different types of cultivation practices such as application of chemical fertilizers and chemical pesticides, improved crop varieties and machineries, etc., are being followed. Among them, synthesized fertilizers, chemical pesticides, and other inputs are being excessively applied for the control of plant diseases and insect pests. Farmers use chemical fertilizers to increase production, but the extensive use of these chemical-based inputs

or fertilizers leads to contamination of soil and groundwater, depletion of soil fertility, greenhouse effect, damage to the ozone layer, acidification and pollution of water resources, destruction of beneficial microorganisms, acidification of soil, and health hazards (Matin et al. 2011). To overcome these problems, several research works in biodegradation of pesticides have been carried throughout the world in order to minimize the residual toxicity in the food and food products.

However, microorganisms play a major role in the degradation of chemical pesticides, and many soilborne bacteria and fungi have the potentiality to breakdown of pesticides into nontoxic elemental compounds in the soil. For biodegradation of pesticides, numbers of microbes have been employed, and among all, plant growth-promoting rhizobacteria (PGPR) are the widely studied bacterial group. PGPR are not only biodegrade pesticides but they are also involved in nitrogen fixation and produce growth-promoting compounds which can help to replace chemical fertilizer for sustainable agriculture (Castillo et al. 2011; Ahmad et al. 2005). PGPR group includes different species of bacteria; among them, diazotrophic *Azotobacter* are free living in rhizosphere soil ecosystem, which are playing different beneficial roles for the plant growth (Page and Shivprasad 1991; Tejera et al. 2005).

The genus *Azotobacter* has the potentiality to produce different types of amino acids, plant growth hormones, antifungal antibiotics, and siderophore and has a unique ability of atmospheric nitrogen fixation in the soil (Myresiotis et al. 2012; Chennappa et al. 2013, 2014, 2016). *Azotobacter* species happens to be the most dominant species in the rhizosphere soil and can biodegrade chlorine-containing pesticide, viz., 2,4,6-trichlorophenol, simple phenols, and substituted phenols used for the management of plant pathogens causing diseases in agricultural crops (Li et al. 1991). In view of these prominent beneficial applications, the review survey of research articles has been carried to know the complete nature and beneficial properties of *Azotobacter* species.

5.2 *Azotobacter* Diversity

Beijerinck (1901) was the first person who isolated and cultured species of *Azotobacter.* Later, several other species of *Azotobacter* have been isolated and described as *Azotobacter vinelandii*, *A. beijerinckii*, *A. insignis*, *A. macrocytogenes*, *A. paspali*, *A. chroococcum*, *A. salinestris*, *A. armeniacus*, *A. brasilense*, *A. agilis*, *A. tropicalis*, and *A. nigricans* (Mulder and Brontonegoro 1974; Page and Shivprasad 1991; Kizilkaya 2009). The diversity and beneficial applications of *Azotobacter* species were well documented by different ecosystems from the last two decades because of its plant growth-promoting activity for sustainable agriculture (Aquilanti et al. 2004; Jimenez et al. 2011). Among different species, *A. chroococcum* and *A. vinelandii* are common habitants found in the rhizosphere soils. The *Azotobacter* are ubiquitous in nature, and its occurrence in soil is influenced by many factors, viz., soil pH, organic matter, calcium, phosphorus, potassium content, and other microorganisms present in soil (Rangaswami et al. 1964).

The occurrence and dominance of *Azotobacter* have been discovered from various rhizospheric soils of agricultural crops such as ragi, sorghum, green gram and soybean, sugarcane, rice, and cereals. *Azotobacter* population was found more in black soil than in red soil, and the number may be decreased with depth, but the decrease was more drastic in black soils (Bagyaraj and Patil 1975; Ramaswami et al. 1977).

5.3 PGPR Properties

The term PGPR was first described by Kloepper and Schroth (1980). PGPR are a group of bacteria that actively colonizes plant roots and promotes plant growth and increases yield (Bin Zakaria 2009). There are several types of rhizobacteria, and the type is depending on the nutrients provided into the soil systems and mechanism used. PGPR are able to increase plant nutrient uptake by introducing nitrogen-fixing bacteria associated with roots (*Azospirillum*) for nitrogen uptake, iron uptake from siderophore-producing bacteria (*Pseudomonas*), sulfur uptake from sulfur-oxidizing bacteria (*Thiobacillus*), phosphorus uptake from phosphate mineral-solubilizing bacteria (*Bacillus*, *Pseudomonas*), and potassium uptake from potassium-solubilizing bacteria (*Bacillus*).

The PGPR promote plant growth and have the potentiality to produce vitamins (riboflavin), amino acids (thiamine), polyhydroxybutyrate (PHB), and phytohormones (nicotin, cytokinin, IAA, and gibberellins), symbiotic and asymbiotic N_2 fixation, production of siderophores, HCN, synthesis of antibiotics and enzymes, and mineralization of phosphates and other nutrients (Gholami et al. 2009; Myresiotis et al. 2012). Enhanced supplies of other plant nutrients such as phytochrome production lead to increases in shoot and root length as well as seed germination of several agricultural crops (Ahmad et al. 2005; Heike 2007). The Production of biologically active substances or plant growth regulators (PGRs) is one of the major mechanisms through which PGPR influence the plant growth and development (Javed et al. 2009). The ability to synthesize phytohormone is widely distributed among plant-associated bacteria, and 80% of the bacteria isolated from plant rhizosphere are able to produce plant growth-promoting substances.

5.3.1 Vitamins

Vitamins are essential for physiological functions of living beings which are produced by several groups of bacteria. *Azotobacter* species produces vitamins under favorable conditions, and *A. vinelandii* and *A. chroococcum* strains produced niacin, pantothenic acid, riboflavin, and biotin which belong to B-group vitamins. They are used to maintain metabolic processes of living beings, but the production of vitamins is controlled by several physical factors such as growth conditions, pH, incubation temperatures, and availability of nitrogen and carbon sources (Revillas et al. 2000). Riboflavin is a vitamin B2 required for a wide variety of cellular processes,

and it plays a key role in metabolism of fats, ketone bodies, carbohydrates, and proteins, respectively (Almon 1958; Revillas et al. 2000).

5.3.2 Amino Acids

Amino acids are also one of the important elements required for the growth and development of cells. Few of the bacterial genera known to produce amino acids, among them *A. vinelandii* and *A. chroococcum*, produced aspartic acid, serine, glutamic acid, glycine, histidine, threonine, arginine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, tryptophan, and phenylalanine (Revillas et al. 2000; Lopez et al. 1981).

5.3.3 HCN

Many bacterial genera have capability of producing HCN. Species of *Azotobacter*, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* produce HCN as a volatile, secondary metabolite that suppresses the growth and development of plant pathogens and that influences the growth of plants (Ahmad et al. 2008). HCN is a powerful inhibitor of many metal enzymes, especially copper-containing cytochrome C oxidases. It is formed from glycine through the action of HCN synthetase enzyme, which is associated with the plasma membrane of certain rhizobacteria.

5.3.4 Siderophore

Siderophore are iron (Fe)-chelating low molecular weight compounds which are produced and utilized by bacteria and fungi. These compounds are produced in response to iron deficiency which normally occurs in neutral to alkaline pH soils, due to low iron solubility at elevated pH (Johri et al. 2003). Species of *Azotobacter* excretes siderophores under limited iron conditions. *A. vinelandii* produces five different siderophore such as 2,3-dihydroxybenzoic acid, aminochelin, azotochelin, protochelin, and the azotobactin which act as antibiotic in nature (Fig 5.1). Siderophores are used as drug delivery agents, which are important main biotechnological applications, antimicrobial agents, and soil remediation (Page and Von Tigerstrom 1988; Mollmann et al. 2009; Kraepiel et al. 2009; Barrera and Soto 2010). Siderophore-producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+} in the vicinity of the root.

5.3.5 Polyhydroxybutyrate (PHB)

Azotobacter species also produces PHB, alginate, and catechol compounds under determined nutritional and favorable environmental conditions (Barrera and

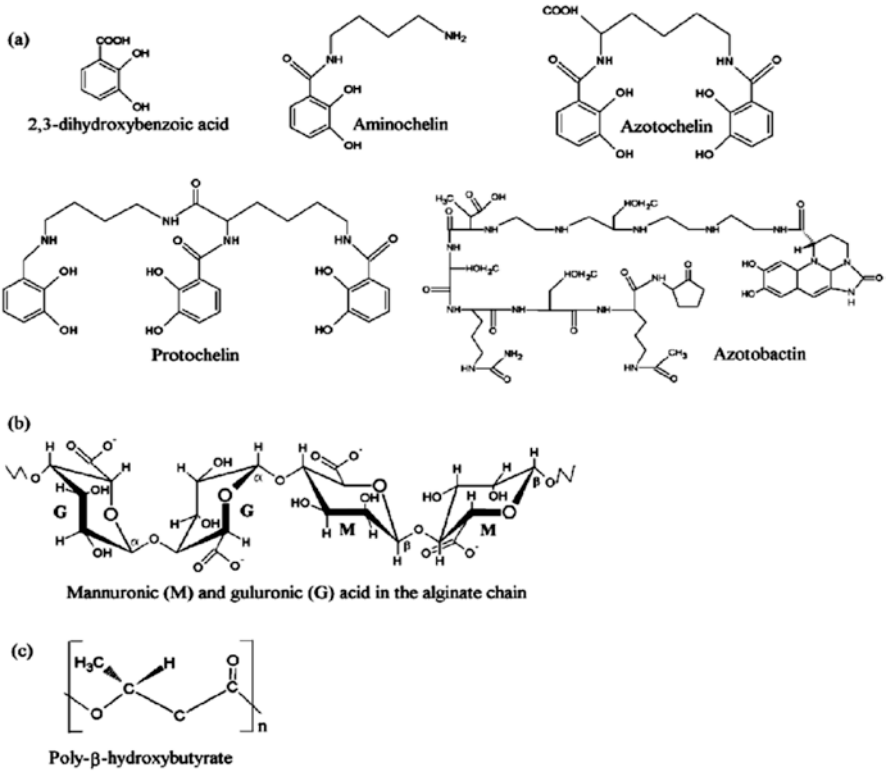


Fig. 5.1 Different types of antibiotics produced by species of *Azotobacter* (Juan et al. 2014)

Soto 2010). PHB are also used in large-scale production of alginic acid which is a biodegradable and biocompatible thermoplastic used in food industry, for thickening soups and jellies.

5.3.6 Enzymes

The production of polyphenol oxidases (PPOs) and phenol oxidases (POs) in members of the family *Azotobacteraceae* is highly presumed and is produced by the group of multi-copper protein bacterial family, respectively (Herter et al. 2011). Few of the reports documented that the production, distribution, occurrence, structural organization, and localization of prokaryotic phenol oxidases seemed to be restricted to some species. *Azotobacter* sp. SBUG 1484 isolated from soil was confirmed for production of phenol oxidases. The presence of phenol oxidases is being exploited in industrial applications such as pulp delignification, textile dye bleaching, and biopolymer synthesis which is highly important. Significant interest in the application of phenol oxidases has also been generated in scientific fields concerning the detoxification and

degradation of environmental pollutants and also concerning with the production of fine chemicals (Herter et al. 2011).

5.3.7 Antifungal Activities

Azotobacter species act as biocontrol agents by the production of antibiotics such as 2,3-dihydroxybenzoic acid, aminochelin, azotochelin, protochelin, and azotobactin for combating plant pathogens (Agarwal and Singh 2002; Mali and Bodhankar 2009; Kraepiel et al. 2009). The production of antibiotics is considered one of the most studied biocontrol mechanisms for combating phytopathogens. The species of *Azotobacter armeniacus* has inhibited root-colonizing *Fusarium verticillioides* which has suppressed fumonisin B1 production. Antifungal activity of *A. vinelandii* showed maximum zone of inhibition (40 mm) against *F. oxysporum* which is commonly known to cause several diseases in agricultural crops, viz., chilli and pigeon pea (Cavaglieri et al. 2005; Bhosale et al. 2013). *Azotobacter* can provide protection against soilborne pathogenic fungi such as *Aspergillus*, *Fusarium*, *Curvularia*, *Alternaria*, and *Helminthosporium* (Khan et al. 2008; Mali and Bodhankar 2009). Nagaraja et al. (2016) have reported the antifungal property of *A. nigricans* against *Fusarium* spp. and its role in decolonizing efficiency against fungal pathogen in rhizoplane soil.

5.3.8 Plant Growth Hormones

5.3.8.1 IAA

Indole acetic acid (IAA) is the important plant auxin produced by different groups of bacteria commonly living in soil (Barazani and Friedman 1999). Saline soil is a rich source of IAA-producing bacteria, whereas 75% of the bacterial isolates are active in IAA production. Many *Azotobacter* species are found to produce IAA in the range of 2.09–33.28 μg/mL (Spaepen et al. 2007; Chennappa et al. 2013, 2014, 2016). Most commonly, IAA-producing PGPR strains are known to increase root length resulting in greater root surface area which enables plants to access more nutrients from soil. IAA is responsible for the division, expansion, and differentiation of plant cells and tissues and stimulates root elongation (Ahmad et al. 2008). These rhizobacteria synthesize IAA from tryptophan by different pathways via tryptophan-independent and tryptophan-dependent pathways.

In contrast, the indole pyruvic pathway appears to be the main pathway present in plant growth-promoting beneficial bacteria (Patten and Glick 2002). Among PGPR species, *Azospirillum* is one of the best studied IAA producers, and other bacteria genera include *Aeromonas*, *Burkholderia*, and *Azotobacter* (Ahmad et al. 2008). *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Rhizobium* (Ghosh et al. 2010) species have been isolated from different rhizosphere soils.

5.3.8.2 Gibberellic Acid

Another important type of auxin produced by *Azotobacter* is gibberellins. GA production was first discovered by Japanese scientist Eiichi Kurosawa, which was produced by the fungi called *Gibberella fujikuroi* under abnormal growth stage in rice plants. GA includes a wide range of chemicals that are produced naturally within plant rhizosphere and by bacteria and fungi. Gibberellins are important in seed germination and enzyme production that mobilizes growth of new cells. GA promotes flowering, cellular division, and seed growth after germination (Upadhyay et al. 2009).

5.3.9 Phosphate Solubilization

Microbes play a significant role in the transformation of phosphorous and referred to as phosphor bacteria. Phosphate-solubilizing bacteria are a group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds. The P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. Phosphate-solubilizing bacteria species such as *A. chroococcum*, *B. subtilis*, *B. cereus*, *B. megaterium*, *Arthrobacter ilicis*, *E. coli*, *P. aeruginosa*, *E. aerogenes*, and *Micrococcus luteus* were identified (Kumar et al. 2000; Garg et al. 2001).

5.3.10 Nitrogen Fixation

The Earth's atmosphere contains 78% nitrogen gas (N_2), and most organisms cannot directly use this resource due to the stability of the compound. Plants, animals, and microorganisms can die of nitrogen deficiency because nitrogen is one of the important N sources. All organisms use the ammonia (NH_3) form of nitrogen to synthesize amino acids, proteins, nucleic acids, and other nitrogen-containing components necessary for life (Lindemann and Glover 2008; Mikkelsen and Hartz 2008). Nitrogen is present in all living organisms, proteins, nucleic acids, and other molecules. It typically makes up around 4% of the dry weight of plant matter.

Inadequate supply of available N_2 frequently results in plants that have slow growth, depressed protein levels, poor yield of low-quality produce, and inefficient water use. The sources of nitrogen used in fertilizers are many, including ammonia (NH_3), diammonium phosphate ((NH_4) $2HPO_4$), ammonium nitrate (NH_4NO_3), ammonium sulfate ((NH_4) $2SO_4$), calcium cyanamide ($CaCN_2$), calcium nitrate (Ca $(NO_3)_2$), sodium nitrate (N_aNO_3), and urea (N_2H_4CO) (Mikkelsen and Hartz 2008; Rifat et al. 2010; Shakhashiri 2003).

5.3.10.1 Nitrogen-Fixing Bacteria

Following photosynthesis, nitrogen fixation is the second most important process in plant growth and development. Nitrogen fixation occurs by the use of nitrogen gas to form ammonium with the help of nitrogenase enzyme. About 300–400 kg N/ha/

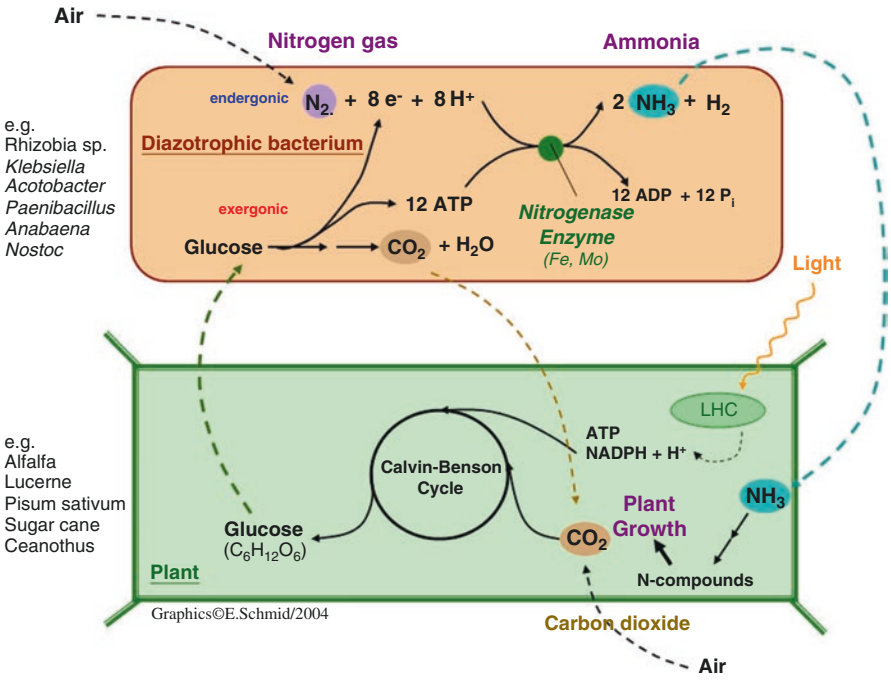


Fig. 5.2 Mechanism of nitrogen fixation by plant growth-promoting rhizobacterial group (**http://classroom.sdmesa.edu/eschmid/Lecture21-Microbio.htm**)

yr of nitrogen fixation has been fixed by nitrogen fixation process in the soil, and the atmosphere comprises of ~78% nitrogen as an inert gas, N_2, which is unavailable to plants. Approximately 80,000 tones of this unavailable nitrogen are present in the soil ecosystem and in the atmosphere. In order to convert to available form of N_2, it needs to be fixed through either the synthetic industrial process (Haber-Bosch process) or through biological nitrogen fixation (BNF). Biological nitrogen fixation (BNF) accounts for 65% of the nitrogen currently utilized in agriculture, and out of that, 80% comes from symbiotic associations, the rest from nonsymbiotic and associative systems (Fig 5.2). PGPR root-colonizing microorganisms are known to fix atmospheric molecular nitrogen through symbiotic, asymbiotic, and associative nitrogen-fixing process.

Symbiotic Nitrogen Fixers

It is estimated that about 80% of symbiotic biological nitrogen fixation available in soil ecosystem and symbiotic nitrogen-fixing bacteria are very specific plant roots of particular legume species for nodulation, invasion, and nitrogen fixation (Chandrasekar et al. 2005). Among different nitrogen-fixing bacteria, *Rhizobia* and *Frankia* have been widely studied, and more than 280 species of woody plants form root nodules which are harbored by *Frankia* (Tilak et al. 2005).

Nonsymbiotic and Associated Nitrogen Fixers

Nonsymbiotic nitrogen fixation is known to be of great agronomic significance, and its main limitation is the availability of carbon and energy source for nitrogen fixation process. This limitation can be compensated by several root-colonizing bacteria living closer or inside the plants. Some of the important nonsymbiotic nitrogen-fixing bacteria include the species of *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas*, and *Xanthobacter* (Tilak et al. 2005). Among all the species, *Azotobacter* is the most studied diazotrophic nonsymbiotic nitrogen-fixing bacterial species and aerobic soil bacteria with a wide variety of metabolic capabilities (Khan et al. 2008; Mirzakhani et al. 2009).

Nitrogen Fixation by *Azotobacter*

Nitrogen fixation is the biological reaction where atmospheric N_2 gas is converted into NH_3. Ammonia is a form of nitrogen that can be easily utilized for biosynthetic pathways; nitrogen fixation is a critical process in the completion of the nitrogen cycle (Murcia et al. 1997; Barrera and Soto 2010). The species of *Azotobacter* are known to fix on an average 10 mg of N/g of carbohydrate under in vitro. *A. chroococcum* happens to be the dominant inhabitant in arable soils capable of fixing N_2 (2–15 mgN_2 fixed/g of carbon source) in culture medium. Most efficient strains of *Azotobacter* would need to oxidize about 1000 kg of organic matter for fixing 30 kg of N/ha. Besides, soil is inhabitated by a large variety of other microbes, all of which compete for the active carbon. Plant needs nitrogen for its growth and *Azotobacter* fixes atmospheric nitrogen nonsymbiotically. Therefore, plants get benefited especially cereals, vegetables, fruits, etc., which are known to get additional nitrogen requirements from *Azotobacter* (Tilak et al. 2005; Tejera et al. 2005; Khan et al. 2008; Mirzakhani et al. 2009).

5.3.11 Abiotic Stress Tolerance

In soil ecosystem, populations of *Azotobacter* sp. are affected by soil physicochemical parameters (organic matter, pH, temperature, soil depth, soil moisture) and microbiological properties (microbial interactions) (Kizilkaya 2009). Owing to the fact that *Azotobacter* is an aerobe, this organism requires oxygen for the biological activity. As many investigators have noted, aeration encourages the propagation of *Azotobacter*. The initiation of growth of nitrogen-fixing *Azotobacter* species was prevented by efficient aeration but preceded normally with gentle aeration (Gul 2003).

5.3.11.1 Salt Tolerance

Many reports related salt, temperature, and pH tolerance of PGPR group of bacteria are available in public database. Among PGPR group, species of *Azotobacter* are known to tolerate maximum salt concentration, and it has been recorded growth rate

up to 10% of NaCl concentration. Similarly, *A. salinestris* was tolerant to 8% NaCl concentration, but the total CFU/mL values were reduced at 8% concentration. The NaCl concentration affected the PGPR activity of *Azotobacter* such as nitrogen fixation in soil. *A. salinestris* was isolated from saline soil samples, and because of this activity, the species has been named as salinestris which is sodium-dependent diazotrophic *Azotobacter* species (Page and Shivprasad 1991).

5.3.11.2 Temperature Tolerance

In relation to temperature, a number of microbes can survive at different temperatures, and *Azotobacter* is a typical mesophilic organism. Most research data predicts that 25–30° is the optimum temperature for the growth and for all the physiological properties of *Azotobacter*. The minimum temperature of growth of *Azotobacter* evidently lies a little above 0 °C. *Azotobacter* cells cannot tolerate high temperatures, but in the form of cysts, they can survive at 45–48 °C and can germinate under favorable conditions (Gul 2003). *A. salinestris* survived up to 45 °C and documented a maximum growth rate at 35 °C, and growth was reduced with increasing temperature.

5.3.11.3 pH Tolerance

The presence of *A. chroococcum* in soil or water is strongly governed by the pH value of these substrates. The presence of *Azotobacter* population in soil ecosystem is controlled by pH concentration, and lower pH (<6.0) decreases the population or is completely absent. The optimum pH between 7 and 7.5 is favorable for the physiological functions of *Azotobacter*, and at this pH population number may fall between 102 and 104 per gram of soil (Becking 2006). *A. chroococcum* survived at a pH of 9.0 and did not observe any inhibition of growth at higher pH range. *A. salinestris* was sensitive to pH of above 9.0 and no growth was observed above this range.

5.4 Bioformulations and Shelf Life

The scientific term bioformulations generally refer to the development of formulations consisting of microorganisms that may substitute the use of chemical fertilizers partially or completely (Naveen et al. 2010). For the sustained availability of the biocontrol formulations, mass production and development of formulation have to be standardized which also increase the shelf life of the bacterial formulations. This is very important since microorganisms with PGPR cannot be applied as cell suspensions to the field. Therefore, organic carrier materials such as talcum powder, lignite, pyrophyllite, and zeolite are used which support and enhance the survival ability of the bacteria for considerable length of time (Nakkeeran et al. 2005).

The viable population of *Azotobacter* in different carrier materials was determined at different storage conditions. FYM formulation recorded highest population (25.66×10^5) by *A. chroococcum*, and the lowest CFU (18.00×10^5) was showed by *A. armeniacus* at 35 °C. More than 40 °C has reduced the survivability of

bacteria and found only half of the population. All the isolates were survived at 4–45 °C of temperature but varied in the total population. As in case of lignite formulations, *A. salinestris* recorded highest CFU/mL of 22.33 × 10^5 at 35 °C, and decreased growth trend was observed above 40 °C at 15 days of intervals. Lignite could be considered as carrier material for *Azotobacter* as biofertilizer formulations. Overall, all the isolates survived up to 12 months of incubation period at 35 °C, and decline in population rate was observed.

In talc formulation, *A. salinestris* isolate showed a steady population throughout the year. Among all, *A. salinestris* recorded a highest CFU (23 to 17.35 × 10^5) up to 12 months of storage at 35 °C. The mean population in FYM formulations, *A. salinestris* and *A. chroococcum* isolate population, was maintained significantly for up to 6 months. Overall, the results depict that talc is the best carrier material to support the *A. salinestris* for longer shelf life at both room temperature and refrigerated temperature conditions, respectively, at the end of a year. Overall, the talc maintained the population *Azotobacter* uniformly.

Talcum-based formulations were developed as method suggested by Vidhyasekaran and Muthamilan (1995). The results revealed the colony-forming units of both *A. nigricans* and *A. salinestris* on Waksman selective media after 6 months of storage in the range of 3 × 107 to 4 × 107, respectively (Nagaraja et al. 2016) (Fig 5.3). This suggests the long-term survival ability of the *Azotobacter* strains and hence can be used as potent biocontrol agents against phytopathogens along with PGPR properties in improving plant growth. The talc-based bioformulation with other bacterial species such as *Pseudomonas fluorescens* strains, *Pseudomonas* strains, and *Rhizobium* sp. has been reported by Vidhyasekaran et al. (1997) and Naik et al. (2013).

5.5 What Are Fertilizers?

Plants, unlike all other living things, need food for their growth and development. They require major essential elements like carbon, hydrogen, and oxygen which are available from the atmosphere, water, and soil. The common essential elements like nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine are available from soil minerals or organic matter or by organic or inorganic fertilizers (Al-Khiat 2006). Most of the soils are not fertile and doesn't contain complete elemental nutrients required for the plant growth. The supply and scarcity of these elemental nutrients can be minimized by the use of fertilizers and other chemical inputs for the growth and development of agricultural crops. Based on the production process and usage, the fertilizers can be roughly categorized into three types: chemical, organic, and biofertilizer (Jen-Hshuan 2006).

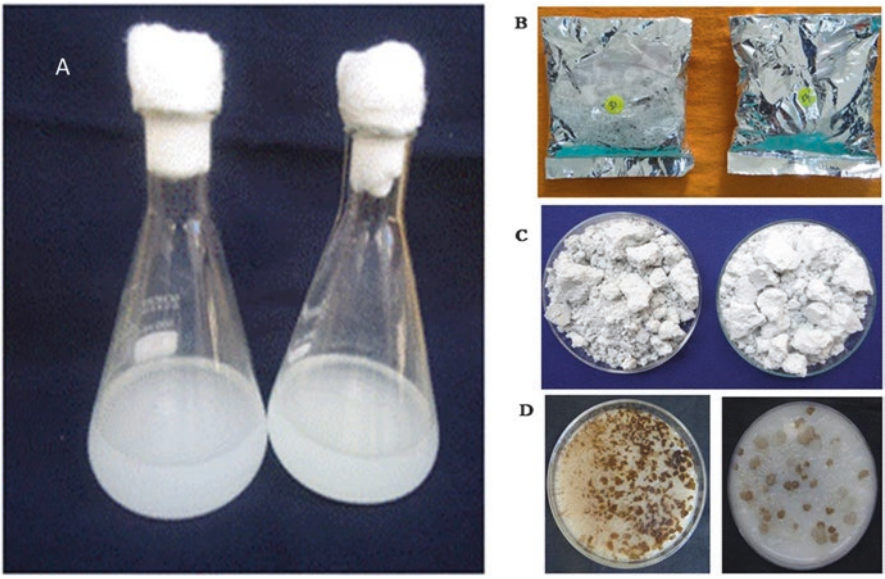


Fig. 5.3 Mass multiplication and formulation of *Azotobacter salinestris* in Waksman broth (**a**) with lignite and talc formulations (**b** and **c**), viable cells of *A. salinestris* by spread plate count method (**d**)

5.6 Types of Fertilizers

5.6.1 Chemical Fertilizer (Synthetic Fertilizer)

Fertilizers play an important role in increasing the yield of agriculture produce. The macronutrients present in inorganic fertilizers include nitrogen, phosphorus, and potassium which influence vegetative and reproductive phase of plant growth (Patil 2010). Chemical fertilizer is often synthesized using Haber-Bosch process, which produces ammonia as the end product. Synthetic fertilizers are soluble and easily available to the plants; therefore, the effect is direct and fast. They are quite high in nutrient content; only relatively small amounts are required for crop growth (Jen-Hshuan 2006).

The use of chemical fertilizers alone has not been helpful under intensive agriculture because it aggravates soil degradation. The degradation is brought about by loss of organic matter which consequently results in soil acidity, nutrient imbalance, and low crop yields. The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion, and acidification of water. These problems can be tackled by use of biofertilizers (Saadatnia and Riahi 2009; Chennappa et al. 2015, 2016). Due to its high solubility, up to 70% of inorganic fertilizer can be lost through leaching, denitrification, and erosion, reducing their effectiveness (Ayoola and Makinde 2007; Alimi et al. 2007). Overapplication can result in negative effects such as leaching,

pollution of water resources, destruction of beneficial microorganisms and friendly insects, crop susceptibility to disease attack, acidification or alkalization of the soil, or reduction in soil fertility, thus causing irreparable damage to the overall system (Jen-Hshuan 2006).

5.6.2 Organic Fertilizer

Organic fertilizer refers to materials (manure, worm castings, compost, seaweed) used as fertilizer that occur regularly in nature, usually as a by-product or end product of a naturally occurring process. Organic fertilizers typically provide the three major macronutrients required by plants: nitrogen, phosphorus, and potassium. Organic fertilizers such as manure have been used in agriculture for thousands of years (Thomas et al. 1990). In addition to increasing yield and fertilizing plants directly, organic fertilizers can improve the biodiversity and long-term productivity of soil. Organic nutrients increase the abundance of soil organisms such as fungal mycorrhiza by providing organic matter and micronutrients and can drastically reduce external inputs of pesticides, energy, and fertilizer, at the cost of decreased yield (wikipedia.org/wiki/Fertilizer).

Organic fertilizers are better sources of nutrient in balanced amounts than inorganic fertilizers where soil is deficient in both macro- and micronutrients. Organic-based fertilizer use is beneficial because it supplies micronutrients and organic components that increase soil moisture retention and reduce leaching of nutrients. Organic fertilizers can be used on acid-tolerant and those better suited to neutral or alkaline conditions (Alimi et al. 2007).

5.6.3 Biofertilizer

Biofertilizers are commonly called microbial inoculants which contain living microorganisms. When biofertilizers are applied to the seed or plant surfaces, they colonize the rhizosphere or interior of the plant and promote expansion of the root system and better seed germination by increasing the supply of primary nutrients to the host plant (Chandrasekar et al. 2005; Selvakumar et al. 2009). Biofertilizers can add 20–200 kg N ha1 by nitrogen fixation, secrete growth-promoting substances, and increase crop yield by 10–50%. They are cheaper, pollution-free, and based on renewable energy sources and also improve soil health (Saeed et al. 2004). For the last one decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status, and enhance crop production by their biological activity in the rhizosphere (Contra 2003; Patil 2010).

Biofertilizers include mainly the nitrogen-fixing, phosphate-solubilizing and plant growth-promoting microorganisms. Among the most extensively used biofertilizers are *Azotobacter*, *Azospirillum*, blue-green algae, *Azolla*, *P*-solubilizing microorganisms, *mycorrhizae*, and *Sinorhizobium* (Selvakumar et al. 2009). Among

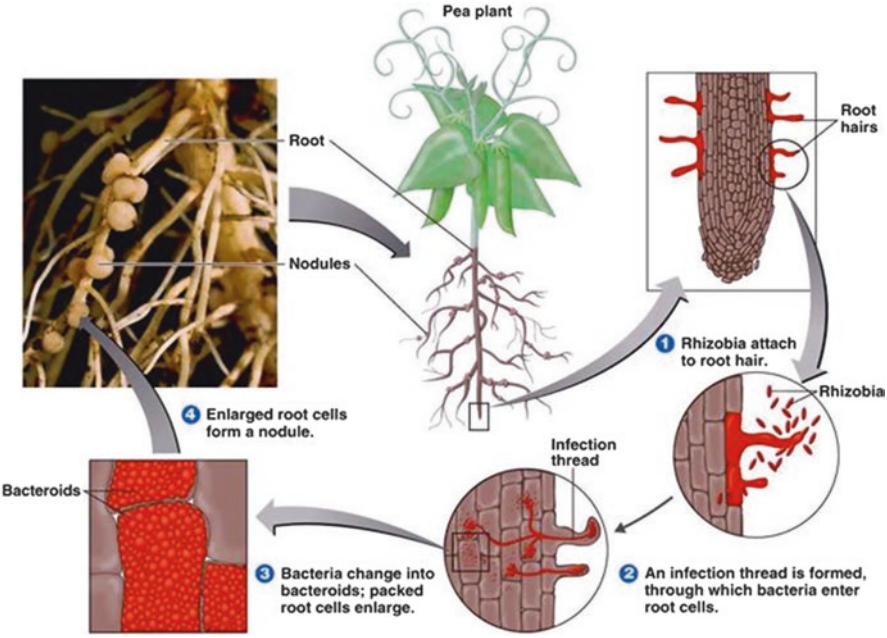


Fig. 5.4 Schematic representation of biofertilizer applications and their mechanisms in plant root ecosystem (https://image.slidesharecdn.com/soilmicrobiologyzarrin-1-140807003503-phpapp01/95/soil-microbiology-33-638.jpg?cb=1407373113)

biofertilizers, *Azotobacter* strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots (Fig 5.4).

5.6.3.1 *Azotobacter* as Biofertilizer

Azotobacter species are used as a biofertilizer for the cultivation of most agricultural crops such as cereals and pulses by direct application, by seed treatment, and by seedling dip methods because of its high nutritional conditions. *Azotobacter* increases seed's germinating ability, and it can increase germination by 20–30% because of the production of the plant growth-promoting compounds, which reduce chemical nitrogen and phosphorus by 25%, stimulating the plant growth. The direct promotion of plant growth by PGPR may include the production and release of secondary metabolites such as plant growth regulators or facilitating the uptake of certain nutrients from the root environment (Glick 1995; Polyanskaya et al. 2002).

The strains of *A. chroococcum* showed their ability to invade the endorhizosphere of wheat and higher production of cellulase and pectinase. *A. chroococcum* is beneficial for plantation as it enhanced growth and induced IAA production and phosphorus solubilization when compared with that of agrochemicals and other biofertilizers on agricultural crops (Sachin 2009). The higher concentration of agrochemical application, the lower is the plant growth (Matin et al. 2011). Different kinds of formulations have been developed from carrier material such as talc, lignite, and vermicompost which are being readily used all over the

world. Among different carrier materials used, vermicompost was the best carrier material for the survival of *A. chroococcum*, and their cells have the most significant effect on improving the growth and yield parameters of summer rice cv. IR-36 (Roy et al. 2010).

Application of PGPR and phosphate-solubilizing bacteria (PSB) combination resulted in a positive effect on plant growth. Combined application of *Azotobacter* and *Azospirillum* bacteria at different levels of nitrogen for sunflower plant showed that these two bacteria increased plant growth characteristics and reduced the application of nitrogen fertilizer by 50%. Similarly, the application of *Azotobacter* can reduce nitrogen fertilizer consumption (Yousefi and Barzegar 2014).

5.6.4 Benefits of Biofertilizers over Chemical Fertilizers

Biofertilizers are used as inoculants and alternatives to chemical fertilizer, and these inoculants increase crop yield, soil fertility, permeability, and organic matter decomposition for sustainable agricultural systems (Silva and Uchida 2000). Biofertilizers maintain the natural habitat of the soil and increase crop yield by 20–30%, and it replaces chemical nitrogen and phosphorus by 25% in addition to stimulating the plant growth. Finally, it can provide protection against drought and some soilborne diseases. They are cost-effective relative to chemical fertilizer and reduce the costs toward fertilizer use. It is an environment-friendly fertilizer that not only prevents damaging the natural source but also helps to some extent clean the nature from precipitated chemical fertilizer and can provide better nourishment to plants.

Biofertilizers provide in addition to nitrogen certain growth-promoting substances like hormones, vitamins, amino acids, etc. On the other hand, biofertilizers supply the nitrogen continuously throughout the entire period of crop growth in the field under favorable conditions over chemical fertilizer (Al-Khiat 2006). Continuous uses of chemical fertilizers adversely affect the soil structure, whereas biofertilizers when applied to soil improve the soil structure. The effects of chemical fertilizers are that they are toxic at higher doses. Biofertilizers, however, have no toxic effects. Chemical fertilizers are expensive; they disturb the ecological balance of agroecosystems and cause pollution to the environment.

References

Agarwal N, Singh HP (2002) Antibiotic resistance and inhibitory effect of *Azotobacter* on soil borne plant pathogens. Indian J Microbiol 42:245–246

Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolated of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Tur J Biol 29:29–34

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

Alimi A, Awosola O, Idowu EO (2007) Organic and inorganic fertilizer for vegetable production under tropical conditions. J Agri Rural Develop 1:120–136

Al-Khiat Ali EH (2006) Effect of cyanobacteria as a soil conditioner and biofertilizer on growth and some biochemical characteristics of tomato (*Lycopersicon esculentum* L.) seedlings. Thesis report, King Saud University. Special Publication 6: 1-4

Almon L (1958) The vitamin B12 content of *Azotobacter vinelandii*. J Nutr:643–648

Aquilanti L, Favilli F, Clementi F (2004) Comparison of different strategies for isolation and preliminary identification of *Azotobacter* from soil samples. Soil Biol Biochem 36:1475–1483

Ayoola OT, Makinde EA (2007) Complementary organic and inorganic fertilizer application: influence on growth and yield of cassava/maize/melon intercrop with a relayed cowpea. Aust J Basic Appl Sci 1(3):187–192

Bagyaraj DJ, Patil RB (1975) *Azotobacter* research in Karnataka. Curr Ther Res 4:181–184

Barazani O, Friedman J (1999) IAA is the major root growth factor secreted from plant growth mediated bacteria. J Chem Ecol 25:2397–2407

Barrera DA, Soto E (2010) Biotechnological uses of *Azotobacter vinelandii* current state limits and prospects. Afr J Biotech 9:5240–5250

Becking JH (2006) The family Azotobacteraceae. Prokaryotes 6:759–783

Beijerinck MW (1901) Fixation of free living atmospheric nitrogen by Azotobacter in pure culture. Koninel Netherlands Academic. Weteucchapp Prac 11:561–582

Bhosale HJ, Kadam TA, Bobade AR (2013) Identification and production of *Azotobacter vinelandii* and its antifungal activity against *Fusarium oxysporum*. J Environ Biol 34:177–182

Bin Zakaria AA (2009) Growth optimization of potassium solubilizing bacteria isolated from biofertilizer. Engg thesis, Universiti Malaysia Pahang

Castillo JM, Casas J, Romero E (2011) Isolation of an endosulfan- degrading bacterium from a coffee farm soil: persistence and inhibitory effect on its biological functions. Sci Total Environ:20–27

Cavaglieri LR, Andres L, Ibanez M, Etcheverry MG (2005) Rhizobacteria and their potential to control *Fusarium verticillioides*, effect of maize bacterisation and inoculums density. Antonie Van Leeuwenhoek 87:179–187

Chandrasekar BR, Ambrose G, Jayabalan N (2005) Influence of biofertilizers and nitrogen source level on the growth and yield of *Echinochloa frumentacea* (Roxb.) link. J Agri Tech 1(2):223–234

Chennappa G, Adkar-Purushothama CR, Suraj U, Tamilvendan K, Sreenivasa MY (2013) Pesticide tolerant *Azotobacter* isolates from paddy growing areas of northern Karnataka, India. World J Microbiol Biotechnol 30:1–7

Chennappa G, Adkar-Purushothama CR, Naik MK, Sreenivasa MY (2014) Impact of pesticides on PGPR activity of *Azotobacter* sp. isolated from pesticide flooded paddy soils. Greener J Biol Sci 4(4):117–129

Chennappa G, Naik MK, Sreenivasa MY (2015) Azotobacter -PGPR activities with special reference to effect of pesticides and biodegradation. Microbial inoculants in sustainable agricultural productivity Vol-II, functional applications (Microbial inoculants as biofertilizers and biopesticides). Springer Book Ser 13(1):229–244

Chennappa G, Naik MK, Adkar-Purushothama CR, Amaresh YS, Sreenivasa MY (2016) PGPR, abiotic stress tolerant and antifungal activity of *Azotobacter* sp. isolated from paddy soils. Indian J Exp Biol 54:322–331

Contra C (2003) Biofertilizers and mycorrhizae. Plant Physiol:1–4

Garg SK, Bhatnagar A, Kalla A, Narula N (2001) *In vitro* nitrogen fixation, phosphate solubilization, survival and nutrient release by *Azotobacter* strains in an aquatic system. Bioresour Technol 80:101–109

Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Acad Sci Eng Technol 49:19–24

Ghosh PG, Sawant NA, Patil SN, Aglave BA (2010) Microbial biodegradation of organophosphate pesticides. Int J Biotech Biochem 6:871–876

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

Gul FS (2003) Growth and nitrogen fixation dynamics of *Azotobacter chroococcum* in nitrogen free and OMW containing medium. The Middle East Technical University. 1–12
Heike B (2007) Microbial biofertilizers and their potential in sustainable agriculture. Rutgers:1–20
Herter S, Schmidt M, Thompson ML (2011) A new phenol oxidase produced during melanogenesis and encystment stage in the nitrogen-fixing soil bacterium *Azotobacter chroococcum*. Appl Microbiol Biotechnol 90:1037–1049
Javed AM, Hafiz NA, Shahzad K, Arshad M (2009) Role of plant growth promoting rhizobacteria applied in combination with compost and mineral fertilizers to improve growth and yield of wheat (*Triticum aestivum* L.) Pak J Bot 41(1):381–390
Jen-Hshuan C (2006) The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International workshop on sustained management of the soil rhizosphere system for efficient Crop production and fertilizer Use 16(20): 1–10
Jimenez DJ, Montana JS, Martinez MM (2011) Characterization of free nitrogen fixing bacteria of the genus *Azotobacter* in organic vegetable grown Colombian soils. Braz J Microbiol 42:846–858
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
Khan HR, Mohiuddin M, Rahman M (2008) Enumeration, isolation and identification of nitrogen fixing bacterial strains at seedling stage in rhizosphere of rice grown in non-calcareous grey flood plain soil of Bangladesh. J Fac Env Sci Tech 13:97–101
Kizilkaya R (2009) Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J Env Biol 30:73–82
Kloepper JW, Schroth MN (1980) Plant growth promoting rhizobacteria on radishes. Proc Int Conf Plant Pathol Bacteriol 2:879–882
Kraepiel A, Bellenger J, Wichard T, Morel F (2009) Multiple roles of siderophores in free living nitrogen-fixing bacteria. Biometals 22:573–581
Kumar V, Behl RK, Narula N (2000) Establishment of phosphate solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol Res 156:87–93
Li DY, Eberspacher J, Wagner B, Kuntzer J, Lingens F (1991) Degradation of 2,4, 6-Trichlorophenol by *Azotobacter* sp. strain GP1. App Env Microbiol:1920–1928
Lindemann WC, Glover CR (2008) Nitrogen fixation by legumes. Electron Distrib 5:1–4
Lopez JG, Toledo MV, Reina S, Salmeron V (1981) Root exudates of maize on production of auxins, gibberellins, cytokinins, amino acids and vitamins by *Azotobacter chroococcum* chemically defined media and dialysed soil media. Toxicol Environ Chem 33:69–78
Mali GV, Bodhankar MG (2009) Antifungal and phytohormone potential of *Azotobacter chroococcum* isolates from ground nut (*Arachis hypogeal* L.) rhizosphere. Asian J Exp Sci 23:293–297
Marianna M, Veres S, Gajdos E, Bakonyi N, Toth B and Levai L (2005) The possible role of biofertilizers in agriculture. Ratarstvo 585–588
Matin XM, Sumathi CS, Kannan VR (2011) Influence of agrochemical and *Azotobacter* spp. application on soil fertility in relation to maize growth under nursery conditions. Eurasian J Biosci 5:19–28
Mikkelsen R, Hartz TK (2008) Nitrogen sources for organic crop production. Better Crops 92:16–19
Mirzakhani M, Ardakani MR, Band AA, Rejali F, Rad SAH (2009) Response of spring safflower to co-inoculation with *Azotobacter chroococcum* and *Glomus intraradices* under different levels of nitrogen and phosphorus. Am J Agri Biol Sci 4:255–261
Mollmann U, Heinisch L, Bauernfeind A, Kohler T, Ankel-Fuchs D (2009) Siderophores as drug delivery agents: application of the Trojan horse strategy. Biometals 22:615–624
Mulder EG, Brontonegoro (1974) Free living heterotrophic nitrogen fixing bacteria. Biol Nitro Fix 57:205–222
Murcia R, Rodelas B, Salmeron V, Toledo MVM, Lopez GJ (1997) Effects of herbicide simazine on vitamin production by *Azotobacter chroococcum* and *Azotobacter vinelandii*. App Soil Eco 6:187–193

Myresiotis CK, Vryzas Z, Mourkidou EP (2012) Biodegradation of soil applied pesticides by selected strains of plant growth promoting rhizobacteria (PGPR) and their effects on bacterial growth. Biodegradation 23:297–310

Nagaraja H, Chennappa G, Rakesh S, Naik MK, Amaresh YS, Sreenivasa MY (2016) Antifungal activity of Azotobacter nigricans against trichothecene-producing Fusarium species associated with cereals. Food Sci Biotechnol 25(4):1197–1204

Naik MK, Rajalaxmi K, Amaresh YS (2013) Search for 2, 4 DAPG positive genes in fluorescent *Pseudomonas* and their exploitation for sustainable disease management. Recent advances in biofertilizer and bio fungicides (PGPR) for sustainable agriculture. Proc 3rd Asian PGPR conference on plant growth promoting rhizobacteria (PGPR) and other microbials. 21–24

Nakkeeran S, Fernando WG, Siddique Z (2005) Plant growth promoting Rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. Biocontrol Biofertilization:257–296

Naveen KA, Samina M, Raffaella B (2010) Bioformulations for sustainable agriculture. Springer, New Delhi, pp 1–30. doi:10.1007/978-81-322-2779-3

Page WJ, Shivprasad S (1991) Azotobacter salinestris spp. nov., a sodium dependent, micro aerophilic and aero adaptive nitrogen fixing bacteria. Int J Syst Bacteriol 41:369–376

Page W, Von Tigerstrom M (1988) Aminochelin, a catecholamine siderophore produced by *Azotobacter vinelandii*. J Gen Microbiol 134:453–460

Patil NM (2010) Biofertilizer effect on growth, protein and carbohydrate content in Stevia *Rebaudiana Var Bertoni*. Recent Res Sci Tech, 2(10):42–44

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

Polyanskaya LM, Vedina OT, Lysak LV, Zuyagintsuv DG (2002) The growth promoting effect of *Beijerinck mobilis* and *Clostridium* sp. cultures on some agricultural crops. Microbiol 71:109–115

Ramaswami P, Mathan K, Nair K (1977) *Azotobacter* population in red and black soils of Tamilnadu. Mys J Agri Sci 11:364–366

Rangaswami G, Sadasivan KV (1964) Studies on occurrence of *Azotobacter* in soil types. J Indian Soci Soil Sci 12:43–49

Revillas JJ, Rodelas B, Pozo C, Toledo MV, Gonalez-Lopez J (2000) Production of B-group vitamins by two *Azotobacter* strains with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. J Appl Microbiol 89:486–493

Rifat H, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion. Ann Microbiol:1–12

Roy DB, Deb B, Sharma GD (2010) Evaluation of carrier based inoculants of *Azotobacter chroococcum* strain SDSA-12/2 in improving growth and yield summer (ahu) rice cv. IR-36. Biofrontiers 1:36–40

Saadatnia H, Riahi H (2009) Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. Plant Soil Environ 55(5):207–212

Sachin DN (2009) Effect of *Azotobacter chroococcum* (PGPR) on the growth of bamboo (*Bambusa bamboo*) and maize (*Zea mays*) plants. Biofrontiers 1:24–31

Saeed AA, Asghari B, Muhammad F, Muhammad A, Aftab A (2004) Comparative study of the effects of biofertilizers on nodulation and yield characteristics of mung bean (*Phaseolus Vulgaris* L.) Int J Agri Biol 6:837–842

Selvakumar G, Lenin M, Thamizhiniyan P, Ravimycin T (2009) Response of biofertilizers on the growth and yield of black gram (vigna mungo L.) Recent Res Sci Technol 1(4):169–175

Shakhashiri (2003) Agricultural fertilizers: nitrogen, potassium, and phosphorus. Chemistry:1–3

Silva JA, Uchida R (2000) Chapter 12, University of Hawaii

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

Tejera NC, Lluch MV, Martinez T, Gonzalez JL (2005) Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. Plant Soil 270:223–232

Thomas MB, David L, Clement, Kevin GW (1990) Organic and inorganic fertilizers. Fact Sheet 837:1–3

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal SC, Mittal S, Tripathi AK, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci 89:136–150

Upadhyay SK, Singh DP, Saikia R (2009) Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Cur Microbiol. https://doi.org/10.1007/s00284-009-9464-1

Vidhyasekaran, Muthamilan (1995) Development of formulations of Pseudomonas fluorescens for control of chickpea wilt. The American phytopathological society

Vidhyasekaran P, Rabindran R, Mathamilan M, Nayar K, Rajappan K, Subraminan N, Vasumathi K (1997) Development of a powder formulation of Pseudomonas Fluorescens for control of rice blast. Plant Pathol 46:291–297

Villa JA, Ray EE, Barney BM (2014) *Azotobacter vinelandii* siderophore can provide nitrogen to support the culture of the green algae *Neochloris oleoabundans and Scenedesmus* sp. BA032. FEMS Microbiol Lett 351(1):70–77

wikipedia.org/wiki/Fertilizer

www.fao.org/docrep/003/x6905e/x6905e.htm

Yousefi, Barzegar (2014) Effect of *Azotobacter* and *Pseudomonas* bacteria inoculation on wheat yield under field condition. Int J Agricul Crop Sci 7(9):616–619

Rhizobacterial Phosphate Solubilizers in Sustainable Agriculture: Concepts and Prospects

6

B.L. Raghunandan

Abstract

Phosphorous is the second most important macroelement in plant nutrition. The availability of the P present in the soil and being applied through chemical fertilizers is very poor due to fixation in acid and alkaline soils and occurs in insoluble state. Soil microorganisms play a central role in biogeochemical cycling of P in soil which converts unavailable form to available form and enables plant for the uptake. Rhizobacterial strains *Bacillus* sp. and *Pseudomonas* sp. and soil fungi *Aspergillus* sp. and *Penicillium* sp. are the key representatives of potential phosphate-solubilizing microbes. Solubilization of P is a complex process which depends on physiological and nutritional attributes of the strain. Organic acid production is the principal mechanism of solubilization carried out by majority of documented phosphate-solubilizing microorganisms (PSM). Besides inorganic acids, siderophores and phosphatases mediate solubilization. In the current scenario, genetic manipulation of the strain to improve P solubilization efficacy is a promising strategy. PSM have also been reported to enhance the plant growth through the production of growth-promoting substances and phytohormones. Moreover, the development of consortia of PSM with other beneficial microflora having multiple benefits would attract the farming community and helps in making the agriculture production system more sustainable.

Keywords

Soil phosphorous • Phosphate solubilization • Organic acids • Plant growth promotion • Biofertilization

B.L. Raghunandan (✉)
AICRP on Biological Control of Crop Pests, Anand Agricultural University, Anand, Gujarat, India
e-mail: raghumic2@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_6

6.1 Introduction

Phosphorous is the 11th most abundant natural element in the Earth's crust and is one among the macroelement essential for plant growth. P is an important key element, and most limiting macronutrient in plant nutrition next to nitrogen makes up about 0.2% of plant dry weight (Schachtmam et al. 1998) and plays an inevitable role in major metabolic processes in plants (Khan et al. 2010) and nitrogen fixation in plants (Saber et al. 2005). Being a component of cell constituents, phosphorous plays a major role in key processes of biological growth and development of plants, viz. photosynthesis, respiration, energy storage and transfer, seed germination, early root formation and flower and fruit formation (Ehrlich 1990).

P is abundant in soils in organic and inorganic forms, and it is a major limiting nutrient element as the great portion of soil P is unavailable for plant uptake. Primary minerals represent the inorganic form of phosphorous in soil such as apatite, hydroxyapatite and oxyapatite, and the main attribute of these minerals is the insolubility and found associated with the surface of hydrated oxides of Fe, Al and Mn which are poorly soluble and assimilable in nature. Inorganic P is present in soil as insoluble mineral complexes, and precipitated forms are cannot be absorbed by plants (Rengel and Marschner 2005). Organic matter of the soil constitutes a second major component of soil P, and organic form of P accounts for 20–80% of total P present in the soil (Richardson 1994a). Inositol phosphate synthesized by microorganisms and plants is the most stable and major component of organic P in the soil (Harley and Smith 1983). Others are phosphomonoesters, phosphodiesters and phosphotriesters.

The quantity of phosphorous present in soil ranging from 400 to 1200 mg/kg of soil (Begon et al. 1990) and in soil solution even at relatively high levels presents in the range of 0.3–3.0 kg/ha (Ross and Middleton 2013), and a large part of this is in insoluble or unavailable form. The P requirement of plants varies with different types of plants. Generally legumes have relatively higher P requirement than grasses ranging between critical values of 0.25–0.30% and 0.20–0.25%, respectively (Richardson 2004).

The plant cell might take up several forms of phosphorous, but the major part absorbed in the forms of HPO_4^{2-} and $H_2PO_4^-$ (Beever and Burns 1980). Generally the concentration of soluble P is very less in soil at the levels of 1 ppm or less (10 M $H_2PO_4^-$) (Goldstein 1996). According to soil pH, the forms of pi exists also change. Under soil pH 6.0, most pi will be present as monovalent species H_2PO_4, and the rate of plant uptake is also high in the pH range of 5.0–6.0 (Furihata et al. 1992). The phosphorous availability to plants not only depends on the amount of phosphorous in soil but also on the solubility in the soil. Out of total P that exists in soluble form in soil, only 0.1% is available for plant uptake because of P fixation in soil. The two main reactions by which P becomes unavailable to plants are fixation and immobilization. Seventy to ninety percent of chemical P fertilizer applied gets fixed in the soil and unavailable for plant uptake (Stevenson 1986; Bhagyaraj and Verma 1995; Holford 1997). Similarly great amount of soil P is also converted to inositol hexaphosphate, a major organic component, thus getting immobilized and not available for plant uptake (Richardson 1994b).

In India about 98% of cultivated land is deficient in available P, and only 1–9% has high P content (Sharma et al. 2013). Due to fixation and immobilization of soluble P in the soil, the available P is supplemented by frequent and regular application of chemical P fertilizers which represent high cost of production and also impose negative impact on soil health, microbial diversity and soil fertility and thereby deterioration of resources in terrestrial freshwater and marine ecosystem eutrophication (Tilman et al. 2001).

The efficiency of phosphatic fertilizers rarely exceeds 30% mainly due to fixation in the form of iron or aluminium phosphate in acidic soils (Norrish and Rosser 1983) or in the form of calcium phosphate in neutral to alkaline soils (Lindsay et al. 1989). It is the fact that only 1% of the total soil P is incorporated into plant biomass during the plant growth which indicates the low availability of P for the plant nutrition (Blake et al. 2000), and it has been calculated that that the world's reserves of high-quality phosphatic rock may be depleted within this century (Cordell et al. 2009). Consequently the processing of the low-grade rocks requires high costs (Isherwood 2000). Hence, all these problems associated with chemical phosphatic fertilizer have led to the search for eco-friendly and economically feasible strategies to improve the crop production. The use of soil microorganisms having phosphate-solubilizing ability is an eco-friendly and environmentally compatible approach. The use of microorganisms has been promoted to ensure reduction in the usage of chemical P fertilizers as they play a central role in improving the soil with concurrent sustenance of crop production and productivity. Microorganisms form an important component of phosphorous cycle which occurs by means of oxidation and reduction reactions (Ohtake et al. 1996) and are integral components for the transfer of P between different pools of soil phosphorous (Fig. 6.1). The phenomenon of mineral phosphate solubilization refers to the conversion of inorganic unavailable phosphate into available, viz. $H_2PO_4^-$ and HPO_4^{2-} for plant uptake. Microorganisms are capable of converting organic and inorganic P (Khan et al. 2009), thereby facilitating the uptake by plant root. Hence the understating of

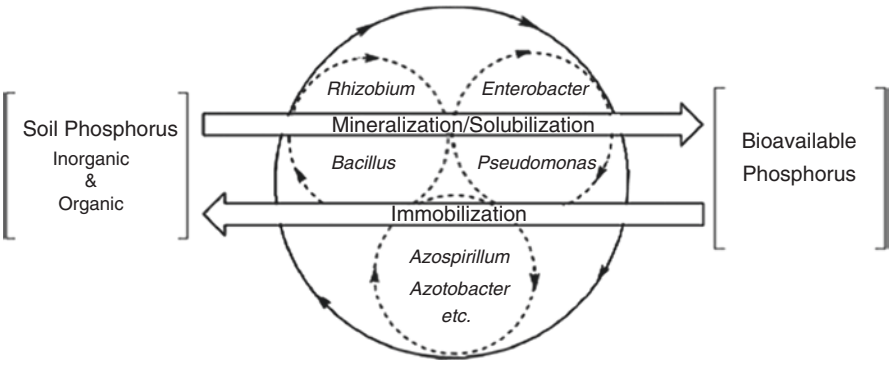


Fig. 6.1 Schematic representation of P solubilization and immobilization by soil microflora (Khan et al. 2007)

plant-soil-microbial P cycle to exploit the rich potential of microorganisms is most needed to achieve sustenance in crop production.

6.2 Phosphate-Solubilizing Microorganisms

Soil microorganisms play a significant and fundamental role in biogeochemical cycling of P in soil. Phosphate-solubilizing microorganisms (PSM) have become an important component of integrated nutrient management system which specifically increases the availability of P for the crops. Nowadays research has focused on the introduction of free-living organisms that form non-specific beneficial association with a wide range of crop plants that can be easily mass produced and have the high persistence potential in the soil environment (Khan et al. 2010). Pikovskaya (1948) reported the solubilization of phosphorous by microorganisms, and different kinds of soil microorganisms are involved in the transformation of soil P. Through the process of solubilization and mineralization, microorganisms release P from inorganic and organic pools of P (Hilda and Fraga 1999). Plant rhizosphere harbours the considerable populations of phosphate-solubilizing microorganisms (Sperber 1958; Alexander 1977). Considerable attention has been received by the agriculturists with regard to phosphate-solubilizing microorganisms as soil inocula which enhance the plant growth and yield (Goldstein et al. 1999; Fasim et al. 2002; Gyaneshwar et al. 2002).

In the last two decades, several reports have thrown light on phosphate-solubilizing microorganisms (Richardson 2004; Rodriguez and Fraga 1999). Various strains of bacteria and fungi have been studied and characterized for P-solubilizing capabilities (Glick 1995; He et al. 1997) (Table 6.1). Fungi *Aspergillus* and *Penicillium* and bacterial species of *Pseudomonas* and *Bacillus* are reported to be the dominant species of phosphate solubilization (Illmer and Schinner 1992; Wakelin et al. 2004). These are ubiquitous and vary in mineral phosphate-solubilizing ability, and generally these organisms are isolated from rhizosphere soils, rhizoplane, phyllosphere and rock phosphate deposits using serial dilution plate method and enrichment culture technique (Zaidi et al. 2009).

During the isolation of phosphate-solubilizing microorganisms, the source of insoluble phosphate in the culture media is a major issue of controversy. The commonly used tricalcium phosphate is relatively weak and unreliable as universal selection factor for the isolation of phosphate-solubilizing microorganisms. The tricalcium phosphate yields many isolates of PSM, and when these isolates are further tested, only few isolates are phosphate solubilizers, because soils generally vary in pH and chemical properties, and there is no metal phosphate compound that can serve as universal selection factor. The selection of metal phosphate will depend on the type of soil where PSM will be used. For alkaline soils, calcium phosphate compounds and rock phosphate, for acidic soils iron or aluminium phosphate compounds, and phytates for organic rich soils are suggested (Bashan et al. 2013a, b).

The strains which are exhibiting P solubilization activity are detected by the formation of clear zone of solubilization around the colonies (Fig. 6.2). By using

Table 6.1 Phosphate solubilizing potential of PSM (Krishnaraj and Dahale 2014)

Sl. no	Strains	Mineral phosphate solubilization (MPS) potential
1	*Acetobacter liquefaciens*	72.9 mg/ml
2	*Acetobacter* sp.	63.8 mg/ml
3	*Acinetobacter* sp.	334–443.26 µg/ml
4	*Azotobacter chroococcum*	1.10–98.11 µg/ml
5	*Bacillus* sp.	236–395 mg/ml
6	*Burkholderia anthina*	>600 µg/ml
7	*Burkholderia cepacia*	250–375 mg/ml
8	*Burkholderia* sp.	0–200 µg/ml
9	*Enterobacter* sp.	568–642 µg/ml
10	*Gluconacetobacter* sp.	180 µg/ml
11	*Micrococcus* sp.	122.4–396.57 µg/ml
12	*Pantoea agglomerans*	62.76–338 mg/ml
13	*Pseudomonas cepacia*	35 mg/ml
14	*Pseudomonas chlororaphis*	493 µg/ml
15	*Pseudomonas fluorescens*	322–520 µg/ml
16	*Pseudomonas gladioli*	68.8 mg/ml
17	*Pseudomonas striata*	156 mg/ml
18	*Rhizobium meliloti*	120–620 µg/ml
19	*Rhizobium* sp.	155–840 µg/ml

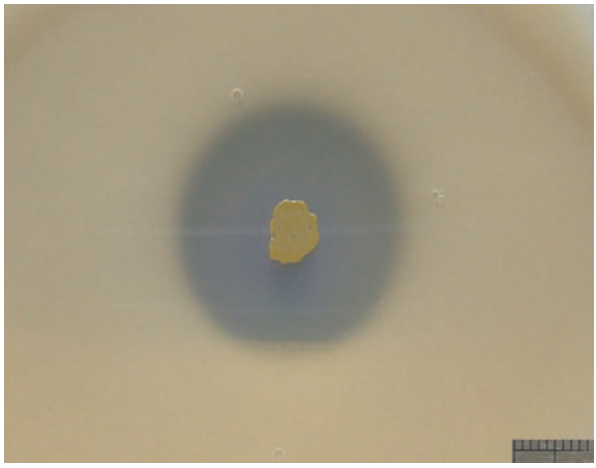


Fig. 6.2 Clear zone of P solubilization by rhizobacterial strain (Zhu et al. 2011)

plate screening methods, the visual detection and semi-quantitative estimation of P solubilization ability can be made, in which clear zone around the microbial colonies in the media contains insoluble mineral source as P source (Ostwal and Bhide 1972), and this method is generally followed for isolation and basic characterization of PSM (Goldstein and Liu 1987). Gupta et al. (1994) reported an improved method

using bromophenol blue dye. In this method, yellow halos are formed around the colonies in response to drop in pH due to production of organic acids, and with this method, more reproducible and correlated results have been obtained than single halo method. The production of zone of solubilization is not only the sole criteria for the consideration. The additional test in liquid media to assay P dissolution should be carried out, and isolates obtained after such tests should be further tested for production of organic acids (Bashan et al. 2013a, b).

The studies on dynamics of phosphate solubilization by bacterial strains have been carried out based on the amount of P release into culture broth using an insoluble compound as the sole source of P. The rate of solubilization is estimated by subtracting the final P concentration from the initial P supplied by the substrate. But this estimation has drawback of not taking into account the P utilized by the cells. Further, the kinetic studies of P accumulation and release would offer a clear picture of phosphate solubilization behaviour. Once a potential strain is identified, it should be further tested for direct contribution to P nutrition in plants and not necessarily to plant growth promotion as PSM can influence plant growth by other mechanisms (Bashan et al. 2013a), and the capability of strain to solubilize insoluble phosphorous is not necessarily correlated with the plant growth promotion attributes (Collavino et al. 2010).

Several bacteria, fungi and actinomycetes and few algae exhibited phosphate-solubilizing capacity. Phosphate-solubilizing bacteria constitute 1–50% of the total microbial population in the soil, and fungi constitute around 0.1–0.5% (Chen et al. 2005). Rhizospheric soil is rich in metabolically active PSM than non-rhizospheric soil (Raghu and MacRae 1966). Among bacterial species, *Pseudomonas* and *Bacillus* are the dominating species, and other bacteria reported are *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Delfia* sp. (Chen et al. 2006), *Azotobacter* (Kumar et al. 2001), *Xanthomonas* (De Freitas et al. 1997), *Enterobacter*, *Pantoea* and *Klebsiella* (Chung et al. 2005). The legume symbiotic bacteria *Rhizobia* have also shown phosphate solubilization activity (Zaidi et al. 2009), and many halophilic bacteria, e.g. *Kushneria sinocarni* isolate from Daqiao salt sediment on the eastern coast of China, exhibited the P solubilization and which may found promising in salt-affected soils (Zhu et al. 2011).

Unlike P-solubilizing bacteria, soil fungi having phosphate-solubilizing ability do not lose the activity even after repeated subculturing in the laboratory. Moreover fungi are able to traverse in the soil more easily than bacteria (Kucey 1983). Generally the P-solubilizing fungi produce more organic acids than bacteria and hence greater P solubilization activity (Venkateswarlu et al. 1984). *Aspergillus* and *Penicillium* are the representative filamentous fungi in the soil involved in phosphate solubilization, although strains of *Trichoderma* (Altomare et al. 1999) have also been reported as P solubilizers. The mycorrhizal fungi are reported to help in phosphorous acquisition which increases the phosphorous uptake, produces plant growth-promoting substances and protects against soilborne plant pathogens (Fankem et al. 2006). Among soil yeasts, *Yarrowia lipolytica* (Vassilev et al. 2001), *Schizosaccharomyces pombe* and *Pichia fermentans* have been reported to have phosphate solubilization activity, but only few studies have been conducted on

yeasts to assess their ability to solubilize phosphates. As more studies are conducted, the great diversity of yeasts and filamentous fungi could be expected. In recent years, the group actinomycetes has attracted the interest of researchers as this group is capable of surviving in extreme environments and also possesses potential benefits such as production of phytohormones and antibiotics that would greatly benefit the plant growth (Fabre et al. 1988; Hamdali et al. 2008a). Hamdali et al. (2008a) reported the genera *Streptomyces* and *Micromonospora* as potential P solubilizers, and they indicated that nearly 20% soil actinomycetes are capable of solubilizing P. In addition to bacteria, fungi and actinomycetes, cyanobacteria and mycorrhiza have also exhibited P solubilization activity (Widada et al. 2007). Hence, the great diversity has been documented in the varied ecological niches, and there is an ample scope to exploit new potent strains from varied ecosystems in the near future.

6.3 Phosphate Solubilization

The microorganisms from diverse ecological niches have the capacity to solubilize unavailable forms of P into available forms of P that can be taken up by crop plants. The major processes of the soil P cycle that affect soil solution P levels are (1) dissolution and precipitation, (2) sorption and desorption and (3) mineralization and immobilization (Sims and Pierzynski 2005). The different mechanisms employed by the microorganisms to solubilize phosphates are (1) release of mineral-dissolving compounds, e.g. organic acids, siderophores, protons, hydroxyl ions and CO_2, (2) release of extracellular enzymes and (3) release of P through substrate degradation (McGill and Cole 1981). The phosphate-solubilizing microbes serve as source of P to plants upon mineralization. Release of immobilized P occurs when the cell dies due to environmental changes, starvation and predation. Drying and wetting and freezing and thawing result in flush events, a sudden release in available P in soil solution due to lysis of microbial cells (Butterly et al. 2009).

6.3.1 Inorganic P Solubilization

Microorganisms solubilize fixed phosphates mainly by organic acid production either by (1) lowering the pH, (2) chelation of cations bound to P, (3) competing for adsorption sites on soil with P and (4) formation of soluble complexes with metal ions associated with insoluble P (Ca, Al and Fe), and consequently P is released. Microorganisms produce organic acids via direct oxidation pathway on the outer cytoplasmic membrane (Zaidi et al. 2009) and lower the pH of the medium indicating P solubilization (Mahila et al. 2004). Organic acids are the products of microbial metabolism mainly by oxidative respiration or by fermentative metabolism of organic carbon (e.g. glucose) (Trolove et al. 2003). The organic acid causes acidification of the microbial cell and eventually decreases pH. The type and amount of acid produced greatly vary with the type of microorganisms and type of carbon

Table 6.2 Organic acids produced by PSM

Organic acids	PSM strain	References
Gluconic acid	*Pseudomonas* sp., *Pseudomonas cepacia*	Illmer and Schinner (1992)
2-Ketogluconic acid	*Rhizobium leguminosarum*, *Rhizobium meliloti*, *Bacillus firmus*	Halder et al. (1990)
Lactic acid	*Bacillus licheniformis*, *Bacillus amyloliquefaciens*	Illmer and Schinner (1992)
Isovaleric acid, isobutyric acid, acetic acid	*Bacillus licheniformis*, *Bacillus amyloliquefaciens*	
Citric acid	*Pseudomonas* sp.	Chen et al. (2006)
Propionic acid	*Bacillus megaterium*	

source in the medium (Patel et al. 2008) (Table 6.2), and the amount of soluble phosphorous released depends on the type and strength of organic acid released.

In gram-negative bacteria, the extracellular oxidation of glucose via the quinoprotein glucose dehydrogenase to gluconic acid is the major mechanism of mineral phosphate solubilization (Puente et al. 2004a, b). The organic acids produced dissociate in pH-dependent equilibrium into respective proton(s) and anion(s) and thereby lowering the pH of the solution. Under laboratory conditions, the production of organic acids in the medium supplemented with calcium phosphate is indicated by the decrease in pH of the media, and the efficiency of pi release is quite dependent on the type of acids like phenolic or aliphatic rather than total acidity. Phenolic acids and citric acids have been found less effective than aliphatic acids. The most frequent acids observed during mineral phosphate solubilization are gluconic acids and 2-ketogluconic acids, and mixture of lactic, isovaleric, isobutyric, acetic, glyoxylic, oxalic, malonic, fumaric, pyruvic, tartaric and succinic acids were also found (Mardad et al. 2013).

The production of organic acids by microorganisms can be detected by enzymatic and high-performance liquid chromatography methods (Parks et al. 1990; Whitelaw 2000). Further it is observed that acidification is not only the mechanism of P solubilization as the reduction in pH levels did not correlate with phosphate-solubilizing ability (Subbarao 1982). Altomare et al. (1999) investigated the biocontrol potential and plant growth-promoting attribute of the strain *Trichoderma harzianum* T-22 under in vitro and identified the P-solubilizing capability of the strain. There was a reduction in pH of the medium, and further organic acids were not detected in the culture filtrate, and hence authors inferred that acidification was probably not the sole mechanism of P solubilization. The solubilization of insoluble P by inorganic acid, e.g. HCl, has also been documented, but the efficacy of solubilization is reported to be less than citric acid or oxalic acid at the same pH (Kim et al. 1997). *Nitrosomonas* and *Thiobacillus* were reported to solubilize phosphate by producing nitric and sulphuric acids (Azam and Memon 1996). Production of H2S (Swaby and Sperber 1958) and microbial sulphur oxidation (Rudolph 1922) have been suggested to solubilize insoluble phosphates. However, the efficacy has been less than organic acids (Kim et al. 1997). The inorganic acids, viz. sulphuric,

nitric and carbonic acids, are reported as inorganic acids involved in phosphate solubilization by some strains (Fankem et al. 2006), but the effectiveness and contribution to P release in soils seem comparatively less than organic acids. Humic and fulvic acids are good chelators of calcium, iron and aluminium present in insoluble phosphates (Gyaneshwar et al. 1999).

Parks et al. (1990) proposed that the concept of excretion of H^+ from NH_4 assimilation could be the alternative mechanism of P solubilization. Krishnaraj et al. (1998) highlighted protons as the major factor involved in P solubilization as they are pumped out of the cell. Besides the chelating substances produced, viz. H_2S, CO_2, mineral acids, siderophores and hormones like indole acetic acid, gibberellins and cytokinins (Kucey et al. 1989), have been correlated with phosphate solubilization.

6.3.2 Organic P Solubilization

Organic P solubilization is also referred to as mineralization of organic phosphorous. Enzymes are mainly involved in the release of P from organic compounds.

Phosphatases (Phosphohydrolase) These enzymes dephosphorylate phosphoester or phosphoanhydride bonds of organic matter. Phosphomonoesterases (phosphatases) (EC. 31.3.1) are the major classes of enzyme release by PSM (Nannipieri et al. 2011). Depending on pH optima, these enzymes are classified as acid phosphatases and alkaline phosphatases, and both can be produced by PSM depending on the external environment (Jorquera et al. 2011). In acidic soils, acid phosphatases predominate, whereas in alkaline soils, alkaline phosphatases are more abundant (Eivazi and Tabatabai 1977). Plant roots can also produce acid phosphatases and rarely produce large quantities of alkaline phosphatases suggesting a potential niche for PSM (Criquet et al. 2004). But some reports indicated that phosphatases of microbial origin have greater affinity for organic phosphate compounds than those of plant root origin (Tarafdar et al. 2001).

Phytases Phytases release P from degradation of phytate. Being a major component of organic P in the soil, phytate is a major source of inositol and a major stored form of P in plant seeds and pollen. The ability of the plants to uptake P from phytate is very limited. Richardson et al. 2001 reported the significant growth and P nutrition in *Arabidopsis* plant supplied with phytate, when they were genetically transformed with phytase gene (*phyA*) of *Aspergillus niger*. Hence, microorganisms play a key role in mineralization of phytate in soil, and their presence in rhizosphere may help plants to acquire P directly from the phytate (Richardson and Simpson 2011).

Phosphatases and C-P Lyases These enzymes are involved in the cleavage of C-P bond of organophosphate compounds (Rodriguez et al. 2006).

6.3.3 Siderophores and EPS in P Solubilization

Siderophores are small molecular weight iron-chelating compounds that scavenge iron from minerals/organic compounds forming Fe^{3+} complexes and absorb through active transport mechanism, e.g. ferrichrome, pyochelin, pyoverdine, etc. These are produced mainly in response to iron limitation (Miethke and Marahiel 2007, Indiragandhi et al. 2008), and about 500 different types of siderophores have been reported which are produced mainly by bacteria and few fungi. Various researchers have reported the production of siderophores by phosphate-solubilizing microorganisms (Vassilev et al. 2006; Caballero-Mellado et al. 2007; Hamdali et al. 2008b). Although the direct role of siderophores in phosphate solubilization is not been widely implicated, the mechanism of exchange of ligand by organic acid anion plays a role in phosphate solubilization and P availability to the plants (Parker et al. 2005).

Yi et al. (2008) studied the role of exopolysaccharides (EPS) produced by microorganisms in relation to phosphate solubilization. EPS are polymers either homo- or heteropolysaccharides excreted by microbial cells outside of their cell wall. The structure and composition of EPS vary, having different organic and inorganic constituents (Sutherland 2001). The bacterial strains having TCP (tricalcium phosphate)-solubilizing capability (*Enterobacter* sp. *(EnHy-401)*, *Arthrobacter* sp. *(ArHy-505)*, *Azotobacter* sp. *(AzHy-510)* and *Enterobacter* sp. *(EnHy-402)*) were used to study the role of EPS in phosphate solubilization and demonstrated the strong EPS production and phosphate solubilization capability. However, further detailed research on role of EPS in P solubilization is inevitable (Fig. 6.3).

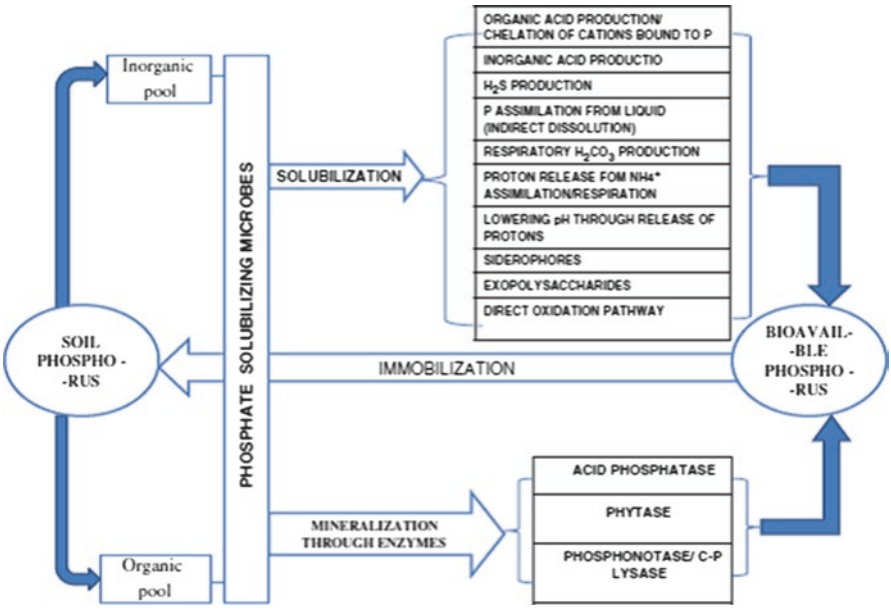


Fig. 6.3 Overview of mechanisms of P solubilization and immobilization by PSM (Sharma et al. 2013)

6.4 Genetics of PSM

In majority of bacteria, the phosphate-solubilizing capacity has been correlated with the production of organic acids (Rodriguez and Fraga 1999). The direct oxidation of glucose to gluconic acid is the key mechanism for mineral phosphate solubilization (Goldstein 1996). The genes involved in mineral and organic phosphate solubilization have been isolated and characterized. The manipulation of genes through genetic engineering followed by expression in selected rhizobacterial strain is a promising perspective to obtain the strain with increased phosphate-solubilizing ability and hence effective use of these inoculants in agriculture.

Goldstein and Liu (1987) were pioneers in genetic engineering of phosphatase genes and first isolated phosphate-solubilizing gene (Mps) from the bacteria *Erwinia herbicola* and isolated gene produced gluconic acid in *E. coli* HB101 and exhibited the solubilization of hydroxyapatite. Similarly *gabY* gene from *Pseudomonas cepacia* was isolated and expressed in *E. coli* HB101 (Babu-Khan et al. 1995). Similarly napA gene phosphatase gene from bacterium *Morganella morganii* and transferred to biofertilizer strain *Burkholderia cepacia* IS-16 and enhanced extracellular phosphatase activity was observed in recombinant strain. Introduction and overexpression of phosphate-solubilizing genes in natural rhizosphere bacteria is a promising approach to improve the efficacy of P solubilization. Cloning and expression of P-solubilizing genes into the strains that do not have the capability may become alternate approach to current need of developing microbial consortium, viz. nitrogen fixers and phosphate solubilizers (Bashan et al. 2000). To achieve successful gene insertions, the barriers such as dissimilarity of metabolic pathways and difference regulatory mechanisms between two strains should be resolved, and despite of all these drawbacks, significant progress has been made to obtain genetically engineered microbes for agriculture (Armarger 2002).

6.5 Biotechnological Applications of PSM

Among the beneficial microorganisms solubilizing insoluble phosphates in soil, *Bacillus* spp., *Pseudomonas* spp., *Azotobacter*, *Rhizobium*, etc. play a significant role in plant nutrition. The use of these potential organisms as biofertilizers becomes an alternative to high-cost chemical fertilizers. Biofertilizers are live or latent cells of microorganisms which facilitate the availability of the essential nutrients to the plants. They are extremely beneficial to the plants by enriching the soil with nutrients. Commercial products and formulations of promising strains of phosphate solubilizers are available in the market making the farming system more sustainable. The use of biofertilizers has certain advantages over chemical fertilizers which include safer than chemical fertilizers, no accumulation in the food chain and not harmful to the ecological processes and the environment.

Besides making soluble P available for plant uptake, there have been numerous reports on plant growth promotion by PSM (Gaur and Ostwal 1972) (Table 6.3). The attributes which promote the plant growth are production of phytohormones,

Table 6.3 Growth promotion by PSM in different crop plants (Krishnaraj and Dahale 2014)

Sl. No	Inoculants	Crop benefited	Effect
1	*Mesorhizobium* sp., *Pseudomonas aeruginosa*	Chickpea	Enhanced uptake of P and N. Increased grain and straw yield
2	*Glomus intraradices*, *Pseudomonas putida*, *P. alcaligenes*, *P. aeruginosa*, *A. awamori*, and *Rhizobium* sp.	Chickpea	Enhanced growth and yield. Increased resistance to diseases
3	*Bacillus* sp.	Cotton	Increase in plant growth parameters and number of bolls per plant and boll weight. Increase in soil available P
4	*Gluconacetobacter* sp. and *Burkholderia* sp.	Cowpea	Increased N and P uptake, root and shoot biomass. Enhanced grain and straw yield
5	*Fluorescent pseudomonas*	Soybean	Increased N and P uptake. Tolerance to abiotic stress, salinity, metal toxicity and pesticide
6	*Rhizobium*, *Pseudomonas striata* and *Bacillus polymyxa*	Gram	Increased nodulation and nitrogen fixation activity. High dry matter content
7	*Pseudomonas* sp.	Gram	Increase in growth and yield
8	*Rhizobium* and *Pseudomonas*	Moth bean	Increase in growth and yield
9	*Pseudomonas* sp. and *Azospirillum* sp.	Rice	Enhanced P uptake and growth parameters. Increase in yield
10	*Pseudomonas* sp.	Soybean	Increase in nodulation, number and dry weight of nodules. Enhanced growth and yield parameters. Increase in nutrient availability and plant uptake
11	*Bacillus* sp.	Sunflower	Increase in growth and yield parameters, oil yield and quality
12	*Pantoea agglomerans Burkholderia anthina*	Tomato	Increase in growth and yield parameters, phosphorous uptake and available phosphorous in soil

i.e. auxin and cytokinins, production of siderophores, nitrogen fixation, ACC deaminase activity and antagonism against plant pathogens (Cattelan et al. 1999). The mechanisms involved in plant growth promotion by PSM are illustrated in Fig. 6.4.

Further, these beneficial organisms have also been exploited in aquaculture for enhancing the fish productivity (Vovk et al. 2013) and for commercial production of organic acids (Behera et al. 2014) and in phytoremediation of contaminated soils. The bacteria *Bacillus megaterium* were used to enhance Cd bioavailability and phytoextractibility. Increased accumulation of Cd by twofold was observed in *Brassica juncea* and *Abutilon theophrasti* inoculated with *Bacillus megaterium* compared to uninoculated control (Jeong et al. 2012).

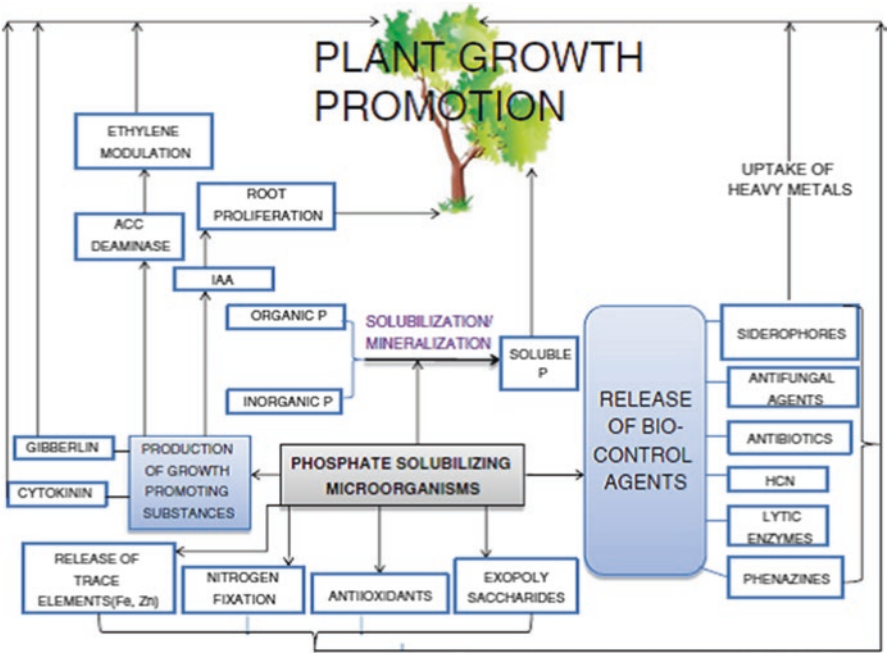


Fig. 6.4 Illustrations of mechanism of plant growth promotion by PSM (Sharma et al. 2013)

6.6 Conclusions

Phosphorous is a key element in plant nutrition. The efficiency of phosphatic fertilizer being applied is very low due to fixation phenomenon in both acidic and alkaline soils. Phosphate-solubilizing microbes play a key role and contribute to biofertilization of agricultural crops, and their inoculation in these soils will be important to restore the overall balance of nutrients and soil health. Hence, it is imperative to isolate and develop the strains to suit different soil types. Further investigation is necessary to improve the efficacy and performance under diverse agroecological conditions.

Greater attention must be given to the research and applications to develop consortia of PSM and other beneficial soil microflora with multiple benefits. Similarly the thermotolerant multifunctional strains capable of surviving in composting temperature may be useful for enrichment of compost. On the other hand, genetic engineering of phosphate-solubilizing bacteria to enhance the solubilization potential or introduction of this trait in other beneficial strains from the perspective of plant growth promotion is not only important, but also practically feasibility is of great concern. An overall look into the different aspects of phosphate-solubilizing microbes as biofertilizers in a concerted manner will enable its acceptance by the farming community and help to achieve a sustainable agriculture.

References

Alexander M (1977) Introduction to soil microbiology. Wiley, New York

Altomare C, Norvell WA, Borjkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Appl Environ Microbiol 65:2926–2933

Armarger N (2002) Genetically modified bacteria in agriculture. Biochimie 84:1061–1072

Azam F, Memon GH (1996) Soil organisms. In: Bashir E, Bantel R (eds) Soil science. National Book Foundation, Islamabad, pp 200–232

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

Bashan Y, Moreno M, Troyo E (2000) Growth promotion of the seawater-irrigated oil seed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* sp. Biol Fertil Soils 32:265–272

Bashan Y, Kamnev AA, de Bashan LE (2013a) A proposal for isolating and testing phosphate solubilizing bacteria that enhance plant growth. Biol Fertil Soils 49:1–2

Bashan Y, Kamnev AA, de Bashan LE (2013b) 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

Beever RE, Burns DJW (1980) Phosphate uptake, storage and utilisation by fungi. Adv Bot Res 8:127–219

Begon M, Harper JL, Townsend CR (1990) Ecology: individuals, populations and communities. Blackwell Scientific Publications, Oxford

Behera BC, Singdevsachan SK, Mishra RR, Dutta SK, Thatoi HN (2014) Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove – a review. Biocata Agric Biotechnol 3:97–110

Bhagyaraj DJ, Verma A (1995) Interaction between arbuscular mycorrhizal fungi and plants: their importance in sustainable agriculture in arid and semiarid topics. In: Jones JG (ed) Advances in microbial ecology. Academic Press, London, pp 119–142

Blake L, Mercik S, Koerschens M, Moskal S, Poulton PR, Goulding KWT, Weigel A, Powlson DS (2000) Phosphorus content in soil, uptake by plants and balance in three European long-term field experiments. Nutr Cycl Agroecosyst 56:263–275

Butterly CR, Bunemann EK, McNeill AM, Baldock JA, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decrease in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41:1406–1416

Caballero-Mellado J, Onofre-Lemus J, De los Santos EP, Martinez-Aguilar L (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

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Chen YP, Rehha PD, Arun AB, Shen FT (2005) Phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chen YP, Rekha PD, Arun AB, Shen FD, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37:1970–1974

Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010) Comparison of *in-vitro* solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol Fertil Soils 46:727–738

Cordell D, Drangert JO, White S (2009) The story of phosphorus: global food security and food for thought. Glob Environ Chang 19:292–305
Criquet S, Ferre E, Farner EM, Le Petit J (2004) Annual dynamics of phosphatase activities in an evergreen oak litter influence of biotic and abiotic factors. Soil Biol Biochem 36:1111–1118
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
Ehrlich HL (1990) Mikrobiologische and biochemische Verfahrenstechnik. In: Einsele A, Finn RK, Samhaber W (eds) Geomicrobiology, 2nd edn. VCH, Verlagsgesellschaft, Weinheim
Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 9:167–172
Fabre B, Armau E, Etienne G, Legendre F, Tiraby G (1988) A simple screening method for insecticidal substances from actinomycetes. J Antibiot 41:212–219
Fankem H, Nwaga D, Deube A, Dieng L, Merbach W, Etoa FX (2006) Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. Afr J Biotechnol 5:2450–2460
Fasim F, Ahmed N, Parson R, Gadd GM (2002) Solubilization of zinc salts by a bacterium isolated from air environment of a tannery. FEMS Microbiol Lett 213:1–6
Furihata T, Suzuki M, Sakur H (1992) Kinetic characterization of two phosphate uptake systems with different affinities in suspension cultured *Catharanthus roseus* protoplasts. Plant Cell Physiol 33:1151–1157
Gaur AC, Ostwal KP (1972) Influence of phosphate dissolving Bacilli on yield and phosphate uptake of wheat crop. Indian J Exp Biol 10:393–394
Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117
Goldstein AH (1996) Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous 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
Goldstein AH, Braverman K, Osorio N (1999) Evidence for mutualism between a plant growing in a phosphate limited desert environment and a mineral phosphate solubilizing (MPS) bacterium. FEMS Microbiol Ecol. 3:295–300
Gupta R, Singal R, Sankar A, Kuhad RC, Saxena RK (1994) A modified plate assay for screening phosphate solubilizing microorganisms. J Gen Appl Microbiol 40:255–260
Gyaneshwar P, Parekh LJ, Archana G, Poole PS, Collins MD, Hutson RA et al (1999) Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by *Enterobacter asburiae*. FEMS Microbiol Lett 171:223–229
Gyaneshwar P, Kumar NJ, Pareka LJ, Podle PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245(1):83–93
Halder AK, Mishra AK, Bhattacharyya P, Chakrabartty PK (1990) Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. J General Appl Microbiol 36:81–92
Hamdali H, Bouizgarne B, Hafidi M, Lebrihi A, Virolle MJ, Ouhdouch Y (2008a) Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Appl Soil Ecol 38:12–19
Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y (2008b) Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions. Appl Soil Ecol 40:510–517
Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London/New York
He ZL, Wu J, O'Donnell AG, Syers JK (1997) Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biol Fertil Soils 24:421–428
Hilda R, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adv 17:319–359

Holford ICR (1997) Soil phosphorus its measurement and its uptake by plants. Aust J Soil Res 35:227–239

Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biol Biochem 24:257–263

Indiragandhi P, Anandham R, Madhaiyan M, Sa TM (2008) Characterization of plant growth promoting traits of bacteria isolated from larval guts of diamond back moth Plutella xylostella (Lepidoptera: Plutellidae). Curr Microbiol 56:327–333

Isherwood KF (2000) Mineral fertilizer use and the environment. International Fertilizer Industry Association/United Nations Environment Programme, Paris

Jeong S, Moon HS, Nam K, Kim JY, Kim TS (2012) Application of phosphate solubilising bacteria for enhancing bioavailability and phytoextraction of cadmium (Cd) from polluted soil. Chemosphere 88:204–210

Jorquera MA, Crowley DE, Marschner P, Greiner R, Ferna'ndez MT, Romero D, Menezes-Blackburn D, De La Luz MM (2011) Identification of b-propeller phytase-encoding genes in culturable *Paenibacillus* and *Bacillus* sp. from the rhizosphere of pasture plants on volcanic soils. FEMS Microbiol Ecol 75:163–172

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate solubilizing microorganisms in sustainable agriculture A review. Agron Sus Dev 27:29–43

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M (2009) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric Biol Sci 1(1):48–58

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch Agron Soil Sci 56:73–98

Kim KY, Jordan D, McDonald GA (1997) Solubilization of hydroxyapatite by *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. Biol Fert Soils 24:347–352

Krishnaraj PU, Dahale S (2014) Mineral phosphate solubilization: Concepts and prospects in sustainable agriculture. Proc Indian Nat Sci Acad. Doi: 10.16943/ptinsa/2014/v80i2/55116

Krishnaraj PU, Khanuja SPS, Sadashivam KV (1998) Mineral phosphate solubilization (MPS) and mps genes components in eco-friendly P fertilization. Abstracts of Indo US workshop on application of biotechnology for clean environment and energy. National Institute of Advanced Studies, Bangalore, p 27

Kucey RMN (1983) Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678

Kucey RWN, Tanez HH, Leggett ME (1989) Microbially mediated increases in plant available phosphorus. Adv Agron 42:199–228

Kumar V, Behl RK, Narula N (2001) Establishment of phosphate- solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol Res 156:87–93

Lindsay WL, Vlek PLG, Chien SH (1989) Phosphate minerals. In: Dixon JB, Weed SB (eds) Minerals in soil environment, 2nd edn. Soil Science Society of America, Madison, WI, USA, pp 1089–1130

Mahila R, Samina K, Najma A, Sadia A, Farooq L (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. Pak J Biol Sci 7:187–196

Mardad I, Serrano A, Soukri A (2013) Solubilisation of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit. Afr J Microbiol Res 7:626–635

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26:267–268

Miethke M, Marahiel MA (2007) Siderophore based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71:413–451

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Bunemann E, Oberson A, Frossard E (eds) Phosphorus in action: Biological processes in soil phosphorus cycling, Soil biology, vol 26. Springer, Heidelberg, pp 251–244

Norrish K, Rosser H (1983) Mineral phosphate. In: Soils: an Australian viewpoint. Academic Press/CSIRO, Melbourne/London, pp 335–361

Ohtake H, Kato J, Kuroda A, Taguchi K, Sakai Y (1996) Chemolactic signal transduction in *Pseudomonas aeruginosa* Pseudomonas. In: Nakazawa T, Furukawa K, Hass D, Silver S (eds) Molecular biology and biotechnology. American Society for Microbiology, Washington, DC, pp 188–194

Ostwal KP, Bhide VP (1972) Solubilization of tri-calcium phosphate by soil *Pseudomonas*. Indian J Exp Biol 10:153–154

Parker DR, Reichmann SM, Crowley DE (2005) Metal chelation in the rhizosphere. In: Zobel RW (ed) Roots and soil management: interactions between roots and the soil, Agronomy monograph no. 48. American Society of Agronomy, Madison, pp 57–93

Parks EJ, Olson GJ, Brickman FE, Baldi F (1990) Characterization of high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron one by a fungus. Indian J Microbiol 5:183–190

Patel DK, Archana G, Kumar GN (2008) Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter* sp. DHRSS in the presence of different sugars. Curr Microbiol 56:168–174

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiology 17:362–370

Puente ME, Bashan Y, Li CY, Lebsky VK (2004a) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol 6:629–642

Puente ME, Li CY, Bashan Y (2004b) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol 6:643–650

Raghu K, MacRae IC (1966) Occurrence of phosphate dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. J Appl Bacteriol 29:582–586

Rengel Z, Marschner P (2005) Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytol 168:305–312

Richardson AE (1994a) Soil microorganisms and phosphorus availability. Soil Biota 50:35–39

Richardson AE (1994b) Soil microorganisms and phosphorus availability. In: Pankhurst CE, Doubeand BM, Gupta VVSR (eds) Soil biota: management in sustainable farming systems. CSIRO, Victoria, pp 50–62

Richardson AE (2004) Soil microorganisms and farming systems. In: Pankhrust CE, Doube BM, Gupta VVSR, Grace PR (eds) Management of soil biota is sustainable farming systems. CSIRO, Melbourne, pp 50–62

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21

Ross HM, Middleton A (2013) Phosphorus fertilizer application in crop production. Agri-Facts, Alberta Agriculture and Rural Development Research and Innovation Division Agriculture Centre, Lethbridge

Rudolph W (1922) Influence of S oxidation upon growth of soybeans and its effect on bacterial flora of soil. Soil Sci l4:247–263

Saber K, Nahla LD, Chedly A (2005) Effect of P on nodule formation and N fixation in bean. Agron Sustain Dev 25:389–393

Schachtmam DP, Robert RJ, Ayling SM (1998) Phosphorus uptake by plants: from soil to cell. Plant Physiol 116:447–453

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587–600

Sims JT, Pierzynski GM (2005) Chemistry of phosphorus in soil. In: Tabatabai AM, Sparks DL (eds) Chemical processes in soil, SSSA book series 8. SSSA, Madison, pp 151–192

Sperber JI (1958) The incidence of apatite-solubilizing organisms in the rhizosphere and soil. Aust J Agric Res 9:778–781
Stevenson FJ (1986) Cycles of soil carbon, nitrogen, phosphorus, sulphur and micronutrients. Wiley, New York
Subbarao NS (1982) Advances in agricultural microbiology. IBH Publications Company, Oxford, pp 229–305
Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiol 147:3–9
Swaby R, Sperber JI (1958) Phosphate dissolving microorganisms in the rhizosphere of legume, Nutrition of Legumes. In Proceedings of University of Nottingham 5th Eastern School of Agricultural Science. (CSIRO Adelaide). Soils Fert 22, 286(1959):289–294
Tarafdar JC, Yadav RS, Meena SC (2001) Comparative efficiency of acid phosphatase originated from plant and fungal sources. J Plant Nutr Soil Sci 164:279–282
Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Wackhamer D (2001) Forecasting agriculturally driven global environmental change. Science 292:281–284
Trolove SN, Hedley MJ, Kirk GJD, Bolan NS, Loganathan P (2003) Progress in selected areas of rhizosphere research on P acquisition. Aust J Soil Res 41:471–499
Vassilev N, Vassileva M, Azcon R, Medina A (2001) Preparation of gel-entrapped mycorrhizal inoculum in the presence or absence of *Yarrowia lipolytica*. Biotechnol Lett 23:907–909
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
Venkateswarlu B, Rao AV, Raina P, Ahmad N (1984) Evaluation of phosphorus solubilization by microorganisms isolated from arid soil. J Indian Soc Soil Sci 32:273–277
Vovk NI, Bazaeva AV, Didenko AV (2013) Use of the phosphate solubilizing bacterial preparation polymyxobacterin in pond aquaculture. Tur J Fish Aqua Sci 13:1–9
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
Whitelaw MA (2000) Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv Agron 69:99–151
Widada J, Damarjaya DI, Kabirun S (2007) The interactive effects of arbuscular mycorrhizal fungi and rhizobacteria on the growth and nutrients uptake of sorghum in acid soil. In: Rodriguez-Barrueco C, Velazquez E (eds) First international meeting on microbial phosphate solubilization. Springer, pp 173–177
Yi Y, Huang W, Ge Y (2008) Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24:1059–1065
Zaidi A, Khan MS, Ahemad M, Oves M, Wani PA (2009) Recent advances in plant growth promotion by phosphate-solubilizing microbes. In: Khan MS et al (eds) Microbial strategies for crop improvement. Springer, Berlin/Heidelberg, pp 23–50
Zhu F, Qu L, Hong X, Sun X, (2011) Isolation and characterization of a phosphate- solubilizing halophilic bacterium Kushneriasp. YCWA18 from Daqiao Salternon the Coast of Yellow Sea of China. Evidence Based Complementary and Alternative Medicine, 1–6, https://doi.org/10.1155/2011/615032

Potassium-Solubilizing Microbes: Diversity, Distribution, and Role in Plant Growth Promotion

7

Priyanka Verma, Ajar Nath Yadav, Kazy Sufia Khannam, Anil Kumar Saxena, and Archna Suman

Abstract

Injudicious application of chemical fertilizers in India has a considerable negative impact on economy and environmental sustainability. There is a growing need to turn back to nature or sustainable agents that promote evergreen agriculture. Potassium (K) is an important and well-known constraint to crop production. Very low rates of potash fertilizer application in agricultural production lead to rapid depletion of K in the soil. Depletion of plant-available K in soils results in a variety of negative impacts of the crops yield and soil health. Microorganisms play important role in determining plant productivity. For successful functioning of introduced microbial bioinoculants, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution, and behavior in soil habitats. Soil microorganisms are directly responsible for recycling of nutrients. K is the third major essential macronutrient for plant growth. The concentrations of soluble potassium in the soil are usually very low, and more than 90% of potassium in the soil exists in the form of insoluble rocks. Use of plant growth-promoting microorganisms (PGPMs) helps in increasing yields in addition to conventional plant protection. The most important PGPMs are

P. Verma (✉)
Department of Microbiology, Akal College of Basic Science, Eternal University, Sirmour 173101, India
e-mail: priyanka.iari@gmail.com

A.N. Yadav
Department of Biotechnology, Akal College of Agriculture, Eternal University, Sirmour 173101, India

K.S. Khannam
Department of Biotechnology, National Institute of Technology, Durgapur 713209, India

A.K. Saxena • A. Suman
Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012, India

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_7

Azotobacter, *Azospirillum*, *Acidithiobacillus ferrooxidans*, *Bacillus circulans*, *B. edaphicus*, *B. globisporus*, *B. mucilaginosus*, *B. subtilis*, *Burkholderia cepacia*, *Enterobacter hormaechei*, *Paenibacillus kribensis*, *P. mucilaginosus*, and *Pseudomonas putida* potassium solubilizes; these are eco-friendly and environmentally safe. Therefore, the efficient K-solubilizing microbes (KSM) should be applied for solubilization of a fixed form of K to an available form of K in the soils. This available K can be easily taken up by the plant for growth and development. In this chapter has been discussed isolation, characterization, diversity, and distribution of KSM from diverse stresses such as low and high temperatures, acidity, alkalinity, salinity, drought, and plant-associated applications. These studies elaborate on indigenous K-solubilizing microbes to develop efficient microbial bioinoculant for solubilization of K in different conditions of soil which enhances the plant growth and yield of crops.

Keywords
Abiotic stresses • Bioinoculant • Diversity • Distribution • K-solubilizing microbes

7.1 Introduction

Countries such as Brazil, China, and India are important food producers and consumers of high amounts of potassium-based fertilizers. In Brazil, around 90% of the potassium required for agriculture is imported (Barbosa Filho et al. 2006). Plants can uptake potassium (K) through the soil minerals, organic materials, and synthetic fertilizers. Consumption of K was exceeded 260 lakh tons for 2 consecutive years (2011 and 2012) in India, and all the K fertilizers were imported across the globe to meet the demand for agricultural productivity (Nagendran et al. 2013), indicating the injudicious application of K fertilizers. K deficiency in the rhizosphere of economically important crops has become an important limiting factor responsible for sustainable development of evergreen agriculture in India (Naidu et al. 2011).

Potassium (K) is one of the major plant macronutrients influencing plant growth, development, and grain quality; its plays a key role in the synthesis of cells, enzymes, proteins, starch, cellulose, and vitamins. Moreover, K not only participates in nutrient transportation and uptake but also confers resistance to abiotic and biotic stresses, leading to enhanced production of quality crops and providing resistance to plant diseases (Epstein 1972; Epstein and Bloom 2005; Maqsood et al. 2013; Pettigrew 2008). The K is absorbed by plants in large amount than any other mineral element except nitrogen (N) and, in some cases, calcium (Ca). Chemical or synthetic K fertilizers are the largest available sources of K rhizosphere; therefore, larger amounts of K fertilizers can be used to promote the availability of K for plant uptake (Li et al. 2007). The concentration of K in straw and grain serves as an indicator whether the K status of crop is deficient or sufficient (Rao et al. 2010).

However, K uptake by aboveground parts of plants is assimilated mainly into the straw but not into the grain (Basak and Biswas 2009).

Release of non-exchangeable K to the third exchangeable form occurs when level of exchangeable and solution K is decreased by crop removal, runoff, erosion, and/or leaching (Sparks 1987). With the introduction of high-yielding crop varieties/hybrids and the progressive intensification of agriculture, the soils are getting depleted in potassium reserve at a faster rate. Moreover, due to imbalanced fertilizer application, potassium deficiency is becoming one of the major constraints in crop production. This emphasized the search to find an alternative indigenous source of K for plant uptake and to maintain K status in soils for sustaining crop production (Sindhu et al. 2014; Supanjani et al. 2006).

Plant growth-promoting microbes are heterogeneous groups of microbes associated with plants in diverse ways. The plant-associated microbes colonize the rhizosphere (rhizospheric microbes), the phyllosphere (epiphytes), and inside of the plant tissue (endophytes). The word "endophyte" means "inside the plant" (derived from the Greek words "endon" meaning "within" and "phyton" meaning "plant"). Although there are diverse meanings for the term, endophytes are most commonly defined as those organisms whose "infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonization can be demonstrated to be internal". While microbes are intimately involved in biogeochemical cycling of metals, anthropogenic release of metals has increased bacterial exposure to a high level of metals in some environments (Nies 1999; Suman et al. 2016a, b). A metal may be regarded as toxic if it impairs growth or metabolism of an organism above a certain threshold concentration: both essential and inessential metals may be toxic when supplied at high enough concentrations (Bowen 1966; Gadd 1992).

Many studies on microbial interactions with toxic metals have been made in the context of functions in metalloenzymes, resistance, and transport, but several aspects of metal "metabolism" remain unclear, particularly the mechanisms employed to obtain metals and associated nutrients from insoluble resources (Wakatsuki 1995). Frequently, microorganisms need to solubilize insoluble metal compounds occurring in the natural environment prior to uptake of essential metals and utilization of associated nutrients, e.g., P and S. Different bacterial species, such as species in the genera *Pseudomonas*, *Agrobacterium*, *Bacillus, Rhizobium*, *and Flavobacterium*, have been tested for their ability to solubilize inorganic phosphate compounds, such as tricalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein 1986). Silicate bacteria were found to resolve potassium, silicon, and aluminum from insoluble minerals (Aleksandrov et al. 1967). K-solubilizing bacteria exert beneficial effects upon plant growth. Their uses as biofertilizers or control agents for agriculture improvement and environmental protection have been a focus of recent research (Deng et al. 2003; Glick 1995). Imbalanced or overdose use of chemical fertilizers have the negative environmental impacts and also increasing costs of crop production; therefore, there is an urgent need to imply eco-friendly and cost-effective agro-technologies to increase crop production. Therefore, the utilization of KSM is considered to be a sound strategy in improving the productivity of agricultural lands.

7.2 Isolation and Identification of Potassium-Solubilizing Microorganism

Potassium-solubilizing bacteria have been isolated and purified on Aleksandrov agar plates (Hu et al. 2006). The composition of the medium (g/liter) is 5.0 g glucose, 0.5 g magnesium sulfate, 0.005 g ferric chloride, 0.1 g calcium carbonate, 2 g calcium phosphate, and 2 g potassium-bearing minerals. Aleksandrov agar medium with different pH (3–11), NaCl concentration (5–20%), temperatures (5–50 °C), and PEG 8000 (−0.5 to −1.5 MPa) were used to isolate diverse groups of K-solubilizing microbes, viz., acidophilic, alkaliphilic, halophilic, psychrophilic, thermophilic, or drought tolerant. Plates were incubated at different temperatures, and time as described earlier by Yadav et al. (2015a). Cultures were purified and maintained at 4 °C as slant and glycerol stock (20%) at −80 °C for further use. Potassium aluminosilicates and mica were used as insoluble potassium-bearing minerals. Microbes showed halo zone on plates were selected and measured the diameter of halo zone. Quantitative potassium solubilization was carried out in Aleksandrov broth. Microbial cultures showed the K solubilization qualitatively were inoculated separately in conical flasks (150 mL) containing 40 mL broth. Available potassium in culture supernatant was determined by using flame photometer.

For identification and phylogenetic profiling of K-solubilizing microbes, the genomic DNA should be extracted for the identification of microbes by the method described by Verma et al. (2016a, b)). The amount of DNA was extracted and assessed by electrophoresis on a 0.8% agarose gel. The 16S rRNA/ITS gene should be amplified as described earlier (Verma et al. (2016a, b)) using the universal primers pA and pH for bacteria and ITS 1 and ITS 2 for fungus (Edwards et al. 1989). The PCR-amplified 16S rRNA/ITS gene was purified. The nucleotide sequences of purified 16S/ITS rDNA have been sequenced with fluorescent terminators (BigDye, Applied Biosystems) and run in 3130xl Applied Biosystems ABI prism automated DNA sequencer. The DNA sequence should be double-checked by sequencing both strands using primers forward and reverse reaction, respectively. The partial 16S rRNA/ITS gene sequences of the isolated strains have been compared with those available in the databases. Identification at the species level has determined using a 16S rRNA/ITS gene sequence similarity of ≥97% with that of a prototype strain sequence in the GenBank. Sequence alignment and comparison have been performed, using the program ClustalW. One sequence from each group was selected as a representative operational taxonomic unit (OTU). The phylogenetic tree was constructed on the aligned datasets using the neighbor-joining method implemented in the program MEGA 4.0.2 (Tamura et al. 2007).

7.3 Diversity of Potassium-Solubilizing Microorganism

Microbial world unique in each ecosystem niche forms the basis of the diversity associated. Agriculture is highly on soils and climatic conditions. The ever-increasing need for food to support the growing population in the country demands

a systematic appraisal of its soil and climate resources in order to prepare effective lands. The diversity of K-solubilizing microorganisms inhabiting different environments has been extensively investigated in the past few years. The different groups of microbes have been reported such as bacteria and fungi, which included bacterial phylum *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, α-*Proteobacteria*, β-*Proteobacteria*, and γ-*Proteobacteria* (Fig. 7.1), and only two fungal phyla were reported to solubilize potassium, namely, *Ascomycota* and *Glomeromycota* (Table 7.2). The last few decades, potassium-solubilizing bacterial genera have been recovered, that is, *Acidithiobacillus*, *Agrobacterium*, *Aminobacter*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Clostridium*, *Delftia*, *Enterobacter*, *Klebsiella*, *Methylobacterium*, *Microbacterium*, *Myroides*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, and *Sphingomonas.* Very least research has been done in K-solubilizing fungus, with only five genera reported being *Aspergillus*, *Cladosporium*, *Fusarium*, *Glomus*, and *Penicillium* (Fig. 7.2).

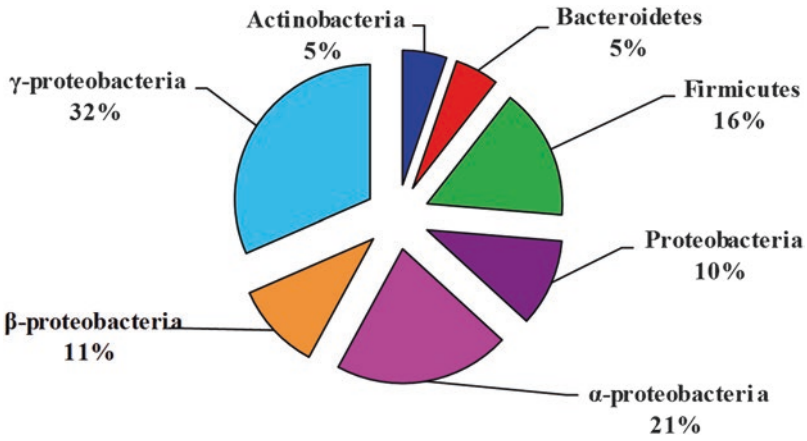


Fig. 7.1 Diversity of potassium-solubilizing bacteria

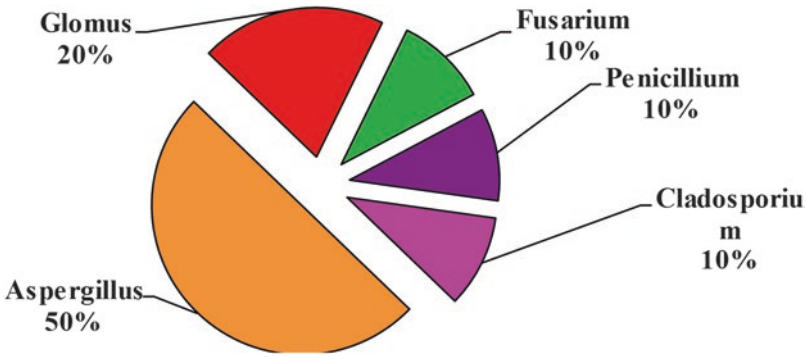


Fig. 7.2 Diversity of plant growth-promoting K-solubilizing fungi

7.3.1 Bacteria

Soil bacteria that colonize plant roots and promote growth when added to seeds, roots, or tubers have been termed plant growth-promoting rhizobacteria (PGPR). Different plant growth-promoting rhizosphere bacteria, including associative bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Enterobacter* groups, have been used for their beneficial effects on plant growth. The mechanisms of plant growth stimulation by associative bacteria are mobilization of nutrients, stimulation of root growth by production of phytohormones, and antagonism against soil-borne plant pathogens. Several studies clearly showed the effect of plant growth-promoting bacteria on plant growth of different crops at different climates and soils. The survival of inoculated PGPR in the plant rhizosphere is in most cases a precondition for a potential plant stimulation effect during the vegetation time or at least during early plant development.

A wide range of rhizospheric bacteria reported as K solubilizers included *B. mucilaginosus* (Zarjani et al. 2013), *B. edaphicus* (Sheng 2005), *B. circulans* (Lin et al. 2002), *Burkholderia*, *Acidithiobacillus ferrooxidans*, *B. mucilaginosus* (Zhang and Kong 2014), *Bacillus edaphicus* (Sheng and He 2006), *Arthrobacter* spp. (Zarjani et al. 2013), *Enterobacter hormaechei* (Prajapati et al. 2013), *Paenibacillus mucilaginosus* (Liu et al. 2012; Hu et al. 2006), *P. frequentans*, *Cladosporium* (Argelis et al. 1993), *Aminobacter*, *Sphingomonas*, *Burkholderia* (Uroz et al. 2007), and *Paenibacillus glucanolyticus* (Sangeeth et al. 2012). These microbial strains have the ability to solubilize K from K-bearing minerals, but only few bacteria, such as *B. edaphicus and B. mucilaginosus*, have high capacity for mobilizing and solubilizing of K from minerals (Zhao et al. 2008).

Verma et al. (2016a, b) reported that most of the bacilli solubilized potassium such as *Bacillus aerophilus*, *Bacillus atrophaeus, Bacillus cereus*, *Bacillus circulans*, *Bacillus horikoshii*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus mojavensis*, *Bacillus pumilus*, *Bacillus sphaericus*, *Exiguobacterium antarcticum*, *Paenibacillus amylolyticus*, *Paenibacillus dendritiformis*, *Paenibacillus polymyxa*, *Planococcus citreus*, *and Planococcus salinarum*. The K-solubilizing bacteria may have use in the amelioration of K-deficient soil in agriculture. Diversity analysis of potassium solubilizing bacteria has been reported in different phylum such as 32% γ-*Proteobacteria*, 21% α-*Proteobacteria*, 16% *Firmicutes*, 11% β-*Proteobacteria*, 10% *Proteobacteria*, and 5% both *Actinobacteria* and *Bacteroidetes*. Maximum potassium-solubilizing bacterial genera have been report from γ-*Proteobacteria*.

7.3.2 Fungi

The alteration of rock minerals in natural environments is a well-known process mainly caused by the action of water and organic acids produced by plant roots and by microorganisms that accelerate this alteration. Molds are capable of solubilizing elements immobilized in silicates during the decomposition of organic matter, resulting in the production of organic acid. Potassium solubilization has been

obtained using molds such as *Aspergillus, Penicillium,* and *Fusarium*. The filamentous fungus *Aspergillus niger* is an exceptionally efficient producer of organic acids, which is one of the reasons for its relevance to industrial processes and its commercial importance. The production of organic acids by *A. niger* is dependent on the pH of the medium, since the greatest quantities of oxalic acid are produced at a pH between 5 and 8, while it is completely absent below pH 3.0.

Arbuscular mycorrhiza can increase the solubility of the mineral form of potassium by releasing protons, H^+, or CO_2 and organic acid anions such as citrate, oxalate, and malate. This also increased the nitrogen, potassium, calcium, and iron in the plant leaves and fruits (Veresoglou et al. 2011; Yousefi et al. 2011). The inoculants of the two arbuscular mycorrhizal fungi (AMF) species *G. intraradices* and *G. mosseae* was applied in soil on a weight basis, and the increasing potassium uptake by maize crop was recorded (Wu et al. 2005). Ectomycorrhizal fungi particularly isolated UFSC-Pt22 and UFSC-Pt186 and contributed to the increase of the efficiency of alkaline breccias as a source of P and K to the plant growth of *Eucalyptus dunnii* seedlings, respectively (Alves et al. 2010).

Prajapati et al. (2012) reported that potassium-solubilizing fungi (KSF) strains such as *Aspergillus terreus* and *Aspergillus niger* were isolated from various K-rich soil samples and observed that *A. terreus* and *A. niger* could solubilize insoluble potassium and showed the highest available potassium in liquid medium by using two various insoluble sources of potassium, i.e., feldspar and potassium aluminum silicate. *Aspergillus* spp., *Aspergillus terreus* (Prajapati et al. 2013), *Aspergillus niger* (Prajapati et al. 2012), and *Penicillium* spp. (Sangeeth et al. 2012) enhanced K solubilization by mobilizing inorganic and organic K and release of structural K from rocks and minerals (Fig. 7.2). Diverity analysis of potassium solubilizing fungi has been reported in two phylum *Ascomycota* and *Glomeromycota* with five genera: *Aspergillus* 50%, *Glomus* 20%, *Cladosporium* 10%, *Fusarium* 10%, and *Penicillium* 10%. *Aspergillus* has been the more frequent potassium-solubilizing fungal genera.

7.4 Distribution of Potassium-Solubilizing Microorganisms

Microbial communities are found in most diverse conditions, including extremes of temperature, salinity, water deficiency, and pH. In order to survive under such extreme conditions, these organisms, referred to as extremophiles, have developed adaptive features that permit them to grow optimally under one or more environmental extremes, while polyextremophiles grow optimally under multiple conditions (Rothschild and Mancinelli 2001). Global work on PGPR for different crops is brief carried out on a hypothesis that PGPR can overcome the burden caused by chemical fertilizer on environment. There are diverse conditions for crops growing in different abiotic stresses of pH, salinity, temperature, and drought (Glick et al. 1999; Verma et al. 2015a).

In an efforts to understand the diversity and distribution of culturable K-solubilizing microbes associated with different crops growing in the diverse environments which included saline soil, acidic soil, water deficiency/drought stress, high temperature, and

low temperature, many researchers isolated, enumerated, and characterized potassium-solubilizing microbes for tolerances to abiotic stresses. Tolerance to stress provided by microbial inoculants become more significant with the perspectives of crop production that has losses due to the severity of abiotic stresses (Grover et al. 2011).

7.4.1 Acidophiles

Acidophilus study indicated that lower pH, increase in number of cells, and the consequent increase in viscosity due to EPS are allied factors affecting K solubilization from feldspar. Fourier-transform infrared spectroscopic spectra also showed the functional groups related to them which in turn indicated the presence of EPS, organic acids, and proteins (Cao et al. 2011; Yadav et al. 2011). Numerous studies have shown that *Bacillus* sp. can promote the release of K from silicate minerals (Badar et al. 2006; Barker et al. 1998). Acidophilic microorganisms has been numerously studied from different crops, rhizospheric soil, cold deserts, etc. in which some microbes were reported as acidophiles such as *Bacillus aerophilus*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus pumilus*, *Lysinibacillus fusiformis*, *Paenibacillus polymyxa*, and *Planomicrobium* sp. could grow at 3 pH (Yadav et al. 2015b). Verma et al. (2013) reported K-solubilizing microbes *Bacillus cereus*, *Bacillus pumilus*, *Bacillus thuringiensis*, *Lysinibacillus fusiformis*, *Planococcus salinarum*, *Pseudomonas rhodesiae*, and *Variovorax soli*.

7.4.2 Alkaliphiles

Alkaliphilic organisms have a pH optimum for growth above pH 9 and no growth at pH 7. Spore-forming alkaliphilic organism growing at pH 8–10 but not at pH 7 has been described which was so peculiar in its properties that the authors established a new genus for it: *Amphibacillus xylanzls* (Niimura et al. 1990). Its lack of cytochromes, quinones, or catalase and its ability to form spores under aerobic as well as anaerobic conditions clearly distinguished this organism from the genera *Bacillus*, *Clostridium*, and *Sporolactobacillus.* A wide range of rhizospheric alkaliphilic microorganisms are reported as potassium solubilizers including *Achromobacter*, *Aerobacter*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Duganella*, *Exiguobacterium*, *Klebsiella*, *Lysinibacillus*, *Micrococcus*, *Paenibacillus*, *Planococcus*, *Pseudomonas*, *Psychrobacter*, *Rhizobium*, *Stenotrophomonas*, and *Variovorax* (Meena and Kanwar 2015; Verma et al. 2016a, b). These bacteria have been isolated from a variety of rhizospheric and non-rhizospheric soils including sugarcane (Rosa-Magri et al. 2012), tea (Bagyalakshmi et al. 2012), tobacco (Zhang and Kong 2014), and wheat (Verma et al. 2013, 2014, 2015a, 2016a, b).

7.4.3 Halophiles

Salinity affects nearly a third of the agricultural land area worldwide. Due to the upward movement of salts in soil solution in arid and semiarid climates, it is a particular problem in irrigation agriculture under those conditions (Shabala and Cuin 2007). Salinity exerts a twofold stress on the crop (Munns and Tester 2008): it causes an osmotic stress due to decreased soil water potential and an accumulation of salts in the plant cell walls. Microbial research in saline environments has also attracted the interest of researchers due to various biotechnological applications (Sahay et al. 2012). *Bacillus alcalophilus*, *Bacillus aquimaris*, *Bacillus siamensis*, *Halobacillus*, *Paenibacillus dendritiformis*, and *Lysinibacillus xylanilyticus* were reported as halophiles (Yadav et al. 2015c).

A study of potassium transport in the haloarchaeon, *Haloferax volcanii*, has shown, however, that the intracellular concentrations of potassium observed in this organism cannot be accounted for by passive processes alone and ATP hydrolysis is required to actively transport potassium into the cell to reach the 3.6 M intracellular concentrations that are maintained by *Hfx. volcanii* (Meury and Kohiyama 1989; Oren 1999). Presently, some K-solubilizing halophilic archaea have been reported: *Haloarcula marismortui*, *Haloarcula vallismortis*, and *Haloferax volcanii* (Ouellette et al. 2015). These microbes have been used for composting of different waste products and materials.

7.4.4 Psychrophiles

Cold-adapted microbes have attracted the attention of the scientific community due to their ability to promote plant growth and produce cold-active enzymes, with potential biotechnological applications in a broad range of industrial, agricultural, and medical processes. Psychrotrophic microbes could be valuable in agriculture as bioinoculants and biocontrol agents for low-temperature habitats. Many cold-tolerant PGPBs have been reported from low-temperature environments including *Arthrobacter*, *Bacillus*, *Exiguobacterium*, *Pseudomonas*, and *Providencia* (Mishra et al. 2011; Selvakumar et al. 2011; Bisht et al. 2013; Yadav et al. 2014). Psychrophiles as biofertilizers, biocontrol agents, and bioremediators would be of great use in agriculture under cold climatic conditions.

Cold-adapted microorganisms have been reported from Antarctic subglacial, permanently ice-covered lakes, cloud droplets, ice cap cores from considerable depth, snow, and ice glaciers (Yadav et al. 2015a, b). Many K-solubilizing microbes have been sorted out from different crops growing in cold environments such as *Achromobacter piechaudii*, *Bacillus amyloliquefaciens*, *Bacillus horikoshii*, *Bacillus megaterium*, *Bacillus* sp., *Exiguobacterium antarcticum*, *Klebsiella* sp., *Stenotrophomonas maltophilia*, and *Stenotrophomonas* sp. (Verma et al. 2015c). Among the isolated microbes, four efficient lignocellulolytic psychrotrophic microbes *Eupenicillium crustaceum, Paecilomyces* sp., *Bacillus atrophaeus*, and *Bacillus* sp.

and commercial fungal consortia *Aspergillus awamori*, *Aspergillus nidulans*, *Trichoderma viride*, and *Phanerochaete chrysosporium* were used in the present study.

7.4.5 Thermophiles

Global warming and its associated effects are expected to impose abiotic stresses, such as extremes of temperatures, drought, and flooding, which are bound to have adverse effects on food production. Climate change affects agriculture and the food production system in many ways (Godfray et al. 2011). Crop production is affected by climatic variables such as rising temperatures, changing precipitation regimes, and increased atmospheric CO_2 levels. It is also affected by biological variables such as the lengths of the crop growth periods and the crop cycle. Over the past decades, climate change has directly affected the plant growth with different abiotic stresses and change ecosystems.

Thermotolerant microbes are used as plant growth promoters to protect the diverse stresses have resulted in more production and yield in many crops. Verma et al. (2016a, b)) have reported thermotolerant K-solubilizing microbes represented by *Bacillus altitudinis*, *Bacillus siamensis*, *Bacillus subtilis*, *Delftia acidovorans*, *Delftia* sp., *Methylobacterium* sp., *Methylobacterium mesophilicum*, *Pseudomonas aeruginosa*, and *Salmonella bongori* from wheat crop growing in peninsular zone of India. *Bacillus altitudinis* were also reported as thermotolerant bacteria from thermal springs (Verma et al. 2015b). These bacteria produced different hydrolytic types of enzymes at high temperature.

7.4.6 Xerophiles

Being the quantitatively most important osmoticum in plants, K is a main determinant of cell turgor (White 2013). Since an adequate turgor pressure is required for cell expansion, this parameter is particularly important in growing plants (Mengel and Busch 1982). However, for a crop growing in an increasingly dry soil, the maintenance of turgidity and water uptake from the soil requires a further reduction of the plant's osmotic potential by an increase in cellular osmolyte concentration. This "osmotic adjustment" may be accomplished by the synthesis of compatible solutes, such as sugar alcohols or amino acids (Hu and Schmidhalter 2005). However, as this process is dependent on the provision of photoassimilates, it is very costly to the plant. In contrast, the uptake and storage of increased amounts of K is an energetically "cheaper" alternative. Accordingly, hyperosmotic treatments, imitating a low soil water potential, cause a sustained K uptake into roots, e.g., of barley (Chen et al. 2005). In the field, an ample K supply will thus support osmotic adjustment and sustain cell expansion at low soil water potentials (Grzebisz et al. 2013).

Microbes have solubilized potassium in water stress condition; these are some species of microbes reported: *Paenibacillus polymyxa, Sporosarcina* sp., *Planococcus salinarum*, *Bacillus pumilus*, *Acidithiobacillus ferrooxidans*, *Bacillus*

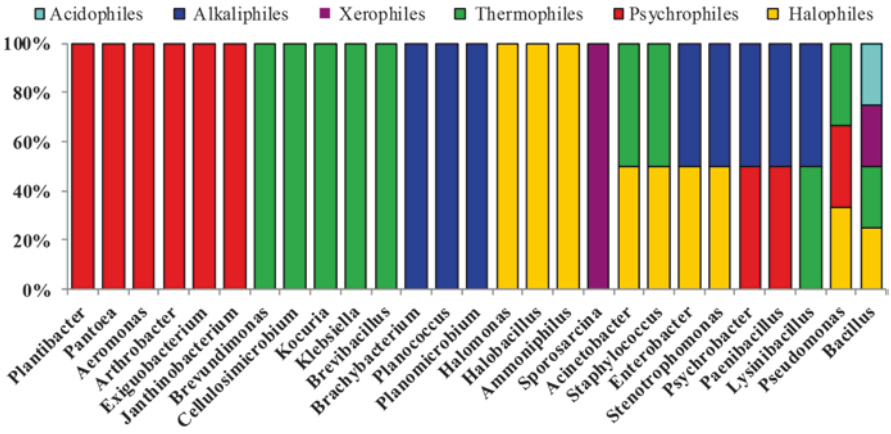


Fig. 7.3 Distribution of potassium-solubilizing microbes in diverse environments (Acidophiles: (Barker et al. 1998; Badar et al. 2006; Verma et al. 2013; Yadav et al. 2015b) Alkaliphiles: (Rosa-Magri et al. 2012; Bagyalakshmi et al. 2012; Zhang and Kong 2014; Meena and Kanwar 2015; Verma et al. 2013, 2014, 2015a, 2016a, b), Halophiles: (Meury and Kohiyama 1989; Oren 1999; Yadav et al. 2015c; Ouellette et al. 2015), Psychrophiles: (Mishra et al. 2011; Selvakumar et al. 2011; Bisht et al. 2013; Yadav et al. 2014; Verma et al. 2015c), Thermophiles: Verma et al. 2015b, 2016a, b), Xerophiles: (Sheng et al. 2002; Verma et al. 2014))

mucilaginosus, *Bacillus edaphicus*, and *Bacillus megaterium* (Sheng et al. 2002). Verma et al. (2014) have identified drought-tolerant K-solubilizing microbes such as *Bacillus megaterium*, *Duganella violaceusniger*, *Paenibacillus dendritiformis*, *Paenibacillus amylolyticus*, *Pseudomonas thivervalensis*, *Psychrobacter fozii*, *Pseudomonas monteilii*, *Pseudomonas lini*, *Stenotrophomonas maltophilia*, and *Stenotrophomonas* sp. from wheat crops growing on central zone of India. These types of microbes protect plants from water deficiency (Fig. 7.3).

7.5 Potassium Availability in the Soil and Its Relevance for Crop Production

Since the 1960s, the world population has doubled from three to seven billion, and this trend will persist in the coming decades. Because of this rapid expansion, a massive increase in crop production is required to meet the food and energy demands of future generations, while also preserving the ecological and energy-related resources of our planet. Additionally, recent climate models predict that incidences and duration of drought and heat stress periods are increasing in many regions, negatively affecting our major crops and thus our food security. Therefore, major challenges for agriculture are to enhance crop yields in more resource-efficient systems and to stabilize plant development and yield formation under biotic and abiotic stress conditions.

7.5.1 Potassium in Soils

Many soils which were initially rich in K have become deficit due to luxurious utilization by crops and inadequate application of K fertilization, soil fixation, runoff, leaching, and soil erosion by different sources (Sheng and Huang 2002; Archana et al. 2012). As mineral soils contain 0.04–3% K, the total K content of the upper 0.2 m of most agricultural soils generally ranges between 10 and 20 g kg^{-1} (Jackson, 1964; Sparks 1987). However, in most of the soil, K (90–98%) is incorporated in the crystal lattice structure of minerals and thus not directly available for plant uptake. The availability of K differs greatly with soil type and is affected by physicochemical properties of the soil. To simplify the complex K dynamics in soil, K in soil is often classified into four groups depending on its availability to plants: water-soluble, exchangeable, non-exchangeable, and structural forms (Fig. 7.1).

Water-soluble K is directly available for plants and microbes and potentially subjected to leaching. Exchangeable K is electrostatically bound as an outer-sphere complex to the surfaces of clay minerals and humic substances (Barre et al. 2008). Both fractions are often considered to be easily available for crops. However, the size of both pools is very small. They make up only about 0.1–0.2% and 1–2% of the total K in soil, respectively (Sparks 1987). Non-exchangeable and structural forms are considered to be slowly- or non-available K sources for plants. However, these pools may also contribute significantly to the plant supply in the long term (Pal et al. 2001) (Fig. 7.4).

Most of the K in soil is in the structural form, mainly comprised of K-bearing primary minerals such as muscovite, biotite, and feldspars. K-feldspars may directly release K to the soil solution, whereas interlayer K of micas is held tightly by electrostatic forces. Weathering of K-feldspars and micas inherited from soil parent materials produces secondary soil minerals which represent the potential sources of plant-available K in soils (Singh and Goulding 1997). K in trioctahedral micas (such as biotite and phlogopite) is reported to be more readily released by weathering, and to stabilize plant development and yield formation under biotic and abiotic stress conditions (Reynolds et al. 2011). In this context, among the many plant nutrients,

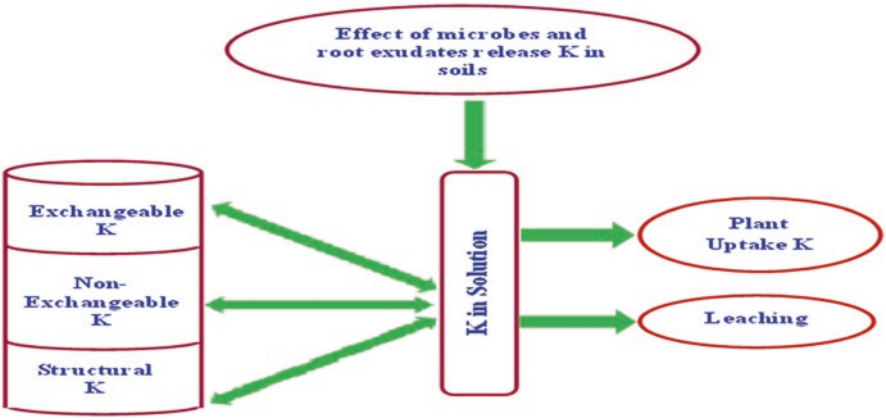


Fig. 7.4 Effects of microbes and root exudates release different forms of K in the soil

potassium (K) plays a particularly crucial role in a number of physiological processes vital to growth, yield, quality, and stress resistance of all crops.

7.5.2 Mechanisms of Potassium Solubilization

Mechanism of potassium solubilization means by which the insoluble potassium and structurally unavailable form of potassium compounds are mobilized and solubilized due to the production of various types of organic acids which are accompanied by acidolysis and complexolysis exchange reactions and these are key processes attributed to the conversion in a soluble form. The efficiency of the K solubilization by various microorganisms was found to vary according to the nature of potassium-bearing minerals and aerobic conditions (Uroz et al. 2009). The release of various types of organic acids were reflected by microorganisms to solubilized the insoluble K to an available form of K. Solubilization of feldspar and illite via rhizospheric microorganisms is due to the production of organic acids like citric acid, tartaric acids, 2-ketogluconic acid, oxalic acid, gluconic acid, malic acid, propionic acid, and fumaric acid, which is easily taken up by the plant. Glycolic and succinic acid seems to be the most frequent agent of K solubilization of mineral (Prajapati and Modi 2012; Zarjani et al. 2013).

Potassium solubilizing microbe solubilized K is done by lowering the pH or by enhancing chelation of the cations bound to K and acidolysis of the surrounding area of microorganism. Such acidolysis by organic acids produced by the rhizospheric microorganisms can either directly dissolve the mineral K as a result of slow releases of exchangeable K, readily available exchangeable K, or can chelate both Si and Al ions associated with K minerals (Romheld and Kirkby 2010). Thus, the synthesis and discharge of organic acids by the microorganisms into the surrounding environment acidify the microbe's cells and their surrounding environment that ultimately leads to the release of K ions from the mineral by protonation and acidification (Goldstein 1994).

7.5.3 Role of Potassium in Plant Growth Promotion

Plant species are known to differ in their K requirement and in their ability to take up K. The differences in absorption of K among different plant species are attributed to variations in root structure, such as root density, rooting depth, and root hair length for more details on the mechanisms of K uptake in plant roots (Nieves-Cordones et al. 2014) and for aspects on the distribution of K throughout the plant (Ahmad and Maathuis 2014; Wigoda et al. 2014). Positive correlations between K uptake efficiency and root hair length or density in K-depleted soils have been reported for maize, oilseed rape, tomato (Jungk 2001), pea, red clover, barley, rye, and perennial ryegrass (Hogh-Jensen and Pedersen 2003). Mengel and Steffens (1985) hypothesized that rye grass competes for K more effectively than red clover due to its longer root hairs and denser root system. Both morphological parameters may also deplete the K in larger volumes of soil solution, and this depletion of K can initiate the release of non-exchangeable K.

In intact plants, K uptake by leaves is stimulated by light (Blum et al. 1992). However, literature reports comparing the effect of K on photosynthesis in different plant species tend to be inconsistent. Tsonev et al. (2011) showed positive effects of K nutrition on the rate of photosynthesis only in crops subjected to some drought treatment. Similarly, Sen Gupta et al. (1989) also reported that plants supplied with elevated K levels showed similar levels of photosynthetic rates. However, when similar plants were exposed to drought, rates of photosynthesis were positively correlated with application rates of K. There are no clear explanations for how K starvation or suboptimal K nutrition downregulate photosynthesis, e.g., under drought conditions. Therefore, further research is needed to explain these findings, especially for crops. In this context, techniques such as chlorophyll fluorescence imaging may be used for noncontact detection of key physiological parameters regulating photosynthesis (e.g., quantum yield, electron transport rate) and stress defense mechanisms (heat dissipation, chlorophyll fluorescence) from the microscopic to the remote sensing scale (Chaerle et al. 2007). Capturing critical threshold values for potassium deficiencies (and quantifying optimum or slight super-optimum potassium nutrition) under high light, drought stress, or heat stress may be possible at early stages of the vegetation period. In the agronomic literature, high K concentrations in crops have often been termed "luxury consumption" which may be considered as an "insurance strategy" to enable the plant to better survive a sudden environmental stress (Kafkafi 1990).

7.5.4 Effects of Potassium-Solubilizing Microorganisms on Different Crops

The application of organo-minerals with a combination of silicate bacteria for enhancing plant growth and yield of maize and wheat was first reported by Aleksandrov et al. (1967). More importantly, research investigation conducted under field level test crops such as wheat, forage crop, maize, and Sudan grass crops has revealed that KSMs could drastically reduce the usage of chemical or organic fertilizers (Xie 1998). KSMs have been isolated from rhizospheric soil of various plants and from K-bearing mineral (Parmar and Sindhu 2013; Zhang et al. 2013); feldspar (Sheng et al. 2008); potato-soybean-cropping sequence (Biswas 2011); Iranian soils (Zarjani et al. 2013); ceramic industry soil (Prajapati and Modi 2012); mica core of Andhra Pradesh (Gundala et al. 2013); common bean (Kumar et al. 2012); biofertilizers (Zakaria 2009); sorghum, maize, bajra, and chili (Archana et al. 2013); cotton, tomato, soybean, groundnut, and banana (Archana et al. 2012); soil of Tianmu Mountain, Zhejiang Province (China) (Hu et al. 2006); rice (Muralikannan 1996); tea (Bagyalakshmi et al. 2012); Valencia orange (Shaaban et al. 2012); black pepper (Sangeeth et al. 2012); potato (Abdel-Salam and Shams 2012); thyme (Yadegari et al. 2012); eggplant (Han and Lee 2006); peanut and sesame (Youssef et al. 2010); and tobacco (Subhashini and Kumar 2014). Better crop performance was reported to be achieved from several horticultural plants, vegetables, and cereals, which were successfully inoculated with KSMs (Singh

et al. 2010; Basak and Biswas 2012; Prajapati et al. 2013). Inoculation with KSMs has been reported to exert beneficial effects on growth of cotton and rape (Sheng 2005), pepper and cucumber (Han et al. 2006), khella (Hassan et al. 2010), sorghum (Badr 2006), wheat (Sheng and He 2006), tomato (Lin et al. 2002), chili (Ramarethinam and Chandra 2005), Sudan grass (Basak and Biswas 2010), and tobacco (Zhang and Kong 2014) Table 7.1.

Prajapati et al. (2012) isolated four different potassium-solubilizing fungi from soils nearby ceramic industries and found that *Aspergillus niger* and *A. terreus* possess a greater potassium-solubilizing activity. *Aspergillus*, *Penicillium*, and *Fusarium* were reported for their remarkable activity to solubilize different kinds of insoluble mineral salts in rocks including phosphates, zinc, and potassium salts (Gour 1990; Simine et al. 1998). Lopes-Assad et al. (2010a, b) reported that *Aspergillus niger* has a better ability to solubilize silicates of potassium and aluminum. Rock powder has been solubilized by *Aspergillus niger* as a source of potassium for agroecological systems Table 7.2.

According to Archana et al. (2012), the efficient K-solubilizing bacteria *Bacillus* spp. showed increase in growth and yield of maize. It indicates that the KSMs significantly increased yield, plant growth, and nutrient uptake component over absolute fertilizer control. Supanjani et al. (2006) reported that integration of P and K rocks with inoculation of K- and P-solubilizing bacteria increased K availability from 13 to 15% and P availability from 12% to 21%, respectively. Soil application of KSMs on plant has ~16% photosynthesis and 35% higher leaf area to control. The overall result of this experiment is the treatment of P and K rocks with P- and K-solubilizing bacterial strains that were sustainable and alternative of chemical fertilizer for crop production. Bagyalakshmi et al. (2012) reported that K-solubilizing strains were isolated from rhizosphere of tea and used as biofertilizers of K in tea that have a solubilizing capacity of muriate of potash (MOP) was increased as compared to mineral K sources. Supplementation of glucose and ammonium nitrate was found to be highly effective in solubilization of MOP as compared to the other sources which should be considered prior to the application of these strains in tea soils as bioinoculants.

K-solubilizing microbes as biofertilizers for agriculture improvement can reduce the use of agrochemicals and support eco-friendly crop production (Archana et al. 2012, 2013; Kloepper et al. 1989; Requena et al. 1997; Sheng et al. 2003; Sindhu et al. 2010; Prajapati et al. 2012, 2013). Therefore, it is imperative to isolate more species of mineral-solubilizing bacteria to enrich the pool of microbial species and genes as microbial fertilizers, which will be of great benefit to the ecological development of agriculture (Liu et al. 2012). Plant growth promoting bioinoculants were assumed to have greater importance in sustainable crop protection which could increase the shelf life providing tolerance to increase adverse conditions (Suman et al. 2016a, b). K-solubilizing microorganisms develop efficient indigenous microbial consortia which are required for enhancing plant growth and yield of various crops as well as improving the soil fertility. This type of microbial consortium is cost-effective and environmentally friendly for enhancing the sustainable agriculture.

Table 7.1 Beneficial effect of potassium-solubilizing bacteria in different plants

KSM	Phylum	Source	References
Acidithiobacillus ferrooxidans	*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Agrobacterium tumefaciens	α-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Aminobacter	*Proteobacteria*	Rhizosphere	Uroz et al. (2007)
Arthrobacter sp.	*Actinobacteria*	Iranian soils	Zarjani et al. (2013)
Azotobacter chroococcum	γ-*Proteobacteria*	Wheat and maize	Singh et al. (2010) and Sheng and He (2006)
Bacillus	*Firmicutes*	Cotton, tomato, soybean, groundnut, banana	Archana et al. (2012)
Bacillus altitudinis	*Firmicutes*	Wheat	Verma et al. (2015a, b, c)
Bacillus amyloliquefaciens	*Firmicutes*	Wheat	Verma et al. (2015a, b, c)
Bacillus amyloliquefaciens	*Firmicutes*	Mica core of Andhra Pradesh	Gundala et al. (2013)
Bacillus circulans	*Firmicutes*	Potato	Abdel-Salam and Shams (2012)
Bacillus edaphicus	*Firmicutes*	Rhizosphere	Sheng (2002)
Bacillus globisporus	*Firmicutes*	Weathered feldspar	Sheng et al. (2008)
Bacillus licheniformis	*Firmicutes*	*Oryza sativa*, *Zea mays*, *Sorghum bicolor*, and wheat	Sheng et al. (2008), Singh et al. (2010) and Basak and Biswas (2012)
Bacillus megaterium	*Firmicutes*	Valencia orange	Shaaban et al. (2012)
Bacillus mucilaginosus	*Firmicutes*	Eggplant, black pepper, maize, wheat	Han and Lee 2006, Sangeeth et al. (2012) and Prajapati et al. (2013)
Bacillus sp.	*Firmicutes*	Rice	Muralikannan (1996)
Bacillus sp. BPR7	*Firmicutes*	Common bean	Kumar et al. (2012)
Bacillus subtilis	*Firmicutes*	Wheat	Verma et al. (2016a, b)
Bacillus thuringiensis	*Firmicutes*	Cold desert	Yadav et al. (2016)
Burkholderia	β-*Proteobacteria*	Tobacco	Uroz et al. (2007) and Zhang and Kong (2014)
Burkholderia cepacia	β-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Clostridium pasteurianum	*Firmicutes*	Rhizosphere	Reitmeir (1951)
Delftia acidovorans	β-*Proteobacteria*	Wheat	Verma et al. (2016a, b)
Delftia sp.	β-*Proteobacteria*	Wheat	Verma et al. (2016a, b)
Enterobacter aerogenes	γ-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Enterobacter asburiae	γ-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Enterobacter cloacae	γ-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Enterobacter hormaechei	γ-*Proteobacteria*	Ceramic industry soil	Prajapati and Modi (2012)

(continued)

Table 7.1 (continued)

KSM	Phylum	Source	References
Klebsiella variicola	γ-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Methylobacterium mesophilicum	α-*Proteobacteria*	Wheat peninsular zone	Verma et al. (2016a, b)
Methylobacterium sp.	α-*Proteobacteria*	Wheat peninsular zone	Verma et al. (2016a, b)
Microbacterium foliorum	α-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Myroides odoratimimus	*Bacteroidetes*	Tobacco	Zhang and Kong (2014)
Paenibacillus frequentans	*Firmicutes*	Rhizosphere	Argelis et al. (1993)
Paenibacillus glucanolyticus	*Firmicutes*	Rhizosphere	Sangeeth et al. (2012)
Paenibacillus kribensis CX-7	*Firmicutes*	Rhizosphere soil, wheat soil of Chang'an, Shanxi Province	Parmar and Sindhu (2013) and Zhang et al. (2013)
Paenibacillus mucilaginosus	*Firmicutes*	Soil of Tianmu Mountain, Zhejiang Province (China)	Hu et al. (2006)
Paenibacillus spp.	*Firmicutes*	Rhizosphere	Sheng et al. (2008), Singh et al. (2010) and Basak and Biswas (2012)
Pantoea agglomerans	γ-*Proteobacteria*	Tobacco	Zhang and Kong (2014)
Pseudomonas azotoformans	γ-*Proteobacteria*	*Oryza sativa*, *Zea mays*, *Sorghum bicolor*, and *Triticum aestivum*	Sheng et al. (2008), Singh et al. (2010) and Basak and Biswas (2012)
Pseudomonas	γ-*Proteobacteria*	Sorghum, maize, bajra, chili	Archana et al. (2013)
Pseudomonas putida	γ-*Proteobacteria*	Tea	Bagyalakshmi et al. (2012)
Rhizobium	α-*Proteobacteria*	Wheat and maize	Sheng and He (2006)
Salmonella bongori	γ-*Proteobacteria*	Wheat	Verma et al. (2016a, b)
Sphingomonas	α-*Proteobacteria*	Rhizosphere	Uroz et al. (2007)

Table 7.2 Beneficial effect of potassium-solubilizing fungi in different sources

KSM	Phylum	Source	References
Aspergillus fumigatus	*Ascomycota*	Waste disposal	Lopes et al. (2010)
Aspergillus awamori	*Ascomycota*	Compost	Biswas DR (2011) and Shukla et al. (2016)
Aspergillus niger	*Ascomycota*	Rock powder, tea	Prajapati et al. (2012) and Nath et al. (2015)
Aspergillus spp.	*Ascomycota*	K-rich soil	Prajapati et al. (2013)
Aspergillus terreus	*Ascomycota*	K-rich soil	Prajapati et al. (2012)
Cladosporium	*Ascomycota*	Tobacco	Zhang and Kong (2014)
Fusarium solani	*Ascomycota*	Cutinase enzymes	Sebastiao et al. (1993)
Glomus intraradices	*Glomeromycota*	Maize	Wu et al. (2005)
Glomus mosseae	*Glomeromycota*	Maize	Wu et al. (2005)
Penicillium spp.	*Ascomycota*	K-rich soil	Sangeetha et al. (2012)

7.6 Conclusions

In this book chapter, we summarize current knowledge regarding the importance of K in plant growth and quality in changing climate and discuss also the factors controlling K availability in soil. Potassium solubilizing microorganisms play an important role in plant nutrition that enhances the K acquisition of plants through soil which increase plant growth promotion activities; these KSM contributions play an important role to bio-fertilization of agricultural crops. Accordingly, further investigation is required to improve the performance and use of potassium-solubilizing microorganism as efficient microbial bioinoculants. The greater attention is needed for studies and application of new efficient combinations of potassium-solubilizing microorganisms and other plant growth-promoting microorganisms for improved results. The mechanisms explaining the synergistic interaction among KSM required further research to elucidate the molecular basis of these interactions. On the other hand, the application of biotechnological tools for genetic manipulation of potassium solubilizing microorganism increases their potassium-solubilizing efficiency/ability/capabilities and/or the insertion of this trait into other strains of plant growth-promoting effects.

7.7 Future Prospects

The K fertilizer supply is often inadequate due to economic reasons, unavailability of fertilizers, or limited knowledge. Fertilizer application techniques may be still better adjusted to the prevailing crop and growth conditions, e.g. as foliar sprays. An increased utilization of the large plant-non-available pool of soil K could decrease the fertilization requirements and improve crop performance, in particular in low-input systems. Ways to tap this resource would be the introduction of competitive K-mobilizing bacterial strains and the design of more K-efficient crop genotypes by

conventional breeding or targeted biotechnological strategies. Promising targets for an improvement of K uptake are root morphology and anatomy, transporter kinetic sand regulation, as well as the release of root exudates. There is considerable variation among species and cultivars in those traits. Optimized K fertilizer application in K limited soils is crucial in order to enhance plant response especially to drought stress via enhancing adaptive/resistance mechanisms of crop plants. Especially, because the demand of K is expected to increase significantly, in particular in developing regions of the world.

Acknowledgment The authors are thankful to the Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi, and the Department of Biotechnology (DBT), Ministry of Science and Technology, for providing the facilities and financial support, to undertake the investigations.

References

Abdel-Salam MA, Shams AS (2012) Feldspar-K fertilization of potato (*Solanum tuberosum* L.) augmented by biofertilizer. J Agric Environ Sci 12(6):694–699

Ahmad I, Maathuis FJM (2014) Cellular and tissue distribution of potassium; physiological relevance, mechanisms and regulation. J Plant Physiol 171:708–714

Aleksandrov VG, Blagodyr RN, Iiiev IP (1967) Liberation of phosphoric acid from apatite by silicate bacteria. Mikrobiyol Zh (Kiev) 29:111–114

Alves L, Oliveira VL, Filho GNS (2010) Utilization of rocks and ectomycorrhizal fungi to promote growth of eucalypt. Braz J Microbio 41:676–684

Archana DS, Nandish MS, Savalagi VP, Alagawadi AR (2012) Screening of potassium solubilizing bacteria (KSB) for plant growth promotional activity. Bioinfolet 9(4):627–630

Archana DS, Nandish MS, Savalagi VP, Alagawadi AR (2013) Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil. Bioinfolet 10:248–257

Argelis DT, Gonzala DA, Vizcaino C, Gartia MT (1993) Biochemical mechanism of stone alteration carried out by filamentous fungi living in monuments. Biogeo Chem 19:129–147

Badar MA, Shafei AM, Sharaf El-Deen SH (2006) The dissolution of K and phosphorus bearing minerals by silicate dissolving bacteria and their effect on sorghum growth. Res J Agric Biol Sci 2:5–11

Bagyalakshmi B, Ponmurugan P, Marimuthu S (2012) Influence of potassium solubilizing bacteria on crop productivity and quality of tea (*Camellia sinensis*). Afr J Agri R 7:4250–4259

Barbosa Filho MP, Fageria NK, Santos DF, Couto PA (2006) Aplicação de rochas silicáticas como fontes alternativas de potássio para a cultura do arroz de terras altas. Revista Espaço e Geografia 9(1)

Barker WW, Welch SA, Chu S, Banfield JF (1998) Experimental observations of the effects of bacteria on aluminosilicate weathering. Am Mineralogist 83(11):1551–1563

Barre P, Montagnier C, Chenu C, Abbadie L, Velde B (2008) Clay minerals as a soil potassium reservoir: observation and quantification through X-ray diffraction. Plant Soil 302:213–220

Basak B, Biswas D (2009) Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. Plant and Soil 317:235–255

Basak BB, Biswas DR (2010) Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. Biol Fertil Soils 46:641–648

Basak B, Biswas D (2012) Modification of waste mica for alternative source of potassium: evaluation of potassium release in soil from waste mica treated with potassium solubilizing bacteria (KSB). ISBN 978-3659298424

Bisht SC, Mishra PK, Joshi GK (2013) Genetic and functional diversity among root-associated psychrotrophic *Pseudomonads* isolated from the Himalayan plants. Arch Microbiol 195:605–615

Biswas DR (2011) Nutrient recycling potential of rock phosphate and waste mica enriched compost on crop productivity and changes in soil fertility under potato-soybean cropping sequence in an Inceptisol of Indo-Gangetic Plains of India. Nutr Cycl Agroecosyst 89(1):15–30

Blum DE, Elzenga JTM, Linnemeyer PA, Van Volkenburgh E (1992) Stimulation of growth and ion uptake in bean leaves by red and blue light. Plant Physiol 100:1968–1975

Bowen H (1966) Of the trace elements. Nuclear activation techniques in the life sciences, p 393

Cao Y, Wei X, Cai P, Huang Q, Rong X, Liang W (2011) Preferential adsorption of extracellular polymeric substances from bacteria on clay minerals and iron oxide. Coll Surf B Biointerf 83(1):122–127

Chaerle L, Leinonen I, Jones HG, Van der Straeten D (2007) Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. J Exp Bot 58:773–784

Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S (2005) Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. Plant Cell Environ 28:1230–1246

Deng S, Bai R, Hu X, Luo Q (2003) Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. App Microbiol Biotechnol 60:588–593

Epstein E (1972) Mineral nutrition of plants: principles and perspectives, p 412

Epstein E, Bloom A (2005) Inorganic components of plants. Mineral nutrition of plants: principles and perspectives, 2nd edn. Sinauer Associates, Inc, Massachusetts, pp 44–45

Gadd GM (1992) Metals and microorganisms: a problem of definition. FEMS Microbiol Lett 100:197–203

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

Glick B, Patten C, Holguin G, Penrose D (1999) Overview of plant growth-promoting bacteria. In: Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London, pp 1–13

Godfray H, Pretty J, Thomas S, Warham E, Beddington J (2011) Linking policy on climate and food. Science 331:1013–1014

Goldstein AH (1986) Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Altern Agric 1:51–57

Goldstein, A. H. (1994). Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington, DC, pp 197–203

Gour AC (1990) Physiological functions of phosphate solubilizing micro-organisms. In: Gour AC (ed) Phosphate solubilizing micro-organisms as biofertilizers. Omega Scientific Publishers, New Delhi, pp 16–72

Grover M, Ali SZ, Sandhya V, Rasul A (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240

Grzebisz W, Gransee A, Szczepaniak W, Diatta J (2013) The effects of potassium fertilization on water-use efficiency in crop plants. J Plant Nutr Soil Sci 176:355–374

Gundala PB, Chinthala P, Sreenivasulu B (2013) A new facultative alkaliphilic, potassium solubilizing, *Bacillus* Sp. SVUNM9 isolated from mica cores of Nellore District, Andhra Pradesh, India. Research and reviews. J Microbiol Biotechnol 2(1):1–7

Han HS, Supanjani, Lee KD (2006) Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ 52:130–136

Han HS, Lee KD (2006) Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ 52:130–136

Hassan EA, Hassan EA, Hamad EH (2010) Microbial solubilization of phosphate–potassium rocks and their effect on khella (*Ammi visnaga*) growth. Ann Agric Sci 55(1):37–53

Hogh-Jensen H, Pedersen MB (2003) Morphological plasticity by crop plants and their potassium use efficiency. J Plant Nutr 26:969–984

Hu Y, Schmidhalter U (2005) Drought and salinity: a comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sci 168:541–549

Hu X, Chen J, Guo J (2006) Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J Microbiol Biotechnol 22:983–990. https://doi.org/10.1007/s11274-006-9144-2

Jackson ML (1964) Chemical composition of soils. In: Bear FE (ed) Chemistry of the soil. Van Nostrand Reinhold, New York, pp 71–141

Jungk A (2001) Root hairs and the acquisition of plant nutrients from soil. J Plant Nutr Soil Sci 164:121–129

Kafkafi U (1990) The functions of plant K in overcoming environmental stress situations. In: Development of K-fertilizer recommendations: proceedings 22nd Colloquium of the International Potash Institute, pp 81–93

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43

Kumar P, Dubey RC, Maheshwari DK (2012) *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 67:493–499

Li Q, Li Z, Huang Y (2007) Status of potassium studies and approaches to improving potassium content in tobacco leaves in China. Agric Sci Technol 35:452–455

Lin Q, Rao Z, Sun Y, Yao J, Xing L (2002) Identification and practical application of silicate-dissolving bacteria. Agric Sci China 1:81–85

Liu D, Lian B, Dong H (2012) Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. Geomicrobiol J 29(5):413–421

Lopes-Assad M L, Avansini SH, Erler G, Rosa MM, de Carvalho JRP, Ceccato-Antonini SR (2010a) Rock powder solubilization by Aspergillus niger as a source of potassium for agroecological systems. 19th World Congress of Soil Science

Lopes-Assad A M L, Avansini A S H, Erler A G, Rosa A M M, Porto J R (2010b). Rock powder solubilization by *Aspergillus niger* as a source of potassium for agroecological systems. In 19th World Congress of Soil Science

Maqsood M, Shehzad MA, Wahid A, Butt AA (2013) Improving drought tolerance in maize (Zea mays) with potassium application in furrow irrigation systems. Int J Agr Biol 15:1193–1198

Meena KR, Kanwar SS (2015) Lipopeptides as the antifungal and antibacterial agents: Applications in food safety and therapeutics. Biomed Res Int 2015

Mengel K, Busch R (1982) The importance of the potassium buffer power on the critical potassium level in soils. Soil Sci 133:27–32

Mengel K, Steffens D (1985) Potassium uptake of rye grass (*Lolium perenne*) and red clover (*Trifolium pratense*) as related to root parameters. Biol Fertil Soils 1:53–58

Meury J, Kohiyama M (1989) ATP is required for K^+ active transport in the archaebacterium Haloferax *volcanii*. Arch Microbiol 151:530–536

Mishra PK, Bisht SC, Ruwari P, Selvakumar G, Joshi GK, Bisht JK, Bhatt JC, Gupta HS (2011) Alleviation of cold stress in inoculated wheat (*Triticum aestivum* L.) seedlings with psychrotolerant *Pseudomonads* from NW Himalayas. Arch Microbiol 193:497–513

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Muralikannan M (1996) Biodissolution of silicate, phosphate and potassium by silicate solubilizing bacteria in rice ecosystem. M. Sc.(Agric) thesis, Tamil Nadu Agricultural University, Coimbatore

Nagendran K, Karthikeyan G, Peeran MF, Raveendran M, Prabakar K, Raguchander T (2013) Management of bacterial leaf blight disease in rice with endophytic bacteria. World App Sci J 28:2229–2241

Naidu L, Sidhu G, Sarkar D, Ramamurthy V (2011) Emerging deficiency of potassium in soils and crops of India. Karnat J Agricult Sci 24(1)

Nath R, Sharma GD, Barooah M (2015) Plant growth promoting endophytic fungi isolated from tea (Camellia sinensis) shrubs of Assam, India. Appl Ecol Environ Res 13:877–891
Nies DH (1999) Microbial heavy-metal resistance. App Microbiol Biotechnol 51:730–750
Niimura Y, Koh E, Yanagida F, K4 S, Komagata K, Kozaki M (1990) Amphibaciffus xyfanus gen. nov., sp. nov., a facultatively anaerobic sporeforming xylan-digesting bacterium which lacks cytochrome, quinone, and catalase. Int J Syst Bacteriol 40:297–301
Oren A (1999) Bioenergetic aspects of halophilism. Mircobiol Mol Biol Rev 63:334–348
Ouellette M, Jackson L, Chimileski S, Papke RT (2015) Genome-wide DNA methylation analysis of Haloferax volcanii H26 and identification of DNA methyltransferase related PD-(D/E) XK nuclease family protein HVO_A0006. Front Microbiol 6
Pal DK, Srivastava P, Durge SL, Bhattacharyya T (2001) Role of weathering of fine-grained micas in potassium management of Indian soils. Appl Clay Sci 20:39–52
Parmar P, Sindhu SS (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. J Microbiol Res 3(1):25–31
Pettigrew WT (2008) Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiologia Plantarum 133:670–681
Prajapati KB, Modi HA (2012) Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. CIBTech. J Microbiol 1:8–14
Prajapati K, Sharma MC, Modi HA (2012) Isolation of two potassium solubilizing fungi from ceramic industry soils. Life Sci Leaflets 5:71–75
Prajapati K, Sharma MC, Modi HA (2013) Growth promoting effect of potassium solubilizing microorganisms on *Abelmoschus esculentus*. Int J Agric Sci 3(1):181–188
Ramarethinam S, Chandra K (2005) Studies on the effect of potash solubilizing/mobilizing bacteria *Frateuria aurantia* on brinjal growth and yield. Pestology 11:35–39
Rao C, Rao AS, Rao K, Venkateswarlu B, Singh A (2010) Categorization of districts based on non-exchangeable potassium: implications in efficient K fertility management in Indian agriculture. Ind J Fert 6:40–54
Reitmeir RF (1951) Soil potassium. In: Norman AG (ed) Advances in agronomy II. Academic Press, New York, pp 113–164
Requena BN, Jimenez I, Toro M, Barea JM (1997) Interactions between plant growth promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in Mediterranean semi-arid ecosystem. New Phytologist 136:667–677
Reynolds M, Bonnett D, Chapman SC, Furbank RT, Manes Y, Mather DE (2011) Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. J Exp Bot 62:439–452
Romheld V, Kirkby EA (2010) Research on potassium in agriculture: needs and prospects. Plant Soil 335:155–180
Rosa-Magri MM, Avansini SH, Lopes-Assad ML, Tauk-Tornisielo SM, Ceccato-Antonini SR (2012) Release of potassium from rock powder by the yeast. Torulaspora globosa. Braz Arch Biol Technol 55:577–582
Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101
Sahay H, Mahfooz S, Singh AK, Singh S, Kaushik R, Saxena AK, Arora DK (2012) Exploration and characterization of agriculturally and industrially important haloalkaliphilic bacteria from environmental samples of hyper saline Sambhar lake, India. World J Microbiol Biotechnol 28:3207–3217
Sangeeth KP, Bhai RS, Srinivasan V (2012) *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. J Spic Aromat Crops 21(2):118–124
Sebastiao MJ, Cabral JMS, Aires-Barros MR (1993) *Fusarium solani* pisi recombinant cutinase partitioning in PEG/potassium phosphate aqueous two-phase systems. Biotechnol Techn 7(9):631–634
Selvakumar G, Joshi P, Suyal P, Mishra PK, Joshi GK, Bisht JK, Bhatt JC, Gupta HS (2011) *Pseudomonas lurida* M2RH3 (MTCC 9245), a psychrotolerant bacterium from the Uttarakhand

Himalayas, solubilizes phosphate and promotes wheat seedling growth. World J Microbiol Biotechnol 27:1129–1135
Sen Gupta A, Berkowitz GA, Pier PA (1989) Maintenance of photosynthesis at low leaf water potential in wheat role of potassium status and irrigation history. Plant Physiol 89:1358–1365
Shaaban EA, El-Shamma IMS, El Shazly S, El-Gazzar A, Abdel-Hak RE (2012) Efficiency of rock-feldspar combined with silicate dissolving bacteria on yield and fruit quality of Valencia orange fruits in reclaimed soils. J Appl Sci Res 8:4504–4510
Shabala S, Cuin TA (2007) Potassium transport and plant salt tolerance. Physiol Plant 133:651–669
Sheng X (2005) Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol Biochem 37:1918–1922
Sheng XF, He LY (2006) Solubilization of potassium-bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. Can J Microbiol 52:66–72
Sheng XF, Huang WY (2002) Mechanism of potassium release from feldspar affected by the strain NBT of silicate bacterium. Acta Pedol Sin 39(6):863–871
Sheng XF, He LY, Huang WY (2002) The conditions of releasing potassium by a silicate dissolving bacterial strain NBT. Agric Sci China 1:662–666
Sheng XF, Zhao F, He H, Qiu G, Chen L (2008) Isolation, characterization of silicate mineral solubilizing *Bacillus globisporus* Q12 from the surface of weathered feldspar. Can J Microbiol 54:1064–1068
Simine CDD, Sayer JA, Gadd GM (1998) Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated form a forest soil. Biol Fert soils 28:87–94
Sindhu SS, Dua S, Verma MK, Khandelwal A (2010) Growth promotion of legumes by inoculation of rhizosphere bacteria. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer-Wien, New York, pp 95–235
Sindhu SS, Parmar P, Phour M (2014) Nutrient cycling: potassium solubilization by microorganisms and improvement of crop growth. In: Geomicrobiol and biogeochemistry. Springer, pp 175–198
Singh B, Goulding KWT (1997) Changes with time in the potassium content and phyllosilicates in the soil of the Broad balk continuous wheat experiment at Rothamsted. Eur J Soil Sci 48:651–659
Singh G, Biswas DR, Marwaha TS (2010) Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (*Zea mays*) and wheat (*Triticum aestivum* L.): a hydroponics study under phytotron growth chamber. J Plant Nutr 33(8):1236–1251
Sparks DL (1987) Potassium dynamics in soils. Adv Soil Sci 6:1–63
Sparks DL, Huang PM (1985) Physical chemistry of soil potassium. In: Munson RD (ed) Potassium in agriculture. Madison, WI: American Society of Agronomy, pp 201–276
Subhashini DV, Kumar AV (2014) Phosphate solubilizing *Streptomyces* spp. obtained from the rhizosphere of *Ceriops decandra* of Corangi mangroves. Indian J Agric Sci 84(5):560–564
Suman A, Verma P, Yadav AN, Srinivasamurthy R, Singh A, Prasanna R (2016a) Develop lications in food safety and therapeutics. ment 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
Suman A, Yadav AN, Verma P (2016b) Endophytic microbes in crops: diversity and beneficial impact for sustainable agriculture. In: Microbial Inoculants in Sustainable Agricultural Productivity. Springer, New Delhi, pp 117–143
Supanjani HH, Jung JS, Lee KD (2006) Rock phosphate potassium and rock solubilizing bacteria as alternative sustainable fertilizers. Agron Sustain Dev 26:233–240
Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
Tsonev T, Velikova V, Yildiz-Aktas L, Gurel A, Edreva A (2011) Effect of water deficit and potassium fertilization on photosynthetic activity in cotton plants. Plant Biosyst 145:841–847

Uroz S, Calvaruso C, Turpault MP, Pierrat JC, Mustin C, Frey-Klett P (2007) Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. Appl Environ Microbiol 73:3019–3027

Uroz S, Calvaruso C, Turpault MP, Frey-Klett P (2009) Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol 17:378–387

Veresoglou SD, Mamolos AP, Thornton B, Voulgari OK, Sen R, Veresoglou S (2011) Medium-term fertilization of grassland plant communities masks plant species-linked effects on soil microbial community structure. Plant Soil 344:187–196

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2013) Elucidating the diversity and plant growth promoting attributes of wheat (*Triticum aestivum*) associated acidotolerant bacteria from southern hills zone of India. Natl J Life Sci 10(2):219–226

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2014) Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (*Triticum aestivum*) growing in central zone of India. Int J Curr Microbiol Appl Sci 3(5):432–447

Verma P, Yadav AN, Khannam KS, Panjiar N, Kumar S, Saxena AK, Suman A (2015a) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann Microbiol 65(4):1885–1899

Verma P, Yadav AK, Khanna KS, Kumar S, Saxena AK, Suman A (2015b) Alleviation of cold stress in wheat seedlings by *Bacillus amyloliquefaciens* IARI-HHS2-30 an endophytic psychrotolerant K-solubilizing bacterium from NW Indian himalayas. Nat J Life Sci 12:105–110

Verma P, Yadav AN, Shukla L, Saxena AK, Suman A (2015c) Hydrolytic enzymes production by thermotolerant *Bacillus altitudinis* IARI-MB-9 and *Gulbenkiania mobilis* IARI-MB-18 isolated from Manikaran hot springs. International. J Adv Res 3(9):1241–1250

Verma P, Yadav AN, Khannam KS, Kumar S, Saxena AK, Suman A (2016a) Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. J Basic Microbiol 56:44–58

Verma P, Yadav AN, Khannam KS, Mishra S, Kumar S, Saxena AK, Suman A (2016b) Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. Saudi J Biol Sci. https://doi.org/10.1016/j.sjbs.2016.01.042

Wakatsuki T (1995) Metal oxidoreduction by microbial cells. J Ind Microbiol 14:169–177

White PJ (2013) Improving potassium acquisition and utilisation by crop plants. J Plant Nutr Soil Sci 176:305–316

Wigoda N, Moshelion M, Moran N (2014) Is the leaf bundle sheath a "smart flux valve" for K^+ nutrition? J Plant Physiol 171:715–722

Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH (2005) Effects of biofertilizer containing N fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166

Xie JC (1998) Present situation and prospects for the world's fertilizer use. Plant Nutri Fertil Sci 4:321–330

Yadav V, Prappulla SG, Jha A, Poonia A (2011) A novel exopolysaccharide from probiotic *Lactobacillus fermentum* CFR 2195: production, purification and characterization. Biotechnol Bioinform Bioeng 1:415–421

Yadav AN, Sachan SG, Verma P, Saxena AK (2014) Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. J Biosci Bioeng 119(6):683–693. https://doi.org/10.1016/j.jbiosc. 2014.11.006

Yadav AN, Sachan SG, Verma P, Tyagi SP, Kaushik R, Saxena AK (2015a) Culturable diversity and functional annotation of psychrotrophic bacteria from cold desert of Leh Ladakh (India). World J Microbiol Biotechnol 31(1):95–108

Yadav AN, Verma P, Kumar M, Pal KK, Dey R, Gupta A, Padaria JC, Gujar GT, Kumar S, Suman A, Prasanna R (2015b) Diversity and phylogenetic profiling of niche-specific Bacilli from extreme environments of India. Ann Microbiol 65(2):611–629

Yadav AN, Sachan SG, Verma P, Kaushik R, Saxena AK (2015c) Cold active hydrolytic enzymes production by psychrotrophic Bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. J Basic Microbiol 56(3):294–307

Yadav AN, Sachan SG, Verma P, Saxena AK (2016) Bioprospecting of plant growth promoting psychrotrophic Bacilli from cold desert of north western Indian Himalayas. Indian J Exp Biol 54:142–150

Yadegari M, Farahani GHN, Mosadeghzad Z (2012) Biofertilizers effects on quantitative and qualitative yield of Thyme (Thymus vulgaris). Afr J Agric Res 7(34):4716–4723

Yousefi AA, Khavazi K, Moezi AA, Rejali F, Nadian NH (2011) Phosphate solubilizing bacteria and arbuscular mycorrhizal fungi impacts on inorganic phosphorus fractions and wheat growth. World Appl Sci J 15(9):1310–1318

Youssef GH, Seddik WMA, Osman MA (2010) Efficiency of natural minerals in presence of different nitrogen forms and potassium dissolving bacteria on peanut and sesame yields. J Am Sci 6(11):647–660

Zakaria AAB (2009) Growth optimization of potassium solubilizing bacteria isolated from biofertilizer. Malaysia Pahang: Bachelor of Chemical Engeneening (Biotech.), Faculty of Chemical and Natural Resources Engineering. Univ, p 40

Zarjani JK, Aliasgharzad N, Oustan S, Emadi M, Ahmadi A (2013) Isolation and characterization of potassium-solubilizing bacteria in some Iranian soils. Arch Agron Soil Sci 59:1713–1723

Zhang C, Kong F (2014) Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. App Soil Ecol 82:18–25

Zhang A, Zhao G, Gao T, Wang W, Li J, Zhang S, Zhu BC (2013) Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: a soil microorganism with biological control potential. Afr J Microbiol Res 7(1):41–47

Zhao F, Sheng X, Huang Z, He L (2008) Isolation of mineral potassium-solubilizing bacterial strains from agricultural soils in Shandong Province. Biodiv Sci 16:593–600

Bacterial Volatile Organic Compounds: A New Insight for Sustainable Agriculture

8

D.G. Panpatte, Y.M. Shukla, H.N. Shelat, R.V. Vyas, and Y.K. Jhala

Abstract

Plant growth-promoting rhizobacteria (PGPR) improve plant growth by improved nutrient acquisition and guarding plants from biotic and abiotic stress. PGPR stimulate plant defense system by induction of systemic resistance or tolerance (ISR/IST). A large number of elicitors are known to stimulate plant defense system, and VOCs are one of the most studied elicitors for ISR/IST response which excites plant defense system without direct physical contact. In this chapter review about the current development regarding interactions of PGPR volatiles and plants is discussed. The mechanisms of action of volatile compounds for plant growth promotion as well as stimulation of plant defense to withstand abiotic and biotic stress are also being elaborated to explain elicitation of plant's self-immunity against various stresses.

Keywords

PGPR • ISR • IST • Volatile organic compounds • Self-immunity • Stress

D.G. Panpatte (✉) • H.N. Shelat • R.V. Vyas • Y.K. Jhala
Department of Agricultural Microbiology, B. A. College of Agriculture, Anand Agricultural University, Anand 388 110, Gujarat, India
e-mail: dgpanpatte@gmail.com

Y.M. Shukla
College of Agriculture, Vaso, Anand Agricultural University, Anand, Gujarat, India

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_8

8.1 Introduction

Living organisms like plants, animals, and microorganisms comprise of a large number of natural chemicals like enzymes, hormones, proteins, and volatile compounds that empower them to survive in nature and play significant roles in organism's metabolism, nutrition, establishment, and conservation in definite ecological location. Volatiles are the compounds having high vapor pressure, which falls into two categories, viz., organic and inorganic. Among inorganic and organic volatiles, volatile organic compounds can travel far from the point of production. Microbial volatile organic compounds (VOC) are found to play key role in antagonism and mutualism. Moreover, various intra- and interspecies cellular and developmental processes are governed by microbial volatiles. Till date the exact mechanism of action of microbial volatiles is to be explained. Since the diversity of volatile-producing microorganisms is huge in nature, if mechanism of action of microbial volatile as an interphase between plant health and microbes can be revealed, then it is likely to disclose unique mechanisms for governing various biological processes critical to plant fitness and will also propose concrete benefits while addressing agricultural and environmental problems.

8.2 Sources of Volatiles in Nature

Biologically produced volatiles comprise of the compounds originating from plants, animals, and microbes. As per general belief, volatile compounds seem to be characteristically linked to the atmosphere, but soil is also considered as a large reservoir of biogenic volatile organic compounds. Volatile organic compounds of biological origin belong to chemical classes such as alcohols, thiols, aldehydes, esters, terpenoids, and fatty acid derivatives which are lipophilic in nature, having low molecular weight and high vapor pressure (Schulz and Dickschat 2007). Usually inside the soil, all the organisms use linkage of signaling pathways to feel the environmental stimuli. This signaling pathway confirms cellular homeostasis which facilitates systematic growth and development as well as controls performance.

8.3 Bacterial Volatile Organic Compounds

In soil, microorganisms like bacteria, fungi, and actinomycetes emit large amount of volatile compounds, among which bacteria are found in higher amount (10^{11} cells/g of soil). Soil bacteria colonize roots, organic residues, and soil particles (Burmolle et al. 2007) as well as the rhizosphere (Mendes et al. 2013). Humans have exploited the potential of microbial volatiles for providing aroma to food and beverages like cheese, sauerkraut, yogurt, wine, etc. The inoculated bacteria release specific odor during fermentation of foodstuffs which is dependent on environmental conditions (Kai et al. 2009). Scientists have discovered more than 1000 different bacterial volatile compounds (Lemfack et al. 2014, http://bioinformatics.

charite.de/mvoc) which are employed by bacteria as communication signals with other organisms which in turn decide positive or negative influence on both the interacting communities (Kai et al. 2009; Romoli et al. 2011). Such volatiles enable the organisms to overcome competitive pressure within the same niche. For example, albaflavenone and dimethyl disulfide are having negative effect on fungal pathogens, whereas geosmin, 2,3-butanediol, acetoin, and tridecane are having positive effect on plant growth. Stress and antibiotic resistance phenotypes of some of the bacteria are attributed to production of volatile compounds. Recently the role of bacterial volatiles in biofilm formation has also been elucidated. Such volatiles attract the nearby bacterial cells to link together to formulate biofilm. Moreover, some of the bacterial volatiles, viz., ammonia and trimethylamine, can alter gut cell physiology in humans and thereby confer immunization against pathogens. Besides these beneficial effects, some of the volatiles of pathogenic microorganisms are responsible for pathogenesis of the strain. In general bacterial volatiles are having tremendous effect on growth, differentiation, and stress resistance in living organisms (Kai et al. 2009; Kai and Piechulla 2009; Effmert et al. 2012; Wenke et al. 2012; Davis et al. 2013).

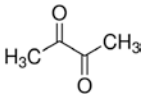
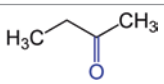
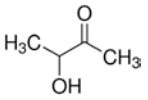
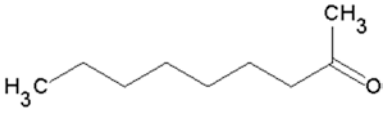
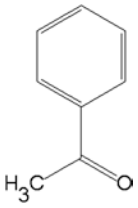
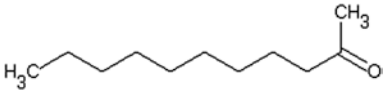
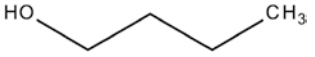
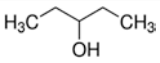
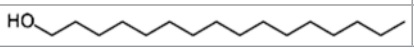
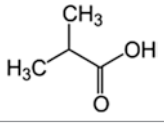
8.3.1 Types of Bacterial Volatile Organic Compounds

Bacterial volatile compounds belonging to different chemical classes are generally produced through catabolic pathways such as glycolysis, protein, and lipid degradation pathways (Schulz and Dickschat 2007; Penuelas et al. 2014). Bacterial volatile compounds derived from organic molecules include numerous chemical classes such as fatty acid derivatives (hydrocarbons, ketones, alcohols), acids, sulfur- and nitrogen-containing compounds, and terpenes (Table 8.1).

8.4 Biological Role of Bacterial Volatiles

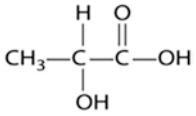
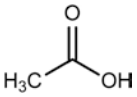
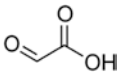
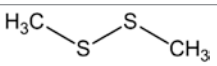
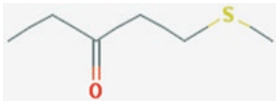
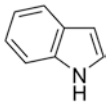
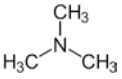
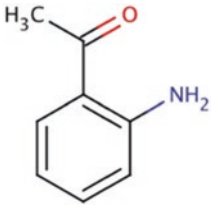
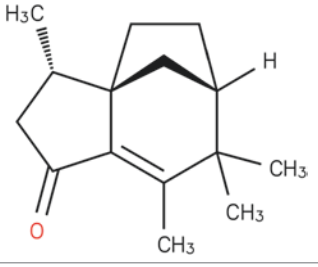
Bacterial volatiles are diverse and complex as compared to that of plants and fungi. Bacterial volatiles are expected to be analogous to other volatiles and possibly assist as communication signals during inter- and intra-organismic communication as well as cell-to-cell communication. It may also act as possible carbon release valve and growth-promoting or growth-inhibiting agents. Volatiles also play an important role in establishment and survival of bacterial populations in ecological niche and for development of different communities. Volatiles can diffuse through aqueous solutions and also travel for far distance in the atmosphere and thereby not only act above ground but also act below ground.

Table 8.1 Types of bacterial volatile organic compounds

Sr. No.	Name of bacterial volatile organic compounds	Structure	Molecular weight
Hydrocarbon			
5	Hexadecane	$H_3C-(CH_2)_{14}-CH_3$	226.45 g/mol
6	Tridecane	$CH_3(CH_2)_{11}CH_3$	184.36 g/mol
Ketones			
7	2,3-Butanedione		86.0892 g/mol
8	2-Butanone		72.11 g/mol
9	Acetoin		88.11 g/mol
10	2-Nonanone		142.23862 g/mol
11	Phenylethanone		120.151 g/mol
12	2-Undecanone		170.30 g/mol
Alcohols			
13	1-Butanol		74.12 g/mol
14	3-Pentanol		88.148 g/mol
15	Hexadecanol		242.4406 g/mol
Acids			
16	Isobutyric acid		88.11 g/mol

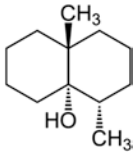
(continued)

Table 8.1 (continued)

Sr. No.	Name of bacterial volatile organic compounds	Structure	Molecular weight
17	Lactic acid		90.08 g/mol
18	Acetic acid		60.05 g/mol
19	Glyoxylic acid		74.04 g/mol
Sulfur-containing compound			
20	Dimethyl disulfide		94.2 g/mol
21	1-(Methylthio)-3-pentanone		132.23 g/mol
Nitrogen-containing compound			
22	Indole		117.15 g/mol
23	Trimethylamine		59.11 g/mol
24	2-Aminoacetophenone		135.16 g/mol
Terpenes			
25	Albaflavenone		218.34 g/mol

(continued)

Table 8.1 (continued)

Sr. No.	Name of bacterial volatile organic compounds	Structure	Molecular weight
26	Geosmin		182.31 g/mol

8.5 Role of Bacterial Volatiles in Agriculture

Food safety is adversely affected by climate change and growing pathogens which reduce crop yield. Use of agrochemicals like synthetic pesticides and fertilizers ensures protection against disease and high crop yield, but ultimately, they significantly affect the health of human and environment. In the present era, biological inputs like biopesticides, biofertilizers, and biodegraders are gaining momentum as appropriate alternatives of synthetic agro-inputs. Limiting factors for polarization of such bioinputs include less efficiency, high costs, and inconsistent performance under field conditions (Glare et al. 2012). Researchers have demonstrated that exposure of plants to bacterial volatiles has significant effect on modulation of plant metabolism, physiology, and genetic status which leads to belief that the plants are capable to recognize and react to microbial volatiles. Till date majority of research regarding plant-bacterial volatile interactions are conducted under laboratory conditions, but recently few of the field trials demonstrating efficiency of bacterial volatiles for sustainable crop protection and production have been conducted (Cortes-Barco et al. 2010a, b; Song and Ryu 2013). These studies undoubtedly establish the essentiality for application of bacterial volatiles in open field conditions and emphasize their various roles to escalate pathogen resistance, defense against herbivores, and as biocontrol agents. Operational distribution of bacterial volatiles still remains a major task.

8.5.1 Bacterial Volatile Compounds as Biostimulants

Bacterial volatile compounds are having a major role in promotion of plant growth. Without direct physical contact between plant and microorganism, bacterial volatile compounds can stimulate plant growth (Ryu et al. 2003). Among various PGPR tested, *Bacillus subtilis* GB03 and *B. amyloliquefaciens* IN937a were found to stimulated plant growth by emission of volatile compounds. To reveal a signaling pathway for VOC-mediated plant growth promotion, a series of mutant lines were tested (Ryu et al. 2003). Upon contact with volatile compounds produced by *B. subtilis* GB03, the total leaf surface area was found to increase in mutant lines ethylene insensitive (*etr*1), auxin-transporter-deficient and ethylene insensitive (*eir*1),

gibberellic acid-insensitive (*gai*2), and brassinosteroid-insensitive (*cbb*1). These altogether thereby disprove the necessity of brassinosteroid, gibberellic acid, or ethylene signaling in the plant growth promotion by volatile compounds. Under field condition, *B. subtilis* GB03 is assumed to persist on seeds before planting and then after it uses seed exudates during seed germination and multiply to finally reach up to growing roots where they will conserve a healthy population through plant–microbe interactions (Kloepper et al. 2004). Required bacterial strength to start plant response is recommended to be 10^4 colony-forming units (cfu)/root. *B. subtilis* GB03 was reported to maintain soil populations of 10^5 cfu/root up to 60 days after planting (Kokalis- Burelle et al. 2006).

8.5.2 Bacterial Volatile Compounds as Bio-protectants Against Abiotic Stress

Bacterial volatile compounds induce systemic tolerance response against abiotic stress such as nutrient deficiency, salinity, and drought (Yang et al. 2009). Induced systemic tolerance is physical and chemical alterations in plants stimulated by PGPR which culminate in improved tolerance to abiotic stresses.

Salt Tolerance

Under saline conditions, the plant faces osmotic stresses which results in reduction of crop growth and yield. The basic mechanism underlying induced systemic tolerance in plants against saline condition mediated by bacterial volatiles comprise of decreased sodium uptake in roots and increased discharge of sodium ions from shoots through regulation of various transport proteins including HKT1 and SOS1. Bacterial volatile organic compound (VOC) upregulates HKT1 gene which in turn increases elimination of sodium ions from xylem sap, thereby expediting elimination of sodium ions from plant leaves. Similarly HKT1 is downregulated in the roots. This mechanism was discovered by a thorough study of *B. amyloliquefaciens* GB03 showing VOC-mediated systemic tolerance (Mayak et al. 2004; Barriuso et al. 2008; Zhang et al. 2008a). During the studies it was revealed that *Arabidopsis* plants treated with GB03 VOC showed increased biomass and less sodium ion content as compared to untreated plants (Zhang et al. 2008a). Similar type of induced systemic tolerance was observed in wild-type plants but not in the *hkt1* mutant, proposing a crucial role of HKT1 in facilitating the salinity tolerance activated by GB03 VOCs. Moreover, increasing the shoot-to-root recirculation of sodium ions too can lead to a greater amount of sodium ions in the roots and lower concentration of sodium ions in the shoots. GB03 VOCs concomitantly inhibit and escalate HKT1 expression in roots and shoots, respectively, which assist in VOC-induced salt tolerance (Zhang et al. 2008b). SOS3 (calcium-signaling sensor) may contribute in VOC- mediated salinity tolerance. GB03 VOCs exhibited 50% decrease in sodium ion concentration in whole wild-type plant, whereas sos3 mutant showed 15% reduction in sodium ion accumulation (Zhang et al. 2008b), proposing that AtSOS3-dependent Na+ exudation is also essential for the reduced buildup of sodium ions in

VOC-treated plants. Moreover, VOCs produced by GB3 also cause acidification of the rhizosphere (Zhang et al. 2009), thus generating a proton gradient that could hypothetically aid in SOS1-mediated transfer of Na+ from roots. Under saline condition, plants modify their metabolism to survive in osmotic stress triggered by the increased sodium ion concentration. *Pseudomonas simiae* strain AU volatile-induced salt tolerance was observed in soybean plants wherein volatile compounds not only decreased root Na^+ levels but also increased the buildup of proline, which defend cells from osmotic stress (Vaishnav et al. 2015). Moreover, plants treated with AU volatiles showed higher level of vegetative storage protein (VSP) and numerous other proteins that are known to assist plants to withstand under stress conditions (Vaishnav et al. 2015).

Drought Tolerance

Under dehydrating conditions, raised accumulation of osmoprotectants in plants can increase cellular osmotic pressure to lower the free water potential of cells which thereby avoid water loss and can also stabilize structure of proteins and membrane. Under osmotic stress, *Arabidopsis* plants exposed to GB03 volatiles accumulated greater level of choline and glycine betaine than plants without volatile treatment (Zhang et al. 2010). 2,3-Butanediol is the most common volatile organic compound found in *P. chlororaphis* strain O6. *Arabidopsis* plants inoculated with *P. chlororaphis* O6 or exposed to 2,3-butanediol exhibited increased drought stress tolerance, which clearly leads to increased stomatal closure and reduced water loss (Cho et al. 2008). Upon application of *P. chlororaphis* O6 or 2,3-butanediol, concentration of salicylic acid (SA) was significantly increased which showed dependence of induced systemic tolerance pathway on SA (Cho et al. 2008). Certain bacterial volatiles such as acetic acid are able to induce formation of biofilms containing higher amount of exopolysaccharides (Chen et al. 2015) which indirectly increase plant's drought tolerance by conservation of moisture.

Inoculation of wheat with *B. thuringiensis* AZP2 under drought stress leads to enhanced plant biomass and fivefold increase in persistence under severe drought due to significant reduction evaporation and maintenance of higher rate of photosynthesis (Timmusk et al. 1999). Detection of volatiles provides promising technique for rapid, noninvasive assay of crop's drought stress and its mitigation (Timmusk et al. 1999). Occupation of roots by *P. chlororaphis* O6 stops water loss by stomatal closure which is mediated by bacterial volatile compound 2R,3R-butanediol, whereas mutant strain deficient in 2R,3R-butanediol production showed no induction of drought tolerance (Cho et al. 2008). Further, *Arabidopsis* mutant lines indicated that induced drought tolerance required the salicylic acid (SA), ethylene, and jasmonic acid-signaling pathways. Both induced drought tolerance and stomatal closure were dependent on Aba-1 and OST-1 kinase (Cho et al. 2008). PGPR can also change morphology of plant roots under drought stress. Rhizobacteria affects the physiological processes at plant's cell membrane. Inoculation of wheat seedlings with *Azospirillum brasilense* reduced membrane potentials as well as phospholipid content in the cell membranes of cowpea due to the changes in proton efflux activities (Bashan et al. 1992). Under water stress conditions, there occur an

increase in phosphatidylcholine and a decrease in phosphatidylethanolamine content (Sueldo et al. 1996), but inoculation with *Azospirillum* reverts these changes in wheat seedlings (Pereyra et al. 2006). Rhizobacterial inoculation also stimulates changes in the elasticity of root cell membranes which seems to be the first steps toward enhanced tolerance to drought (Dimkpa et al. 2009). PGPR also strengthen plant cell membranes by activating the antioxidant defense system which in turn enhance drought tolerance in plants (Gusain et al. 2015).

Nutrient Acquisition

Bacterial volatiles generally help in the acquisition of sulfur and iron. Dimethyl disulfide (DMDS) is an S-containing volatile compound commonly produced by many soil bacteria and fungi (Kanchiswamy et al. 2015). Emission of DMDS from *Bacillus* sp. strain B55, a natural symbiont of *Nicotiana attenuata* plants, rescued plant growth retardation caused by S-deprivation (Meldau et al. 2013). The incorporation of bacteria-emitted S into plant proteins was demonstrated by adding radiolabeled ^{35}S to the bacterial growth medium. In addition to detecting DMDS, Meldau et al. (2013) also detected the S-containing compound S-methylpentanethioate in *Bacillus* sp. B55 VOCs. The authors attributed most of the S-nutrition provided by *Bacillus* sp. B55 VOCs to DMDS rather than to S-methylpentanethioate for two reasons. First, DMDS was detected as a major component of the volatile emissions, while S-methylpentanethioate was present in only trace amounts. Second, synthetic DMDS was superior to the natural VOC blends in rescuing S-starvation phenotypes of N. attenuata plants (Meldau et al. 2013). Sulfur in SO42 is in an oxidative state and thus requires an energy-consuming reduction process for biological assimilation (Takahashi et al. 2011). In contrast, sulfur in DMDS is in a chemically reduced state. Therefore, it appears that DMDS may not only provide S to plants but may also help plants avoid expending energy on sulfate reduction. Consistent with this hypothesis, DMDS supplementation significantly decreased the expression of S-assimilation genes as well as methionine biosynthesis and recycling (Meldau et al. 2013). Like DMDS in *Bacillus* sp. B55 VOCs, other S-containing volatile compounds such as dimethyl sulfide and dimethyl trisulfide have been detected in high concentrations in other microbial VOC blends (Kanchiswamy et al. 2015). Whether these microbial VOCs may also enhance S-assimilation by plants remains to be determined.

The transition between ferrous iron (Fe2+) and ferric iron (Fe3+) generates a redox potential that is important for electron transfer reactions including photosynthesis. Deprivation of Fe severely impairs the photochemical capacity and is accompanied by leaf chlorosis. Graminaceous monocots produce siderophores that increase Fe3+ mobility in soil and directly uptake Fe3+ without reduction, while non-graminaceous monocots and dicots not only acidify the rhizosphere to increase Fe3+ mobility but also use plasma membrane ferric reductase to reduce Fe3+ and subsequently transport Fe2+ into the roots (Curie and Briat 2003). Augmented Fe uptake was observed in *Arabidopsis* exposed to GB03 VOCs, which do not contain any known siderophores (Farag et al. 2006; Zhang et al. 2009). Under Fe-sufficient growth conditions, plants treated with GB03 VOCs displayed typical Fe deficiency

responses, including transcriptional upregulation of the root Fe3+ reductase gene FRO2 and of the Fe2+ transporter gene IRT1, increases in FRO2 enzyme activity, and rhizosphere acidification(Zhang et al. 2009). As a result, Fe levels were elevated in VOC-treated plants, consistent with greater amounts of Fe-rich photosynthetic apparatus (Zhang et al. 2008b). GB03 VOC-triggered gene induction of IRT1 and FRO2 requires the transcription factor FIT1, because VOC failed to induce IRT1 or FRO2 in the fit1 knockout mutant (Zhang et al. 2009). VOC treatment also failed to increase iron uptake or photosynthesis in the fit1 mutant. Still, it remains unknown how VOC-treated plants initiate the inducible iron deficiency responses. One possibility is that a demand for more iron may result from VOC-induced leaf cell expansion (Zhang et al. 2007) and/or photosynthesis augmentation (Zhang et al. 2008b). Also unclear is the identity of the component(s) in GB03 VOCs that induce plant iron deficiency responses. On the other hand, acid component such as diethyl acetic acid possibly accounts for the rhizosphere acidification that is directly caused by VOC exposure (Farag et al. 2006; Zhang et al. 2009).

8.5.3 Bacterial Volatile Organic Compounds to Fight Against Biotic Stress

Phytopathogens are major and chronic threat for agricultural production world over, and losses due to pathogen account for about 13% of the total production losses. Due to increasing production, the producers are becoming more and more dependent on agrochemicals for plant disease management. That's why these agrochemicals dominate the global market of phytosanitary products. But nowadays due to increasing awareness of consumers about pesticide-free safer food, this leads to reduction in the use of these agrochemicals which leads to the development of a new strategy comprising the use of biocontrol agents for plant disease management. Various types of biocontrol agents are presently accessible in the market which differ by the composition of microorganisms within it, namely, bacteria, fungi, viruses, and nematodes. Among which, bacterial biocontrol agents exerts their activity in three ways:

1. Competition: here rhizobacteria due to their fast chemotactic movement toward root exudates outcompete pathogen population in the acquisition of nutrients and specific niche and thereby reduce pathogen population.
2. Antibiosis: the rhizobacteria having capacity to produce antibacterial and antifungal compounds directly inhibit pathogen growth.
3. Plant immunization: here due to plant colonization by rhizobacteria, the plant's innate defense system is activated to respond strongly to the pathogen attack which can be called as induced resistance.

In all these three mechanisms, bacterial volatiles are having major roles. Volatile compounds can travel across membranes unrestrictedly and get released into the atmosphere or soil in the absence of a diffusion barrier (Pichersky et al. 2006).

Moreover mass movement of water through the soil facilitates quick movement of volatile compounds all over the system (Wheatley 2002). Due to its ability to penetrate membranes easily as well as efficient delivery through soil, it improves antagonistic potential of a volatile against target organism.

Nematicidal Activity of Bacterial Volatile Organic Compounds

Meager efforts were done for testing antagonistic potential of bacterial volatile organic compounds against phytopathogenic nematodes. Till date laboratory tests were done to determine influence of bacterial volatile organic compounds on second-stage juvenile (J2) of plant parasitic nematodes. Gu et al. (2007) evaluated the nematicidal activity (NA) of 200 bacterial isolates against *Panagrellus redivivus* in using compartmentalized petri dishes and found more than 20% nematicidal activities by 149 isolates wherein 49 isolates showed more than 80% NA including *B. weihenstephanensis*, *B. simplex*, *B. subtilis*, and *Serratia marcescens.* Same bacterial strains were also tested against *Bursaphelenchus xylophilus* wherein 165 bacterial strains showed more than 20% NA. Six bacterial strains (two of *B. simplex*, three of *weihenstephanensis*, and one of *S. marcescens*) revealed strong NA (80%) against both tested nematode species. Huang et al. (2010) reported that volatile organic compounds produced by *B. megaterium* showed 100% mortality of *Meloidogyne incognita* J2 and strong inhibition of egg hatching. It was observed that same isolates showed significant variation in their nematicidal activity because of their VOC production pattern. Among the 81 different VOCs identified in the 15 bacterial isolates by Gu et al. (2007), 46 VOCs were not having any NA and 18 showed strong NA and 2 VOCs (benzaldehyde and trimethylpyrazine) occurred in all samples at high concentrations. Among all the tested 20 VOCs, 9 VOCs, viz., 2-undecanone, 2-octanol, decanol, benzaldehyde, 2-nonanone, dimethyl disulfide, benzeneacetaldehyde, cyclohexene, and phenol, showed 100% NA against tested nematodes. Huang et al. (2010) identified a total of 17 VOCs from *B. megaterium* which were tested in vitro, against *M. incognita*, by using commercial compounds. Among a total of 17 compounds tested, 2-nonanone, 2-undecanone, decanal, dimethyl disulfide, and benzeneacetaldehyde showed more than 80% nematicidal activities.

Control of Phytopathogenic Fungi by Bacterial Volatiles

Presently many of the researchers have evaluated the role of bacterial volatiles in fungicidal activity. Fernando et al. (2005) isolated various bacterial strains, viz., *Pseudomonas chlororaphis* (five isolates), *P. corrugate* (one isolate), *P. fluorescens* (three isolates), and *P. aurantiaca* (one isolate), from canola and soybean plants, which showed production of antifungal VOCs which inhibited sclerotia and ascospore germination as well as mycelial growth of *Sclerotinia sclerotiorum* in laboratory and soil tests. Similarly, cyanide produced by *P. fluorescens* CHAO inhibits tobacco rot caused by the fungus *Thielaviopsis basicola* (Voisard et al. 1989). Liu et al. (2008) reported the production of volatiles by bacterium species *Paenibacillus polymyxa*, *B. pumilus*, and *B. subtilis* isolated from cucumber rhizosphere. These volatiles showed 20–100% inhibitory effect on phytopathogenic fungi, viz., *S. sclerotiorum*, *B. cinerea*, *A. brassicae*, *A. solani*, *Ascochyta citrullina*, *F.*

oxysporum, *F. graminearum*, *Cercospora kikuchii*, *Rhizoctonia solani*, *Phoma arachnidicola*, and *Verticillium dahiae*. Moreover, Arrebola et al. (2010) reported that *B. subtilis* and *B. amyloliquefaciens* obtained from Valencia and Shamouti oranges produced volatile organic compounds having 25–50% inhibitory effect on *Penicillium crustosum* and *P. italicum.* Wan et al. (2008) reported that VOCs produced by *Streptomyces platensis* F-1 reduce mycelial growth of *R. solani*, *S. sclerotiorum*, and *B. cinerea* and controlled the disease caused by them in rice, oilseed rape, and strawberry, respectively. Huang et al. (2012) reported that application of DMDS produced by *B. cereus* C1L significantly protected tobacco against *Botrytis cinerea* under greenhouse conditions.

Baysal et al. (2013) detected the production of 2,3-butanediol by *B. subtilis* strains, FZB24, QST713, and EU07, which can efficiently control *Fusarium oxysporum* f.sp. *radices-lycopersici.*

Giorgio et al. (2015) reported that six strains of volatile-producing rhizobacteria inhibited the growth of *Sclerotinia sclerotiorum* strain. The presence of 1-undecene, 2-nonanone, 2-undecanone, 2-propanone, 1-tetradecanol, acetic acid, m-cymene, dl-limonene, dimethyl disulfide, and dimethyl trisulfide was detected in bacterial culture filtrate through GC–MS analysis.

Mackie and Wheatley (1999) detected that there exist variations in inhibitory effects of single bacterial isolate against various fungal pathogens which may be attributed to the facts that different fungi may respond to different component(s) of the volatile mixture as the site for reaction may be different; some of the fungi have developed mechanism to detoxify the volatile metabolite(s) (Kai et al. 2007). Mechanism of action of bacterial VOCs includes inhibition of fungal mycelial growth or enzyme activity (Wheatley 2002). Exposure to both larger and older bacterial populations greatly increases both the degree and the rate of inhibitory effects on the fungi (Mackie and Wheatley 1999). VOCs can be fungicidal or fungistatic and water soluble. Mackie and Wheatley (1999) found that the inhibitory effects on many fungi by the bacterial VOCs were not fungicidal and the persistence of the effects due to VOC adsorption into agar medium indicated that the active compounds are water soluble. VOCs produced by microorganisms played an important role during their evolution in the context of their interactions, community population, and functional dynamics. Such interactions will result in functional responses by the organisms involved to some community members and coincidental disadvantage to others. The substrate-dependent variation in VOC production will result in variations in microbial, and consequently systemic, response (Wheatley 2002).

Bactericide Activities of VOC Substances Produced by Microorganisms

Gram-positive *Bacillus* sp. strains producing volatile compounds, viz., acetoin and butanediol, induced systemic resistance in tobacco against *Erwinia carotovora* SCC1 and promoted plant growth (Ryu et al. 2003, 2004). Han et al. (2006) reported colonization of cucumber roots by *P. chlororaphis* O6 deliberates defense against *Corynespora cassiicola*. Rudrappa et al. (2010) reported that *Arabidopsis thaliana* (Col-0) plants, inoculated with *B. subtilis* strain FB17, showed lower disease severity against *P. syringae* pv. tomato DC3000 compared to plants without FB17

treatment as *B. subtilis* produced acetoin (3-hydroxy-2-butanone), which triggers induced systemic resistance (ISR) and protects plants against DC3000 pathogenesis. To further confirm the role of acetoin, *B. subtilis* acetoin biosynthetic mutants were created, and it showed reduced emission of acetoin which in turn showed reduction on protection. Further analysis suggested that resistance to DC3000 occurs through NpR, salicylic acid (SA)-/ethylene (ET)-mediated pathway. Choi et al. (2014) indicated that *B. amyloliquefaciens* strain IN937a encourages induced systemic resistance (ISR) against bacterial spot disease caused by *Xanthomonas axonopodis* pv. *vesicatoria* in pepper through VOC emission. Among all the volatiles tested, 3-pentanol was tested. Treatment receiving 3-pentanol significantly reduced disease severity in field trials over 2 years. To further elucidate the role of bacterial volatile in stimulation of plant defense, expression of defense genes was studied and revealed that the expression of CaPR1, CaPR2, and CaPIN2 increased in 3-pentanol-treated pepper plants. Dandurishvili et al. (2011) reported that VOCs produced by the *Serratia plymuthica* IC1270, *P. fluorescens* Q8r1-96, and *P. fluorescens* B-4117 inhibited the growth of *Agrobacterium tumefaciens* and *A. vitis* under laboratory conditions. Further analysis revealed presence of dimethyl disulfide (DMDS) as the major volatile produced by antagonistic bacterial strains as well as emitted by tomato plants treated with bacterial strains. Further to rule out possibility of involvement of antibiotics in suppression of pathogen, mutants of *P. fluorescens* Q8r1-96 and *S. plymuthica* IC1270 deficient in 2,4-diacetylphloroglucinol or pyrrolnitrin production, respectively, were tested and revealed that mutants also showed suppression of pathogens and thereby established the role of bacterial volatile in protection of plants against crown gall disease.

8.6 Future Prospects

Exploration, implementation, and adoption of BVOCs for crop production and protection should be emphasized for sustainable crop production. Till date majority of research pertaining to BVOCs is carried out under laboratory conditions and only few species of volatile-producing microorganisms are explored, but still BVOCs showed considerable influence on plant growth, development, and defense. If we want to explore the potential of BVOCs as low-cost, eco-friendly bioinoculant, then more experiments should be conducted under field trial conditions to provide scientific evidence. Generally BVOCs are most attractive as biological pesticides; their use was restricted up to 4% of the global pesticide market. We need to recognize the multidimensional communication of BVOCs with other microorganisms and crops. Research on BVOCs is in its infancy, but in the future, BVOCs will outcompete chemical pesticides and fertilizers as natural products which benefit crops.

References

Arrebola E, Sivakumar D, Korsten L (2010) Effect of volatile compounds produced by *Bacillus* strains on postharvest decay in citrus. Biol Control 53:122–128

Barriuso J, Solano BR, Gutierrez-Manero FJ (2008) Protection against pathogen and salt stress by four plant growth-promoting rhizobacteria isolated from *Pinus* sp. on *Arabidopsis thaliana*. Phytopathology 98:666–672

Bashan Y, Alcaraz-Menéndez L, Toledo G (1992) Responses of soybean and cowpea root membranes to inoculation with *Azospirillum brasilense*. Symbiosis 13:217–228

Baysal O, Lai D, H-H X, Siragusa M, Carimi F, Silva JA, Tor M (2013) A proteomic approach provides new insights into the control of soil-borne plant pathogens by *Bacillus* species. PLoS One 5(1):1–12

Burmølle M, Hansen LH, Sørensen SJ (2007) Establishment and early succession of a multispecies biofilm composed of soil bacteria. Microb Ecol 54:352–362

Chen Y, Gozzi K, Yan F, Chai Y (2015) Acetic acid acts as a volatile signal to stimulate bacterial biofilm formation. Microbiology 6:e00392

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH (2008) 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. Mol Plant-Microbe Interact 21:1067–1075

Choi HK, Song GC, Yi H-S, Ryu C-M (2014) Field evaluation of the bacterial volatile derivative 3-pentanol in priming for induced resistance in pepper. J Chem Ecol 40:882–892

Cortes-Barco AM, Hsiang T, Goodwin PH (2010a) Induced systemic resistance against three foliar diseases ofAgrostis stolonifera by (2R, 3R)-butanediol or an isoparrafin mixture. Ann Appl Biol 157:179–189

Cortes-Barco AM, Goodwin PH, Hsiang T (2010b) Comparison of induced resistance activated by benzothiadiazole, (2R,3R)-butanediol and an isoparaffin mixture against anthracnose of Nicotiana benthamiana. Plant Pathol 59:643–653

Curie C, Briat JF (2003) Iron transport and signaling in plants. Annu Rev Plant Biol 54:183–206

Dandurishvili N, Toklikishvili N, Ovadis M, Eliashvili P, Giorgobiani N, Keshelava R, Tediashvili M, Vainstein A, Khmel I, Szegedi E, Chernin L (2011) Broad-range antagonistic rhizobacteria Pseudomonas fluorescens and Serratia plymuthica suppress Agrobacterium crown gall tumours on tomato plants. J Appl Microbiol 110:341– 352

Davis TS, Crippen TL, Hofstetter RW, Tomberlin JK (2013) Microbial volatile emissions as insect semiochemicals. J Chem Ecol 39:840–859

Dimkpa C, Wein T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32:1682–1694

Effmert U, Kaldéras J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703

Farag MA, Ryu CM, Sumner LW (2006) GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry 67:2262–2268

Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk SC (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem 37:955–964

Giorgio A, De Stradis A, Lo Cantore P, Iacobellis SN (2015) Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on *Sclerotinia sclerotiorum*. Front Microbiol 6:1–13

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L, Stewart A (2012) Have biopesticides come of age? Trends Biotechnol 30:250–258

Gu YQ, Mo MH, Zhou JP, Zou CS, Zhang KQ (2007) Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol Biochem 39:2567–2575

Gusain YS, Singh US, Sharma AK (2015) Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.) Afr J Biotechnol 14:764–773

Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ, Kim YC (2006) Gac S-dependent production of 2R, 3R-Butanediol by *P. chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *P. syringae* pv. *tabaci* in tobacco. MPMI 19(8):924–930

http://bioinformatics.charite.de/mvoc

Huang Y, Xu C, Ma L, Zhang K, Duan C, Mo M (2010) Characterization of volatiles produced from *Bacillus megaterium* YFM 3.25 and their nematicidal activity against *Meloidogyne incognita*. Eur J Plant Pathol 126:417–422

Huang CJ, Tsay JF, Chang SY, Yang HP, Wu WS, Chen CY (2012) Dimethyl disulfide is an induced systemic resistance-elicitor produced by Bacillus cereus C1L. Pest Manag Sci 68(9):1306

Kai M, Piechulla B (2009) Plant growth promotion due to rhizobacterial volatiles – an effect of CO_2? FEBS Lett 583:3473–3477

Kai M, Effmert U, Berg G, Piechulla B (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch Microbiol 187:351–360

Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B (2009) Bacterial volatiles and their action potential. Appl Microbiol Biotechnol 81:1001–1012

Kanchiswamy CN, Malnoy M, Maffei ME (2015) Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Front Plant Sci 6:151

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94:1259–1266

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006) Plant growth-promotion rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl Soil Ecol 31:91–100

Lemfack MC, Nickel J, Dunkel M, Preissner R, Piechulla B (2014) mVOC: a database of microbial volatiles. Nucleic Acids Res 42:D744–D748

Liu W, Wu W, Zhu B, Du Y, Liu F (2008) Antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens. Agril Sci China 7(9):1104–1114

Mackie AE, Wheatley RE (1999) Effects and incidence of volatile organic compound interactions between soil bacterial and fungal isolates. Soil Biol Biochem 31:375–385

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

Meldau DG, Meldau S, Hoang LH, Underberg S, Wunsche H, Baldwin IT (2013) Dimethyldisulfide produced by the naturally associated bacterium Bacillus spB55 promotes Nicotiana attenuata growth by enhancing sulfur nutrition. Plant Cell 25:2731–2747

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

Penuelas J, Asensio D, Tholl D (2014) Biogenic volatile emissions from the soil. Plant Cell Environ 37:1866–1891

Pereyra MA, Zalazar CA, Barassi CA (2006) Root phospholipids in Azospirillum-inoculated wheat seedlings exposed to water stress. Plant Physiol Biochem 44:873–879

Pichersky E, Noel JP, Dudareva N (2006) Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science 311:808–811

Romoli R, Papaleo MC, dePascale D, Tutino ML, Michaud L, LoGiudice A (2011) Characterization of the volatile profile of Antarctic bacteria by using solid-phase microextraction-gas chromatography-mass spectrometry. J Mass Spectrom 46:1051–1059

Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Pare PW, Bais HP (2010) The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Community Biol 3(2):130–138

Ryu CM, Farag MA, CH H (2003) Bacterial volatiles promote growth in Arabidopsis. Natl Acad Sci 100:4927–4932

Ryu CM, Farag MA, CH H, Reddy MS, Kloepper JW, Paré PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Schulz S, Dickschat JS (2007) Bacterial volatiles: the smell of small organisms. Nat J Prod Reprod 24:814–842

Song GC, Ryu CM (2013) Two volatile organic compounds trigger plant self-defense against a bacterial pathogen and a sucking insect in cucumber under open field conditions. Int J Mol Sci 14:9803–9819

Sueldo RJ, Invernati A, Plaza SG, Barassi CA (1996) Osmotic stress in wheat seedlings: effects on fatty acid composition and phospholipid turnover in coleoptiles. Cereal Res Commun 24:77–84

Takahashi H, Kopriva S, Giordano M, Saito K, Hell R (2011) Sulfur assimilation in photosynthetic organisms : molecular functions and regulations of transporters and assimilatory enzymes. Annu Rev Plant Biol 62:157–184

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31:1847–1852

Vaishnav A, Kumari S, Jain S, Varma A, Choudhary DK (2015) Putative bacterial volatile-mediated growth in soybean (*Glycine max* L. *Merrill*) and expression of induced proteins under salt stress. J Appl Microbiol 119:539–551

Voisard C, Keel C, Haas D, Défago G (1989) Cyanide production by *P. fluorescens* cells helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8(2):351–358

Wan M, Li G, Zhang J, Jiang D, Huang H (2008) Effect of volatile substances of *Streptomyces platensis* F-1 on control of plant fungal diseases. Biol Control 46:552–559

Wenke K, Weise T, Warnke R (2012) Bacterial volatiles mediating information between bacteria and plants. In: Witzany G, Baluska F (eds) Biocommunication of plants. Springer, Berlin/Heidelberg, pp 327–347

Wheatley RE (2002) The consequences of volatile organic compound mediated bacterial and fungal interactions. Any Van Leeuw 81:357–364

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Zhang H, Kim MS, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu CM, Allen R, Melo IS, Paré PW (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in Arabidopsis. Planta 226:839–851

Zhang H, Kim MS, Sun Y, Dowd SE, Shi H, Paré PW (2008a) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mol Plant-Microbe Interact 21:737–744

Zhang H, Xie X, Kim S, Kornyeyev DA, Holaday S, Pare PW (2008b) Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in plant. Plant J 56:264–273

Zhang H, Sun Y, Xie X, Kim MS, Dowd SE, Paré PW (2009) A soil bacteria regulates plant acquisition of iron via deficiency-inducible mechanisms. Plant J 58:568–577

Zhang H, Murzello C, Kim MS, Xie X, Jeter RM, Zak JC (2010) Choline and osmotic stress tolerance induced in Arabidopsis by the soil microbe *Bacillus subtilis* (GB03). Mol Plant-Microbe Interact 23:1097–1104

Perspectives of Plant-Methylotrophic Interactions in Organic Farming

9

Vadivukkarasi Ponnusamy, Jayashree Shanmugam, Mayakkannan Gopal, and Seshadri Sundaram

Abstract

Almost all plant functions are directly affected by stress components like adverse climate, drought, temperature, salinity, heavy metals, pesticides, and soil pH, which are considered to be major limiting factors in crop production. Prevalence of intensive infections in crops retards the yield with reduced market acceptance leading to double-headed crisis with the high cost of production and incidence of high level of microbial contamination, including mycotoxin in the end product. Alteration in the agricultural practices is the need of the hour, i.e., switching from synthetics to biological inputs to effectively promote soil fertility, plant tolerance, and crop productivity. Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. The study of the interactions between plants and their microbial communities is important for developing sustainable management practices. Methylotrophic bacteria occupy different habitats like soil, water, leaf surfaces, nodules, grains, and air due to their great phenotypic plasticity. They can reach populations of 10^4 to 10^6 col-

V. Ponnusamy
Plant Pathology Lab, Crop Protection Division, ICAR-Indian Institute of Spices Research (IISR), Kozhikode, Kerala 673012, India

V. Ponnusamy • J. Shanmugam • S. Sundaram (✉)
Indigenous and Frontier Technology Research Centre (IFTR), Chennai, Tamil Nadu, India
e-mail: Seshadri@iftr.in; tsseshadri@gmail.com

M. Gopal
NOCH International Sdn Bhd, Kuala Lumpur, Malaysia

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_9

ony-forming units (CFU) per gram of plant tissue. They can function as a plant-beneficial microbe through production of biological active compounds which might explain their capacity to stimulate plant growth and protect them from various pathogens. This chapter discusses the merits of utilizing *Methylobacterium* as biofertilizers/bioprotectants for crops production and protection.

Keywords
Methylobacterium • Biofertilizer • Plant-microbe interaction • Organic farming

9.1 Introduction

Plant growth in nature is always constrained by genetic and environmental factors. Among the environmental factors, biotic and abiotic factors play equal role in deciding the growth of plants. They include all external conditions and influences affecting the life and development of plant that include temperature, moisture supply, radiant energy, composition of the atmosphere, soil aeration and soil structure, soil reaction, biotic factors, supply of mineral nutrients, and presence/absence of growth-restricting substances. Among these factors, the characteristics of soil play a big part in the plant's ability to extract water and nutrients. If plants are to grow to their potential, the soil must provide a satisfactory environment for plant growth. Managing soil health is a formidable challenge to ensure productivity, profitability, and production target. In the recent past, intensive agronomic practices, to optimize the crop production that calls for high yield and quality to achieve food security, resulted in extensive dependence on synthetic fertilizers and pesticide application in soils. The use of agrochemicals in agriculture is under the scanner constantly today in view of the ever-increasing health and environmental concerns. While the agricultural soils witnessed indiscriminate use of chemicals, particularly nitrogen (N), phosphorus (P), potash (K), the conventional mode of farming, which catered these major along with other minor nutrient deficiencies of soils, concomitantly intensified the decline of soil ecosystem functioning (Schultz et al. 1995) and in turn causes loss in soil productivity. Owing to this, more emphasis is given to the use of biofertilizers which can be a good supplement/alternative to chemical fertilizers.

Loss of soil quality is related to soil organic matter (SOM) depletion that is increased by continuous cropping without rotations, frequent soil tillage, and large use of both inorganic chemical fertilizers and nonselective pesticides (Pane et al. 2015). Besides being costly, the repeated use of these fertilizers leads to reduction in pH and exchangeable bases, thus making them unavailable to crops and consequently impacting the crop productivity (Zainol et al. 1993; Savci 2012). To obviate this problem, of late, there was a resurgence of sustainable organic agricultural practices as an alternative for the production of nutrient-rich, harmless, high-quality food. Taking these into account, scientists are desperate to innovate inexpensive, environmentally benign, and easy-to-use options to overcome fertilizer use in agriculture. One such strategy is the use of biofertilizers for maintaining the soil fertility as part of organic farming to ensure food safety to add biodiversity to soil (Shetty et al. 2014).

The restricted availability of major nutrients like nitrogen and phosphorus limits plant growth and yield. While it is estimated that about 175 million tons of nitrogen per year is added to soil worldwide through the biological fixing process, approximately 95–99% soil phosphorus are available in the form of insoluble phosphates and hence cannot be utilized by plants (Vassilev and Vassileva 2003). These gaps can be bridged with the use of biofertilizers. The possibility of using soil microorganisms as biofertilizer can increase both the nutrient uptake capacity and water use efficiency (Armada et al. 2014). There are several microorganisms, which can add nitrogen to the soil by symbiotic or asymbiotic N_2 fixation and also solubilize the cheaper sources of phosphorus, such as rock phosphate, silicates, potassium, etc. (Seshadri 2003). Thus, biofertilizers are a major players for improving productivity in organic farming on a global scale.

9.2 Biofertilizers in Organic Farming

Biofertilizers are defined as microbial products that contain live inoculants or latent cells or extracted metabolites causing no adverse effects to ecosystem. When applied to seeds, plant surfaces, or soil, they colonize the rhizosphere or the interior of the plant and promote plant growth by increasing the supply or availability of primary nutrients to the host plant (Vessey 2003). They exert beneficial effects from direct influence mechanisms to an indirect on plant growth encouraging mobilizing nutrients, producing plant growth regulators, protecting plants from phytopathogens, improving soil structure, sequestering heavy metal toxicity, degrading xenobiotic compounds, or increasing the efficiency of other mutualistic beneficial microorganisms (Muthukumarasamy et al. 2002; Rajkumar et al. 2010; Ahemad and Kibret 2014). Thus, usage of microbial inoculants with versatile plant-beneficial traits reduces fertilizer application (Kloepper et al. 1989; Adesemoye et al. 2008; Yim et al. 2013).

9.3 Methylotrophs

Several bacterial genera play a vital role in enriching nutrients in the soil as biofertilizers either singly or as consortia. One such microbial group is methylotrophs, known since the late nineteenth century, which has the ability to grow on single-carbon compounds as their sole source of energy (Peel and Quayle 1961). They were able to grow at the expense of reduced carbon compounds containing one or more carbon atoms with no carbon-carbon bonds. They include bacteria, yeasts, fungi, and archaea. Obligate methylotrophs grow only on C_1 compounds, whereas facultative methylotrophs can grow on methanol and methylamine, as well as C_2, C_3, and C_4 compounds (Anthony 1982; Lidstrom 2006). On the basis of their carbon substrate utilization pattern, they are divided into three classes: (1) obligates (methane, methanol, and methylamines), (2) restricted facultative (besides C_1 limited range of other simple compounds like glucose, fructose), and (3) less restricted facultative (C_1 and variety of simple and complex organic substrates) (Goldberg and

Rokem 1991). Two most occurring C_1 substrates in the terrestrial environment are methanol (CH_3OH) and methane (CH_4), and they are the important intermediates in the global carbon cycle which are utilized directly or indirectly by methylotrophs (Anthony 1982; Goldberg and Rokem 1991; Chistoserdova et al. 2003).

Biotically, methane is produced by methanogenic archaea and abiotically by biomass burning, coal mining, and the oil industry. Recent studies show the production of methane by plant cells (12–370 ng per g dry weight h^{-1}) (Keppler et al. 2006), which is distinct from the microbially produced methane that is transported by hydrophytes from underground. They are reported to be produced significantly from pectin and correspond to 2%–12% of the total global methane release (Bruhn et al. 2012). Methane-oxidizing methylotrophs are also called as methanotrophs, the only recognized biological drivers of methane fluxes in terrestrial ecosystem that has the capability to oxidize methane by hydroxyl radicals before it reaches the atmosphere (Dunfield et al. 2007; Trotsenko and Murrell 2008; Conrad 2009). The organic compounds that result in the conversion process are further utilized by other organisms. Therefore, methanotrophs have a critical role in incorporating the carbon atom of methane into the carbon cycle. They are a physiologically distinct group of mostly aerobic gram-negative bacteria that belong to members of two phyla: *Proteobacteria* and *Verrucomicrobia*. The *Proteobacteria* are broadly divided into type I and type II methanotrophs (*Gammaproteobacteria* and *Alphaproteobacteria*) (Dedysh et al. 2002; Knief 2015; Fradet et al. 2016). Currently type I methanotrophs are classified into the family *Methylococcaceae*, and type II includes two distinct families—*Methylocystaceae* and *Beijerinckiaceae*. The phylum *Verrucomicrobia* was proposed lately after the isolation of three extremophile thermoacidophilic methanotrophs, "*Methylokorus*," "*Acidimethylosilex*," and "*Methyloacida*," which grow at pH 1.5 and 65 °C (Dunfield et al. 2007; Pol et al. 2007; Semrau et al. 2008). However, to unify they were currently proposed with remarkable new genus name "*Methylacidiphilum*" (Op den Camp et al. 2009). Most of the methanotrophs are aerobic with few exceptional, e.g., "*Candidatus Methylomirabilis oxyfera*" which consumes methane anaerobically arising from subsurface reservoirs before it reaches the sea level (Orphan et al. 2002: Ettwig et al. 2010; Nazaries et al. 2013). There are circumstantial evidences available on the physiological potential of methanotrophs cultures to grow on methanol (Radajewski et al. 2002) suggesting that they may consume methanol in soils and thrive under micro-/millimolar concentrations (Kolb 2009).

The second most abundant organic C_1 compound utilized by methylotrophs is methanol (0.1–10 p.p.b.), a volatile carbon compound but chemically more reactive than methane. Principally about 3×10^{12} mol/year and 25×10^{12} mol/year of methanol are generated during plant growth and decay of plants by methylation of the methoxy groups of cell wall-associated pectin polymers and lignin (Nemecek-Marshall et al. 1995; Fall and Benson 1996). Apparently, the methanol produced from plant biomass is higher (26 Tmol per year) than the observed rates (Galbally and Kirstine 2002; Jacob et al. 2005). Most of the known species of methylotrophs belonging to diverse phyla are facultative but particularly feed on methanol (Lidstrom 2006; Chistoserdova et al. 2009). About 83% soil-derived aerobic methylotrophic isolates are reported to utilize methanol (Kolb 2009). Higher percentages

of methanol are emitted from leaves particularly growing leaves that amount more than adult leaves (Nemecek-Marshall et al. 1995). Though methanol fluxes from leaf surfaces were correlated with stomatal distribution and conductance, there are evidences that show the presence of lower fractions of methanol from the non-stomatal surface compared to stomatal surface (Nemecek-Marshall et al. 1995).

9.3.1 *Methylobacterium*-Plant Interaction

Bacteria-plant interactions have been well documented by various researchers. This intimate relationship is guided by molecular communication between bacteria-bacteria and bacteria-plants and are regulated by specific exuded compounds by bacteria or by many metabolites released by the host plant (Hardoim et al. 2008), ethanol (Williams and Yavitt 2009), and methanol (Sy et al. 2005).

The phyllosphere, defined as the aerial part of plants, is common niche for synergism between bacteria and plant and has been recognized as a well-known habitat for methanol-utilizing methylotrophs. Phyllospheric methanol-utilizing methylotrophs were discovered in the 1980s (Corpe and Basile 1982), and the first aerobic methanol-utilizing bacterium was *Bacillus methylicus* (later renamed *Bacterium methylicum*, but no longer available in culture) (Loew 1892). Since then many novel species have been isolated including the *Alpha-*, *Beta-*, and *Gammaproteobacteria*, *Verrucomicrobia*, *Cytophagales*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Madhaiyan et al. 2012), and the leaf surfaces were recorded to colonize by large population of bacteria of the genera *Methylobacterium*, *Methylophilus*, *Methylibium*, and *Hyphomicrobium* (Lopez-Velasco et al. 2011), thus suggesting that methylotrophs of terrestrial ecosystems are likely to have a role in consuming methanol as C_1 compound and thereby partially mitigating its emission into the atmosphere (Kolb 2009). *Methylobacterium* has been reported to associate with more than 70 species of plants (Omer et al. 2004). Among the methanol utilizers, the C_1 metabolism of the genus *Methylobacterium* provides a selective advantage upon phyllosphere colonization (Sy et al. 2005). Often they are termed as "pink-pigmented facultative methylotrophs" or "PPFMs" because of their distinctive pink pigmentation, which falls under the α-subclass of the *Proteobacteria*, order *Rhizobiales,* and family *Methylobacteriaceae* (Green 2001). The pigment is nondiffusible and non-fluorescent and is a carotenoid (Urakami et al. 1993). Urakami et al. (1993) and Jourand et al. (2004) also reported some colorless nonpigmented colonies in the genus *Methylobacterium*. They constitute a group of strictly aerobic, gram-negative, rod-shaped bacteria (0.8–1.2 × 1.0–8.0 μm) (Trotsenko et al. 2001; Green 2006). Figure 9.1 shows the PPFM culture in solid and liquid media. They are found in a variety of habitats including phyllosphere, rhizosphere, root nodules, dust, contaminated water, marine water, freshwater, drinking water, lake sediments, etc. (Corpe and Rheem 1989; Vadivukkarasi et al. 2014, 2015; Jayashree et al. 2016). They form about 0.5–69.4 colony-forming unit/cm^2 on the leaf surfaces (Chanprame et al. 1996). Figure 9.2 shows the colony formation of PPFM on leaf surfaces.

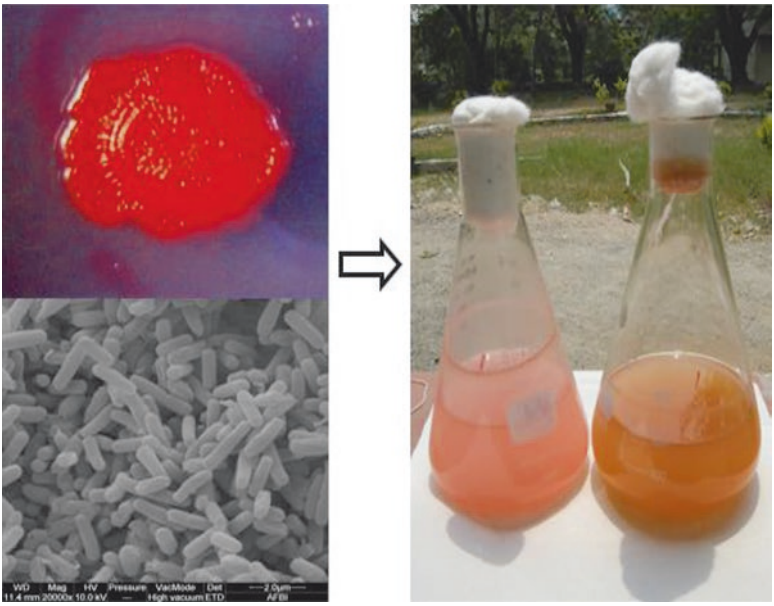


Fig. 9.1 Growth and morphology of methylobacteria. Clockwise: Growth of methylobacteria on media (solid and liquid) containing methanol as sole carbon source and their morphology as viewed under scanning electron microscope (SEM)

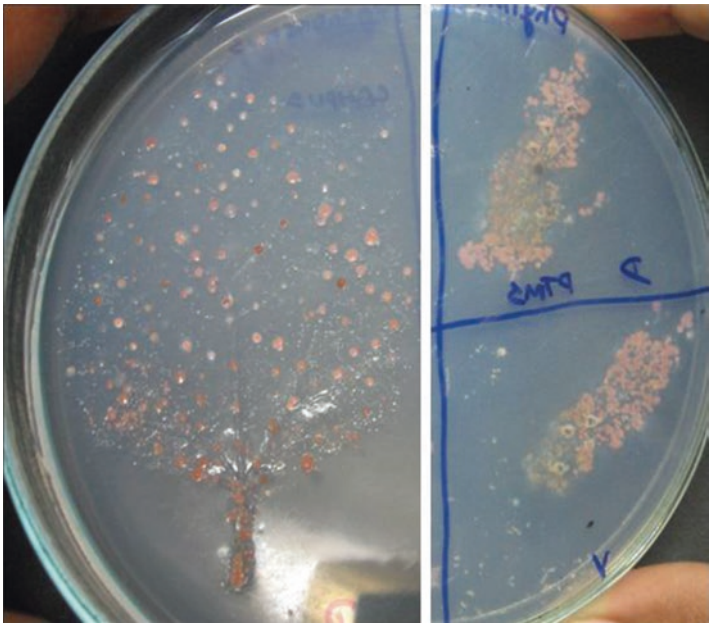


Fig. 9.2 Leaves collected from different plants were impregnated on media containing methanol as the sole carbon source

The genus *Methylobacterium* interacts symbiotically which may also be endophytic within the plants in intercellular spaces or as epiphytic attached on plant surfaces like phyllosphere, in the nearby soil around the roots (rhizosphere) with the formation of root biofilm or nodules (Sy et al. 2005; Andreote et al. 2006; Yates et al. 2007; Vorholt 2012). The degrees of plant-*Methylobacterium* association vary from very strong, in the form of symbioses, to semi-tight, as demonstrated by endophytic association, to loose, as demonstrated by epiphytic association on plant surfaces (Dourado et al. 2015). *Methylobacterium* occupies specific niches of the plants which could possibly have arisen from an intimate coevolution process with host plants. An example of this co-evaluation process is the bacterial capacity to mediate high photosynthetic activity in the host by the induction of a higher number of stomata, increased chlorophyll concentration and greater amount of malic acid (Cervantes-Martinez et al. 2004). In this process, the epiphytic colonization of bacteria is the first stage toward developing an association with plants (Andreote et al. 2006). *While Methylobacterium funariae* (type strain F3.2) was reported to inhabit the leaf surfaces of "primitive" land plants like such as mosses (common cord moss, *Funaria hygrometrica* Hedw.) interact with its host organism via the secretion of phytohormones (cytokinins, auxins), it was also further established that the bacterial isolate uses methanol emitted from the stomatal pores as principal carbon source and amino acids leached from the surface of the epidermal cells of the host as nitrogen source for cell metabolism (Schauer and Kutschera 2011).

After plant recognition, endophytic colonization likely depends on traits such as adhesins, pili, and EPS (exopolysaccharides) to attach to the cells on the surface. Several genes and proteins have been reported to be upregulated during phyllosphere and endophytic colonization. Several studies show *Methylobacterium* as a putative endophyte of different host plants, such as cotton (Lacava et al. 2004), peanut (Madhaiyan et al. 2006), citrus (Araújo et al. 2002), *Pinus* (Pohjanen et al. 2014), eucalyptus (Andreote et al. 2009), sunn hemp (Sy et al. 2001), *Catharanthus roseus*, tobacco (Andreote et al. 2006), strawberry (Abanda-Nkpwatt et al. 2006), and even mangrove plants (Dourado et al. 2012). They can reach populations of 10^4–10^6 colony-forming units (CFU) per gram of plant tissue in a mucilaginous layer in meristematic tissue (Doronina et al. 2002; Rossetto et al. 2011) with the species of this genus exhibiting vast diversity inside the host plants (Andreote et al. 2009). The complex mechanisms of plant-*Methylobacterium* interactions are controlled by bacterial genes responsible for metabolism, stress defense, and pathogenicity. Presence of genes responsible for type IV pilus biosynthesis and hemolysin-type adhesions in *Methylobacterium oryzae* CBMB20 suggests their mode of plant colonization (Kwak et al. 2014).

Members of the *Methylobacterium* genus produce AHL (N-acyl-homoserine lactones), the quorum sensing (QS) systems, with an increase in bacterial cell density responsible for bacterial cell-to-cell communication (Nieto Penalver et al. 2006; Poonguzhali et al. 2007; Pomini et al. 2009). Gram-negative bacteria living in association with plants use quorum sense (QS) systems, as signaling molecules, which are regulated by the LuxI/LuxR system that allows bacteria to function as a

multicellular organisms (Barnard et al. 2007). They regulate the transcription of different genes related to the secretion of virulence factors, biofilm formation, sporulation, exchange of DNA, and others (Zhu and Sun 2008). In *M. mesophilicum* SR1.6/6, an increase in cell density along with the long-chain homoserine lactones (HSLs) that upregulate the expression of several genes related to plant-bacteria interactions, such as bacterial metabolism (mxaF), adaptation to stressful environments (crtI and sss), interactions with plant metabolism compounds (acdS), and pathogenicity (patatin and *pho*U), has been reported (Dourado et al. 2013). Expression of *acd*S gene has been related to the increased ACC deaminase enzyme activity besides being regulated by the promoter responsible for the transcription activation of *nif* genes encoding nitrogen fixation (Tittabutr et al. 2008; Madhaiyan et al. 2015).

Genes that encode enzymes related to auxin biosynthesis, such as amine oxidase, aldehyde dehydrogenase, nitrilase/cyanide hydratase, N-acyltransferase, nitrile hydratase, and amidase, are also reported from the genus *Methylobacterium*. *M. oryzae* CBMB20 was screened for genes encoding amine oxidase and aldehyde dehydrogenase involved in the KEGG pathway where indole-3-acetic acid is produced from tryptophan through tryptamine and indole-3-acetaldehyde. The same strain was also screened for 21 genes encoding the components of the urea ABC transporters along with urease operon containing the structural genes *ureAB* and the accessory genes *ureD*, *ureE*, *ureF*, and *ureG*. These genes allow the bacterium to synthesize and degrade urea thereby promoting the plant growth (Kwak et al. 2014). Zeatin production by *Methylobacterium* was related to the presence of *miaA* genes required for the isopentenylation of adenosine residue of tRNA by the action of several hydrolase and isopentenyl tRNA transferase (Koenig et al. 2002). A gene encoding acid phosphatase, two genes encoding phytase, and the *phn* operon encoding the C-P lyase system which enhances phosphate solubilization have also been reported from *M. oryzae* (Kwak et al. 2014). Sulfur is a one of the important ingredients for increasing the quality and yield of crops. While the sulfur oxidation pathway of *Methylobacterium* has been reported to be species specific (Friedrich et al. 2001), some *sox* genes were reported in *M. extorquens*, *M. nodulans*, and *Methylobacterium* sp. (Anandham et al. 2007).

Apart from the above, expression of several antioxidant-related and stress regulator (*phy*R) genes and proteins also has been reported from *M. extorquens*. In addition to this, PhyR regulon has a central role in the adaptation of *Methylobacterium* to the plant environment by dealing various stresses that are likely to encounter in the phyllosphere (Gourion et al. 2006). PhaA, which initiates synthesis of the reserve polyhydroxybutyrate (PHB), has also been reported to get upregulated when the bacterium is in association with the plant leaf (Gourion et al. 2008). In addition, *M. oryzae* CBMB20 has been reported to possess clusters of genes *cobPOQD*, *cobF*, *cobTS*, and *cobWNGHIJKLEMB* involved in vitamin B_{12} biosynthesis (Kwak et al. 2014).

Methylobacterium genera are also able to demonstrate mutual synergistic effects with other groups of bacteria and arbuscular mycorrhizal fungi on various crops and improve growth and nutrient uptake (Kim et al. 2010). In citrus, the endophytic *Methylobacterium* was found to interact with *Xylella fastidiosa*, the causal agent of

citrus variegate chlorosis (CVC), suggesting that this bacterium can act in plant, influencing the microbial balance in the plant host and participating on the plant development (Araújo et al. 2002; Lacava et al. 2004; Gai et al. 2009). Recent observation on the trophic interactions between methanotrophs and heterotrophs in terrestrial and aquatic environments where heterotrophic bacteria function as stimulators of methane oxidation by methanotrophs, e.g., through cobalamin production throws open this area wide open for studying microbe-microbe interactions in field conditions (Iguchi et al. 2015).

9.3.2 *Methylobacterium*-Plant Growth Promotion

Abundance of PPFMs in various plants such as apple, Arabidopsis sp., black gram, coffee, cotton, Crotalaria sp., cucumber, *Ginkgo biloba*, groundnut, *Lotononis bainesii*, maize, mustard, *Nicotiana* sp., pepper, pigeon pea, pine, poplar, papaya, potato, radish, rice, soybean, strawberry, sunflower, tobacco, tomato, wheat, soybean and sugarcane have been reported by many researchers along with their unique plant growth promoting abilities viz. fix atmospheric nitrogen (Sy et al. 2001; Yates et al. 2007), enzyme and siderophore production (Jayashree et al. 2011a, b), and antagonistic effects, (Holland and Polacco 1994; Araújo et al. 2002; Lacava et al. 2004). Methylobacterial strains have also been proven to induce morphogenic calli and shoot formation in plants (Vadivukkarasi 2013). Sugar quality and cane yield of sugarcane have been reported to get enhanced with the association of PPFMs (Madhaiyan et al. 2005). There are several reports (Ivanova et al. 2001; Hornschuh et al. 2006; Omer et al. 2004) on the addition of tryptophan as an inducer and a precursor of auxin for the production of indole acetic acid (IAA) by the bacterial strains. Molecular plant-*Methylobacterium* interaction studies have also confirmed the excretion of another key phytohormone, cytokinins (trans-zeatin), by these bacteria at low levels in culture medium (Koenig et al. 2002). Germination of seeds and plant growth has been related to the induction by the exocellular phytohormones produced by *Methylobacterium* (Corpe and Basile 1982). Recently, *M. populi* isolated from the soil of the ex-industrial site ACNA (Aziende Chimiche Nazionali Associate) in Cengio (Savona, Italy) and able to grow on minimal selective medium with a complex mixture of different classes of xenobiotic compounds as the sole carbon source was found to show multiple plant growth promotion activities, viz., produce indole-3-acetic acid (IAA) and siderophores, solubilize phosphate, produce a biofilm in the presence of phenanthrene, and alleviate phenanthrene stress in tomato seeds (Ventorino et al. 2014). In another instance, endophytic *Methylobacterium mesophilicum* SR1.6/6 could significantly promote biomass production and height of aerial part of rootstocks of both *Citrus limonia* and *Citrus sunki* which was attributed to the presence of indole-3- acetic acid (IAA) biosynthesis pathway in the strain (Bogas et al. 2016). Interestingly, the team also could recover the strains from rootstocks in culture medium and confirmed the endophytic colonization of rootstocks by *Methylobacterium*.

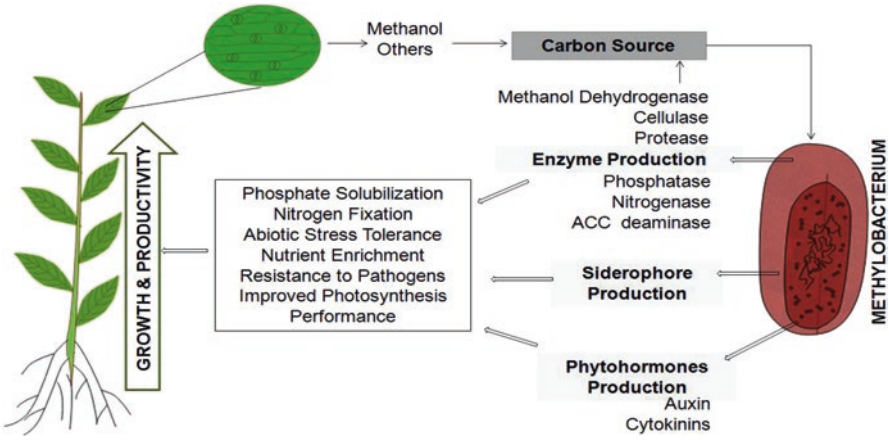


Fig. 9.3 Relationship in *Methylobacterium*: plant interaction and growth promotion

When bacteria come in contact with the plant exudates, array of genes involved in metabolic pathways may guide the bacteria to colonize the plant and trigger the sequential expression of beneficial genes to promote plant growth or induce systemic resistance, increasing plant health. *Methylobacterium*-plant interactions have proven to be potential for the environmental sustainability affected over the time with the use of synthetic chemical-oriented agricultural practices. Significance of *Methylobacterium* as plant growth promoter can be categorized by their three possible underlying mechanisms, (1) as a bio-stimulator influencing directly plant growth by producing plant growth hormones, (2) as a biofertilizer providing micronutrients such as nitrogen and phosphate to the plants, and (3) as a bio-controller suppressing the growth of pathogens by producing antifungal metabolites and inducing systemic resistance in plants (Kwak et al. 2014; Jayashree et al. 2014). Their ubiquitous nature and their association with more than 70 plant species make them a model to study the particular traits that these bacteria have on plant growth promoting attribute and prove them as potential agents for plant growth promotion and biocontrol agents against various plant diseases (Holland and Polacco 1994). *Methylobacterium* is termed as "little farmers," nurturing and protecting plant at every stage (Holland et al. 2002). Although many methylotrophic bacteria are known, both aerobic and anaerobic, based on the ecological roles, functional capabilities, and cultivation strategies, this chapter will cover the prospects of aerobic PPFM in organic farming. Figure 9.3 depicts the possible relationship between methylobacterium interaction and plant growth promotion.

Methylobacterium establishes an association with the host either by the production of a number of compounds that affect plant metabolism, e.g., cytokinins, auxins, Vitamin B_{12}, and osmoprotectants, and several fungal cell wall-degrading enzymes, siderophores, lytic enzymes, nitrogen fixation, antibiotics, and cyanide (Raaijmakers and Mazzola 2012; Ongena and Jacques 2008; Lacava et al. 2008; Madhaiyan et al. 2014) or by the consumption of plant metabolic wastes (Trotsenko

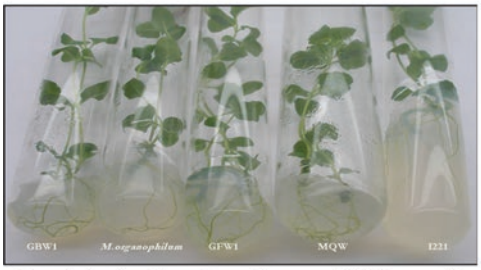
et al. 2001). Specific positive effects such as germination of seed, crop yield, resistance to pathogen, etc. through PPFMs-plant association were reported by many researchers (Kalyaeva et al. 2001; Irvine et al. 2012).

9.4 *Methylobacterium* as Biofertilizers/Bioprotectants

Methylobacterium as inoculants in plant growth and agriculture have been demonstrated in both in vitro and field experiments (Fig. 9.4). Enhanced root and shoot induction, elongation, growth and yield as a result of *Methylobacterium* inoculation in plants have been reported by many researchers in the in-vitro, pot as well as field experiments (Vadivukkarasi et al. 2008; Chinnadurai et al. 2009; Krishnamoorthy et al. 2011) that include antagonistic effect against phytopathogens in Maize (Romanovskaya et al. 2001), better shelf life and stress abatement in Tomato (Joe et al. 2014), enhanced iron translocation in broad bean and corn (Bishop et al. 2011), ethylene emission and pathogenesis-related proteins synthesis in tomato (Yim et al. 2013), improved nodulation in soybean (Parthiban et al. 2012), increased dry biomass and macronutrient accumulation in maize and sorghum-Sudan grass hybrid (Kim et al. 2014), red pepper plant growth and yield with or without additional

Multiple shoot production in *Spilanthes calva* grown in MS medium supplemented with Methylobacterial culture filtrates.

Comparisons on the impact of culture filtrate on the growth of plants



Root induction by culture filtrates of different pink pigmented Methylobacterial isolates.

Field trials using *Methylobacterium* on Paddy

Fig. 9.4 Evaluation of *Methylobacterium* as bio-fertilizer under *in vitro* and field conditions

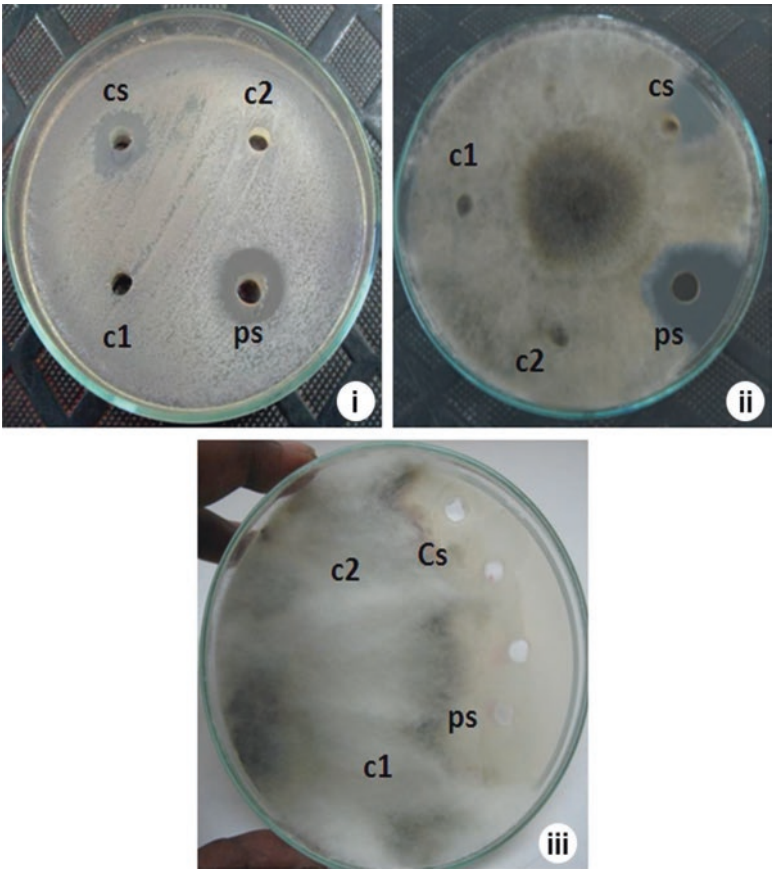


Fig. 9.5 Inhibitory effect of siderophores produced by *Methylobacterium* against plant pathogenic microbes (i) *Xanthomonas oryzae* (ii) *Botrytis cinerea* (iii) *Fusarium oxysporum*. *C1*, *C2* control, *CS* crude sample, *PS* purified sample

methanol spray (Ryu et al. 2006; Sa 2006; Madhaiyan et al. 2010), and nutrient translocation in plant leaves (Bishop et al. 2011). A strain, *Methylobacterium extorquens* MM2 isolated from the phyllosphere of mustard plant (*Brassica nigra*) was reported to produce IAA and increase the seed vigor and promote the growth of *Lycopersicon esculentum* L. (Subhaswaraj et al. 2017).

Methylobacterium sp. can protect host plants by the synthesis of a large spectrum of molecules (Fig. 9.5), by nutrient competition with pathogens (Berg 2009), or by inducing systemic resistance (ISR) (Nigris et al. 2013). ISR can be induced by volatile organic compounds released from some bacteria (Madhaiyan et al. 2004; Naznin et al. 2013) and by genes of bacteria that encode plant cell wall degradation enzymes such as glycosidases, cellulases (or endoglucanase), hemicellulase, phosphatases, (Filho et al. 2012; Pedrosa et al. 2011), pectinase (Lee et al. 2006), phosphatase and cellulase (Jayashree et al. 2011a, b), protease (Jayashree et al. 2014) plant growth regulators

(Vadivukkarasi et al. 2008), siderophores (Jayashree et al. 2011b), and induced plant growth and protected plants against pathogens (Madhaiyan et al. 2006). *Methylobacterium* sp. even at a low density was able to induce potato resistance against *Pectobacterium atrosepticum* by activating the plant antioxidant system (Ardanov et al. 2012). Defense response was induced in tomato challenged with *Ralstonia solanacearum* after treatment with *Methylobacterium* (Yim et al. 2013), and significant protection against *Aspergillus niger* and *Sclerotium rolfsii* in groundnut under pot-culture conditions has also been reported (Madhaiyan et al. 2006, 2010).

Antagonistic properties of phyllosphere available methylobacteria were reported to prevent infection and maintain the health of the plants by symbiotic association (Patkowska 2003; Poorniammal et al. 2010). Methylotrophs isolated from the rhizosphere soil, phyllosphere, and roots of *Capsicum annum* inhibited the growth of the phytopathogens such as *Colletotrichum capsici*, *S. rolfsii*, *Fusarium oxysporum*, *Cercospora capsici*, and *Xanthomonas campestris* established through dual culture technique (Savitha et al. 2015) with maximum zone of inhibition observed in *C. capsici*, *S. rolfsii*, and *F. oxysporum*. Production of siderophores by methylobacteria (Jayashree et al. 2008; Vaidehi and Sekar 2012) is an added trait for their use in organic agriculture to control phytopathogens. PPFMs have been reported to act as biocontrol agents against tomato root pathogens (Janahiraman et al. 2016) through induction of systemic resistance to a great extent (Madhaiyan et al. 2006; Indiragandhi et al. 2008). Capacity of siderophore production was reported to give a natural competitive advantage while limiting the supply of iron and essential trace elements, in turn preventing the pathogens to grow further by production of salicylic acid (Indiragandhi et al. 2008).

Apart from acting as biological control agents to reduce the development of plant diseases caused by plant pathogenic fungi, bacteria, and viruses, methylobacteria are also used to control even nematodes (Chinnadurai et al. 2009; Prabhu et al. 2009; Poorniammal et al. 2010; Krishnamoorthy et al. 2011; Verma et al. 2014). Root-knot nematode, *Meloidogyne incognita*, and fungal pathogens *Fusarium udum*, *F. oxysporum*, *Pythium aphanidermatum*, and *S. rolfsii* were effectively controlled by the PPFMs (Poorniammal et al. 2009).

A recent study on inoculation of *Methylobacterium* in pot as well as field growth tests for rice and barley has shown that the inoculation has resulted in better ripening of rice seeds and increased size of barley grains with little impact on the total yield. Further the studies also suggested that there is a strong selection pressure at the species level of *Methylobacterium* residing on a given plant species and that selection of appropriate species that can persist on the plant is important to achieve growth promotion (Tani et al. 2015).

9.5 Conclusion

With the rising awareness on the deleterious effects of using chemical fertilizers, the demand for biofertilizers is increasing steadily all over the world. Microbes have been used as inoculants into agricultural fields for more decades. Recent approaches on the use of plant growth-promoting rhizobacteria (PGPR) as inoculants have

given thrust to the identification of more microbes with novel potentials for plant growth and development. Yet, there is a little knowledge about the methods which are used for identifying the best bacteria for the task, and even less is known about their competence in various domains. Hence, the world is continuously working on isolation and characterization of microbes with different characteristics. In addition to the identification of potential microbes, there lies the challenge of taking the same into more numbers in a given particular environment.

Methylotrophs offer an advantage of using them in both rhizosphere and phyllosphere for sustainable agriculture. Empirical studies show that Methylotrophs to possess multiple traits for plant growth, which makes them a suitable and promising candidate for use in organic and sustainable agricultural practices. While they participate in the biogeochemical cycling in soil ecosystems their ability to colonize the phyllosphere in huge numbers, produce phytohormones for plant growth, nodulate plants, fix nitrogen, help plant acquire nutrients, solubilizing difficult to solubilize phosphates, silicates, siderophore production to combat pathogens, and their ability to induce systemic resistance in plants makes them a promising and effective candidates for organic agriculture and as alternatives or supplements to chemical fertilizers. Their ability to colonize phyllosphere has led to the idea of using them profusely as foliar microbial sprays to different crops. However, it requires coordinated work by microbiologists, agronomists, organic enthusiasts, voluntary organizations and farmers to promote the adaptation of methylotrophs as biofertilizers in different agricultural systems. Development of microbial consortia with methylotrophs and other microorganisms with different benefits for different crops, thereby combining different traits into one product, with several yield-promoting effects is another strategy to convince the end users. In addition, technologies leading to the development of low-cost carriers with more active cells, long shelf life, and ability to store at ambient temperatures are essential for their extended use.

Acknowledgments Authors thank Shri AMM Murugappa Chettiar Research Centre (MCRC), Tharamani, Chennai, for providing necessary facilities for conducting the research work; Life Sciences Research Board (LSRB), Defence Research Development Organization (DRDO), and Department of Biotechnology, Government of India, for the financial support; Dr. Mary E. Lidstorm, University of Washington, Seattle, for providing the bacterial standard strains; and Dr. P. V. Sujithkumar for the help in the preparation of the manuscript.

References

Abanda-Nkpwatt D, Müsch M, Tschiersch J, Boettner M, Schwab W (2006) Molecular interaction between *Methylobacterium extorquens* and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site. J Exp Bot 57(15):4025–4032

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

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspectives. J King Saud Univ – Sci 26:1–20

Anandham R, Indiragandhi P, Madhaiyan M, Kim K, Yim W, Saravanan VS, Chung J, Sa T (2007) Thiosulfate oxidation and mixotrophic growth of *Methylobacterium oryzae*. Can J Microbiol 53:869–876

Andreote FD, Lacava PT, Gai CS, Araújo WL, Maccheroni W Jr, Overbeek LSV, Elsas JDV, Azevedo JL (2006) Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella fastidiosa*. NRC Res Press 52:419–426

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. Microb Ecol 57:82–93

Anthony C (1982) The biochemistry of methylotrophs. Academic Press, London

Araújo WL, Marcon J, Maccheroni JW, 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(10):4906–4914

Ardanov P, Sessitsch A, Haggman H, Kozyrovska N, Pirttila AM (2012) *Methylobacterium*-induced endophyte community changes correspond with protection of plants against pathogen attack. PLoS One 7(10):e46802

Armada E, Portela G, Roldan A, Azcon R (2014) Combined use of beneficial soil microorganism and agrowaste residue to cope with plant water limitation under semiarid conditions. Geoderma 232:640–648

Barnard AML, Bowden SD, Burr T, Coulthurst SJ, Monson RE, Salmond GPC (2007) Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. Philos Trans R Soc Lond Ser B Biol Sci 362(1483):1165–1183

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84(1):11–18

Bishop YM, Barton LL, Johnson GV (2011) Influence of *Methylobacterium* on iron translocation in plants. Biometals 24:575–580

Bogas AC, Aguilar-Vildoso CI, Camargo-Neves AA, Araújo WL (2016) Effects of growth-promoting endophytic *Methylobacterium* on development of *Citrus* rootstocks. Afr J Microbiol Res 10(19):646–653

Bruhn D, Moller IM, Mikkelsen TN, Ambus P (2012) Terrestrial plant methane production and emission. Physiol Plant 144:201–209

Cervantes-Martinez J, Lopez-Diaz S, Rodriguez-Garay B (2004) Detection of the effects of *Methylobacterium* in *Agave tequilana* Weber Var. azul by laser-induced fluorescence. Plant Sci 166:889–892

Chanprame S, Todd JJ, Widholm JM (1996) Prevention of pink-pigmented methylotrophic bacteria (*Methylobacterium mesophilicum*) contamination of plant tissues cultures. Plant Cell Rep 16:222–225

Chinnadurai C, Balachandar D, Sundaram SP (2009) Characterization of 1-amino cyclopropane-1-carboxylate deaminase producing *Methylobacteria* from phyllosphere of rice and their role in ethylene regulation. World J Microbiol Biotechnol 25:1403–1411

Chistoserdova L, Chen SW, Lapidus A, Lidstrom ME (2003) Methylotrophy in *Methylobacterium extorquens* AM1 from a genomic point of view. J Bacteriol 185:2980–2987

Chistoserdova L, Kalyuzhnaya MG, Lidstrom ME (2009) The expanding world of methylotrophic metabolism. Annu Rev Microbiol 63:477–499

Conrad R (2009) The global methane cycle: recent advances in understanding the microbial processes involved. Environ Microbiol Rep 1:285–292

Corpe WA, Basile DV (1982) Methanol utilizing bacteria associated with green plants. Dev Ind Microbiol 23:483–493

Corpe WA, Rheem S (1989) Ecology of the methylotrophic bacteria on living leaf surfaces. FEMS Microbiol Ecol 62:243–250

Dedysh SN, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Liesack W, Tiedje JM (2002) *Methylocapsa acidiphila* gen. Nov., sp. nov., a novel methane-oxidizing and dinitrogen-fixing acidophilic bacterium from sphagnum bog. Int J Syst Evol Microbiol 52(1):251–261

Doronina NV, Trostsenko YA, Kuznetsov BB, Tourova TP, Salkinoja-Salonen MS (2002) *Methylobacterium suomiense* sp. nov. and *Methylobacterium lusitanum* sp. nov., aerobic, pink-pigmented, facultatively methylotrophic bacteria. In J Syst Evol Microbiol 52(3):773–776

Dourado MN, Ferreira A, Araújo WL, Azevedo JL, Lacava PT (2012) The diversity of endophytic methylotrophic bacteria in an oil-contaminated and an oil-free mangrove ecosystem and their tolerance to heavy metals. Biotechnol Res Int 2012:759865

Dourado MN, Bogas AC, Pomini AM, Andreote FD, Quecine MC, Marsaioli AJ, Araújo WL (2013) Methylobacterium-plant interaction genes regulated by plant exudate and quorum sensing molecules. Braz J Microbiol 44(4):331–339

Dourado MN, Neves AAC, Santos DS, Araújo WL (2015) Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic *Methylobacterium* spp. Biomed Res Int 2015:909016

Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S, Ly B, Saw JH, Zhou Z, Ren Y, Wang J, Mountain BW, Crowe MA, Weatherby TM, Bodelier PL, Liesack W, Feng L, Wang L, Alam M (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MM et al (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548

Fall R, Benson AA (1996) Leaf methanol: the simplest natural product from plants. Trends Plant Sci 1:296–301

Filho SF, Quecin MC, Bogas AC (2012) Endophytic *Methylobacteriumextorquens* expresses a heterologous -1,4- endoglucanase a (EglA) in *Catharanthus roseus* seedlings, a model host plant for *Xylella fastidiosa*. World J Microbiol Biotechnol 28(4):1475–1481

Fradet DT, Tavormina PL, Orphan VJ (2016) Members of the methanotrophic genus *Methylomarinum* inhabit inland mud pots. Peer J 4:e2116

Friedrich CG, Rother D, Bardischewsky F, Quentmeier A, Fischer J (2001) Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism. Appl Environ Microbiol 67:2873–2882

Gai CS, Lacava PT, Quecine MC, Auriac MC, Lopes JR, Araújo WL, Miller TA, Azevedo JL (2009) Transmission of *Methylobacterium mesophilicum* by *Bucephalogonia xanthophis* for paratransgenic control strategy of citrus variegated chlorosis. J Microbiol 47:448–454

Galbally IE, Kirstine W (2002) The production of methanol by flowering plants and the global cycle of methanol. J Atmos Chem 43(3):195–229

Goldberg I, Rokem JS (1991) Biology of methylotrophs. Butterworth-Heinemann, Madison

Gourion B, Rossignol M, Vorholt JA (2006) A proteomic study of *Methylobacterium extorquens* reveals a response regulator essential for epiphytic growth. Proc Natl Acad Sci U S A 103:13186–13191

Gourion B, Francez-Charlot A, Vorholt JA (2008) PhyR is involved in the general stress response of *Methylobacterium extorquens* AM1. J Bacteriol 190:1027–1035

Green PN (2001) Methylobacterium. In: Dworkin M (ed) The prokaryotes, Release 3.5, 3rd edn. Springer, New York

Green PN (2006) Methylobacterium. In: Dorkin MM, Falkow S, Rosenburg E, Schleifer K-H, Erko-Stackbrandt (eds) The prokaryotes, a handbook on the biology of bacteria, proteobacteria: alpha and beta subclass, vol 5. Springer, New York, pp 257–265

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

Holland MA, Polacco JC (1994) PPFMs and other covert contaminants: is there more to plant physiology than just plant? Annu Rev Plant Physiol Plant Mol Biol 45:197–209

Holland MA, Long RLG, Polacco JC (2002) *Methylobacterium* spp.: phylloplane bacteria involved in cross-talk with the plant host. In: Lindow SE, Hecht-Poinar EI, Elliott VJ (eds) Phyllosphere microbiology. APS Press, St Paul

Hornschuh M, Grotha R, Kutschera U (2006) Moss-associated *Methylobacteria* as phytosymbionts: an experimental study. Naturwissenschaften 93(10):480–486

Iguchi H, Yurimoto H, Sakai Y (2015) Interactions of methylotrophs with plants and other heterotrophic bacteria. Microorganisms 3:137–151

Indiragandhi P, Anandham R, Kim KY, Yim WJ (2008) Induction of systemic resistance by modulating ethylene biosynthesis pathway by ACC deaminase containing *Methylobacterium oryzae* against *Pseudomonas syringae* in tomato. World J Microbiol Biotechnol 24:1037–1045

Irvine IC, Brigham CA, Suding KN, Martiny JBH (2012) The abundance of pink-pigmented facultative methylotrophs in the root zone of plant species in invaded coastal sage scrub habitat. PLoS One 7(2):e31026

Ivanova EG, Doronina NV, Trotsenko IA (2001) Aerobic *Methylobacteria* are capable of synthesizing auxins. Microbiology 70:392–397

Jacob DJ, Field BD, Li Q, Blake DR, de Gouw J, Warneke C, Hansel A, Wisthaler A, Singh HB, Guenther A (2005) Global budget of methanol: constraints from atmospheric observations. J Geophys Res 110(D08303):1–17

Janahiraman V, Anandham R, Kwon SW, Sundaram S, Karthik Pandi V, Krishnamoorthy R, Kim K, Samaddar S, Sa T (2016) Control of wilt and rot pathogens of tomato by antagonistic pink pigmented facultative methylotrophic *Delftia lacustris* and *Bacillus* spp. Front Plant Sci 7:1626

Jayashree S, Ershath Ali M, Vadivukkarasi P, Seshadri S (2008) Screening of pink pigmented facultative methylotrophs (PPFM's) for siderophore production. International symposium on microbial biotechnology: diversity, genomics and meta genomics, Association of Microbiologist of India, University of Delhi, New Delhi

Jayashree S, Lalitha R, Vadivukkarasi P, Kato Y, Seshadri S (2011a) Cellulase production by pink pigmented facultative methylotrophic strains (PPFMs). Appl Biochem Biotechnol 164:666–680

Jayashree S, Vadivukkarasi P, Anand K, Kato Y, Seshadri S (2011b) Evaluation of pink-pigmented facultative methylotrophic bacteria for phosphate solubilization. Arch Microbiol 193:543–552

Jayashree S, Annapurna B, Jayakumar R, Sa T, Seshadri S (2014) Screening and characterization of alkaline protease produced by a pink pigmented facultative methylotrophic (PPFM) strain, MSF 46. J Genet Eng Biotech 12(2):111–120

Jayashree S, Vadivukkarasi P, Mayakkannan G, Sundaram S (2016) Population dynamics and seasonal variation of bacterial system utilizing single carbon from river Cooum and river Adyar, Chennai, Tamilnadu, India. Int J Curr Microbiol App Sci 5(3):466–477

Joe MM, Saravanan VS, Islam MR, Sa T (2014) Development of alginate-based aggregate inoculants of *Methylobacterium* sp. and *Azospirillum brasilense* tested under *in vitro* conditions to promote plant growth. J Appl Microbiol 116:408–423

Jourand P, Giraud E, Bena G, Sy A, Willems A, Gillis M (2004) *Methylobacterium nodulans* sp. nov.,for a group of aerobic, facultatively methylotrophic, legume root nodule forming and nitrogen-fixing bacteria. Int J Syst Evol Microbiol 54:2269–2273

Kalyaeva MA, Zacharchenko NS, Doronina NV, Rukavtsova EB, Ivanova EG, Alekseeva VV, Trotsenko YA, Buryanov YI (2001) Plant growth and morphogenesis *in vitro* is promoted by associative methylotrophic bacteria. Russ J Plant Physiol 48(4):514–517

Keppler F, Hamilton JTG, Braß M, Röckmann T (2006) Methane emissions from terrestrial plants under aerobic conditions. Nature 439:187–191

Kim K, Yim W, Trivedi P, Madhaiyan M, Deka Boruah HP, Islam MR, Lee G, Sa TM (2010) Synergistic effects of inoculating arbuscular mycorrhizal fungi and *Methylobacterium oryzae* strains on growth and nutrient uptake of red pepper *Capsicum annuum* L. Plant Soil 327:429–440

Kim K, Kwak C, Lee Y, Sa T (2014) Effect of co-inoculation of *Brevibacterium iodinum* RS16 and *Methylobacteriumoryzae* CBMB20 on the early growth of crop plants in *Saemangeum* reclaimed soil. Korean J Soil Sci Fertil 47:1–7

Kloepper JW, Lifshitz R, Zablotwicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43

Knief C (2015) Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. Front Microbiol 6:1346

Koenig RL, Morris RO, Polacco JC (2002) tRNA is the source of low-level trans-zeatin production in *Methylobacterium* spp. J Bacteriol 184:1832–1842

Kolb S (2009) Aerobic methanol-oxidizing bacteria in soil. FEMS Microbiol Lett 300:1–10

Krishnamoorthy R, Chauhan PS, Parthiban S, Hong B, Joe MM, Sa T (2011) Co-inoculation effect of *Methylobacterium fujisawaense* and *Azospirillum brasilense* CW301 on early growth of rice. Korean J Soil Sci Fertil 5:141–142

Kwak M-J, Jeong H, Madhaiyan M, Lee Y, Sa T-M, TK O (2014) Genome information of *Methylobacterium oryzae*, a plant-probiotic methylotroph in the phyllosphere. PLoS One 9(9):e106704

Lacava PT, Araújo WL, Marcon J, Maccheroni JW, Azevedo JL (2004) Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus variegated chlorosis. Lett Appl Microbiol 39:55–59

Lacava PT, Silva-Stenico ME, Araujo WL (2008) Detection of siderophores in endophytic bacteria *Methylobacterium* sp. associated with *Xylella fastidiosa* sub sp. pauca. Pesq Agropec bras Brasilia 43(4):521–528

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY, Sa TM (2006) Physiological enhancement of early growth of rice seedlings (*Oryza sativa* L.) by production of phytohormone of N_2-fixing methylotrophic isolates. Biol Fertil Soils 42(5):402–408

Lidstrom ME (2006) Aerobic methylotrophic prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, vol. 2: ecophysiology and biochemistry. Springer-Verlag, New York

Loew O (1892) Ueber einen Bacillus, welcher Ameisensäure und Formaldehyd assimilieren kann. Centralbl Bakteriol 12:462–465. (in German)

Lopez-Velasco G, Welbaum GE, Boyer RR, Mane SP, Ponder MA (2011) Changes in spinach phylloepiphytic bacteria communities following minimal processing and refrigerated storage described using pyrosequencing of 16S rRNA amplicons. J Appl Microbiol 110:1203–1214

Madhaiyan M, Poonguzhali S, Senthilkumar M, Seshadri S, Chung H, Yang J, Sundaram S, Sa TM (2004) Growth promotion and induction of systemic resistance in rice cultivar co-47 *Oryza sativa* L. by *Methylobacterium* sp. Bot Bull Acad Sin 45:315–324

Madhaiyan M, Poonguzhali S, Lee HS, Hari K, Sundaram SP, Sa T (2005) Pink pigmented facultative methylotrophic bacteria accelerate germination, growth and yield of sugarcane clone Co86032 *Saccharum officinarum* L. Biol Fertil Soils 41:350–358

Madhaiyan M, Reddy BV, Anandham R, Senthilkumar M, Poonguzhali S, Sundaram SP, Sa TM (2006) Plant growth promoting *Methylobacterium* induces defense responses in groundnut *Arachis hypogaea* L. compared with rot pathogens. Curr Microbiol 53:270–276

Madhaiyan M, Poonguzhali S, Kang BG, Lee YJ, Chung JB, Sa TM (2010) Effect of co-inoculation of methylotrophic *Methylobacteriumoryzae* with *Azospirillum brasilense* and *Burkholderia pyrrocinia* on the growth and nutrient uptake of tomato, red pepper and rice. Plant Soil 328:71–82

Madhaiyan M, Poonguzhali S, Senthilkumar M, Lee J-S, Lee K-C (2012) *Methylobacterium gossipiicola* sp. nov., a pink-pigmented facultative methylotrophic bacteria isolated from cotton phyllosphere. Int J Syst Evol Microbiol 62:162–167

Madhaiyan M, Chan KM, Ji L (2014) Draft genome sequence of *Methylobacterium* sp. strain L2-4, a leaf- associated endophytic N-fixing bacterium isolated from *Jatropha curcas* L. Genome Announc 2:e01306–e01314

Madhaiyan M, Alex THH, Ngoh ST, Prithiviraj B, Ji L (2015) Leaf-residing methylobacterium species fix nitrogen and promote biomass and seed production in *Jatropha curcas*. Biotechnol Biofuels 8:222

Muthukumarasamy R, Revathi G, Seshadri S, Lakshminarasimhan C (2002) *Gluconacetobacter diazotrophicus* (syn. *Acetobacter diazotrophicus*), a promising diazotrophic endophyte in tropics – a review. Curr Sci 83(2):137–145

Nazaries L, Murrell JC, Millard P, Baggs L, Singh BK (2013) Methane, microbes and models: fundamental understanding of the soil methane cycle for future predictions. Environ Microbiol 15:2395–2417

Naznin HA, Kimura M, Miyazawa M, Hyakumachi M (2013) Analysis of volatile organic compounds emitted by plant growth-promoting fungus *Phoma* sp. GS8-3 for growth promotion effects on tobacco. Microbes Environ 28(1):42–49

Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski CL, Fall R (1995) Methanol emission from leaves. Enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development. Plant Physiol 108:1359–1368

Nieto Penalver CG, Morin D, Cantet F, Saurel O, Milon A, Vorholt JA (2006) *Methylobacterium extorquens* AM1 produces a novel type of acyl-homoserine lactone with a double unsaturated side chain under methylotrophic growth conditions. FEBS Lett 580:561–567

Nigris S, Baldan E, Zottini M, Squartini A, Baldan B (2013) Is the bacterial endophyte community, living in Glera (*Vitis vinifera*) plants, active in biocontrol? In: Schneider C, Leifert C, Feldmann F (eds) Endophytes for plant protection: the state of the art. Deutsche Phytomedizinische Gesellschaft, Braunschweig

Omer ZS, Tombolini R, Gerhardson B (2004) Plant colonization by pink-pigmented facultative methylotrophic bacteria (PPFMs). FEMS Microbiol Ecol 47(3):319–326

Ongena M, Jacques P (2008) *Bacillus*lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 16:115–125

Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, Jetten MS, Birkeland NK, Pol A, Dunfield PF (2009) Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environ Microbiol Rep 1:293–306

Orphan VJ, House CH, Hinrichs KU, Mckeegan KD, Delong EF (2002) Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc Natl Acad Sci U S A 99:7663–7668

Pane C, Celano G, Piccolo A, Villecco D, Spaccini R, Palese AM, Zaccardelli M (2015) Effects of on-farm composted tomato residues on soil biological activity and yields in a tomato cropping system. Chem Biol Technol Agri 2:4

Parthiban S, Chauhan PS, Tipayno S, Krishnamoorthy R, Lee S, Sa T (2012) ACC deaminase producing *Methylobacteriumoryzae* CBMB20 improves plant growth and nodule activity in soybean on co-inoculation with *Bradyrhizobium japonicum* USDA110. Korean J Soil Sci Fertil 6:47–48

Patkowska E (2003) The effect of phyllosphere microorganisms on the healthiness of aboveground parts of soybean (*Glycine max* (L.) Merrill). Hortorum Cultus 2:65–71

Pedrosa FO, Monteiro RA, Wassem R, Cruz LM, Ayub RA, Colauto NB (2011) Genome of *Herbaspirillum seropedicae* strain SmR1, a specialized diazotrophic endophyte of tropical grasses. PLoS Genet 7(5):e1002064

Peel D, Quayle JR (1961) Microbial growth on C1 compounds. 1. Isolation and characterization of pseudomonas AM1. Biochem J 81:465–469

Pohjanen J, Koski mäkil JJ, Sutela S, Ardanov P, Suorsal M, Niemi K, Sarjala T, Häggman H, Pirttilä AM (2014) Interaction with ectomycorrhizal fungi and endophytic methylobacterium affects nutrient uptake and growth of pine seedlings *in vitro*. Tree Physiol 34(9):993–1005

Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, den Camp HJMO (2007) Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature 450:874–878

Pomini AM, Cruz PLR, Gai C, Araújo WL, Marsaioli AJ (2009) Long-chain acyl-homoserine lactones from *Methylobacterium mesophilicum*: synthesis and absolute configuration. J Nat Prod 72:2130–2134

Poonguzhali S, Madhaiyan M, Sa T (2007) Production of acyl-homoserine lactone quorum-sensing signals is widespread in gram-negative *Methylobacterium*. J Microbiol Biotechnol 17:226–233

Poorniammal R, Sundaram SP, Kumutha K (2009) *In Vitro* biocontrol activity of *Methylobacterium extorquens* against fungal pathogens. Int J Plant Prot 2(1):59–62

Poorniammal R, Sundaram SP, Kumutha K (2010) Induced systemic resistance by *Methylobacterium extorquens* against *Rhizoctonia solani* in cotton. Int J Plant Protec 2:199–204

Prabhu S, Kumar S, Subramanian S, Sundaram SP (2009) Suppressive effect of *Methylobacterium fujisawaense* against root-knot nematode, *Meloidogyne incognita*. Indian J Nematol 39(2):165–169

Raaijmakers JM, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu Rev Phytopathol 50:403–424

Radajewski S, Webster G, Reay DS, Morris SA, Ineson P, Nedwell DB, Prosser JI, Murrell JC (2002) Identification of active methylotroph populations in an acidic forest soil by stableisotope probing. Microbiology 148:2331–2342

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Romanovskaya VA, Stolyar SM, Malashenko YR, Dodatko TN (2001) The ways of plant colonization by *Methylobacterium* strains and properties of these bacteria. Microbiology 70:221–227

Rossetto PB, Dourado MN, Quecine MC, Andreote DF, Welington L, Araújo WL, João L, Azevedo JL, Pizzirani-Kleiner AA (2011) Specific plant induced biofilm formation in *Methylobacterium* species. Braz J Microbiol 42(3):878–883

Ryu CM, Kima J, Choi O, Kima SH, Park CS (2006) Improvement of capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. Biol Control 39:282–289

Sa T (2006) Plant growth substances produced by *Methylobacterium* sp. and their effect on the growth of tomato *Lycopersicon esculentum* L. and red pepper *Capsicum annuum* L. J Microbiol Biotechnol 16:1622–1628

Savci S (2012) An agricultural pollutant: chemical fertilizer. Int J Envtl Sci Dev 3(1):77–80

Savitha P, Sreenivasa MN, Nirmalnath JP (2015) *In vitro* screening for biocontrol activity of pink pigmented facultative methylotrophs against phytopathogens. Karnataka J Agric Sci 28(2):286–287

Schauer S, Kutschera U (2011) A novel growth-promoting microbe, *Methylobacterium funariae* sp. nov., isolated from the leaf surface of a common moss. Plant Signal Behav 6(4):510–515

Schultz RC, Colletti JP, Faltonson RR (1995) Agroforestry opportunities for the United States of America. Agroforestry Sys 31:117–142

Semrau JD, DiSpirito AA, Murrell JC (2008) Life in the extreme: thermoacidophilic methanotrophy. Trends Microbiol 16:190–193

Seshadri S (2003) Biofertilizers, production, utilization and future prospects. In: Forward edges of microbial resources and development. The Institute of of Microbial Ecology and Resources, Mokwon University, Korea, 19 Nov 2003

Shetty PK, Alvares C, Yadav AK (2014) Organic farming and sustainability National Institute of Advanced Studies Indian Institute of Science Campus, Bangalore. ISBN: 978–93–83566–03–7

Subhaswaraj P, Jobina R, Parasuraman P, Siddhardha B (2017) Plant growth promoting activity of pink pigmented facultative methylotroph – *Methylobacterium extorquens* MM2 on *Lycopersicon esculentum* L. J Appl Biol Biotechnol 5(01):42–46

Sy A, Giraud E, Jourand P, Garcia N, Willems A, de Lajudie P, Prin Y, Neyra M, Gillis M, Boivin-Masson C, Dreyfus B (2001) Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. J Bacteriol 183(1):214–220

Sy A, Timmers ACJ, Knief C, Vorholt JA (2005) Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. Appl Environ Microbiol 71:7245–7252

Tani A, Sahin N, Fujitani Y, Kato A, Sato K, Kimbara K (2015) Methylobacterium species promoting rice and barley growth and interaction specificity revealed with whole-cell matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF/MS) analysis. PLoS One 10(6):e0129509

Tittabutr P, Awaya JD, Li QX, Borthakur D (2008) The cloned 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene from *Sinorhizobium* sp. strain BL3 in *Rhizobium* sp. strain TAL1145 promotes nodulation and growth of *Leucaena leucocephala*. Syst Appl Microbiol 31:141–150

Trotsenko YA, Murrell JC (2008) Metabolic aspects of aerobic obligate methanotrophy. Adv Appl Microbiol 63:183–229

Trotsenko YA, Ivanova EG, Doronina NV (2001) Aerobic Methylotrophic bacteria as phytosymbionts. Microbiology 70:623–632

Urakami T, Araki H, Suzuki K, Komogata K (1993) Further studies of the genus *Methylobacterium* and description of *Methylobacterium aminovorans* sp. nov. Int J Syst Bacteriol 43:504–513

Vadivukkarasi P (2013) Studies on isolation, characterization and applications of pink pigmented facultative methylotrophs (PPFMs) from diverse environments of Chennai. PhD Dissertation, Shri AMM Murugappa Chettiar Research Centre (MCRC), University of Madras, Tamil Nadu, India

Vadivukkarasi P, Priyadarshini M, Karthic R, Jayashree S, Seshadri S (2008) Plant growth regulators in pink pigmented facultative methylotrophs and its effect on *in vitro* propagation of *Spilanthes calva*. International symposium on microbial biotechnology: diversity, genomics and meta genomics, association of microbiologist of India, University of Delhi, New Delhi

Vadivukkarasi P, Jayashree S, Seshadri S (2014) Studies on the influence of climatic conditions on pH and temperature of southeast coast, Chennai, bay of Bengal. IJE R T 3(8):1478–1482

Vadivukkarasi P, Jayashree S, Seshadri S (2015) Population of methanol utilizing bacteria in southeast coast, bay of Bengal, Chennai, Tamil Nadu, India. AARJMD 2(7):2319–2801

Vaidehi K, Sekar C (2012) Amino acid conjugated hydroxamate type of siderophore production in *Methylobacterium* phyllosphaerae MB-5. Cibtech J Microbiol 1:24–30

Vassilev N, Vassileva M (2003) Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. Appl Microbiol Biotechnol 61:435–440

Ventorino V, Sannino F, Piccolo A, Cafaro V, Carotenuto R, Pepe O (2014) *Methylobacterium populi* VP2: plant growth-promoting bacterium isolated from a highly polluted environment for polycyclic aromatic hydrocarbon (PAH) biodegradation. Sci World J 2014:931793

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2014) Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (*Triticum aestivum*) growing in central zone of India. Int J Curr Microbiol App Sci 3(5):432–447

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

Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–840

Williams CJ, Yavitt JB (2009) Temperate wetland methanogenesis: the importance of vegetation type and root ethanol production. Soil Sci Soc Am J 74:317–325

Yates RJ, Howieson JG, Reeve WG, Nandasena KG, Law IJ, Bra UL, Ardley JK, Nistelberger HM, Real D, O'Hara GW (2007) *Lotononis angolensis* forms nitrogen fixing, lupinoid nodules with phylogenetically unique, fast-growing, pink-pigmented bacteria, which do not nodulate *L. bainesii* or *L. listii*. Soil Biol Biochem 39:1680–1688

Yim W, Seshadri S, Kim K, Lee G, Sa T (2013) Ethylene emission and PR protein synthesis in ACC deaminase producing Methylobacterium sp. inoculated tomato plants (*Lycopersicon esculentum* mill.) challenged with *Ralstonia solanacearum* under greenhouse conditions. Plant Physiol Biochem 67:95–104

Zainol E, Mahmud AW, Sudin M (1993) Effect of intercropping system in surface processes in an acid Ultisol 2. Changes in soil chemical properties and their influence on crop production. J Natural Rubber Res 8(2):124–136

Zhu H, Sun SJ (2008) Inhibition of bacterial quorum sensing-regulated behaviors by *Tremella fuciformis* extract. Curr Microbiol 57:418–422

10 Phytostimulating Mechanisms and Bioactive Molecules of *Trichoderma* Species: Current Status and Future Prospects

Lakshmi Tewari, Raj Kumar Pandey, Raj Shekher Sharma, Naveen Kumar, and Salil K. Tewari

Abstract

Ever-increasing pressure on the agricultural land due to various biotic and abiotic stresses made agriculture a nonprofitable venture. In order to bring back the lost glory to agriculture, there is an urgent need to reclaim this eroded agriculture with sustainable practices, one among them is the use of plant growth-promoting microorganisms such as rhizosphere-competent *Trichoderma* sp. In this chapter, the major mechanisms and bioactive molecules involved in plant growth promotory activity of *Trichoderma* sp. are described in detail. *Trichoderma* sp. is also known to produce growth-regulating phytohormones and other bioactive molecules which are known to protect them against antimicrobial compounds secreted by plant, but they also help the plants in overcoming various stresses. Various hydrolytic enzymes such as chitinases, glucanases, and proteinases are produced by *Trichoderma* which aid in its mycoparasitic response. The fungus is also able to enhance plant growth through nutrient solubilization and its uptake. It mobilizes phosphates from fixed organic/inorganic phosphorus sources through both enzymatic (phosphatases, phytases) and nonenzymatic mechanisms (production of organic acids and siderophores). *Trichoderma* produces a wide array of secondary metabolites and volatile compounds which are mainly responsible for its biocontrol action. Suppression of fungal plant pathogens through mycoparasitism involves signal transduction and G protein signaling in *Trichoderma*. Secondary metabolites and volatile compounds produced by this fungus are very

L. Tewari (✉) • R.K. Pandey • R.S. Sharma • N. Kumar
Department of Microbiology, College of Basic Sciences & Humanities, G.B. Pant University of Agriculture & Technology, US Nagar, Pantnagar 263145, Uttarakhand, India
e-mail: lakshmi_tewari@yahoo.co.in

S.K. Tewari
Department of Genetics & Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture & Technology, US Nagar, Pantnagar 263145, Uttarakhand, India

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_10

diverse in their occurrence and mode of action against phytopathogens. Recent developments in molecular biology, metabolomics, and proteomics have opened an insight for the use of secondary metabolites as biopesticides rather than the application of whole organisms.

Keywords
Trichoderma • Biocontrol • PGP activity • Secondary metabolites • Plant defense mechanisms

10.1 Introduction

Agriculture is the largest private enterprise in India and will continue to be the lifeline of Indian economy in the future. Present population growth rate together with diversions of fertile land for non-agriculture uses exerts tremendous pressure to expand agriculture. Fast-changing environment, chemical-intensive agricultural practices, and several other soil factors are imposing a paramount pressure on sustaining the agricultural production. Continuous agricultural practices have resulted in depletion of nutrients in soil; moreover, several other abiotic stresses such as drought or water logging, high or low temperature, soil pH, and salinity and biotic (phytopathogens, insects) factors also affect the crop yield (Nagaraj kumar et al. 2005); farmers are, therefore, facing several problems especially scarcity of cultivable land and excessive demand for chemical fertilizers and pesticides. The agrochemicals cannot increase crop yield beyond a threshold level, rather their excessive use not only adversely affects the environment and human health but also disturbs the natural microbial flora of soil. This has resulted in new challenges for agricultural productivity. Hence, during recent years, there has been a growing concern for environmental hazards caused by these agrochemicals. Under these circumstances, focus on eco-friendly, climate-resilient, sustainable organic agricultural practices assumes lot of importance as an alternative to chemical-driven agriculture. The use of microbial inoculants having dual potential for biocontrol of phytopathogens as well as plant growth enhancement is an important approach in this direction (Srivastva et al. 2004). The use of microorganisms as plant growth-promoting agents is not a new concept, since from ancient days microbes are known to play a key role in enhancing plant growth through various mechanisms such as nitrogen fixation, phosphate solubilization, ACC deaminase activity, induction of plant immune response, tolerance to abiotic stress, and suppression of phytopathogens (Shoresh et al. 2010).

Rhizosphere, the most dynamic region of the soil, is well known for its microbial diversity and is colonized with several plant growth-promoting microorganisms (PGPMs) such as mycorrhizal fungi; species of *Rhizobium*, *Pseudomonas*, *Bacillus*, *Azotobacter*, *Trichoderma*, *Aspergillus*; and others which have also been reported to stimulate plant growth by suppressing plant diseases (Wees et al. 2008). These soil bacteria and fungi are known to mediate processes such as nutrient immobilization

and mineralization, nitrogen fixation, and denitrification (Rashid et al. 2004). Among the fungi, members belonging to the genus *Trichoderma* are outstanding due to their high adaptability to various ecological conditions and variety of lifestyles. They live in soil interacting with animals and plants and also grow saprophytically on wood, bark, and many other substrates. These fungi can form endophytic associations with plants and also interact with other microbes in the rhizosphere, thereby influencing disease protection, plant growth, and yield. Several *Trichoderma* sp. (e.g., *T. harzianum*, *T. viride*, *T. virens*, *T. atroviride*, *T. koningii*, etc.) have been identified as potential biocontrol agents which are also having other plant growth-enhancing abilities (Harman 2000). Recent progress in molecular biology has opened the door to uncover the vast mechanisms of biocontrol action of *Trichoderma* as well as the responses induced in plants upon its colonization (Shinozaki and Shinozaki 2006). Due to these reasons, today *Trichoderma* is used worldwide as a potential biopesticide. They are also well-known producers of several secondary metabolites and other bioactive molecules, and their role in activating plant defense mechanisms has been studied recently in depth (Vinale et al. 2008). Different types of bioactive molecules such as siderophores, peptaibols, pyrones, antibiotics, volatile organic compounds, and polyketides are synthesized by *Trichoderma* spp. Secondary metabolites, which are produced during the later growth phases, are mainly responsible for various plant growth-promoting and biocontrol abilities of the fungus as well as inducing stress tolerance and immune response of plants. The recent in-depth understanding of the functioning of the bioactive molecules of *Trichoderma* sp., has opened a vast scope to formulate efficient biopesticides and biofertilizers involving secondary metabolites.

10.2 The Genus *Trichoderma*: A Potential Rhizosphere-Competent Fungus

The genus *Trichoderma* consists of asexually reproducing fungi that are commonly found in nearly all types of soils, root ecosystems, and other natural habitats especially those containing high organic matter throughout the world. These are free-living fungi that are highly interacting with other rhizosphere microflora. The fungus grows fast in culture and produces numerous green spores. It is considered as one of the most important soilborne plant growth-promoting fungi. Mycoparasitic activity and antibiotic-producing potential were first demonstrated in *Trichoderma lignorum* by Weindling (1932). One of the most interesting aspects of studies on *Trichoderma* is its potential to employ varied mechanisms for disease control. In general the fungus exhibits a preference for wet soil. They show a high level of genetic diversity and can be used to produce a wide range of products of commercial and ecological interest. *Trichoderma* are effective root colonizers by which they deplete the nutrients and make pathogenic microbes to starve, produce organic acids causing the release of macro- and micronutrients for uptake by plants, release volatile substances and secondary metabolites that act as antimicrobial agents, are

capable of producing plant hormones such as zeaxanthin (maize) and gibberellins that accelerate seed germination, trigger the plant immunity, and provide tolerance to plants against abiotic stress.

The genus *Trichoderma* belongs to the phylum *Ascomycetes*, class *Sordariomycetes*, order *Hypocreales*, and family *Hypocreaceae*. The fungus belonging to the genus *Trichoderma* was isolated for the first time from soil and decomposing organic matter and introduced by Persoon in 1794; in 1865, a link to the sexual state of a *Hypocrea* species was suggested (Tulasne and Tulasne 1865). It was difficult to distinguish different species assigned to the genus *Trichoderma*/*Hypocrea* morphologically, and it took until 1969 that development of a concept for identification was initiated (Rifai 1969; Samuels 2006). Thereafter, numerous new species of *Trichoderma*/*Hypocrea* were discovered, and by 2006, the genus contained more than 100 phylogenetically defined species (Druzhinina et al. 2006). *Trichoderma*, for the most part, was classified as *imperfect fungi*, in that they produce only asexual spores. The sexual stage, when found, is within the *Ascomycetes* in the genus *Hypocrea* (Harman 2002). These fungi also colonize woody and herbaceous plant materials, in which the sexual teleomorph (genus *Hypocrea*) has most often been found. Rifai (1969) outlined the speciation concept within the genus *Trichoderma* and described nine species aggregates: *T. piluliferum* Webster & Rifai, *T. polysporum* (Link) Rifai, *T. virens* Gidden & Foster, *T. hamatum* (Bon.) Bain. *T. koningii* Oudem. Apud Oudem. Et Koning, *T. aureoviride* Rifai, *T. harzianum* Rifai, *T. longibrachiatum* Rifai, *T. pseudokoningii* Rifai, and *T. viride* Pers. However, with the use of molecular approaches particularly sequence polymorphism with internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA), several new species have been identified (Hayes et al. 1994). The mycelium is hyaline with septate, profusely branched and smooth-walled hyphae (Fig. 10.1a, b). Chlamydospores are present in most species. The conidiophores are highly ramified and phialides are flask shaped or ovoidal (Hermosa et al. 2000). Safe identification of new species was significantly facilitated in recent years, by development of an oligonucleotide barcode (TrichOKEY) and a customized similarity search tool (TrichoBLAST), both available online (Druzhinina et al. 2011; Kopchinskiy et al. 2005).

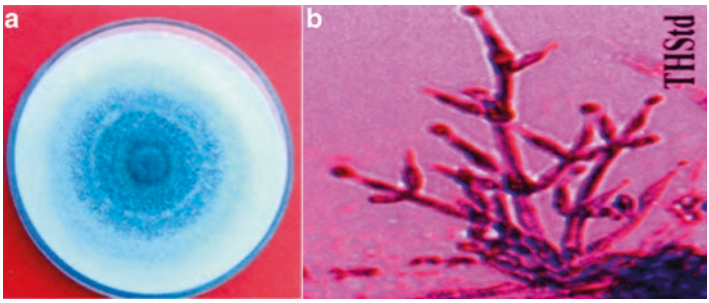


Fig. 10.1 *Trichoderma harzianum*: colony morphology on culture medium (**a**) and microscopic view of mycelial structure with conidiospores (**b**)

10.3 Establishment of Plant-*Trichoderma* Association

Colonization is the principal activity needs to be performed by any beneficial or pathogenic microorganism in order to achieve its goals. Colonization involves the ability to adhere and recognize plant roots, penetrate the plant, and withstand toxic metabolites produced by the plants in response to invasion by a foreign organism whether it is a pathogen or beneficial organism (Brotman et al. 2008). In this regard, *Trichoderma* is the most effective rhizosphere colonizer, which can establish long-lasting relationship with the plants and induce many beneficial responses such as localized or systemic plant resistance responses, nutrient acquisition/mobilization, tolerance to abiotic stress, and deactivation of toxic compounds secreted by phytopathogens, increasing the population of beneficial microflora. These beneficial attributes of *Trichoderma* are due to its unique properties such as resistant to antimicrobial compounds, i.e., phytoalexins, flavonoids, terpenoids, and phenolic derivatives synthesized by plant. *Trichoderma* in turn synthesizes many bioactive molecules which not only react with the antimicrobial products of plant, but they also aid in the plant growth promotion, in various cell-to-cell communication processes, morphogenesis of fungus, adhesion, and hyphal aggregation process. For example, *T. koningii* suppresses the production of phytoalexins during colonization of roots of lotus. Tolerance to antimicrobial compounds by plant is achieved by the presence of ABC transport systems in *Trichoderma* (Ruccco et al. 2009). Some *Trichoderma* strains can colonize only local sites on roots, but rhizosphere-competent strains can colonize entire root surfaces for several weeks or months. *Trichoderma* modifies the rhizosphere by secreting growth-regulating hormones such as auxins that promote the root growth which in turn facilitates colonization by increasing the available surface area. Some of the chemicals are reported to be secreted by *Trichoderma* such as cysteine-rich hydrophobin-like proteins that facilitate anchoring/attachment, Tastdy1 from *T. asperellum* and Qid74 of *T. harzianum*, and expansin-like proteins with cellulose-binding molecules and endopolygalacturonases to facilitate root penetration (Viterbo and Chet 2006). Driving force for colonization, coordination of defense mechanism, and increased rate of photosynthesis are the plant-derived sucrose. Interestingly, *Trichoderma* strains can also colonize leaf surfaces under some conditions, but biocontrol activity might not be dependent on the growth of *Trichoderma* on leaf surfaces (Hermosa et al. 2012). According to recent reports, *Trichoderma* sp. is not confined to outer root tissues only but can also live in the plant as *true endophytes* (Bae et al 2009). Interestingly, most of the endophytic *Trichoderma* discovered are "new species (*T. stromaticum*, *T. amazonicum*, *T. evansii*, *T. martiale*, *T. taxi*, *T. theobromicola*)" (Chaverri et al. 2011). The benefits offered by the endophytic *Trichoderma* species are much better than non-endophytic *Trichoderma*, as they are directly involved in the induction of the transcriptomic changes in plants and protect plants from diseases and abiotic stresses (Bailey et al. 2006, 2009); these endophytes deploy various modes of entry into the plants and form appressorium-like structures. Hence, this interaction is mutually beneficial, but since *Trichoderma* spp. are also capable of living freely in soil, they are considered as opportunistic plant symbionts (Vargas et al. 2009).

10.4 Effect of *Trichoderma* Colonization on Plant Metabolism

Significant changes are observed after the colonization of *Trichoderma* in the plant metabolism, wide range of compounds are released by *Trichoderma* sp. into zone of interaction, and they are known to play a key role in plant growth promotion. Increase in levels of fungal proteins such as xylanases, cellulases, and swollenin by *Trichoderma* induces disease resistance in plants; products of avirulence-like (Avr) genes, peptaibols, also aid in this process. There is also induction of pathogenesis-related (PR) proteins. Mainly systemic acquired resistance (SAR) and induced systemic resistance (ISR) are triggered by *Trichoderma* colonization. SAR is usually triggered by local infection, and ISR is known to result from colonization of roots. Fungal colonization is also known to increase the percentage of germination and photosynthetic capacity of plants; increase dry matter content, starch, and total and soluble sugars; and reduce sugar content in leaves of different parts. The fungal colonization also increases the root proliferation by enhancing the levels of growth-promoting hormones such as auxins and cytokinins and hence provides suitable niche for *Trichoderma*. There is an increased level of antimicrobial compounds upon colonization by *Trichoderma*. In cucumber, root colonization by strain T-203 causes an increase in phenolic glucoside levels; their aglycones (which are phenolic glucosides with the carbohydrate moieties removed) are strongly inhibitory to a range of bacteria and fungi. Thus, root colonization by these fungi induces significant changes in the plant metabolic machinery (Sivan and Chet 1989).

10.5 Plant Growth Promotory Mechanisms

The plants colonized with *Trichoderma* are benefited in many ways such as increased rate of metabolism, i.e., photosynthesis, activation of plant defense mechanism, and deactivation of harmful microbial compounds secreted in and around their occurrence, increase in root growth, accumulation of antimicrobial compounds, increased resistance to the abiotic stress, enhanced nutrient acquisition capacity such as nitrogen use efficiency, phosphorous solubilization, and micronutrient mobilization and uptake (Fig. 10.2).

10.5.1 Direct Mechanisms of Plant Growth Promotion

The rhizosphere-competent fungus *Trichoderma* sp. like other beneficial root-colonizing microorganisms also enhances plant growth and productivity in different ways. Responses to application of *Trichoderma* sp. are characterized by reducing germination time especially in case of vegetables, increased germination percentage, and increased plant development and metabolism and crop yield. These responses may be due to one or more of the attributes like increased uptake and translocation of minerals, solubilization of nutrients and their release from the soil or organic matter, vitamin production or conversion of materials to a form useful to

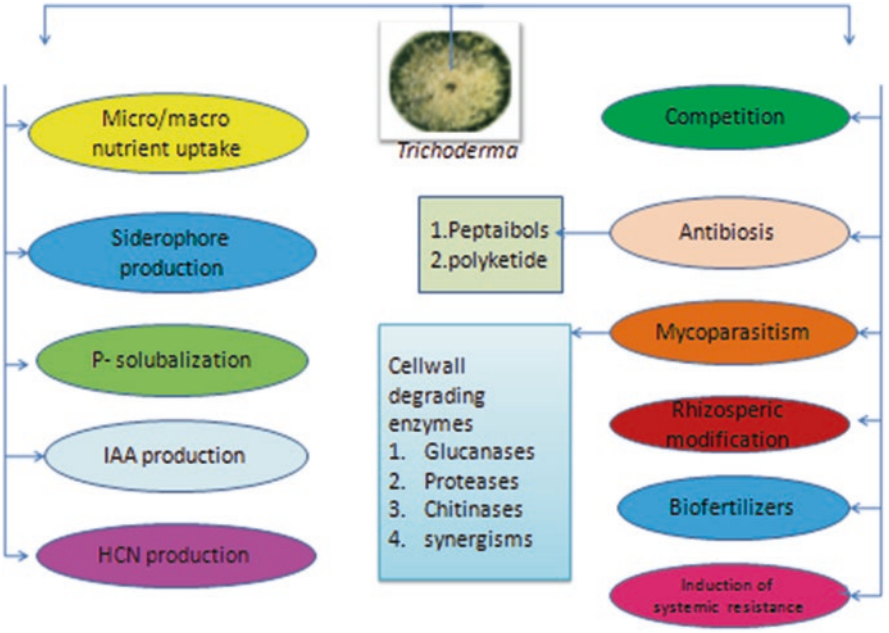


Fig. 10.2 Schematic representation of plant growth-promoting activities of *Trichoderma* sp.

the plants, suppression of deleterious root microflora including those not causing obvious disease, production of growth-stimulating factors (hormones), and synthesis of metal-chelating agents, i.e., ionophores/siderophores. Root colonization by plant growth-enhancing strains of *Trichoderma* results in increased development of root and/or aerial systems and crop yields (Glick 1995).

10.5.1.1 Increase in Nutrient Uptake Efficacy

Recent studies have proved that seed treatment with *Trichoderma* strains results in an increase in nitrogen use efficiency of plants. This effect was first noticed with *T. harzianum* T-22 strain in maize during field trials in the late-1990s; the treated plants were more green and healthy as compared to untreated plants. One of the major threats faced by present-day agriculture is "yield plateauing" frequently observed phenomenon in almost all crop plants. The plants which are engineered in such a manner to respond to high levels of fertilizers and inputs now started showing this phenomenon. To counter this phenomenon, seeds are treated with *Trichoderma* strains which will increase the plant nitrogen use efficiency, and it is a long-term effect that persists for the whole productive lifetime of crop (Yedidia et al. 2001). In the case of maize, the presence of *T. harzianum* T-22, yield plateau was reached with 40–50% less nitrogen fertilizer. This particular mechanism is commercially exploited in the United States, and approximately 0.3 million hectares of wheat are being planted with seeds treated with *T. harzianum* strain T-22 (Porras et al. 2007).

10.5.1.2 Enhancing Nutrient Availability for Plants

Soil is the most complicated dynamic ecosystem in which both micro- and macronutrients undergo a complex dynamic equilibrium of soluble and insoluble forms which is greatly influenced by soil pH and microflora. Soil microbes play an important role in maintaining the equilibrium between soluble and insoluble forms of nutrients by carrying out the processes of mineralization and immobilization in soil. Phosphorus is one of the key macronutrients limiting plant growth and metabolism as approximately 95 to 99% is present in the form of insoluble phosphates in soil and cannot be utilized by plant. Moreover, a major portion (more than 80%) of the phosphatic fertilizers added to soil becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to insoluble fixed inorganic form (Hiolford 1997). It is generally fixed as tricalcium phosphate (TCP) in alkaline soil (at pH above 7.0) and as ferric phosphate ($FePO_4$) and $AlPO_4$ in acidic soil (at pH $\leq$ 5.0), which needs to be solubilized where phosphate-solubilizing microorganisms play an important role (Fankem et al. 2006). Numerous soil bacteria and fungi have been reported to mineralize and mobilize nutrients from soil. *Trichoderma* spp. strongly influence the complex transitions of various plant nutrients from insoluble forms to soluble forms, thereby enhancing accessibility and absorption by roots (Saravanan et al. 2007). Several species of *Trichoderma*, e.g., *T. harzianum*, *T. virens*, *T. viride*, and *T. atroviride*, have been reported to solubilize various forms of inorganic plant nutrients and thus play an important role in nutrient management (Fig. 10.3). *Trichoderma* sp. can solubilize and store phosphate in its biomass that is released in readily available form in close proximity of

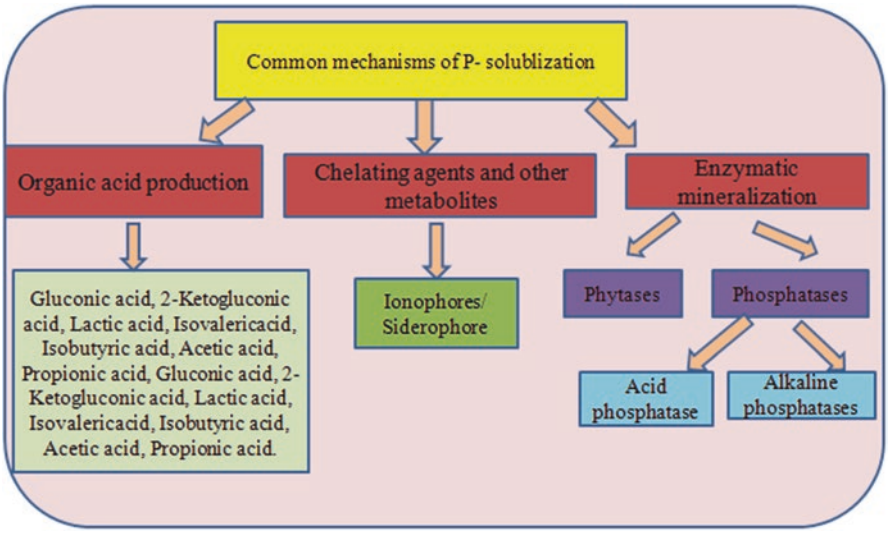


Fig. 10.3 Schematic representation of possible mechanisms of phosphate solubilization adopted by *Trichoderma* spp.

roots after lysis of the mycelium with age. *Trichoderma harzianum* is a potential phosphate solubilizer, but strain variability is always observed (Sakia et al. 2015). It is also capable of solubilizing other nutrients such as iron, MnO_2, and metallic zinc; iron and manganese in particular have been investigated with regard to both solubilization and their influence on plant disease.

Nutrient-solubilizing processes. Three possible mechanisms for in vitro solubilization of some insoluble or sparingly soluble minerals by *T. harzianum* (Rifai) have been proposed: (a) acidification of the medium through production of organic acids, (b) production of chelating metabolites such as ionophores or siderophores, and (c) redox activity.

Acidic Solubilization

Production of organic acids as a mechanism for phosphate solubilization by many PSMs has been reported by many workers (Rodriguez and Fraga 1999; Nautiyal et al. 2000); it results in lowering of pH which ultimately solubilizes the insoluble phosphorous source (Gaur and Sachar 1980; Gaind and Gaur 1991; Gaur 1990; IIlmer et al. 1995; Puente et al. 2004). The most common organic acid produced by gram-negative bacteria is gluconic acid; the bacteria oxidizes glucose from the medium (environment) to gluconic acid resulting in the acidification of the medium and solubilization of insoluble phosphate (Goldstein 1996). Phosphate-solubilizing microorganisms have been shown to produce monocarboxylic acid (acetic, formic); monocarboxylic hydroxy acids (lactic, gluconic, glycolic); monocarboxylic keto acids (2-keto gluconic); dicarboxylic acid (oxalic, succinic); dicarboxylic hydroxy acids (malic, maleic); and tricarboxylic hydroxyl acids (citric) in liquid media from simple carbohydrates (Goldstein 1986; Iyamurimye et al. 1996; Gyaneshwar et al. 1998; Kim et al. 1998; Vinay kumar 2003; Puente et al. 2004). Therefore, release of organic acids that sequester cations and acidify the microenvironment near root zone is thought to be a major mechanism of solubilization of nutrients such as phosphorous, manganese, iron, and zinc by several phosphate-solubilizing microorganisms (PSM).

Enzymatic Solubilization

The term "phosphatases" has been used broadly to describe a wide group of enzymes that hydrolyze organic phosphorus (P) compounds, pyrophosphates, metaphosphates, and inorganic polyphosphates that occur in plenty amounts in soil. Three different groups of enzymes are involved in solubilization of insoluble phosphate sources, namely, phosphatases, phytases, and phosphonatases (C-P lyases). As acid phosphatases and phytases are dominant in soil, therefore, they are mainly responsible for solubilization of fixed phosphorous present in organic matter of soil. The phosphatases attack the phospho-ester or phospho-anhydride bond of organic matter, causing its dephosphorylation. Phytases work particularly on phytate/phytic acid (inositol hexakisphosphate), while C-P lyases perform C-P cleavage in organo-phosphonates.

- **Phosphatases** (**phosphohydrolase**: **esterases**) are the enzymes that hydrolyze phosphoric acid monoesters from substrate by cleaving phosphor-ester bond and release phosphate ions. Due to their wide potential for biotechnological applications, phosphatases have gained the attention of the present era (Rodríguez et al. 2006). On the basis of pH optima, phosphatases may be divided into two broad groups: alkaline phosphatases and acid phosphatases. Both acid and alkaline phosphatases exist in soil and are distinguished on the basis of pH ranges at which they are active (Malcolm 1983). Phosphatases have been reported to be secreted in response to signals of the absence of soluble phosphates (Peleg et al. 1996).
- **Alkaline phosphatases** or basic phosphatases are the enzymes having pH optima greater than 7.0; that means, they work well in alkaline or basic environments (Tamas et al. 2002). The enzyme alkaline phosphatase (Alp, EC 3.1.3.1.) that catalyzes the cleavage of monophosphate groups from inorganic or organic backbones is frequently used in soil ecology as a marker for microbial activities (Kuperman and Carreiro 1997). The enzyme is homodimeric metalloenzyme with molecular weight of 86,000 kDa. To each monomer, one magnesium (Mg) and two zinc (Zn) ions are attached. Bacterial alkaline phosphatases are highly resistant to several environmental adversities as they are present in the periplasmic space in gram-negative bacteria. The enzyme dephosphorylates many molecules like sugar phosphates, phenols, alkaloids, etc. with the help of some accepter molecule; it can transphosphorylate alcohols. Bacterial alkaline phosphatases are highly active with several applications as in epitope mapping, immunoblotting, expression, analysis of mutants, etc. Induced expression of alkaline phosphatase in *Trichoderma* sp. in the presence of insoluble phosphorus source (tricalcium phosphate) has been well reported (Kapri and Tewari 2010). *Trichoderma* spp. can also retain its phosphate-solubilizing potential under abiotic stress conditions, such as under phosphorus-deficient conditions and in the presence of heavy metals (Rawat and Tewari 2011).
- **Acid phosphatase**: Acid phosphatases have been described in several bacteria, fungi, and yeast. Several fungi, e.g., *Aspergillus* and *Penicillium* spp., have the ability to synthesize acid phosphatase enzyme, but there is scarcity of literature on acid phosphatase activity of *Trichoderma* strains. These are the phosphatase enzymes that work in acidic pH range, i.e., pH < 6.0. They are dominant in soil solutions; thus major P solubilization in soil is performed by acid phosphatases. Many genes for acid phosphatases have been isolated from different species of gram-negative bacteria such as acpA gene for acid phosphatase with pH optima at 6 which having a wide range of substrate has been isolated from *Francisella tularensis*. Three molecular families (class A, class B, and class C) form the nonspecific acid phosphatases in bacteria. They are located inside the cell and thus contribute to hydrolyze organic phosphor-ester bond of nucleotide, phosphate sugars, etc. (Rodríguez et al. 2006).

- **Phytase (*myo*-inositol hexakisphosphate phosphohydrolase) enzyme**: The enzyme phytase catalyzes hydrolysis of *myo*-inositol hexakisphosphate (phytic acid) to inorganic monophosphate and lower *myo*-inositol phosphates and in some cases to free *myo*-inositol. The Enzyme Nomenclature Committee of the International Union of Biochemistry distinguishes two types of phytases: 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). This classification is based on the first phosphate group attacked by the enzyme. Thus, bioavailability of inositol phosphate depends on their mineralization by both types of extracellular phytases, which have many biological sources. Most phytases come under high molecular weight acid phosphatases. Microbial phytase activity is most frequently detected in fungi, particularly in *Aspergillus* species. Shieh and Ware (1968) screened over 2000 microorganisms isolated from soil for phytase production. Most of the positive isolates produced only intracellular phytase. Extracellular phytase activity was observed only in 30 isolates. All extracellular phytase producers were filamentous fungi. Twenty-eight belonged to the genus *Aspergillus*, one to *Penicillium*, and one to *Mucor* (Aowson and Davis 1983). Generally, phytate is the primary source of inositol, and in the plant seeds, and pollen is found as the major stored form of phosphate. Phytases that are optimal for improving animal nutrition have been in focus of many genetic engineering studies. Another attractive property of these enzymes that is not currently utilized is solubilization of soil organic phosphorus through phytate degradation. Phytate is the main component of organic forms of P in soil (Rodríguez et al. 2006). Plants are not able to obtain phosphorus directly from phytate efficiently. The mechanisms employed by the *Trichoderma* in P solubilization are almost similar to the mechanisms employed by the general P-solubilizing rhizobacteria. Some of the mechanisms employed by *Trichoderma* are acidification of the medium, production of chelating metabolites, and redox activity. *T. asperellum* has been shown to enhance the availability of P and Fe to plants with significant increases in dry weight, shoot length, and leaf area (Yedidia et al. 2001).

10.5.1.3 Enhancing Micronutrient Availability

Iron availability to plants is a unique kind of phenomenon; it is neither assimilated by bacteria nor plants in aerobic soils, because ferric ion, which is the predominant form in nature, is only sparingly soluble so that the amount of iron availability for assimilation by living organisms is extremely low. Under these critical circumstances, microorganisms have evolved special mechanisms for the assimilation of iron, such as the production of low-molecular-weight compounds known as siderophores (the iron-specific ionophores), which transport this element into their cells. Siderophores are divided into three main families depending on the characteristic functional group, i.e., hydroxamate, catecholates, and carboxylates. At present more than 500 different types of siderophores are known, of which 270 have been structurally characterized (Ali and Vidhale 2013). Interestingly, siderophores play a key role in both direct and indirect enhancement of plant growth. Fungal siderophores mainly are fusarinins, coprogens, and ferrichromes that all belong to the group of

hydroxamate type of siderophores that share the structural unit N-5-acyl-N-5 hydroxy ornithine (Renshaw et al. 2002). Coprogen, Coprogen B, and ferricrocin were excreted from all the six *Trichoderma* species tested under iron-deficient conditions by Anke et al. (1991).

10.5.1.4 Secretion of Phytohormones

Highly effective molecules, which are known to influence the most essential stages of plant growth and development, are produced by plant in minute quantities, which is insufficient for meeting the needs of large requirement of plants; several plant growth-promoting rhizospheric microorganisms are able to synthesize phytohormones such as auxins (indole acetic acid), cytokinins, gibberellins, abscisic acid, and ethylene and thus enhance the plant growth. The first report on discovery of gibberellins was made from the fungus *Gibberella fujikuroi*. Indole acetic acid (IAA) is also produced by *Pseudomonas fluorescens*. Also, an increase of IAA, gibberellin, and cytokinin level was observed in *G. fasciculatum*-inoculated *Prosopis juliflora*. However, these mechanisms are not well studied in the case of *Trichoderma*. *Trichoderma* strains are capable of enhancing plant biomass production, promoting lateral root growth and development through an auxin-dependent mechanism or able to produce indole-3-acetic acid or auxin analogues (Contreras-Cornejo et al. 2009); an auxin-like effect has been observed in etiolated pea stems treated with harzianolide and 6-pentyl-a-pyrone, the major secondary metabolite produced by different *Trichoderma* strains (Vinale et al. 2008). The growth-promoting activity of *T. atroviride* on tomato seedlings has been suggested to be associated with the reduced ethylene production resulting from a decrease in its precursor l-amino cyclopropane-1-carboxylic acid (ACC) through the microbial degradation of IAA in the rhizosphere or through the ACC deaminase (ACCD) activity present in the microorganisms (Gravel et al. 2007).

10.5.2 Indirect Mechanisms (Biocontrol Action)

Trichoderma spp. is well known today for its biocontrol potential against soilborne fungal phytopathogens such as *Fusarium*, *Pythium*, *Sclerotium*, *Rhizoctonia*, *Sclerotinia*, *Macrophomina* sp., etc. which are the major wilt-causing pathogens. Among various *Trichoderma* sp., the most widely reported and commonly used biocontrol species are *T. harzianum*, *T. viride*, and *T. virens* (Cook and Baker 1983). They are known to produce a wide range of antibiotic substances and parasitize fungal phytopathogens. They also compete with other soil microorganisms for space, nutrients, and key exudates from seed and roots that stimulate the germination of propagules of plant pathogenic fungi in soil. *T. harzianum* has high potential to control sheath blight of rice by antagonizing the pathogen *Rhizoctonia solani* (Tewari and Bhanu 2004). Today, *Trichoderma* strains are used for biological control, either alone or in combination with other microbes or chemical adjuvants. They are also known to produce certain lytic enzymes that degrade the cell wall of the pathogen. These versatile fungi are highly efficient producers of many extracellular

enzymes like cellulases, chitinases, glucanases, proteases, etc. *Trichoderma* sp. is the most extensively used fungal biocontrol agent for the management of plant pathogens affecting seed, root, and aerial plant parts. *Trichoderma* uses a variety of mechanisms to provide protection against several plant pathogens and/or plant diseases and enhance plant growth, such that it may (1) directly kill the pathogen by mycoparasitism and/or antibiosis; (2) adversely affect the growth and development of the pathogen by competing for the nutrients, oxygen, or space; (3) alter fitness of the pathogen; (4) induce systemic plant resistance; (5) enhance plant growth and its tolerance to stress; (6) metabolize plant exudates supporting pathogen; (7) inactivate enzymes produced by the pathogens; and (8) synthesize cell wall-degrading enzymes (lytic enzymes) that degrade the cell wall of pathogen. Some of the most common mechanisms involved in biocontrol of fungal phytopathogens by *Trichoderma* spp. are described below:

10.5.2.1 Mycoparasitism

Mycoparasitism is a complex process that involves tropic growth of biocontrol agent toward the target (pathogen) organism and finally attack and dissolution of the pathogen's cell wall by the activity of various enzymes, which may be associated with physical penetration of cell wall (Rawat and Tewari 2010). Thus, in this process, the antagonist exists in intimate association with the other target fungi from which it derives some or all its nutrients. Mycoparasitism is a well-known phenomenon in biocontrol action of *Trichoderma*. In general, the overall process of mycoparasitization of fungal pathogen involves four steps: (1) The first stage is the chemotropic growth of the biocontrol fungus toward the target fungi that produce chemical stimuli, for example, a volatile or water-soluble substance produced by the host fungus serves as a chemoattractant for parasite. (2) The next step is recognition of the target pathogen, in which lectins of the host (pathogen) and carbohydrate receptors on the surface of the biocontrol fungus might be involved. (3) The third step is attachment, secretion of lytic enzymes, and cell wall degradation. Mycoparasites can usually either coil around host hyphae or grow alongside it and produce cell wall-degrading enzymes to attack the target fungus, and (4) the final step is penetration of the biocontrol agent into host by forming appressorium-like structures to penetrate the target fungus cell wall. A large number of mycoparasitic *Trichoderma*-based formulations are commercially available in the market as a promising alternative to chemical pesticides for the use of farmers in countries like the United States, India, Israel, New Zealand, and Sweden (Howell 2003).

Signal Transduction Pathways Involved in Mycoparasitism

Environmental signaling plays an important role in cellular organisms. Understanding of the mechanisms of cell signaling in *Trichoderma* spp. is limited compared to "model" fungi like *Neurospora crassa*, but there has been significant progress based on genetic approaches. The seven transmembrane G protein-coupled receptor Gpr1 of *Trichoderma* is involved in sensing the fungal prey; silencing of the gpr1 gene in *T. atroviride* rendered the mycoparasite unable to respond to the presence of the host fungus (Omann et al. 2012). Binding of a ligand to such receptors leads to

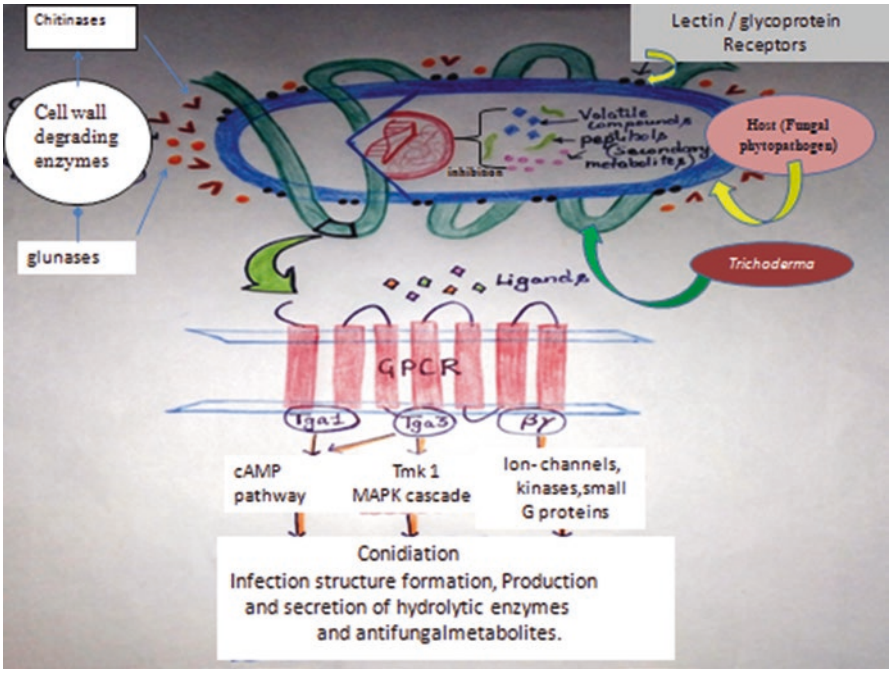


Fig. 10.4 Overall process of mycoparasitization of fungal phytopathogen by *Trichoderma* spp. showing involvement of various compounds and G protein signaling (Source: modified from Omann and Zeilinger 2010)

downstream signaling events via activation of G protein cascades. The overall process of mycoparasitism of fungal pathogen by Trichoderma sp. is illustrated in Fig. 10.4. Indeed, deletion of the Tga3 Gα protein-encoding gene affected the mycoparasitic abilities of *T. atroviride* in a similar way to loss of Gpr1 (Zeilinger et al. 2005). Deletion of the adenylate cyclase gene tac1 severely impaired growth and mycoparasitic abilities of *T. virens* (Mukherjee et al. 2007). Like most other filamentous fungi, *Trichoderma* spp. have three MAP kinase cascades comprising MAPKKK, MAPKK, and MAPK (Schmoll 2008), and MAPK pathways may act in mycoparasitism and biocontrol (Kumar et al. 2010). These reports imply important functions of signaling cascades in mycoparasitism and related biocontrol properties (Mukherjee et al. 2012).

The Role of G Protein Signaling in Biocontrol Action of *Trichoderma*

The heterotrimeric G protein signaling in *Trichoderma* sp. consisted of three parts: a G protein-coupled receptor (GPCR), a heterotrimeric G protein (α, ß, γ subunits), and an effector molecule (Neer 1995). More than 1000 GPCR-encoding genes have been identified and characterized from different organisms; most of them were of vertebrate origin (Kolakowski 1994). All these receptor proteins have seven transmembrane domains and have the N-terminus outside and the C-terminus inside the

cytoplasm. When ligand binds to the receptor, it changes the conformation that leads to the release of the G proteins and exchange of GDP for GTP on the Gα subunit. GTP bound α dissociates from its ß γ partner, allowing both signaling units to regulate the activities of downstream effectors (Kaziro et al. 1991; Gutkind 1998). Highly conserved heterotrimeric G proteins act as signal transducers that couple cell surface receptors to cytoplasmic effector proteins. G proteins are necessary during sexual and pathogenic development and during secondary metabolism. In fungi, they are part of the pheromone signaling cascade and also affect a number of developmental and morphogenetic processes which determine the virulence of fungi and plant-fungal pathogen interactions (Bölker 1998). Rocha-Ramirez et al. (2002) reported that *T. atroviride* subgroup I Gα subunit Tga1 is involved in both coiling and conidiation. This has been shown by overexpression of tga1 gene and by tga1 gene silencing. These results were confirmed by tga1 gene deletion mutant study (Reithner et al. 2005).

10.5.2.2 Antibiosis

Antibiosis is one of the most powerful mechanisms employed by *Trichoderma* sp. as its biocontrol strategy against fungal pathogens. Antibiosis occurs during interactions involving low-molecular-weight diffusible compounds or antibiotics produced by *Trichoderma* strains that inhibit the growth of other microorganisms. There is a wide diversity of antibiotics produced by both bacteria and fungi which are known to have profound effect on pathogens. *Trichoderma* produces wide variety of secondary metabolites, which play predominant role in biocontrol activity. It releases more than 43 substances that have antibiotic activity. Most *Trichoderma* strains produce volatile and nonvolatile toxic metabolites that impede colonization by antagonized microorganisms. Secondary metabolites are chemically different natural compounds that play an important role in regulating interactions between organisms, such as phytotoxins (secondary metabolites produced by fungi that attack plants), mycotoxins (toxins produced by fungi that colonize crops capable of causing diseases and death in humans and other animals), pigments (colored compounds also with antioxidant activity), and antibiotics (natural products capable of inhibiting or killing microbial competitors. Weindling (1934) characterized the "lethal principal" excreted by a strain of *T. lignorum* into the medium as "gliotoxin" and demonstrated that it was toxic to both *R. solani* and *Sclerotinia americana*. At present *Trichoderma* species are reported to produce a number of antibiotics, such as gliotoxin, glivirin, glioviridin, viridin, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, and sesquiterpenes, and some steroids.

10.5.2.3 Production of Hydrolytic Enzymes

Attachment of *Trichoderma* to the host (pathogen) is followed by a series of degenerating events and degradation of cell wall of the pathogen by synthesizing various cell wall-degrading enzymes (CWDEs); among them, chitinases, glucanases, and proteinases are the major ones. *Trichoderma* are good producers of hydrolytic enzymes, and the most intensively studied of these belong to the chitinolytic system (chitinases and NAGase), glucanases followed by proteinases (de Almeida et al. 2010).

Trichoderma strains generally produce β-1, 3 and β-1, 6 glucanases that hydrolyze the glucan polymer of the cell wall of the pathogen. *T. harzianum* produces at least four β-1, 3-glucanase isoenzymes under different in vitro culture conditions. Some *Trichoderma* strains also secrete β-1, 6 glucanases which are also involved in cell lysis, along with chitinase and proteinase activity. The enzyme chitinases act on chitin, a major component of fungal cell wall. Fungal cell wall contains chitin and/or β-glucan fibrils that are embedded in protein matrix. Thus, extracellular proteases, synthesized by *Trichoderma*, hydrolyze these proteins present in pathogens' cell walls and play a significant role in mycoparasitism. Extracellular hydrolytic enzymes of *Trichoderma*, e.g., *T. harzianum*, act synergistically as shown by in vitro studies (Harman 2000).

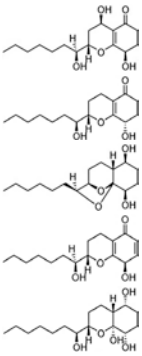
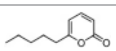
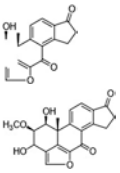
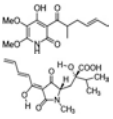
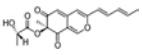
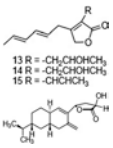
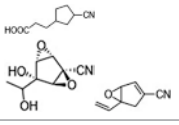
10.5.2.4 Production of Secondary Metabolites and Volatile Compounds

Trichoderma sp. produces different types of volatile compounds and secondary metabolites as means of its biocontrol activity. The production of secondary metabolites by *Trichoderma* sp. is strain dependent and includes antifungal substances belonging to a variety of chemical compounds (Table 10.1). They have been classified into three categories: (1) volatile antibiotics, i.e., 6-pentyl-a-pyrone (6PP), and most of the isocyanide derivatives; (2) water-soluble compounds, i.e., heptelidic acid or koningic acid; and (3) peptaibols, which are linear oligopeptides of 12–22 amino acids rich in a-aminoisobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol at the C-terminus (Howell 2003). The production of low-molecular-weight, nonpolar, volatile compounds (i.e., 6PP) results in a high concentration of antibiotics in the soil environment that have a relatively long-distance range of influence on the microbial community, while a short distance effect may be due to the polar antibiotics and peptaibols acting in close proximity to the producing hyphae. Although the role and the effects of peptaibols are clear, the mode of action of other *Trichoderma* secondary metabolites (i.e., pyrones) and their possible synergisms with other compounds are yet to be elucidated. Viride pyrone showed antagonistic activity against *Sclerotium rolfsii*, whereas δ-decanolactone was found to control *B. cinerea*, *Phytophthora* spp., *Aspergillus niger*, and *Candida albicans* (Hill et al. 1995; Kishimoto et al. 2005). Plant growth promotion activity of *Trichoderma* is a result of combined activities of primary and secondary metabolites, but the role of secondary metabolites is largely appreciated because they exhibit several biological functions and play an important role in regulating interactions between organisms.

10.5.2.5 Competitive Inhibition of Pathogen

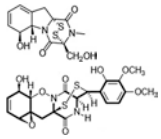
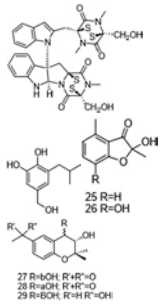
Competition seems to be an important mechanism of biocontrol, but it is difficult to assess its actual contribution in biological control. Competition is considered as a "classical" mechanism of biological control that involves competition between antagonist and plant pathogen for space and nutrients (Chet 1987). The omnipresence of *Trichoderma* in agricultural and natural soils throughout the world proves that it is an excellent competitor for space and nutritional resources. Neither

Table 10.1 List of secondary metabolites produced by *Trichoderma* sp.

Secondary metabolites	Synthesizing organism	Molecular structure	Effective against	References
1. **Koninginins**	*T. koningii* and *T. harzianum*		*Rhizoctonia solani*, *Phytophthora cinnamomi*, *Pythium middletonii*, *Fusarium oxysporum*, and *Bipolaris sorokiniana*	Almassi et al. (1991) and Ghisalberti and Rowland (1993)
2. Pyrones	*T. atroviride* and *T. harzianum*		*Rhizoctonia solani*, *Fusarium oxysporum*, and some bacteria	Scarselletti and Faull (1994) and Worasatit et al. (1994)
3. Viridins	*T. viride* and *T. virens*		*Botrytis allii*, *Colletotrichum lini*, *Fusarium caeruleum*, *Penicillium expansum*, *Aspergillus niger*, and *Stachybotrys atra*	Reino et al. (2008) and Brian et al. (1944)
4. Nitrogen heterocyclic compounds	*T. harzianum*		*Botrytis cinerea*, *R. solani*, *G. graminis* var. *tritici*, and *Pythium ultimum*	Dickinson et al. (1989) and Vinale et al. (2006)
5. Azaphilones	*T. harzianum*		*R. solani*, *P. ultimum*, and *G. graminis* var. *tritici*	Vinale et al. (2006)
6. Butenolides and hydroxy-lactones	*T. harzianum*		*P. ultimum*, *R. solani*, *and B. cinerea*	Almassi et al. (1991) and Vinale et al. (2006)
7. Isocyano metabolites	*T. hamatum*, *T. viride*, and *T. koningii*		*Phytophthora* sp.	Tamura et al. (1975)

(continued)

Table 10.1 (continued)

Secondary metabolites	Synthesizing organism	Molecular structure	Effective against	References
8. Diketo-piperazines	*T. virens*		*R. solani*, *P. ultimum*	Howell (1998)
9. Peptaibols	*T. harzianum*		*P. ultimum*, *R. solani*	Daniel and Filho (2007)

antibiosis nor mycoparasitism is mainly involved in biocontrol of seedling disease in cotton, but competition is the main mechanism in this case. Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens. Indirect mechanisms in plant growth promotion include the competition for nutrient (Fe^{+3}), in which the siderophore secreted by *Trichoderma* scavenges iron from the environment thus making it unavailable for competing microorganisms, and it is described as one of the key factors in antagonism of *T. asperellum* against *F. oxysporum* f.sp. lycopersici (Segarra et al. 2010). A recent study further reported the detection of an average 12–14 siderophores by isotope-assisted screening of *T. atroviride*, *T. asperellum*, *T. gamsii*, *T. hamatum*, *T. virens*, *T. harzianum*, *T. polysporum*, and *T. reesei* with dimerum acid, coprogren, fusigen, fusarinine A, and the intracellular siderophore ferricrocin being produced by all examined species (Lehner et al. 2013).

10.5.2.6 Stimulation of Plant Immune Responses

Another very important indirect means of plant growth promotion is the induction of host resistance; plants are known to respond to variety of environmental stimuli, including gravity, light, temperature, physical stress, water, and nutrient availability; and they also respond to the chemical stimuli produced by soil-/plant-associated microbes. Those stimuli are known to induce the resistance in plants against wide range of pathogens. This resistance in plants is achieved by various mechanisms such as systemic acquired resistance (SAR); is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection; and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins

include a variety of enzymes, some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death and another mechanism known as induced systemic resistance (ISR) that is mediated by jasmonic acid (JA) and ethylene, which are produced following applications of some nonpathogenic rhizobacteria (Kloepper et al. 1992). The defense responses may also include the physical thickening of cell wall by lignifications, deposition of callose, accumulation of antimicrobial low-molecular-weight substance (e.g., phytoalexins), and synthesis of various proteins (e.g., chitinases, glucanases, peroxidases). The ISR triggered by *Trichoderma* occurs through the JA/ET signaling pathway similarly to PGPR ISR. Recent studies have shown the colonization of Arabidopsis roots by *T. atroviride* that induces a delayed and overlapping expression of the defense-related genes of the SA and JA/ET pathways against biotrophic and necrotrophic phytopathogens, both locally and systemically (Salas-Marina et al. 2011). *Trichoderma* is able to trigger a long-lasting upregulation of SA gene markers in plants unchallenged by pathogens, although when plants are infected by a pathogen such as *B. cinerea*, the pretreatment with *Trichoderma* may modulate the SA-dependent gene expression, and, soon after infection, the expression of defense genes induced through the JA signal transduction pathway occurs, causing ISR to increase over time (Tucci et al. 2011).

- *Role of bioactive* Neither antibiosis nor mycoparasitism is mainly involved in biocontrol of seedling disease in cotton, but competition is the main mechanism in this case. Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens.

10.5.2.7 Elicitors in Plant Stress Response

Various abiotic stresses such as drought, low/high temperatures, salinity and acidic conditions, light intensity, submergence, anaerobiosis, and nutrient starvation are the main factors that are impacting agricultural production. Speaking numerically, water deficient (drought) has affected 64% of the land area, flood (anoxia) 13%, salinity 6%, mineral deficiency 9%, acidic soils 15%, and cold 57%. But any accurate estimation of agricultural loss in terms of ecological disturbances due to abiotic stress could not be made. Recently, several PGPR have been shown to efficiently help plants to overcome abiotic stress such as salinity and drought in both field crops and trees (Contreras-Cornejo et al. 2014). The role played by *Trichoderma* in mitigating the problems caused by abiotic stress is very significant. *T. harzianum* when treated to seeds (tomato) or soil treatment (Arabidopsis) largely improved the germination at osmotic potentials of up to 0.3 MPa (Mastouri et al. 2010, Harman 1991). *T. harzianum* treated to maize seeds showed the enhanced deep rooting ability thus surviving under water-deficit conditions. Further in *Trichoderma*-inoculated cacao seedlings, drought-induced changes such as stomatal closure and reduction of net photosynthesis were delayed under drought compared with non-inoculated plants; *Trichoderma*-treated squash plants showed higher fresh weight compared to

untreated seeds. Salt stress is known to cause other inhibitory effects in plant growth such as reduced uptake of potassium ions; as potassium is a compatible solute, its uptake is essential for osmotic adaptation of plants; and it also plays an important role in the closure of stomata; hence trichodermal treatment can ameliorate the salt-induced multiple growth inhibition. *Trichoderma* inoculation also increased calcium content under salinity compared with nonsaline condition. *Trichoderma harzianum* has recently been shown to improve resistance to heat and cold (seedlings of tomato were imbibed at 25 °C for 1 day, then exposed either 10 °C or 35 °C, and then returned to 25 °C). Seedlings were much less damaged by the temperature extremes in the presence of T *harzianum* (Hermosa et al. 2011).

10.6 *Trichoderma*-Based Commercial Products

It is estimated that 90% of all antagonistic fungi used in plant protection belong to the genus *Trichoderma* (Benitez et al. 2004). The success of *Trichoderma* as a biocontrol agent is due to the ability of the fungus to produce plethora of secondary metabolites *Trichoderma* interacts with other microorganisms but mainly with pathogenic fungi. Today several Trichoderma-based commercial products are available in the market that can be used as biopesticides and biofertilizer in green and sustainable agriculture (Lorito et al. 2010) (Table 10.2).

Table 10.2 List of some of the commercial *Trichoderma*-based biopesticides

Organism name	Trade name	Mode of action	Effective against
T. harzianum + *T. viride*	Trichodex Trichopel Trichojet Trichodowels	Mycoparasitic	Effective against *Armillaria* and *Botryosphaeria* and others
Trichoderma sp.	Trichodry Trichoflow Trichogrow Trichopel	Mycoparasitic	Suppresses root pathogens
Trichoderma viride	Ecosom TV Tricon	Mycoparasitic	Effective against rot diseases
Trichoderma harzianum	Root shield BioTrek 22 g Supresivit	Mycoparasitic antagonistic	Effective against variety of soil pathogens and wound pathogens
Trichoderma viride	Bioderma	Mycoparasitic	*Fusarium* wilt and *Verticillium* wilt and all types of leaf spot and leaf blight
Trichoderma harzianum	Bioderma-H	Mycoparasitic	*Pythium*, *Rhizoctonia*, *Schlerotinia*, *Fusarium*, and *Verticillium* wilt, all types of leaf spot and leaf blight

10.7 Biotechnological Applications of *Trichoderma* Bioactive Molecules

1. Various compounds produced by *Trichoderma* such as secondary metabolites, volatile compounds, and antimicrobial compounds are known to have great application in the field of agriculture and industry such as drug and cosmetics.
2. *Trichoderma* are known to produce wide range of hydrolytic enzymes which are great application in industry and agriculture.
3. Trichodermal mycoparasitism provides valuable biotechnological tools to understand the basic process and in vitro biocontrol studies.
4. Various volatile and secondary metabolites produced by *Trichoderma* serve as a starting material for synthesis of chemicals which are effect against phytopathogens.

10.8 Conclusion

Increased pressure on agriculture with abiotic and biotic stress and the use of *Trichoderma* as phytostimulant reduce the pressure on the use of the chemicals. *Trichoderma* provide various direct and indirect mechanisms of plant growth promotion and hence can be used as phytostimulant in various crops as it offers a sustainable alternative to chemical agriculture. *Trichoderma* are known to produce a wide range of antimicrobial compounds which do not benefit them, but they also induce plant resistance to pathogens. *Trichoderma* are known to synthesize secondary metabolites, and volatile compounds are directly responsible for antagonistic properties against phytopathogens, and they are also responsible for various plant growth promotion activities. Recent studies have concluded that secondary metabolites from *Trichoderma* offer a wide range of application in biocontrol. In fact, treatment with *Trichoderma* metabolites produces extensive changes of the plant expressome, proteome, and metabolome by acting on specific pathways involved in synthesis of major hormones resistance to biotic and abiotic stresses and nutrient uptake.

10.9 Future Prospects

With this current understanding about *Trichoderma* and its various plant growth-promoting characters, there is a need to understand these mechanisms at molecular levels to gain deeper insights in these mechanisms. It is necessary to elucidate the mechanisms of mycoparasitism in detail such as how biotic and abiotic interactions affect the mycoparasitic activity and how it can give us information regarding the biocontrol activity of the *Trichoderma*. So far our knowledge concerning mycoparasitism's genomics is based on single genome per species, and there is a need to address the long-standing axiom of strain variation of biocontrol relevant mycoparasitic traits. Recent breakthroughs also point out the importance of assessing and

delineating the ecological niche and life histories of mycoparasites to better interpret data emerging from comparative genomics and to allow highly targeted application of respective strains. Study of secondary metabolites produced by *Trichoderma* as biopesticides rather than whole organism needs special attention, and it can revolutionize the biopesticide industry.

References

Almassi F, Ghisalberti EL, Narbey MJ, Sivasithamparam K (1991) New antibiotics from strains of *Trichoderma harzianum*. J Nat Prod 54:396–402

Ali SS, Vidhale NN (2013) Bacterial siderophore and their application. J Curr Microbiol App Sci 2(12):303–312

Anke H, Kinn J, Berquist KE, Sterner O (1991) Production of siderophores by strains of the genus *Trichoderma*-isolation and characterization of the new lipophilic coprogen derivative, palmitoylcoprogen. Biol Met 4:176–180

Aowson SJ, Davis RP (1983) Production of phytate hydrolyzing enzyme by some fungi. Enzym Microb Technol 5(5):377–382

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA (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(11):3279–3295

Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J et al (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta 224(6):1449–1464

Bailey BA, Strem MD, Wood D (2009) *Trichoderma* species form endophytic associations within Theobroma cacao trichomes. Mycol Res 113(12):1365–1376

Benitez T, Rincon MCL, Codon AC (2004) Biocontrol mechanism of Trichoderma strains. Int Microbiol 7:249–260

Bolker M (1998) Sex and crime: heterotrimeric G proteins in fungal mating and pathogenesis. Fungal Genet Biol 25(3):143–156

Brian PW (1944) Production of gliotoxin by *Trichoderma viride*. Nature 154:667

Brotman Y, Briff E, Viterbo A, Chet I (2008) Role of swollenin, an expansin-like protein from *Trichoderma*, in plant root colonization. Plant Physiol 147(2):779–789

Chaverri P, Gazis RO, Samuels GJ (2011) *Trichoderma* Amazonicum, a new endophytic species on Hevea Brasiliensis and H. Guianensis from the Amazon basin. Mycologia 103(1):139–151

Chet I (1987) *Trichoderma*: application, mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi. In: Chet I (ed) Innovative approaches to plant disease control. Wiley, New York, pp 137–160

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J (2009) *Trichoderma* virens, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. Plant Physiol 149(3):1579–1592

Contreras-Cornejo HA, Macías-Rodríguez L, Alfaro-Cuevas R, López-Bucio J (2014) *Trichoderma* spp. improve growth of Arabidopsis seedlings under salt stress through enhanced root development, osmolite production, and Na+ elimination through root exudates. Mol Plant-Microbe Interact 27(6):503–514

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

Daniel JF, Filho ER (2007) Peptaibols of *Trichoderma*. Nat Prod Rep 24:1128–1141

de Almeida RP, van Lenteran JE, Stouthames R (2010) Does wolbachia behavior? Braz J Biol 70:435–442

Druzhinina IS, Kopchinskiy AG, Kubicek CP (2006) The first 100 *Trichoderma* species characterized by molecular data. Mycoscience 47(2):55–64

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Kubicek CP (2011) *Trichoderma*: the genomics of opportunistic success. Nat Rev Microbiol 9(10):749–759

Fankem HD, Niwaga A, Deubel L, Dieng W, Merbach W, Etoa FX (2006) Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (Elaus guingenesis) rhizosphere in Cameroon. African J Biotech 5:2450–2460

Gaind S, Guar AC (1991) Thermotolerant phosphate solublizing microorganisms and their interaction with mung bean. Plant Soil 133:141–149

Gaur AC, Sachar S (1980) Effect of rock phosphate and glucose concentration on phosphate solublization by Aspergillus awamori. Curr Sci 49:553–554

Gaur AC (1990) Phosphate solublizing microorganisms as biofertilizer. Omega Scientific Publishers, New Delhi, p 176

Ghisalberti EL, Rowland CY (1993) Antifungal metabo lites from Trichoderma harzianum. J Nat Prod 56:1799–1804

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41(2):109–117

Goldstein AH (1986) Bacerial solubilization of mineral phosphate: historical perspectives and future prospects. Am J Altern Agric 1:57–65

Goldstein AH (1996) Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram negative bacteria. In: Torriani-Gorini A, Yagil E, Silver S (eds) Phosphate in microorganisms: cellular and moleular biology. ASM Press, Washington, DC, pp 197–203

Gutkind JS (1998) Cell growth control by G protein-coupled receptors: from signal transduction to signal integration. Oncogene 17(11 Reviews):1331–1342

Gyaneshwar P, Naresh Kumar G, Parekh lJ (1998). Effect of buffering on the phosphate solubilizing ability of micro-organisms Microbiol Biotechnol14(5):699–673

Harman GE (1991) Seed treatments for biological control of plant disease. Crop Prot 10(3):166–171

Harman GE (2000) Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma* harzinum T-22. Plant Dis 84(4):377–393

Harman GE (2002) Trichoderma sp including T. harzinium,T. viridae, T. koningii, T. hamatum and other sp. Deutoromycetes, Monnilaes (Asexual classification system). In: Biological Control. Cornell University, Genevea, Newyork, pp 144–156

Hayes CK, Klemsdal S, Lorito M, Di Pietro A, Peterbauer C, Nakas JP et al (1994) Isolation and sequence of an endochitinase-encoding gene from a cDNA library of *Trichoderma harzianum*. Gene 138(1):143–148

Hermosa MR, Grondona I, Iturriaga ET, Diaz-Minguez JM, Castro C, Monte E, Garcia-Acha I (2000) Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. Appl Environ Microbiol 66(5):1890–1898

Hermosa R, Botella L, Keck M, Jimenez JA, Montero Barrientos M, Arbona V, Gomez Cadenas A, Monte E, Nicolas C (2011) The overexpression in Arabidopsis Thaliana of a *Trichoderma harzianum* gene that modulates glucosidase activity, and enhances tolerance to salt and osmotic stresses. J Plant Physiol 168:1295–1302

Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of *Trichoderma* and of its genes. Microbiology 158(1):17–25

Hill RL, O'Donnell DJ, Gourlay AH, Speed CB (1995) The suitability of *Agonopterix ulicetella* (Lepidoptera: Oecophoridae) as a control for *Ulex europaeus* (Fabaceae: Genisteae) in New Zealand. Biocontrol Sci Tech 5:3–10

Hiolford ICR (1997) Soil phosphorous, its measurement, its uptake by plants. Aust J Soil Res:227–239

Howell CR (1998) The role of antibiosis in biocontrol. In: Harman GE, Kubicek CP (eds) *Trichoderma and Gliocladium*, Vol 2. Taylor and Francis, London, pp 173–184

Howell CR (2003) Mechanisms employed by T. harzianum species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis 87(1):4–10
IIlmer P, Barbato A, Schinner F (1995) Solublization of hardly-soluble AlPO4 with P-solublizing microorganisms. Soil Biol Biochem 27(3):265–270
Iyamurimye F, Dick RP, Baham J (1996) Organic amendments and phosphorous dynamic. I. Phosphorous chemistry sorption (J). Soil Sci 161(7):426–435
Kapri A, Tewari L (2010) Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp. Braz J Microbiol 41(3):787–795. ISSN 1517-8382
Kaziro Y, Itoh H, Kozasa T et al (1991) Structure and function of signal-transducing GTP-binding proteins. Annu Rev Biochem 60:349–400
Kim KY, Jordan D, Mcdonald GA (1998) Enterobacter agglomerans, phosphate soluliblizing bacteria and microbial activity in soils effect of carbon sources. Soil Biol Biochem 30:995–1003
Kishimoto K, Matsui K, Ozawa R, Takabayashi J (2005) Volatile C6-aldehydes and allo-ocimene activate defense genes and induce resistance against Botrytis cinerea in Arabidopsis thaliana. Plant Cell Physiol 46:1093–1102
Kloepper JW, Tuzun S, Kuć JA (1992) Proposed definitions related to induced disease resistance. Biocontrol Sci Tech 2(4):349–351
Kolakowski LF Jr (1994) GCRDb: a G-protein-coupled receptor database. Receptors Channels 2(1):1–7
Kopchinskiy A, Komoń M, Kubicek CP, Druzhinina IS (2005) Tricho BLAST: a multilocus database for *Trichoderma* and Hypocrea identifications. Mycol Res 109(6):658–660
Kumar G, Karthik L, Rao KVB (2010) Antimicrobial activity of latex of Calotropis Gigantea against pathogenic microorganisms. Pharmacologyonline 3:155–163
Kuperman RG, Carreiro MM (1997) Soil heavy metals concentration, microbial biomass and enzyme activities in a contaminated grasslands ecosystems. Soil Biol Biochem 29:179–190
Lehner SM, Alanasova L, Neumann NK (2013) Isotope-assited screening for iron- containing metabolites reveals high divetrsity among known and unknown siderophores produced by *trichoderma* spp. Appl Environ Microbiol 79:18–31
Lorito M, Woo SL, Harman GE, Monte E (2010) Translational research on *Trichoderma*: from'omics to the field. Annu Rev Phytopathol 48:395–417
Malcolm RE (1983) Assessment of soil phosphatase activity in soils. Soil Biol Biochem 15(4):403–408
Mastouri F, Björkman T, Harman GE (2010) Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100(11):1213–1221
Mukherjee M, Mukherjee PK, Kale SP (2007) cAMP signalling is involved in growth, germination, mycoparasitism and secondary metabolism in *Trichoderma virens*. Microbiology 153:1734–1742
Mukherjee M, Mukherjee PK, Horwitz BA, Zachow C, Berg G, Zeilinger S (2012) *Trichoderma*–plant–pathogen interactions: advances in genetics of biological control. Indian J Microbiol:1–8
Nagarajkumar M, Jayaraj J, Muthukrishnan S, Bhaskaran R, Velazhahan R (2005) Detoxification of oxalic acid by Pseudomonas fluorescens strain PfMDU2: implications for the biological control of rice sheath blight caused by Rhizoctonia solani. Microbiol Res 160(3):291–298
Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal K, Verma D (2000) Stress induced phosphate soulubilization in bacteria isolated from alkaline soils. EMS Microbiol Lett 182(2):291–296
Neer EJ, Heterotrimeric G (1995) Proteins: organizers of transmembrane signals. Cell 80(2):249–257
Omann M, Zeilinger S (2010) How a Myco parasite employs G-protein signalling: using the example of Trichoderma , J Signal Transduct 123126, 8
Omann MR, Lehner S, Escobar Rodriguez C, Brunner K, Zeilinger S (2012) The seven-transmembrane receptor Gpr1 governs processes relevant for the antagonistic interaction of Trichoderma atroviride with its host. Microbiology 158:107–118

Peleg Y, Addison R, Aramaya R, Metzenberg RL (1996) Translocation of *Neurospora crassa* transcription factor NUC-1 into the nucleus is induced by phosphate limitation. Fungal Genet Biol 20:185–191

Porras M, Barraeu C, Romero F (2007) Effects of soil solorization and Trichoderma on strawberry production. Crop Prot 26:782–789

Puente ME, Lebskyvk BY (2004) Microbial populations and activities in the rhizoplane of rock weathering desert plants. Plant Biol 6:620–642

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

Rawat R, Tewari L (2010) Transmisssion electron microscopic study of cytological changes in Sclerotium rolfsii parasitized by biocontrol fungus trichoderma sp. Mycology 1(4):237–241

Rawat R, Tewari L (2011) Effect of abiotic stress on phosphate solubilization by biocontrol fungus *Trichoderma* sp. Curr Microbiol. https://doi.org/10.1007/s00284-011-9888-2

Reino JL, Guerrero RF, Hernandez-Galan R, Collado IG (2008) Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochemistry 7:89–123

Reithner B, Brunner K, Schuhmacher R et al (2005) The G protein alpha subunit Tga1 of *Trichoderma atroviride* is involved in chitinase formation and differential production of antifungal metabolites. Fungal Genet Biol 42(9):749–760

Reithner B, Schuhmacher R, Stoppacher N et al (2007) Signaling via the *Trichoderma atroviride* mitogen-activated protein kinase Tmk1 differentially affects mycoparasitism and plant protection. Fungal Genet Biol

Renshaw JC, Robson GD, Trini APJ (2002) Fungalsiderophores: structures, functions and applications. Mycol Res 106:1123–1142

Rifai MA (1969) Revision of the genus *Trichoderma*. United Nations. 2009. World population prospects: the 2008 revision

Ruccco M, Lanzuise S, Vinale F, Marra R, Turra D, Lois Woo S, Lorito M (2009) Identification of new biocontrol gene in Trichoderma atroviridae; the role of an ABC transporter membrane pump in interaction with different plant pathogenic fungi. Mol Plant-Microbe Interact 22(3):291–301

Rocha-Ramirez V, Omero C, Chet I et al (2002) *Trichoderma atroviride* G-protein α-subunit gene *tga1* is involved in mycoparasitic coiling and conidiation. Eukaryot Cell 1:594–605

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role I plant growth promotion. Bitechnol Adv 17(4–5):319–339

Rodriguez-Escudero I, Rotger R, Cid VS, Molina M (2006) Inhibition of cdc42- dependent signaling in Saccharomyces cerevisiae by phosphatase-dead sig D/SoPB from Salmonella typhimurium. Microbiology 152(11):3437–3452

Sakia S, Pandey RK, Tewari Laksmi (2015). Phosphate solubilizing potential of fungal isolates from bamboo rhizosphrere 13 (3): 492–496

Salas-Marina MA, Silva-Flores MA, Uresti-Rivera EE, Castro-Longoria E, Herrera-Estrella A, Casas-Flores S (2011) Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. Eur J Plant Pathol 131:15–26

Samuels GJ (2006) *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96(2):195–206

Saravanan R, Pavani Devi V, Shanmugan A, Satish Kumar D (2007) Isolation and partial purification if extracellular enzymes (1, 3) – D-glucanase from Trichoderma Ressi. (3929). Bioitechnology 6(86–98):13

Scarselletti R, Faull JL (1994) In vitro activity of 6-pentyl- a -pyrone, a metabolite of Trichoderma harzianum , in the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum f. sp. Lycopersici*. Mycol Res 98:1207–1209

Schmoll M (2008) The information highways of a biotechnological workhorse—signal transduction in *Hypocrea jecorina*. BMC Genomics 9:430

Shieh TR, Ware JH (1968) Survey of micro-organisms for the production of extracellular phytase. Appl Microbiol 16(9):1348–1351
Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43
Sivan A, Chet I (1989) The possible role of competition between Trichoderma harzianum and Fusarium oxysporum on rhizosphere colonization. Phytopathology:198–203
Srivastva S, Yadav KS, Kundu BS (2004) Prospects of using phosphate solublizing pseudomonas as biofugicides. Indian J Microbiol 44(2):91–94
Tamas L, Huttov AJ, Mistrki I, Kogan G (2002) Effect of carbon Chittin- Glucan on the activity of some hydrolytic enzymes in maize plants. Chem Pep 56(5):326–329
Tamura A, Kotani H, Naruto S (1975) Trichoviridin and dermadin from *Trichoderma* sp. Tk-1. J Antibiot 28:161–162
Tewari L, Bhanu C (2004) Evaluation of agro-industrial wastes for conidia bases incoulum production of biocontrol agent: Trichoderma harzanium. J Sci Ind Res 6:807–812
Tucci M, Ruocco M, De Masi L, De Palma M, Lorito M (2011) The beneficial effect of *Trichoderma spp*. on tomato is modulated by the plant genotype. Mol Plant Pathol 12:341–354
Tulasne LR, Tulasne C (1865) Selecta fungorum carpologia 3:197–198
Vargas WA, Mandawe JC, Kenerley CM (2009) Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma* virens and maize plants. Plant Physiol 151(2):792–808
Vinale F, Marra R, Scale F, Ghisalberti EL, Lorito M, Sivasithamparam K (2006) Major secondary metabolites produced by two commercial *Trichoderma strains* active different phytopathogens. Lett Appl Microbiol 43:143–148
Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma*–plant–pathogen interactions. Soil Biol Biochem 40(1):1–10
Viterbo ADA, Chet I (2006) TasHyd1, a new hydrophobin gene from the biocontrol agent *Trichoderma asperellum*, is involved in plant root colonization. Mol Plant Pathol 7(4):249–258
Wees S, CM, van der Ent S, Pieterse CMJ (2008) Plant immune response trigerred by beneficial microbes. Curr Opin Plant Biol 11:443–448
Weiendling R (1932) Trichoderma lignorum as a parasite of other soil fungi. Phytopathology 22:834–845
Weindling R (1934) Studies on a lethal principle effective in the parasitic action of Trichoderma lignorum on Rhizactonia and other soil fungi. Phytopathology 24:1153–1169
Worasatit N, Sivasithamparam K, Ghisalberti EL, Row- land C (1994) Variation in pyrone production, pectic enzymes and control of rhizoctonia root rot of wheat among single-spore isolates of *Trichoderma koningii*. Mycol Res 98:1357–1363
Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803
Yedidia I, Srivastva AK, Kajalnik Y, Chet I (2001) Effect of T. harzinum on microelement concentration and increase in growth of cucumber plant. Plant Soil:235–242
Zeilinger S, Reithner B, Scala V et al (2005) Signal transduction by Tga3, a novel G protein alpha subunit of *Trichoderma atroviride*. Appl Environ Microbiol 71(3):1591–1597

Biofertilizer Application in Horticultural Crops

11

D.V. Pathak, Mukesh Kumar, and Kusum Rani

Abstract

India is the second largest producer of horticultural crops in the world. But the productivity and quality need to be enhanced to fulfil the demand of increasing population. It needs ecofriendly technology which can increase production for ensuring national food security and sustainable production system. Excessive use of non-renewable exhaustive petroleum product-based chemicals in horticultural production system and their residual effect on soil, environment and human health is very harmful. Ecofriendly, cost-effective and organic-based inputs such as botanical pesticides, biofertilizers, FYM, vermicompost, biogas slurry, disease and pest-resistant varieties in cultivation of horticultural crops will be safeguarding soil health and quality production. The use of various bioinoculants like *Azotobacter*, *Azospirillum* and VAM along with PGPRs not only will supplement various nutrients in the soil but also improve the quality and quantity of fruits.

Keywords

Horticultural crops • Ecofriendly • Non-renewable • Biofertilizer • FYM • Vermicompost

D.V. Pathak (✉)
CCS Haryana Agricultural University, Regional Research Station,
Bawal, Rewari, Haryana 123501, India
e-mail: pathak_dv@rediffmail.com

M. Kumar
CCS Haryana Agricultural University, Krishi Vigyan Kendra,
Bawal, Rewari, Haryana 123501, India
e-mail: sabharwalmk@gmail.com

K. Rani
Department of Botany and Plant Physiology, CCS Haryana Agricultural University,
Hisar, Haryana 125004, India
e-mail: sabharwalkrs@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_11

11.1 Introduction

Biofertilizers are microorganisms which are capable of mobilizing nutrients from nonusable form to usable form through biological process. They are a cost-effective and inexpensive source of plant nutrients, do not require non-renewable source of energy during their production, improve crop growth and quality of the product by producing plant hormones and help in sustainable crop production through maintenance of soil productivity. They are also useful as biocontrol agents, since they control many plant pathogens.

Biofertilizer is used in live formulation of beneficial microorganism which on application to seed, root or soil mobilize the availability of nutrients particularly by their biological activity, help build up the lost microflora and in turn improve the soil health, in general. Hence, the use of biofertilizer is increasing day by day due to increase in the prices of chemical fertilizers, its beneficial effect on soil health and increase in productivity of the crop. Biological microorganisms used as biofertilizers increase the growth of plants either by enhancing the availability of nutrients, releasing plant growth-stimulating hormones, reducing the damage caused by pathogen/pest or improving resistance to environmental stress/pollutants.

Being one of the integrated components of agricultural production system, the horticultural crops (fruits, vegetables, ornamentals, plantation crops, etc.) are among the key contributors for economic development in the country. The horticultural industry is growing at a very fast pace with India being the second largest producer of fruits and vegetable in the world. The horticultural crops need ecofriendly technology for enhancing the efficiency of production and for ensuring nutritional food security for sustainable agriculture production system. The application of biofertilizers offer an economically attractive and sustainable means of reducing external inputs of chemical fertilizers and for improving the quality of natural land resources. The beneficial use of nitrogen-fixing microorganisms, viz. *Azotobacter*, and phosphate-solubilizing bacteria as biofertilizers used as a supplementary source of plant nutrition on agricultural crops is well documented (Gajbhiye et al. 2003). The application of two-thirds of recommended dose of N along with 10 kg/ha *Azospirillum* as soil application alone or in combination with 2 kg/ha *Azospirillum* as vine dipping gave highest marketable tuber yield and dry of tuber (Anonymous 2004).

11.2 Need of Biofertilizers

A better understanding of these processes is critical for maintaining the health of the plant and feeding the organisms that live on soil and prolong soil productivity and biodiversity of the environment (Morrissey et al. 2004). There is a small but concerted effort under way to harness the root system of plants in an attempt to increase yield potentials of staple food crops in order to meet the projected doubling in global food demand in the next 50 years (Zhang et al. 2010; Giles et al. 2008). These efforts are being done in the face of a changing global climate and increasing global population, which will inevitably require more productively grown food, feed and

fibre on less optimal and often infertile lands, which already prevails in many developing countries (Tilman et al. 2002). Meeting the global challenges of climate change and population growth with a better understanding and control of rhizosphere processes will be one of the most important science frontiers of the next decade for which a diverse, interdisciplinary trained workforce will be required (Bora et al. 2016).

Nutrients play an important role in quality and yield production of horticultural crops. Nutrients status of soil is the most important factor affecting the productivity of crops. Production efficiency of crops depends upon the supply of synthetic fertilizers and agrochemicals. The chemical fertilizers play a key role by contributing 50–60% increase in productivity. Due to imbalanced use of these chemical fertilizers, problems like soil deterioration, groundwater contamination and environment pollution result. Production of chemical fertilizers based in non-renewable exhaustive petroleum products and their use increase the production cost. Non-judicious use of chemical fertilizers results in loss of soil fertility and soil health. This situation emphasized the need for developing alternate production systems that are friendlier to the environment and is more judicious in managing soil health. Ecofriendly or organic farming includes biological and natural inputs such as botanical pesticides, biofertilizers, FYM, vermicompost, poultry manure, disease-resistant varieties and different pest management practices to bring sustainability in agriculture. Thus, the use of biofertilizers in cultivation of horticultural crops will help in safeguarding the soil health and also the quality of production. These offer an economically sustainable means of reducing external inputs and improving the quality and quantity of natural land resources.

Demand is much higher than the availability. It is estimated that by 2020, to achieve the targeted production of 321 million tonnes of food grains, the requirement of nutrient will be 28.8 million tonnes, while their availability will be only 21.6 million tonnes, having a deficit of about 7.2 million tonnes (Arun 2007). This gap can be fulfilled by judicious use of biofertilizers along with chemical nutrients.

11.3 Plant Growth-Promoting Rhizobacteria

The beneficial rhizosphere microorganisms include *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, *Serratia* and mycorrhizal fungi. This microbial population interacts with each other and with the plant through symbiotic, associative, neutralist or antagonism effect.

Thus biofertilizers or microbial inoculants are defined as a preparation containing efficient microbial/bacterial strain with nitrogen-fixing, phosphate-solubilizing or plant growth-promoting ability that are used to inoculate the seeds, seedlings or soil to augment the availability of certain nutrients in the plant utilizable form. Through intensive selection and screening procedures, specific strains of these microorganisms have been recognized for their specific role in plant nutrient management and promotion of plant growth. Biofertilizers could be grouped into three major categories:

1. Nitrogen fixing (*Rhizobium*, *Azotobacter*, *Azospirillum*, blue-green algae)
2. Phosphate solubilizing (*Pseudomonas*, *Bacillus*, *Aspergillus*, mycorrhizal fungi)
3. Potash mobilizer (*Bacillus* sp., *Pseudomonas* sp.)
4. Sulphur uptake (*Thiobacillus*)
5. Zinc solubilizer (*Bacillus subtilis*, *Thiobacillus thiooxidans* and *Saccharomyces* sp.).
6. Iron uptake (*Pseudomonas fluorescens*)
7. Plant growth promoters (*Pseudomonas*, *Bacillus*, *Serratia*)

11.3.1 Nitrogen-Fixing Microorganism

Free-living (*Azotobacter*, *Clostridium*, *Anabaena*, *Nostoc*), symbiotic (*Rhizobium, Frankia, Anabaena azollae*) and associative symbiotic (*Azospirillum*).

Biological nitrogen fixation (BNF), which provides nitrogen inputs into agricultural soils, involves highly specialized and intricately evolved interactions between soil microorganisms and higher plants for harnessing the atmospheric elemental nitrogen. Four important N_2-fixing associations, i.e. *Rhizobium*–leguminous plants, *Frankia*–actinorhizal plants, *Anabaena–Azolla* symbiosis and lichen symbiosis involving cyanobacteria, have been studied in great detail. The legume–*Rhizobium* symbiotic association reduces about 70–80% of the total of 17.2×10^7 tonnes of biologically fixed nitrogen per year. The symbiotic rhizobia have been found to fix nitrogen ranging from 24–584 kg N/ha annually. The *Azolla–Anabaena* symbiotic system has been reported to contribute 45–450 kg N/ha, and *Frankia–actinorhizal* symbiosis provides 2–362 kg N/ha (Elkan 1992). The production of biofertilizers and their commercialization is focused on the creation and support of sustainable production system. They occupy an important place as they help in making important plant nutrients, thus, providing a scope for reduction in the use of costly chemical fertilizers, which can pollute soil in long-term use. Moreover, other properties such as auxin production have been attributed to biofertilizers (Bora et al. 2016).

The inoculation with free-living bacteria *Azotobacter* and associative bacteria *Azospirillum* has been reported to increase the yield by 5–10% depending upon the cereal crop used (Sturz et al. 2000). *Azotobacter* has been reported to contribute 15 Kg N ha^{-1} per year. It has been observed that inoculation of soil or seed with *Azotobacter* causes increase in yield of different crops (Sindhu et al. 2010). *Azotobacter* inoculation saves addition of nitrogenous fertilizers by 10–20%. The method of inoculation of *Azotobacter* is similar to that of *Rhizobium* (Sukhada 1999). *Azotobacter* cells are not usually present on the root surface but are present in rhizosphere. *A. chroococcum* and *A. vinelandii* are deemed to be the most commonly occurring species *Azotobacter* is capable of converting nitrogen to ammonia, which in turn is taken up by the plants (Kamil 2008). *Azotobacter* sp. can also produce antifungal compounds to fight against many plant pathogens (Chen 2006).

Azospirillum is the associative nitrogen fixer, aerobic bacteria, which have the ability to associate with growing root system of a variety of crop plants. This nitrogen-fixing *Azospirillum* when applied to the soil undergoes multiplication and

fixes atmospheric nitrogen in the soil for utilization of various crops. It also promotes root development and vegetative growth. *Azospirillum* sp. has the ability to fix 20–40 Kg N ha^{-1} and its inoculation results in average increase in yield of 5–10%. It is recommended for paddy, millets, oilseeds, fruits, vegetables, sugarcane, banana, coconut, oil palm, cotton, chilly, lime, coffee, tea, rubber, spices, herbs, ornaments, trees, etc. *Azosprillum* fix nitrogen from 10 to 40 kg /ha. They are found colonizing the root system of many vegetable plants. *Azospirillum* inoculation helps the plants in better vegetative growth and also in saving input of nitrogenous fertilizers by 10–20% (Sukhada 1999). The most important *Azospirillum* spp. is *A. brasilense*, which has a wide range of tolerance against abiotic stresses. The bacteria stimulate plant growth even in the presence of several stresses such as drought (Noshin et al. 2008).

11.3.2 *Azolla*

Azolla being green manure can substitute 40–50 kg nitrogen/ha. It is a source of nutrients to poultry, fish and water animals (Bora et al. 2016). BGA forms symbiotic association capable of fixing nitrogen with fungi, liverworts, ferns and flowering plants, but the most common symbiotic association has been found between the free-floating aquatic fern *Azolla* and *Anabaena azollae* (BGA). *Azolla* contains 4–5% N on dry basis and 0.2–0.4% on wet basis and can be the potential source of organic manure and nitrogen in rice production (Mishra et al. 2013).

11.3.3 Phosphate-Solubilizing Microorganisms

Bacteria (*Bacillus megaterium* var. *phosphaticum*, *Bacillus circulans*, *Pseudomonas striata*), fungi (*Penicillium* sp., *Aspergillus awamori*), arbuscular mycorrhizal (*Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp. and *Sclerocystis* sp.) and ectomycorrhiza (*Laccaria* sp., *Pisolithus* sp., *Boletus* sp., *Amanita* sp.).

Phosphatic biofertilizers were first prepared in USSR using *Bacillus megaterium* var. *phosphaticum* as P-solubilizing bacteria and the product was named as 'phosphobacterin'. It was extensively used in collective farming of seed and soil inoculation to cover an area of million hectares annually and reported to give 5–10% increase in crop yields. Inoculation experiments conducted with phosphobacterin and other phosphate-solubilizing microorganisms (PSM) for various crops like oat, wheat, potatoes, groundnut, peas, soybean, tomatoes and tobacco showed an average 10–15% increase in yields in about 30% of the experiments conducted (Dubey 1997). The application of phosphorus fertilizers should be reduced by 25–50% depending upon the crop. The plants get colonized up to 80–90% within 3–8 months. The nutrients contents like P, Zn and Cu are increased in the leaves; there is a saving in P by 25–50% without reduction in yield of plants (Sukhada 1999).

The most important phosphate-solubilizing bacteria (PSB) belong to genera *Bacillus* and *Pseudomonas*, though species of *Achromobacter*, *Alcaligenes*,

Azotobacter, *Brevibacterium*, *Burkholderia*, *Corynebacterium*, *Enterobacter*, *Rahnella*, *Serratia*, *Synechococcus*, *Thiobacillus* and *Xanthomonas* have also been reported to be active in solubilizing insoluble P (Cattelan et al. 1999). Another important group of microorganisms which help in improving phosphate uptake by plants are fungi, viz. *Aspergillus awamori*, *A. niger*, *Penicillium digitatum* and *Schwanniomyces occidentalis*. Application of phosphate-solubilizing microorganisms has shown a promising response in improving yields of various crops.

Important phosphate-solubilizing organisms are *Pseudomonas striata*, *Bacillus polymxa*, *Aspergillus* and *Penicillium digitatum*. These microorganisms can grow in insoluble phosphatic sources. It is reported that PSB culture increased yield up to 200–500 kg/ha, and thus 30–50 kg of superphosphate can be saved (Chen 2006). These organisms solubilize the unavailable forms of inorganic P like tricalcium, iron, aluminium and rock phosphates into soluble forms by release of a variety of organic acids like succinic, citric, malic, fumaric, glyoxylic and gluconic acids (Venkateswarlu et al. 2008).

11.3.4 PGPR

The plant rhizosphere bacteria belonging to the genera *Pseudomonas* and *Bacillus* have been recognized as early root colonizers, which enhance plant growth by different mechanisms including increased mobilization of insoluble nutrients and enhance iron availability in the rhizosphere by producing siderophores (Klopper et al. 1980) and/or by producing phytohormones including auxins and cytokinins (Dubeikovsky et al. 1993). Some of the rhizobacteria inhibit the growth of pathogenic bacteria and fungi by production of antibiotics, hydrolytic enzymes or hydrocyanic acid (Edward and Seddon 2001), and these antagonistic bacteria have the potential for use as biocontrol agents (Thomashow and Weller 1996).

Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens whereas others trigger beneficial effects. Generally, the formulated mycorrhizal symbiosis significantly improved plant growth performance, such as plant height, stem diameter, shoot, root or total dry weight (Wu et al. 2011). The key effects of AM symbiosis can be summarized as follows: (i) improve rooting and plant establishment, (ii) improve uptake of low mobile ions, (iii) improve nutrient cycling, (iv) enhance plant tolerance to (biotic and abiotic) stress, (v) improve quality of soil structure and (vi) enhance plant community diversity. Obviously, the interest of horticulturists in AM technology is due to the ability of AMF to increase the uptake of phosphorus and other nutrients and to increase resistance to biotic and abiotic stress. Arbuscular mycorrhizal fungi's (AMF) are obligate biotrophs, which can form mutualistic symbiosis with the roots of around 80% of plant species (Abbott and Robson 1982).

Table 11.1 Phytohormones produced by different rhizobacteria

Phytohormones	PGPRs
Indole-3-acetic acid (IAA)	*Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*
Zeatin and ethylene	*Azospirillum* sp.
Gibberellic acid (GA3)	*Azospirillum lipoferum*
Abscisic acid (ABA)	*Azospirillum brasilense*

11.4 Mechanism Involved in Plant Growth Promotion

Microbial population in the rhizosphere has substantial effect on growth and yield of cereals, leguminous crops, fruits, vegetables and flower crops (Glick 1995) and may benefit the plant in a variety of ways, including (i) increased recycling, mineralization and uptake of mineral nutrients; (ii) synthesis of vitamins, amino acids, auxins, gibberellins and plant growth-regulating substances; (iii) bioremediation of heavy metals in contaminated soils; and (iv) antagonism with potential plant pathogens through competition and development of amensal relationship based on production of antibiotic, siderophores and/or hydrolytic enzymes (Stockwell and Stack 2007) (Table 11.1).

So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed plant growth-promoting rhizobacteria (PGPR). There are several PGPR inoculants currently commercialized that promote growth through at least one mechanism: suppression of plant disease (bioprotectants), improved nutrient acquisition (biofertilizers) or phytohormone production (biostimulants) (Bora et al. 2016).

11.5 Inoculation Responses of Biofertilizers on Horticultural Crops

11.5.1 Area and Production Under Horticultural Crops

Fruits and vegetables account for nearly 90% of the total horticulture production in the country. India is now the second largest producer of fruits and vegetables in the world and is the leader in several horticultural crops, namely, mango, banana, papaya, cashew nut, areca nut, potato and okra (lady's finger). Horticultural crops such as fruits, flowers, vegetables, spices and medicinal and aromatic plants occupy only 8.2% of the cultivated area and contribute 33% of GDP and 52% of export value of agriculture in India.

The production contribution of horticultural crops in India is as follows: fruits (31.3%), vegetables (59.4%), flowers and aromatic (1.1%), spices (2.1%) and plantation crops (6.0%) (Anonymous 2015). Horticultural sector contributes in improving land use for food, nutritional value, medicinal value, aesthetic value and nutritional security. It also promotes crop diversification, employment generation and poverty alleviation; apart from these, they also maintain ecological balance. India has

witnessed voluminous increase in horticulture production over the last few years. Significant progress has been made in area expansion resulting in higher production. Over the last decade, the area under horticulture grew by about 2.7% per annum and annual production increased by 7.0%. The highest annual growth of 9.5% is seen in fruit production during 2013–2014. In addition to the beautification of the local landscape, great scope exists for export of flowers; floriculture is important for bee-keeping industry which too provides an alternate source of income to the Indian farmers. Apart from the health improvements, the production of vegetables improves the economy of the country as these are very good source of income and employment.

11.5.2 Need of Biofertilizers in Horticultural Crops

Biofertilizer is a recent concept being used in horticultural crops. Biofertilizers should be integrated with organic manures and chemical fertilizers to enhance the soil organic carbon and maintain sustainability in the field and horticultural crops (Pathak and Kumar 2016). Generally, fruit crops have now received more attention than vegetables and ornamental crops. *Glomus fasciculatum*, *Glomus mosseae*, *Azospirillum*, *Azotobacter* and PSB (phosphorous-solubilizing bacteria) are found useful for different horticultural crops. The use of biofertilizers particularly inoculating with *Azotobacter* could substantiate 50% nitrogen requirement of banana and produce higher yield over full doses of nitrogen application. The absorption of mobile nutrients like nitrogen also increases in association with VAM fungi (Bora et al. 2016). The organically produced fruits and vegetables not only fetch much higher value in the domestic as well as international market but also devoid agrochemical residues, thereby having positive impact on human health.

11.5.3 Inoculation Response in Fruits

India is the second largest producer of fruits after China and contributes 12.55% share of global fruit production. Almost all types of fruits (tropical, subtropical and temperate) are grown in one or the other region of the country. The area under fruit crops in India is 7.22 million hectares with a production of 88.98 m MT. In India, the productivity of fruits crops is quite lower (12.3 MT/ha) as compared to the USA (23.3 MT/ ha), Indonesia (22.3 MT/ha) and Brazil (14.5 MT/ha), however, above the world productivity (11.4 MT/ ha) (Anonymous 2014). In past two decades, there is a twofold increase in area and production of fruit crops in which India occupied first place in production of mango, banana, papaya, pomegranate, sapota and aonla. It is mainly due to development of high-yielding varieties, resistant/tolerant to several biotic stresses, improved production technologies and value addition.

Under Egyptian soil conditions, the inoculation of Washington navel orange with *Pseudomonas fluorescens* strain 843 was not only highly effective in increasing the production of Washington navel orange as well as improving the quality of fruits but also in inhibiting the survival of nematode in the soil concluding that this strain can

be used as biofertilizer and biocontrol of pathogenic nematode-infected citrus trees (Abdelaal et al. 2010).

Entomopathogenic fungi, *M. anisopliae*, were isolated from banana stem weevil (*Odoiporus longicollis*). Application of *Trichoderma viride* @ 20 g/plant, once at planting and after 3 months was found effective in controlling nematodes (*P. coffeae* and *M. incognita*), reducing the incidence of Panama wilt in Rasthali and Virupakshi. Spraying of native stain of *Pseudomonas* sp. 2 at 106 ml in Robusta prevented the occurrence of crown rot disease. *Trichoderma hamatum* strain 4; *T. harzianum* strains 20, 25 and 37; and *T. reesei* strain 7 were found good root growth promoters of citrus. Biological control of guava wilt indicated the possibility of its control with *Aspergillus niger* (Anonymous 2004). The bioinoculants such as *Azotobacter chroococcum*, phosphate-solubilizing bacteria, plant growth-promoting bacteria and mycorrhizae were tested on seed germination, plant height and other growth parameters of guava in the presence of FYM and vermicompost. In this study the maximum seed germination (51.1%) was observed in treatment having FYM+ PGPR or FYM+ *A. chroococcum* at 40 days after sowing followed by the treatment having FYM+ PGPR + PSB+ *A. chroococcum* or vermicompost + PSB + *A. chroococcum* (48.9%) (Pathak et al. 2009).

Growth and yield tomato was significantly higher when the biofertilizers were inoculated with combined treatment (*Azotobacter* and *Azospirillum*) compared to individual inoculation and control. This could be due to the collective effect of biofertilizers (Ramakrishnan and Selvakumar 2012). Similar growth increase was recorded in black pepper earlier also with combined inoculation of biofertilizers (*Azospirillum*, *Azotobacter* and phosphobacteria) (Bopaiah and Khadeer 1989).

Biofertilizer inoculation with strain *Pseudomonas fluorescens* strain 843 growth-promoting rhizobacteria significantly improved fruit quality as well as increased fruit yield, fruit weight, fruit length, TSS and juice volumes, while inoculation with strain *Azospirillum brasilense* strain W24 increased but did not significantly improve fruit quantity and quality of Washington navel orange (Abdelaal et al. 2010). The beneficial effects of biofertilizers have been widely reported in banana (Tiwary et al. 1998; Mohandas 1996).

Singh and Banik (2011) reported that application of 500:250:250 g NPK/tree +50 kg FYM + 250 g *Azospirillum* of INM system was best for achieving better yield and quality in mango cv. Himsagar. The combined biofertilizer application of *Azotobacter* + *Azospirillum* + AM + PSM in mango cv. Himsagar was most effective in improving soil and fruit size. The fruit quality of strawberry cv. Chandler, viz. total soluble solids, total sugars, ascorbic acid and anthocyanin content, was highest in fruits obtained from plants supplied with 25% nitrogen through FYM + 75% nitrogen in the form of urea + *Azotobacter* (Umar et al. 2009).

The main effects of AM inoculation in horticultural crops include: (i) enhanced seedling growth, (ii) reduced phosphate requirements, (iii) increased survival rate and development of micropropagated plantlets, (iv) increased resistance to fungal root pathogens, (v) increased resistance to abiotic stresses, (vi) earlier flowering and fruiting, (vii) increased crop uniformity, (viii) improved rooting of cuttings and (ix) increased fruit production (Chang 1994).

Ruiz (1992) observed that microbial population in the soil increased considerably due to use of *Azotobacter*, mycorrhiza and phosphorins in banana. The commercial yield also increased by 25–30% and saved 50% of inorganic fertilizers. Shen et al. (2013) examined that the compost and biotreatment more effectively controlled *Fusarium* wilt disease in banana. The treatment resulted in higher total soluble sugars (TSS) to titratable acidity (TSS/TA) ratios, yield, culturable and total soil bacteria and culturable actinobacteria population.

VAM fungi are responsible for more than twofold increased acquisition of the less mobile nutrients like P, Ca, S, Zn, Mg and Cu from the rhizosphere. The high efficiency of *Azospirillum* for fixing nitrogen and better mobilization of fixed phosphorus by VAM even at high temperature can make these highly suited for Mosambi (Manjunath et al. 1983). The per cent of wilting in VAM-treated guava was recorded to be lower as compared to untreated trees (Srivastava et al. 2001). The root colonization per cent was higher in *Glomus mosseae* inoculated papaya plants. Nutrient content of N, P, K and also of Fe, Mn, Zn and Cu increased due to VAM inoculation. The improvement in yield parameters in the presence of *Azospirillum* might be due to its dual nature of nitrogen fixation and production of phytohormone substances (Govindan and Purushothaman 1984).

11.5.4 Inoculation Response in Floriculture

Various plant growth parameters of gladiolus were positively influenced by the application of both the biofertilizers in combination with nitrogen, and it was maximum under 75% N + 100% PK (375:200:200 kg NPK ha^{-1}) + *Azotobacter* + *Azospirillum* and at par with the treatment 100% NPK (500:200:200 kg ha^{-1} + *Azotobacter* + *Azospirillum*) (Dalve et al. 2009). Biofertilizer application enhanced various growth parameters at all stages of growth compared to chemical fertilizer application alone. Application of biofertilizers along with 50% NPK brought about results on par with 100% NPK fertilizer with respect to chlorophyll content, floral characteristics such as days taken to 50% flowering, number and weight of flowers per plant, diameter of flowers, ten flower weight and flower yield per plant and shelf life of flowers, indicating replacement of NPK chemical fertilizers to the extent of 50% (Jayamma et al. 2014). Inoculation of *Azotobacter* and PSB improves growth, flowering and yield characteristics of marigold and PSB was judged the best (Kumar 2002).

11.6 Conclusion

Positive response in horticultural crops has been observed by the use of various inoculants alone or in combination by various workers. These bioinoculants not only affect the fruit yield but also improve the fruit quality. The inorganic fertilizers can be supplemented with organic manures and biofertilizers. Various PGPRs can also be used as biopesticides for control of various plant pathogens and insect attack. Vesicular–arbuscular mycorrhiza is the most common inoculants in horticultural

crops and floriculture. But more research work is needed in India for application of biofertilizers in fruit crops so that we may make them completely organic. It is the need of the hour that a microbiologist should work in association with a horticulturist, and this technology should be transferred to the farmer's field.

References

Abbott LK, Robson AD (1982) The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust J Agric Res 33:389–408

Abdelaal S, El-Sheikh MH, Hassan HAS, Kabeil SS (2010) Microbial bio-fertilization approaches to improve yield and quality of Washington navel Orange and reducing the survival of nematode in the soil. J Am Sci 6(12):264–271

Anonymous (2004) Improvement and management of horticultural crops. Annual Report, DARE/ICAR, New Delhi 50–66

Anonymous (2014) Indian horticulture database, Ministry of Agriculture, Govt. of India, Gurgaon

Anonymous (2015) Horticultural statistics at a glance. Horticulture statics division, Department of Agriculture cooperation and farmers welfare, Ministry of Agriculture and farmers welfare, Govt. of India, New Delhi

Arun KS (2007) Bio-fertilizers for sustainable agriculture. Mechanism of P-solubilization, 6th edn. Agribios Publishers, Jodhpur, pp 196–197

Bopaiah BM, Khadeer BA (1989) Effect of biofertilizers on growth of black pepper (*Piper nigrum*). Indian J Agric Sci 59:682–683

Bora L, Tripathi A, Bajeli J, Chaubey AK, Chander S (2016) A review on microbial association : its potential and future prospects in fruit crops. Plant Arch 16(1):1–11

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening of plant growth promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 3:1670–1680

Chang DCN (1994) What is the potential for management of vesicular-arbuscular mycorrhizae in horticulture? In: Robson AD, Abbott LK, Malajczuk N (eds) Management of Mycorrhizas in agriculture, horticulture and forestry. Kluwer, Dordrecht, p 187

Chen JH (2006) The combined use of chemical and organic fertilizers and/ or biofertilizers for crop growth and soil fertility. International workshop on sustained management of the soil rhizosphere system for efficient crop production and fertilizer use land development, Department, Bangkok 10900 Thailand

Dalve PD, Mane SV, Nimbalkar RR (2009) Effect of biofertilizers on growth, flowering and yield of gladiolus. Asian J Hortic 4(1):227–229

Dubeikovsky AN, Mordukhova EA, Kochetkov VV, Polikarpova FY, Boronin AM (1993) Growth promotion of blackcurrant soft wood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased amount of indole-3-acetic acid. Soil Biol Biochem 255:1277–1281

Dubey SK (1997) Coinoculation of phosphate solubilizing bacteria with *Bradyrhizobium japonicum* to increase phosphate availability to rainfed soybean on vertisol. J Indian Soc Soil Sci 45:506–509

Edward SG, Seddon B (2001) Mode of antagonism of *Brevibacillus brevis* against *Botrytis cinerea in vitro*. J Appl Microbiol 91:652–659

Elkan GH (1992) Biological nitrogen fixation systems in tropical ecosystems : an overview. In: Mulongoy K, Gueye M, DSC S (eds) Biological nitrogen fixation and sustainability of tropical agriculture. Wiley, Chichester, pp 27–40

Gajbhiye RP, Sharma RR, Tiwari RN (2003) Effect of biofertilizers on growth and yield of tomato. Indian J Hort 60:368–371

Giles E, Oldroyd D, Downie JA (2008) Coordinating nodule morphogenesis with Rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546

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

Govindan M, Purushothaman D (1984) Biomass production, root colonization and phosphatase activity by VA-mycorrhizal fungi in papaya. Agric Res J Kerala 22:133–138

Jayamma N, Nagaraj M, Naik MN, Jagadeesh KS (2014) Influence of biofertilizer application on growth, yield and quality parameters of jasmine (*Jasminum Auriculatum*). International conference on food, biological and medical sciences (FBMS-2014) January 28–29, 2014 Bangkok (Thailand)

Kamil P (2008) Plant growth promotional effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Rice Plant. Nepal J Sci Technol 9:85–90

Klopper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286:883–884

Kumar M (2002) Effect of different organic manures and biofertilizers on growth flowering and yield of marigold cv. Pusa narangi. M Sc (Horticulture) thesis, Department of Horticulture, CCS HAU, Hisar

Manjunath A, Mohan R, Bagyaraj DJ (1983) Responses of citrus to VAM inoculation in unsterile soils. Canadian. Aust J Bot 61:2779–2732

Mishra DJ, Rajvir S, Mishra UK, Shahi SK (2013) Role of bio-fertilizer in organic agriculture: a review. Res J Recent Sci 2:39–41

Mohandas S (1996) In: Proceedings conference on challenges for banana production and utilization in 21st century. National Research Centre on banana. September 24–25, Trichy, Tamil Nadu, India, pp 883–887

Morrissey J, Dow J, Mark G, Ogara F (2004) Are microbes at the root of a solution to world food reduction. EMBO Rep 5(10):922–926

Noshin I, Asghari B, Sumera I (2008) Variation in *Rhizobium* and *Azospirillum* strains isolated from maize growing in arid and semi arid areas. Int J Agri Biol 10:612–618

Pathak D, Kumar M (2016) Microbial inoculants as biofertilizers and biopesticides. In: Singh DP et al (eds) Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi

Pathak D, Singh S, Saini RS, Sharma JR (2009) Impact of bio-inoculants on germination and plant growth of guava (*Psidium guajava*). Haryana J Hortic Sci 38(1&2):26–28

Ramakrishnan K, Selvakumar G (2012) Effect of biofertilizers on enhancement of growth and yield on tomato (*Lycopersicum esculentum* Mill.) Int J Res Bot 2(4):20–23

Ruiz ML (1992) *Azotobacter mycorrhiza* and phosphorins in banana. Musarama 11:15

Shen Z, Zhong S, Wang Y, Wang B, Mei X, Li R, Ruan Y, Shen Q (2013) Induced soil microbial suppression of banana *Fusarium* wilt disease using compost and biofertilizer to improve yield and quality. Eur J Soil Biol 57:1–8

Sindhu SS, Verma N, Dua S, Chaudhary D (2010) Biofertilizer application for growth stimulation of horticultural crops. Haryana J Hortic Sci 39(1&2):48–70

Singh SR, Banik BC (2011) Response of integrated nutrient management on flowering, fruit setting, yield and fruit quality in mango (*Mangifera indica* L.) cv. Himsagar. Asian J Hortic 6(1):151–154

Srivastava AK, Ahmed R, Kumar S, Sukhada M (2001) Role of VA-mycorrhiza in the management of wilt disease of guava in the alfisols of Chotanagpur. Indian Phytopathol 54(1):78–81

Stockwell VO, Stack JP (2007) Using *Pseudomonas spp* for integrated biological control. Phytopathology 97:244–249

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Res Plant Sci 19:1–30

Sukhada M (1999) Biofertilizers for horticultural crops. Indian Hort 44(1):32–35

Thomashow LS, Weller DM (1996) Current concept in the use of introduced bacteria for biological disease control : mechanism and antifungal metabolites. In: Stacy G, Keen N (eds) Plant-microbe interactions, vol 1. Chapman and Hall, New York, pp 187–235

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677

Tiwary DK, Hasan MA, Chattopadhyay (1998) Studies on the effect of inoculation with *Azotobacter* and *Azospirillum* on growth, yield and quality of banana. Indian Agric 42(4):235–240

Umar I, Wali VK, Kher R, Jamwal M (2009) Effect of FYM, urea and *Azotobacter* on growth, yield and quality of strawberry cv. Chandler. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37:139–143

Venkateswarlu B, Desai S, Prasad YG (2008) Agriculturally important microorganisms for stressed ecosystems : challenges in technology development and application. In: Khachatourians GG, Arora DK, Rajendran TP, Srivastava AK (eds) Agriculturally important microorganisms, vol 1. Academic World, Bhopal, pp 225–246

Wu OS, Li GH, Zou YN (2011) Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (*Prunus persica* L. Batsch). J Anim Plant Sci 21(4):746–750

Zhang F, Shen J, Zhang J, Zuo Y, Li L, Chen X (2010) Rhizosphere processes and management for improving nutrient use efficiency and crop productivity: implications for China. Adv Agron 107:1–32

Fermentation: A Process for Biofertilizer Production

12

Harish Suthar, Krushi Hingurao, Jaysukh Vaghashiya, and Jivabhai Parmar

Abstract

Biofertilizers are the product of fermentation process, constituting efficient living soil microorganisms. They improve plant growth and productivity through supply of easily utilizable nutrients. They are cost-effective and eco-friendly bioinoculants having great potential to enhance agricultural production in sustainable way. Biofertilizers are grouped into different types based on their functions such as nitrogen-fixing, phosphate-solubilizing, phosphate mobilizing, and other plant growth-promoting biofertilizers promoting plant growth by different mechanisms. Solid-state fermentation and submerged fermentation are two main types of fermentation, used for the production of biofertilizers. Each type of biofertilizer is prepared by selection of efficient microbial strain, its cultivation using specific nutrient medium, scale-up, and formulation using solid or liquid base. Knowledge about host specificity of the microbial strain and properties of soil and environmental conditions of the field are the important factors which determine the success of biofertilizer application. Recent developments in the field of microbial taxonomy, molecular biology, genetic engineering, metabolic engi-

H. Suthar (✉) • J. Vaghashiya
Department of Post-Harvest Technology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat 396 450, India
e-mail: harish.s@nau.in; parmarjeeva@yahoo.com

K. Hingurao
Department of Microbiology and Biotechnology Centre, Faculty of Science, The M. S. University of Baroda, Vadodara, Gujarat 390 002, India
e-mail: krushi2311@gmail.com

J. Parmar
Department of Fruit Science, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat 396 450, India
e-mail: jaysukh_v@yahoo.in

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*, Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_12

neering, computer science, and nanotechnology have played a significant role in the advancement of fermentation process of biofertilizer production. Hence, the production of biofertilizers having better efficiency, higher competitive ability, multiple functionality, and longer shelf life has become possible. Biofertilizers with such characteristics can be an effective substitute of chemical fertilizers. The present chapter deals with the types of biofertilizers, their applications and outcomes, types of fermentation processes used for biofertilizer production, and past and present status of fermentation technologies used for biofertilizer production.

Keywords
Fermentation • Biofertilizer • Microorganisms • Scale-up • Plant growth

12.1 Introduction

Fermentation is a metabolic process used by microorganisms to produce energy by breaking down complex organic compounds into simple metabolites under anaerobic conditions. Fermentation is a word derived from the Latin word *fervere*, which means "to boil." The name was first given to foam product obtained by boiling of crushed grapes in large vessel (Bassey 2013). Insights of actual fermentation process were given by French microbiologist Louis Pasteur who is remembered as the father of fermentation. The science of fermentation is known as zymology. Production of specific metabolites using microorganisms grown in specific nutrient medium is also referred as fermentation (Kure et al. 2016). Humans have been using microbial fermentation for many centuries as a technology for large-scale production of metabolites, which are beneficial to them. Batch fermentation and continuous fermentation are the two main types of fermentation process which are used at large scale. Batch fermentation is a discontinuous process in which nutrients are supplied only once to the microorganisms at the start of the fermentation. After a specific time period, the whole content of the fermenter tank would be taken out for the next step of the processing. In continuous fermentation, the nutrients are supplied to the microorganisms continuously at a fixed rate, and similarly products are also removed from the fermenter tank. Continuous fermentation maintains the microorganisms in the exponential growth phase, and hence, production would be higher compared to batch fermentation (Bakri et al. 2012). Similarly, depending upon the state of substrate used, fermentation is classified as (1) solid-state fermentation (SSF), where substrates in solid form such as paper pulp and agricultural waste are used for microbial growth, and (2) submerged fermentation (SmF), where substrates in liquid form such as molasses, corn steep liquor, and nutrient-rich broths are used for the growth of microorganisms (Coelho et al. 2011). For successful application of fermentation technology, selected microorganism is grown using specific nutrient medium under optimum physiological conditions such as temperature, pH, agitation, and aeration. At industrial level, fermentation technology is used for different purposes such as (1)

production of microbial biomass, (2) bioconversion of microbial substrate, (3) production of primary or secondary metabolites, and (4) production of enzymes (Demain 2000). Pharmaceutical, chemical, food, alcohol beverages, and agriculture are the areas where the fermentation technologies are used widely.

In agriculture, microbial fermentation plays a crucial role in enhancing soil fertility and crop production. Millions of soil microorganisms are known to support plant growth and protect them against diseases. Some of them have very good potential to be used as biofertilizers, which support plant growth, or biopesticides, which protect plants against foreign invaders (Nagappan 2013; Pathak and Kumar 2016). Focus of the present chapter would be on the biofertilizers. Biofertilizers are the product of fermentation process, containing specific individual or group of soil microorganisms which improve the plant growth and productivity through supply of easily utilizable form of nutrients. They are also known as bioinoculants (Malusa et al. 2012). Since ancient time, farmers are benefited by biofertilizers through indirect application. Crop rotation with leguminous plants and use of manure are the examples of such application. Leguminous plants and manure both contain microorganisms naturally. Their activities improve the soil fertility and agricultural production (Franche et al. 2009). In modern agriculture practices, biofertilizers prepared in the laboratory using highly efficient microbial strain or consortium of such strains and packed in compatible carrier are used for field applications. As reported, application of biofertilizers is a cost-effective and eco-friendly approach for improving soil fertility and agricultural production (Nalawde and Bhalerao 2015). They have proven potential to replace the costly and hazardous chemical fertilizers (Alam and Seth 2014). Hence, in the present chapter, different types of biofertilizers, their applications, beneficial outcomes, fermentation processes for biofertilizer preparation, and different types of fermentation technologies used in the past and present are discussed in detail.

12.2 Biofertilizers: Types, Application, and Outcome

Fertilizer is a chemical or natural substance added to soil or plant parts to increase the soil fertility and plant growth. As per Fertilizer (Control) Order, 1985 (FCO 1985, amendment November 2009, Ministry of Agriculture, Department of Agriculture and Cooperation, Government of India), there are three types of fertilizers: (1) inorganic fertilizers, inorganic substances of synthetic origin which are also known as synthetic fertilizers; (2) organic fertilizers, substances made up of materials of a biological nature (plant/animal) and may include unprocessed mineral materials that have been altered through microbiological decomposition process; and (3) biofertilizers, material containing efficient living microorganisms which increase the soil fertility and crop productivity (FCO 1985).

Synthetic fertilizers act as instant source of plant nutrients, but biofertilizers work differently. They convert food available in the soil into essential nutrients and supply them to plants. Based on the functions played by individual biofertilizers, to provide specific nutrients to the plants, biofertilizers are grouped into different types such as nitrogen (N_2)-fixing, phosphate-solubilizing, phosphate-mobilizing,

micronutrient-providing, and plant growth-promoting biofertilizers (Nayak and Patangray 2015). Plant growth promoting biofertilizers differ from other types of biofertilizers in their mechanism to promote plant growth but generally stimulate growth by enhancement of nutrient uptake. They also promote growth by different actions such as production of plant growth hormones and inhibition of plant pathogen infection. Further, subgrouping of biofertilizers could be done based on the kind of microorganisms used (bacteria, fungi, algae, etc.) and their style of living (free-living, symbiotic, and associative symbiotic) (Mishra et al. 2013). Application of biofertilizers to the crop is done by seed treatment, soil application, or root dipping method. Information of N_2-fixing, phosphate-solubilizing, phosphate-mobilizing, and plant growth-promoting biofertilizers is covered in this part of the chapter.

12.2.1 N_2-Fixing Biofertilizers

The Earth's atmosphere contains 78% N_2. The atmospheric N_2 is relatively inert; hence, plants cannot use it for the biosynthesis of building blocks such as amino acids, proteins, DNA, RNA, etc. Applications of specific soil microorganisms as biofertilizers fix the N_2 to ammonia in the soil, which can be used by plants (Ghany et al. 2013). Two groups of microorganisms are used for the preparation of N_2-fixing biofertilizers: (1) nonsymbiotic (free-living) microorganisms and (2) symbiotic microorganisms (Doroshenko et al. 2007; Gomare et al. 2013; Waheed et al. 2014).

12.2.1.1 Free-Living N_2-Fixing Biofertilizers

Clostridium and *Azotobacter* are the extensively studied genera of the N_2-fixing bacteria. Bacteria belonging to *Clostridium* genus are anaerobic in nature and have lesser N_2-fixing capacity compared to aerobic *Azotobacter* bacteria. The amount of N_2 fixed by these two genera is around 20–50 lb/acre annually (Carnahan et al. 1960).

Azotobacter Biofertilizers

Azotobacter is a genus of free-living N_2-fixing bacteria and belongs to *Azotobacteriaceae* family. They are Gram-negative, aerobic, motile, heterotrophic, and saccharophilic bacteria which have the highest metabolic rate compared to any other microorganism (Gandora et al. 1998). The first representative of the genus, *Azotobacter chroococcum*, was discovered in 1901 by Martinus Beijerinck. *A. chroococcum* is most commonly found in different types of soil all over the world and widely used as a biofertilizer. Further, *A. vinelandii*, *A. beijerinck*, *A. paspali*, *A. nigricans*, *A. insignis*, and *A. macrocytogenes* are other species of *Azotobacter* found in subsequent years and used as biofertilizers (Gurikar et al. 2016). Strains of *Azotobacter* are reported to use sugars, alcohols, and salts of organic acids and grow well in nitrogen (N)-free medium. They are sensitive to the environmental conditions such as high salinity, acidic pH, and temperature above 35 °C. They are mostly found in neutral and alkaline soil and in rhizosphere of plants such as sugarcane, rice, maize, bajra, wheat, cotton, and mustard (Singh et al. 2016a). Several features of *Azotobacter* bacteria make them highly valuable biofertilizers for commercial

applications: (1) survival under harsh conditions by producing cyst; (2) high slime secretion surround the cells, which add in retaining water; (3) high metabolic rate and full range enzymes for N_2 fixation, which make them able to fix N_2 in the presence of oxygen (O_2) (Maier and Moshiri 2000); (4) ability to fix ~20–40 mg N/g of carbon (C) source used that is equivalent to 20–40 kg N/ha (Patil et al. 2013); (5) production of phytohormones such as gibberellic acid (GA) and indole acetic acid (IAA), which promote plant growth (Kukreja et al. 2004); (6) production of thiamine, riboflavin, pyridoxine, cyanocobalamin, nicotinic acid, and pantothenic acid; and (7) production of antifungal antibiotics that inhibit the growth of several pathogenic fungi in the root zone and help to prevent seedling mortality (Wani et al. 2013). There are several reports available which show positive outcome of *Azotobacter* biofertilizer applications. Jaga and Upadhyay (2013) reported 10–12% increase in the yield of wheat. Increase in grain yield ~15–35% in maize is reported by Baral and Adhikari (2013). Further, in sorghum ~15–19.5% productivity enhancement was reported (Reddy et al. 1977; Abd El-Lattief 2016).

Cyanobacteria Biofertilizers

Cyanobacteria is a phylum of Gram-negative, non-motile, and N_2-fixing bacteria. They obtain their energy through photosynthesis. *Cyanobacteria* phylum belongs to *Cyanophyta* division of kingdom *Eubacteria*. They are also called blue-green algae. They are free-living or form symbiotic relationships with plants or fungi. They are unicellular as well as filamentous. They contain thick-walled heterocysts, which contain the enzyme nitrogenase required for N_2 fixation. *Cyanobacteria* are highly efficient N_2-fixing biofertilizers, with their added abilities to grow on different habitats, to reduce greenhouse gas emission, and to enhance the fertility of soil and short generation time (Singh et al. 2016b). *Anabaena* and *Nostoc* are the two genera of the *Cyanobacteria* phylum which are reported to be used successfully as N_2-fixing biofertilizers in the fields such as rice and wheat (Kaushik 2014). Along with 20–30 kg/ha annual N_2 fixation, *Cyanobacteria* are reported to increase the crop yield by addition of organic matter to the soil (Vaishampayan et al. 2001). It is an economic and vital alternate of costly synthetic N fertilizers for farmers.

12.2.1.2 Symbiotic N_2-Fixing Biofertilizers

Rhizobium Biofertilizers

Rhizobium is a genus of Gram-negative soil bacteria that fix N_2 which belongs to *Rhizobiaceae* family. It contains rod-shaped cells that incite hypertrophies on plants (root nodules, leaf nodules, etc.). *Rhizobium* is the most studied genus of the symbiotic N_2-fixing bacteria (Zahran 1999). *Rhizobium* species forms endosymbiotic association (nodular symbiosis) with roots of legumes. The bacteria attach to the root hairs and penetrate the root cells. The N_2-fixing form of bacteria within root nodule is called "bacteroides" (Yehya et al. 2013). *Rhizobium leguminosarum* was the first identified species of the genus *Rhizobium*. All further species were initially placed in the same genus, but reclassification done using modern methods identified several new genera such as *Sinorhizobium*, *Mesorhizobium*, and *Bradyrhizobium*.

Among these, *Rhizobium* and *Sinorhizobium* are in the *Rhizobiaceae*, while *Mesorhizobium* and *Bradyrhizobium* are the members of *Phyllobacteriaceae* and *Bradyrhizobiaceae* family, respectively. Genus *Rhizobium* consists of 49 rhizobial (root-living) and 11 nonrhizobial species (Weir 2016). Symbiotic relationship of different species of *Rhizobium* genus is host specific. *Rhizobium* species effective for one group of plants can be less or not effective for other groups of plants. *R. leguminosarum* bv. *phaseoli*, *Phaseolus vulgaris*; *R. leguminosarum* bv. *viciae*, *Vicia*; *R. leguminosarum* bv. *trifolii*, *Trifolium*; *R. etli*, *Phaseolus vulgaris*; *R. tropici*, *Teramnus labialis*; and *R. indigoferae*, *Indigofera*, are the examples of N_2-fixing *Rhizobium* species with their specific hosts (van Rhijn and Vanderleyden 1995; Wei 2002). It is reported that application of biofertilizers containing *Rhizobium* species could fix 40–250 kg N/ha annually and increase the agricultural yield ~20% (Pindi and Satyanarayana 2012).

Azolla Biofertilizers

Azolla is a free-floating water fern that floats in water. It fixes N_2 in association with *Cyanobacteria*. Genus *Azolla* contains seven species of aquatic ferns in the family *Salviniaceae*. It includes *Azolla microphylla*, *A. filiculoides*, *A. pinnata*, *A. caroliniana*, *A. nilotica*, *A. rubra*, and *A. mexicana* (Kannaiyan and Kumar 2005). In case of symbiotic relationship, *Cyanobacteria* (*Anabaena azollae*) are generally found within ovoid cavity inside the leaves of the water fern *Azolla*. In this relationship, bacteria receive C and N sources from host fern in exchange for fixed N_2 (Prasanna et al. 2012). The interest in the use of the symbiotic N_2-fixing water fern *Azolla* as an effective N_2-fixing system has been increased in all over the world, esp. in rice-growing areas (Kobiler et al. 1981). It is reported that free-living *Cyanobacteria* could fix ~15–30 kg N/ha/year, while *Azolla-Anabaena* fixes 312–600 kg N/ha annually, in the rice ecosystem (Vaishampayan et al. 2001; Prasanna et al. 2012). The important benefit of *Azolla* to be used as biofertilizer for rice crop is its quick decomposition in the soil and efficient availability of N to rice. *Azolla* as biofertilizer is used for rice cultivation in different countries such as Vietnam, China, Thailand, and the Philippines. It is reported that application of *Azolla* biofertilizers led to increase in rice yield 0.5–2.0 t/ha (Singh et al. 2014).

Azospirillum Biofertilizers

Azospirillum is a genus of Gram-negative, motile, nonspore-forming, microaerophilic, spiral-shaped, and N_2-fixing bacteria, which belongs to *Rhodospirillaceae* family. Beijerinck (1922) isolated *Azospirillum* first time and named it as *Spirillum lipoferum*. Dobereiner and Day (1976) were the first who coined the term "associative symbiosis" for the relationship between N_2-fixing *Spirillum* and forage grass. Tarrand et al. (1978) renamed the genus as *Azospirillum* during reclassification. Associative symbiotic relationship of *Azospirillum* with cereal host plants is different kind of symbiotic relationship in which bacterial cells remain associated with host plant without any visible structure (such as nodule) formation. *Azospirillum* strains form associative symbiosis with many plants, particularly with C_3 and C_4 plants (maize, sugarcane, oilseeds, cotton, sorghum, pearl millet, etc.), and are

reported to be used as biofertilizers for these crops (Adholeya and Das 2012). *Azospirillum* biofertilizers are applied to the field by seed treatment, root dipping, or soil application (Wani et al. 2016). Their application has several beneficial outcomes such as (1) fixing ~20–40 kg N/ha/year (Abd El-Lattief 2016); (2) helping plant roots in mineral and water uptake and hence enhancing crop yield; (3) reducing pathogen damage; (4) reducing germination of parasitic weed; (5) being widely used as phyto-stimulator inoculate for cereal crops; (6) modulating plant hormonal balance; (7) enhancing the growth of rice plants significantly, equivalent to application of 15–20 kg N/ha (Rodrigues et al. 2008); and (8) having ~10–30% higher dry matter and seed yield compared to control plants in sorghum (Kapulnic et al. 1981; Rodrigues et al. 2008).

12.2.2 Phosphate-Solubilizing Biofertilizers

Phosphorus is the second most important nutrient after N, required by plants for their growth. It is required for several physiological processes of plants such as photosynthesis, C metabolism, membrane formation, seed development, root elongation, and proliferation (Kumar et al. 2016). Plant acquires phosphorus from the soil in the form of phosphate (P). Generally, P remains in a precipitated form in the soil as mono- or orthophosphate, and hence, the mobility of the P is very less compared to other macronutrients. Soil microbes which can solubilize the insoluble form of P in soil and make it available to plants are called phosphate-solubilizing microorganisms (PSM) (Roychowdhury et al. 2015). PSM include both bacteria and fungi. They play a key role in making P available to plants. They excrete organic acids which solubilize P by lowering soil pH and enzymes (phosphatase and phytase) produced by them mineralize the P so that it can be easily taken up by plants (Vessey 2003; Ponmurugan and Gopi 2006). Among all the PSM, *Bacillus* species (e.g., *Bacillus megaterium* and *Bacillus circulans*) and *Pseudomonas* species (e.g., *Pseudomonas striata*) are reported to be performing well (Prasad 2014). Further, some fungal genera such as *Penicillium* and *Aspergillus* are also reported to have high potential to be used as P-solubilizing biofertilizers. The phosphate-solubilizing fungi (PSF) are reported to produce more acid compared to phosphate-solubilizing bacteria (PSB), but their population (0.1–0.5%) is very less compared to PSB (1–50%). Hence, their P-solubilizing activity of PSF is also lesser than the PSB, in soil (Alam et al. 2002; Sharma et al. 2013). The PSB isolated from alkaline habitats are reported to perform well under extreme conditions such as high salinity, high pH, and high temperature (Lavania and Nautiyal 2013). Hence, biofertilizers prepared using such kind of bacteria can be applicable for a wide range of crops growing under different environmental conditions. In addition to phosphate solubilization, PSB are also reported to promote plant growth by secreting hormones, vitamins, and other growth factors. They are also reported to enhance the availability of trace elements, modulate plant hormone level, and protect plant against pathogens by antibiotic production (Akhtar and Siddiqui 2009; Tensingh Baliah and Jeeva 2016). Tao et al. (2008) reported that the PSB strains exhibit phosphate-solubilizing

efficiency 25–42 µg/ml and organic phosphate-mineralizing efficiency between 8–18 µg/ml. *Pseudomonas striata* and *Bacillus polymyxa* are reported to solubilize 156 and 116 mg P/l, respectively (Rodriguez and Fraga 1999). The application of phosphate-solubilizing biofertilizers is reported to enhance crop yield by 20–30% (Ghosh 2004).

12.2.3 Phosphate-Mobilizing Biofertilizer

P is abundantly present in soils, but its low mobility makes it a prime limiting factor for plant growth. To overcome this problem, phosphate-mobilizing microorganisms could play an important role. Several soil fungi are reported to mobilize the immobile form of P by its hyphal structure, and hence, they are used as phosphate-mobilizing biofertilizers. *Mycorrhiza* is widely used for this purpose (Javaid 2009).

12.2.3.1 Phosphate-Mobilizing Mycorrhizal Biofertilizers

Mycorrhiza (myco + rrhiza = fungus + root) is a symbiotic association of a fungus and roots of vascular plants. There are two main types of *Mycorrhiza*: (1) *Ectomycorrhiza*, which colonize the host plant roots extracellularly, and (2) *Endomycorrhiza*, which penetrate the root cells and colonize the host plant roots intracellularly (Moore et al. 2011). Both are used as P-mobilizing biofertilizers. After colonization, fungi act as extended roots and hence enhance the coverage and absorption of plants for water and nutrients from the soil.

Arbuscular Mycorrhizal Biofertilizer

Arbuscular mycorrhiza (AM) is one type of *Endomycorrhiza*, found in diverse soil habitats. It forms beneficial symbiosis with the roots of angiosperms and other plants through specialized structures called vesicles and arbuscules and is reported to increase their P uptake efficiency (Brundrett 2002). They are high-affinity P transporters and reported to have mutual relationship with ~80% of crops (Harrison and van Buuren 1995). They are reported to help host plants to get water, nutrients, and protection against adverse conditions and pathogens (Auge et al. 2015). These features make AM potential phosphate-mobilizing biofertilizers. The beneficial outcomes of AM biofertilizer application are (1) saving ~25–50% of phosphatic fertilizers (Sharma et al. 2007), (2) improving soil structure and productivity (Rillig et al. 2015), (3) reducing greenhouse gas (NO_2) emission (Lazcano et al. 2014), and (4) as shown in 82 out of the 112 (92%) experiments carried out using AM as inoculants by different researchers, improving nutrient content and higher yield in plants (Berruti et al. 2016).

12.2.3.2 Plant Growth-Promoting Biofertilizers

It is a specific group of microorganisms that can be found in the rhizosphere. The rhizosphere is the narrow zone of soil surrounding the plant root. The zone is directly influenced by plant root secretions and activity of associated soil

microorganisms. Soil, which is not a part of the rhizosphere, is called bulk soil (Brimhall et al. 1992; Richter and Markewitz 1995). Microbes colonizing the rhizosphere include bacteria, fungi, algae, and protozoa. Among all these, bacteria are the most abundantly found (95%) in the rhizosphere. Bacteria which are colonizing in the rhizosphere and promoting plant growth are named "plant growth-promoting rhizobacteria (PGPR)" by Kloepper and Schroth (1978). *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Azotobacter*, *Variovorax*, *Azospirillum*, and *Serratia* are the examples of the PGPR bacteria. These PGPR species play an important role in enhancing plant growth by different mechanisms such as (1) enhancement of abiotic stress tolerance; (2) secretion of phytohormones and plant growth regulators such as IAA, GA, cytokinins, and ethylene; (3) production of siderophores; (4) production of volatile organic compounds; and (5) production of protective enzymes such as chitinase, glucanase, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase which are used as plant growth-promoting biofertilizers (PGPB) commercially. Apart from that, there are certain rhizospheric fungi (*Trichoderma*, *Penicillium*, *Aspergillus*, etc.) that are also used as PGPB (Glick et al. 2007).

12.3 Production of Biofertilizers by Fermentation

Biofertilizers are microorganisms containing formulations used to supply nutrients to the plants in an eco-friendly manner. N_2 fixers, phosphate solubilizers, phosphate mobilizers, and plant growth promoters are the types of biofertilizers widely used by farmers for the enhancement of soil fertility and agricultural production (Baby 2002; Jayaraj et al. 2004). The contributions of scientists, such as discovery of N_2 fixation by root nodules of legumes in 1886 by Hellriegel and Wilfarth (1888); isolation and cultivation of *Rhizobium* by Beijerinck (1888) from the root of legumes; launching by Nobbe and Hiltner (1895) of "Nitragin, a pure culture of rhizobia," in the market for N_2 fixation; introduction by Pikovskaya (1948) of phosphate solubilizers; discovery of N_2 fixation in blue-green algae by Stewart (1969); and identification of N_2-fixing *Azospirillum* by Dobereiner and Day (1976), established the base of the application of biofertilizers. Afterward, many researchers have developed biofertilizers containing different microbial strains with several useful features (Podile and Kishore 2006; Abdel Ghany et al. 2013).

Fermentation is an important process used for biofertilizer production due to their economic and user-friendly nature. The final product of the fermentation process mainly depends on the types of substrate used. SSF and SmF are the two main substrate-based types of fermentations, widely used for large-scale production of biofertilizers (Sect. 1.1). Substrates which are commonly used in case of SSF are bagasse, paper pulp, wheat bran, rice and rice straw, vegetable and fruit waste, and synthetic media (Pandey et al. 1999). In case of SmF fermentation, soluble sugars, liquid synthetic media, fruit and vegetable extracts, dairy by-products, and wastewater are the commonly used substrates (Subramaniyam and Vimala 2012). Microorganisms are the bioagents which carry out actual conversion of the substrate

into the product of interest. Hence, specific microbial agent has to be selected for specific biofertilizer production. Type of crop and environmental conditions of field are the two key factors decide the selection of specific microbial strain for biofertilizer production for that specific crop (Dodd and Ruiz-Lozano 2012). Microbes are either aerobic or anaerobic in nature; hence, the process of fermentation should be carried out as per the nature of microorganism. For aerobic fermentation, the fermenter should have facility to provide aeration, and for anaerobic fermentation, the design of the fermenter should be in such a way that it can maintain anaerobic conditions for the microbial growth (Rosenberger and Elsdens 1960). Parameters such as pH, temperature, contamination-free environment, and incubation period are also playing important role in the success of fermentation process used for biofertilizer production. It is reported that production of biofertilizer comprises mainly three important steps: (1) development of strains, (2) upscale of biomass, and (3) preparation of inoculants (Sethi and Adhikary 2012). Success of abovementioned three steps depends upon certain sub-steps, which should be followed properly for the large-scale production of biofertilizers. These include selection of suitable and efficient microorganism, selection of suitable nutrient medium, selection of optimum growth conditions, selection of specific method of propagation, pilot-scale study, large-scale production, and quality testing at each level. Further, selection of suitable carrier for biofertilizer formulation, packaging, storage, and transport are also important factors (Biederbeck and Geissler 1993; Albareda et al. 2008; Atieno et al. 2012). It is reported that the carrier material used in biofertilizer formulation should have characteristics such as capacity to maintain high viable count, low soluble salt content, high water-holding capacity, cost-effectiveness, non-toxicity, and biodegradability (Gomare et al. 2013).

To maintain the control over the quality of biofertilizers produced, the government of India has recommended certain quality standards, under the ambit of FCO 1985 (amended in year 2009), which must be followed by manufacturer, for successful commercialization and application of the biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirillum*, phosphate-solubilizing, and phosphate-mobilizing biofertilizers (Yadav and Chandra 2014). This part of the chapter covers information of fermentation processes used for production of the abovementioned biofertilizers.

12.3.1 Production of N_2-Fixing Biofertilizers

Biofertilizers containing microorganisms which can fix N_2 into plant usable form of N in the soil are called N_2-fixing biofertilizers. *Rhizobium*, *Azospirillum*, and *Azotobacter* are the most effective and widely used N_2 fixers.

12.3.1.1 Production of *Rhizobium* Biofertilizers

Selection of the strain is the most important step in *Rhizobium* biofertilizer production. For fermentation process, selection of strain should be host specific, and strain should have the ability to grow actively under the environmental conditions where it is going to be applied. It is reported that each crop variety requires a specific species

of *Rhizobium* to form effective nodules (Sect. 1.2.1.2). Growth of specific plants is enhanced only when nodules are produced by effective strains of *Rhizobium* (Cooper 2004; Abi-Ghanem et al. 2012). Further, selection of culture medium for the cultivation of selected microorganism for biofertilizer production depends upon suitable C and N sources present in the medium and mineral nutrients required for high growth rate of bacteria in that medium. Yeast extract mannitol (YEM) is the widely used medium for *Rhizobium* species. YEM medium for *Rhizobium* contains (g/l): mannitol, 10.0; yeast extract, 1.0; K_2HPO_4, 0.5; $MgSO_4.2H_2O$, 0.2; NaCl, 0.1; $CaCO_3$, 1.0; and pH, 6.8 ± 0.2 (Allen and Allen 1950; Subba Rao 1977). But for large-scale production, the use of YEM can be costly. Hence, commercial-scale producers prefer cost-effective and easily available media for biofertilizer production (Ben Rebah et al. 2002). Many researchers have used agricultural waste and industrial by-products such as molasses, corn steep liquor, deproteinized leaf extracts, cheese whey, jaggery solution, and wastewater sludge as media ingredients for the cultivation of *Rhizobium* species (Chanda et al. 1987; Jain et al. 2000; Estrella et al. 2004; Ben Rebah et al. 2007; Singh et al. 2011). These products are reported to supply nutrients required for *Rhizobium* growth. They can be used for media optimization to develop cost-effective and easily available medium as better alternate of YEM medium. Further, growth parameters such as pH, aeration, agitation, and temperature are needed to be optimized before using these kinds of media for large-scale production. pH between 6 and 8, temperature around 28 °C, and incubation under aerobic conditions were reported to give better results for N_2-fixing *Rhizobium* species (Agarwal and Ahmad 2010; Parthiban et al. 2011). After selection of suitable strain, suitable medium, and optimum growth conditions at laboratory level, the next step is scale-up. It is reported to carry out in two steps: pilot-scale production and large-scale production, using fermenters of different sizes (Bissonnette et al. 1986). Finally, obtained culture is used for either carrier-based formulation or liquid formulation. For carrier-based formulation, the scaled-up pure culture of required *Rhizobium* species is reported to be mixed with suitable carrier material (e.g., peat, charcoal, lignite, vermiculite, kaolin, etc.) (Singh et al. 2012). Then, the formulation is packed in polythene bags under aseptic conditions and supplied to farmers. For liquid formulations, liquid materials such as water, oil, or solvents are used as carriers. Leo Daniel et al. (2013) reported that liquid biofertilizers (*Bacillus*, *Azospirillum*, and *Azotobacter*) formulated with 2% polyvinylpyrrolidone (PVP), 0.1% carboxymethylcellulose (CMC), and 0.025% polysorbate promoted growth and survival of the cells for a longer period of time. After formulation, the final product is analyzed for the quality, and it should fulfill the mandatory specifications of *Rhizobium* biofertilizers for production in India, as mentioned in FCO 1985 (Table 12.1).

12.3.1.2 Production of *Azotobacter* Biofertilizers

Ashby's N-free medium is commonly used for the cultivation of *Azotobacter* species. It contains the following (g/l): sucrose, 20.0; K_2HPO_4, 0.2; $MgSO_4.2H_2O$, 0.2; NaCl, 0.2; K_2SO_4, 0.1; $CaCO_3$, 5.0; and pH, 7.4 ± 0.2 (Subba Rao 1977). In this medium, sucrose is used as a C source and atmospheric N_2 as N source. K_2HPO_4

Table 12.1 Standard specifications of *Rhizobium* biofertilizer (FCO 1985)

S. No.	Parameter	Requirements
(i)	Base	Carrier based[a] in the form of moist/dry powder or granules or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cells/g of powder, granules, or carrier material or 1×10^8 cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5–7.5
(v)	Particle size in case of carrier-based material	All material shall pass through 0.15–0.212 mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30–40%
(vii)	Efficiency character	Should show effective nodulation on all the species listed on the packet

[a]Type of carrier: The carrier materials such as peat, lignite, peat soil, humus, wood charcoal, or similar material favoring growth of organism

Table 12.2 Standard specifications of *Azotobacter* biofertilizer (FCO 1985)

S. No.	Parameter	Requirements
(i)	Base	Carrier based[a] in the form of moist/dry powder or granules or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cells/g of powder, granules, or carrier material or 1×10^8 cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5–7.5
(v)	Particle size in case of carrier-based material	All material shall pass through 0.15–0.212 mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30–40%
(vii)	Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed

[a]Type of carrier: The carrier materials such as peat, lignite, peat soil, humus, wood charcoal, or similar material favoring growth of organism

provides buffering to the system, and other ingredients of the medium provide various ions required for the growth of *Azotobacter*.

Steps used in fermentation process for the large-scale production of *Azotobacter* biofertilizers are commonly used for *Rhizobium*. The medium and growing conditions might differ as selected strain would be of different genus. Table 12.2 shows the specifications required to be fulfilled for the *Azotobacter* biofertilizers produced in India (FCO 1985).

12.3.1.3 Production of *Azospirillum* Biofertilizers

The medium widely used for the growth of *Azospirillum* is OAB medium. It is composed of Solution A and B. Solution A (g/l): malic acid, 5; NaOH, 3; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.02; NaCl, 0.1; NH_4Cl, 1.0; yeast extract, 0.1; $FeCl_3$, 0.01; (mg/l):

Table 12.3 Standard specifications of *Azospirillum* biofertilizer (FCO 1985)

S. No.	Parameter	Requirements
(i)	Base	Carrier based[a] in the form of moist/dry powder or granules or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cells/g of powder, granules, or carrier material or 1×10^8 cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5–7.5
(v)	Particle size in case of carrier-based material	All material shall pass through 0.15–0.212 mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30–40%
(vii)	Efficiency character	Formation of white pellicle in semisolid N-free bromothymol blue medium

[a]Type of carrier: The carrier materials such as peat, lignite, peat soil, humus, wood charcoal, or similar material favoring growth of organism

$NaMoO_4.2H_2O$, 2; $MnSO_4$, 2.1; H_3BO_3, 2.8; $Cu(NO_3)_2.3H_2O$, 0.04; $ZnSO_4.7H_2O$, 0.24; and 900 ml distilled water.

Solution B (g/l): K_2HPO_4, 6; KH_2PO_4, 4; and 100 ml distilled water. After autoclaving and cooling, the A and B solutions are mixed, pH 6.8 (Okon et al. 1977; Bashan et al. 1993). Further, during fermentation using this medium, the design of the fermenter should be in such a way that it can maintain microaerophilic condition for the growth of *Azospirillum*. Table 12.3 shows the specifications required to be fulfilled for the *Azospirillum* biofertilizers, produced in India (FCO 1985).

12.3.2 Production of Phosphate-Solubilizing Biofertilizers

For P-solubilizing biofertilizers, selection of suitable strain is carried out on the basis of its P-solubilizing ability and field of application. Different fields have different soil properties such as physical and chemical nature, organic matter, and P content; hence, it affects the growth activity of phosphate-solubilizing strains (Kim et al. 1998). Next step is selection of suitable production medium. It is a medium in which selected strain grows and increases cell numbers. Pikovskaya's medium is specific and widely used medium for PSB such as *Bacillus* and *Pseudomonas* (Pikovskaya 1948; Roychowdhury et al. 2015). It contains the following (g/l): glucose, 10; tricalcium phosphate (TCP), 5; $(NH_4)_2SO_4$, 0.5; NaCl, 0.2; $MgSO_4.7H_2O$, 0.1; KCl, 0.2; yeast extract, 0.5; $MnSO_4.H_2O$, 0.002; $FeSO_4.7H_2O$, 0.002; and pH, 7.2 ± 0.2. After selection of P-solubilizing strain and production medium, mother culture is prepared by inoculating pure bacterial culture in the sterile medium and incubated under shaking condition till the population reaches to ~10^9 CFU/ml. Further, for scale-up the mother culture is transferred to a pilot-scale fermenter and then to a larger fermenter for bulk production. The capacity of a fermenter depends upon the final volume of biofertilizer required for application. The fermentation process would be carried out with continuous agitation and aeration for ~7 days till

Table 12.4 Standard specifications of phosphate-solubilizing bacterial biofertilizer (FCO 1985)

S. No.	Parameter	Requirements
(i)	Base	Carrier based[a] in the form of moist/dry powder or granules or liquid based
(ii)	Viable cell count	CFU minimum 5×10^7 cells/g of powder, granules, or carrier material or 1×10^8 cells/ml of liquid
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5–7.5 for moist/dry powder, granulated carrier based and 5.0–7.5 for liquid based
(v)	Particle size in case of carrier-based material	All material shall pass through 0.15–0.212 mm IS sieve
(vi)	Moisture per cent by weight, maximum in case of carrier based	30–40%
(vii)	Efficiency character	The strain should have phosphate-solubilizing capacity in the range of minimum 30%, when tested spectrophotometrically
		In terms of zone formation, a minimum of 5 mm solubilization zone in prescribed media having at least 3 mm thickness

[a]Type of carrier: The carrier materials such as peat, lignite, peat soil, humus, wood charcoal, or similar material favoring growth of organism

the population of selected strain cells reach to 10^9 CFU/ml (Pindi and Satyanarayana, 2012). Quality testing would be done each day to check the purity and growth of the selected strain. After that the broth would be harvested, stored under cool temperature, and then mixed with suitable carrier material under aseptic condition. The cell count of the final formulation should be $\geq 10^7$ CFU/g for carrier-based formulation (Table 12.4). The carrier-based biofertilizers are generally stored at cool temperature to maintain viability of culture. The P-solubilizing biofertilizer formulation can be prepared using liquid base also ($\geq 10^8$ CFU/ml). As reported, liquid-based biofertilizers have greater viability, better stability at high temperature, and higher activity in the field (Leo Daniel et al. 2013; Nehra and Choudhary 2015). For production of P-solubilizing biofertilizer in India, the recommended quality standards for both carrier-based and liquid-based formulations should be as per FCO 1985 (amended in year 2009) (Table 12.4).

12.3.3 Production of Phosphate-Mobilizing Biofertilizers

Many researchers have reported that AM fungus has great proven potential to be used as P-mobilizing biofertilizer (Berruti et al. 2016; Rillig et al. 2015). In spite of this, application of AM biofertilizers is limited due to its obligate symbiotic nature. Like other biofertilizers, AM cannot be produced using synthetic media in the laboratory at a large scale. Other constraints in using AM as P-mobilizing biofertilizer are variation in plant genotypes and soil nature. Hence, production of AM is generally carried out in pot cultures under control conditions to avoid contaminants

Table 12.5 Standard specifications of mycorrhizal biofertilizer (FCO 1985)

S. No.	Parameter	Requirements
(i)	Form/base	Fine powder/tablets/granules/root biomass mixed with growing substrate
(ii)	Particle size for carrier-based powder formulations	90% should pass through 250 micron IS sieve (60 BSS)
(iii)	Moisture content per cent maximum	8–12%
(iv)	pH	6.0–7.5
(v)	Total viable propagules/g of product, minimum	100/g of finished product
(vi)	Infectivity potential	80 infection points in test roots/g of mycorrhizal inoculums used

(Klironomos and Hart 2002; Douds et al. 2005). Trap plants such as *Brachiaria* and *Zea mays* are compatible as host to provide massive colonization of AM fungi and hence most commonly used for large-scale crop inoculum development. The inoculum prepared by this method contains a concentrated set of the same kind of propagules generally found in natural soil inocula (Berruti et al. 2016). It is reported that AM biofertilizer application led to increase in nutrient content, growth, and yield of host plants. But the success or failure of the application depends upon growing conditions (greenhouse or open field), origin of inoculum (native or foreign), and method of application (single species or consortium) (Secilia and Bagyaraj 1987; Herrmann and Lesueur 2013). For the production of P-mobilizing biofertilizer in India, the recommended quality standards should be as per FCO 1985 (amended in year 2009) (Table 12.5).

12.4 Past and Present Fermentation Technologies Used for Biofertilizer Production

Current world population is 7.4 billion, and it is going to reach 9.9 million in the year 2050 (Population Reference Bureau, USA). As per the Food and Agriculture Organization (FAO) of the United Nations, the average demand for the agricultural commodities will be 60% higher in year 2030 than today (Mia and Shamsuddin 2010). To provide food to such a high population is a major issue of concern for all the countries. In a country like India, decreasing availability of agricultural land and reduction in soil fertility are two main limiting factors. In the past, production of agricultural commodities was enhanced by application of chemical fertilizers, but it caused severe environmental hazards (Bhardwaj et al. 2014). Hence, development of technologies that can overcome the limiting factors and enhance agricultural production in sustainable manner is the demand of present time. Organic farming and microbial fermentation are the potential technologies which are presently used to achieve the needed target. Application of biofertilizers (N_2 fixing, phosphate solubilizing, phosphate mobilizing, and plant growth promoters), produced using

microbial fermentation technology, is reported to enhance the crop productivity and soil fertility in a sustainable manner (Armada et al. 2014). In the past, farmers were not so much aware about application and advantages of biofertilizers, but since the last decade, farmers have also realized the benefits of biofertilizers. Training programs and subsidies given by the government of different countries played a significant role for this purpose. In the past, knowledge about microbial strains, nutrient media, cultivation conditions, formulation, packaging, storage, application, and behavior of microbes in the field was very limiting. But knowledge about these points has increased in recent years due to extensive research. Information about the properties of soil, host specificity of microbial strain, development of specific media for cultivation, understanding of incubation conditions, up-gradation in fermenter designs, and advancement in bioprocessing is available in the form of published research articles (Zohar-Perez et al. 2005; Malusa et al. 2012).

In the case of N_2-fixing biofertilizers, the N_2-fixing efficiency and survival of selected microbial strain depend on the host plant variety and hosting soil (Morgan et al. 2005). In the past, all nodulating bacteria were classified in one genus, that is, *Rhizobium*, and hence during application of *Rhizobium* biofertilizers in the field, expected results were not obtained. But at present, due to the latest molecular biology techniques, reclassification was done, and nodulating bacteria were separated in several new genera. Application of polyphasic taxonomy identified new genera and species based on symbiotic performance with selected hosts, cultural and morphological characteristics, DNA-DNA relatedness, rRNA-DNA hybridization, 16S rRNA analysis, RFLP, and multilocus enzyme electrophoresis (van Rossum et al. 1995). Hence, application of host-specific strain started developing, and better output in terms of N_2 fixation and crop yield becomes possible (Hameed et al. 2004). Advanced technologies such as genetic engineering and recombinant DNA technology are used to develop new strains with better efficiency. Successful application of genetic engineering is reported for transfer of nitrogenase activity into a variety of non-diazotrophic bacteria (e.g., *Pseudomonas protegens* Pf-5 X940). Inoculation of maize and wheat with the robust rhizosphere colonizer, *P. protegens* Pf-5 X940 that had been engineered to express *P. stutzeri* A1501 *nif* genes, reported to improve N content and growth (Fox et al. 2016; Li et al. 2016). Rafael et al. (2017) manipulated the endogenous regulation for both N_2 fixation and assimilation in *Azotobacter vinelandii*. They developed a single mutant strain (by substitution of native promoter with exogenously inducible promoter) and double mutant strain (by deletion in the *nifL*gene) of *A. vinelandii*. Under special growth conditions, both the single and the double mutant strains consistently released very high levels of ammonium (>20 mM) into the growth medium and are reported to promote growth in cucumber plants in the absence of N fertilizer.

Further, in the past, conventional and time-consuming methods were used for the development of suitable medium for biofertilizer production. But development of statistical media optimization techniques and new statistical software, such as SPSS, Design-Expert, Origin, etc., made media development easy and fast. It also becomes possible to develop a strain-specific and cost-effective medium using agricultural waste and industrial by-products (Peng et al. 2014). Next is development in

understanding of cultivation conditions. To develop efficient biofertilizers, one should simulate the field conditions in the laboratory. In a country like India, commercial-scale production of biofertilizers started around 1970. Before that, people were using imported cultures, with defined medium and growing conditions given by the suppliers. Hence, microbes showing promising results in the laboratory failed to give same performance in the field. Soil properties such as salinity, acidity, alkalinity, moisture, level of available nutrients, and population of native microbes vary with soil to soil and that affect the activity of biofertilizers. It is reported that alkalinity can affect the survival and function of *Rhizobia*, *Mycorrhizae*, and other microorganisms (Paul and Nair 2008). Duraraj et al. (2016) reported that pH above 9.0 restricts the availability of nutrients such P, K, Ca, and Mg. Such kind of information was not available in the past; hence, success rate of biofertilizers application was low. But in present time, due to updated knowledge, strains isolated from the local region are used, and they are cultivated by simulating the actual field conditions. Further, appropriate field trials in the field are taken, and then promising strains are used for large-scale biofertilizer production (Trivedi and Pandey 2007). In the last two decades, new fermenter designs, development of automatic control systems for mainlining parameters (pH, aeration, agitation, foaming, etc.), and mathematical modeling made application fermentation technology precise, user-friendly, and better for the microbial fermentation processes (Saithi et al. 2016). Initially, biofertilizer production was carried out using single microbial strain, but in the last 10 years, biofertilizers containing more than one strain (consortium) are reported to be used effectively in the field. Chang and Yang (2009) developed thermo-tolerant multifunctional phosphate-solubilizing biofertilizers by inoculating six different thermo-tolerant phosphate-solubilizing microbes into agricultural and animal wastes. Zaiadan et al. (2014) developed two consortia ZOB-1 (*Anabaena variabilis*, *Chlorella vulgaris*, and *Azotobacter* sp.) and ZBOB-2 (*Nostoc calcicola*, *Chlorella vulgaris*, and *Azotobacter* sp.). Among these, ZOB-1 showed improved germination and growth of rice plants.

Biofertilizers commercially used for application are of two types: solid based and liquid based. Solid-based biofertilizers, also referred to as carrier-based biofertilizers, are prepared with the help of carrier materials such as activated charcoal, peat, lignite, soil, humus, etc. The material would act as a carrier for microorganism. In the past, carrier-based biofertilizers were widely used. With the use of carrier-based biofertilizers, some drawbacks are reported such as contamination, low shelf life, temperature sensitivity, problem of packaging, and being bulky to transport hence high transport cost (Shanware et al. 2014; Trivedi et al. 2016). In conventional carrier-based methods, sterilization of carriers such as charcoal and lignite (100 mesh size) was used to be done in open-topped stainless steel trays. The method is now improved by FAO; the carrier is sterilized and sealed in packages. Autoclaving the carrier material at 121 °C for 1 h and gamma radiation are reported to be effective methods for sterilization of carriers to prevent contamination (Senoo et al. 2002; Abd El-Fattah et al. 2013). In recent time, biofertilizers prepared by liquid base such as water, oil, emulsion, etc. are widely used. They are reported to have shelf life of ~2 years (longer survival in the field which fulfills the nutrient

demand of crops for the entire life cycle), stability at high temperature (storage up to 55 °C), tolerance to UV radiation, less chances of contamination, easy packaging, higher potential to fight against native population, user-friendly field application, and required dosage that is 10 times lower compared to carrier-based biofertilizers (Verma et al. 2011; Nehra and Choudhary 2015). In present time, nanotechnology-based approach has also developed, called "nanoencapsulation technology." Conventional PGPR biofertilizer preparation is reported to be less effective as 90% of it was lost during application. PGPR prepared using nanoencapsulation technology reported to overcome this problem and gave better results (Pindi and Satyanarayana 2012).

As discussed above, developments taken place in the last few years, in the field of microbial taxonomy, molecular biology, genetic engineering, metabolic engineering, computer science, and nanotechnology, played a significant role in the advancement of fermentation technology and in the understanding of process for biofertilizer production. Hence, large-scale production of biofertilizer having better efficiency and longer shelf life has become possible in user-friendly and cost-effective way. This way the innovative research and technology advancement should continuously grow to develop new biofertilizers which can substitute chemical fertilizers and enhance agricultural production in a sustainable manner to fulfill the demand of increasing human population.

12.5 Conclusion

Fermentation is a process used for production of biofertilizers. Biofertilizers are living microorganisms containing solid- or liquid-based formulations, which play an important role in enhancing soil fertility and crop yield in a sustainable manner. N_2-fixing, phosphate-solubilizing, phosphate-mobilizing, and other plant growth promoting microorganisms with different features are used as biofertilizers. The successful application of these biofertilizers in the field depends on the fermentation technology used for their production. Various parameters such as host plant-specific microbial strain; optimized, cost-effective, and easily available growth medium; growth rate, competency, efficiency, and survival of selected strain under conditions generally prevalent in target field; and properties of carrier material used in formulation are taken into consideration for the development of proficient fermentation bioprocess. In recent years, extensive research in the fields of microbial taxonomy, molecular biology, genetic engineering, metabolic engineering, computer science, and nanotechnology has been carried out. The pool of information available from all the fields has improved the knowledge and understanding about the abovementioned parameters, as a result of which great advancement has taken place in the fermentation technology. Hence, efficiency, stability, cost-effectiveness, ease in application, and multifunctionality of biofertilizers have increased. It has led to enhancement in both soil fertility and productivity of agricultural crops. Furthermore, innovative research is required to produce biofertilizers which can completely replace the chemical biofertilizers and generate a plentiful amount of crops in a sustainable way.

References

Abd El-Fattah DA, Eweda WE, Zayed MS et al (2013) Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculants. Ann Agric Sci 58(2):111–118

Abd El-Lattief EA (2016) Use of *Azospirillum* and *Azotobacter* bacteria as biofertilizers in cereal crops: a review. Int J Res Eng Appl Sci 6(7):36–44

Abdel Ghany TM, Alawlaqi MM, Al Abboud MA (2013) Role of biofertilizers in agriculture: a brief review. Mycopathologia 11(2):95–101

Abi-Ghanem R, Carpenter-Boggs L, Smith JL et al (2012) Nitrogen fixation by US and middle eastern chickpeas with commercial and wild middle eastern inocula. ISRN Soil Sci 2012:1. https://doi.org/10.5402/2012/981842

Adholeya A, Das M (2012) Biofertilizers: potential for crop improvement under stressed conditions. In: Tuteja N, Gill SS, Tuteja R (eds) Improving crop productivity in sustainable agriculture. Wiley-VCH, Weinheim, pp 183–200

Agarwal S, Ahmad Z (2010) Contribution of the *Rhizobium* inoculation on plant growth and productivity of two cultivars of Berseem (*Trifolium alexandrinum* L.) in saline soil. Asian J Plant Sci 9:344–350

Akhtar M, Siddiqui Z (2009) Effects of phosphate solubilizing microorganisms and *Rhizobium* sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. Afr J Biotechnol 8(15):3489–3496

Alam S, Seth RK (2014) Comparative study on effect of chemical and biofertilizer on growth, development and yield production of paddy crop (*Oryza sativa*). Int J Sci Res 3(9):411–414

Alam S, Khalil S, Ayub N et al (2002) In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. Int J Agric Biol 4:454–458

Albareda M, Rodríguez-Navarro DN, Camacho M et al (2008) Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem 40:2771–2779

Allen EK, Allen ON (1950) The anatomy of nodular growths on roots of *Tribulus cistoides* L. Proc Soil Soc Am 14:179–183

Armada E, Portela G, Roldan A et al (2014) Combined use of beneficial soil microorganism and agro waste residue to cope with plant water limitation under semiarid conditions. Geoderma 232:640–648

Atieno M, Herrmann L, Okalebo R et al (2012) Efficiency of different formulations of *Bradyrhizobium japonicum* and effect of co-inoculation of *Bacillus subtilis* with two different strains of *Bradyrhizobium japonicum*. World J Microbiol Biotechnol 28:2541–2550

Auge RM, Toler HD, Saxton AM (2015) *Arbuscular mycorrhizal* symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25:13–24

Baby UI (2002) Biofertilizers in tea planters. Chronicle 98:395–396

Bakri Y, Akeed Y, Thonart P (2012) Comparison between continuous and batch processing to produce xylanase by *Penicillium canescens* 10-10c. Braz J Chem Eng 29(3):441–448

Baral RB, Adhikari P (2013) Effect of *Azotobacter* on growth and yield of maize. SAARC J Agri 11(2):141–147

Bashan Y, Holguin G, Lifshitz R (1993) Isolation and characterization of plant growth-promoting rhizobacteria. In: Glick BR, Thompson JE (eds) Methods in plant molecular biology and biotechnology. CRC Press, Boca Raton, pp 331–345

Bassey EE (2013) Trends in fermentation process, purification and recovering of biomolecules. Inter J Agri Biosci 2(6):340–343

Beijerinck MW (1888) Culture des *Bacillus radicola* aus den. Knöllchen. Bot Ztg 46:740–750

Beijerinck MW (1922) *Azotobacter chroococcum* als indikator van de vruchtbarrheid van den grond. K Ned Akad Wet Versl GewoneVergad Afd Natuurkd 30:431–438

Ben Rebah F, Tyagi RD, Prevost D (2002) Wastewater sludge as a substrate for growth and carrier for rhizobia: the effect of storage conditions on survival of *Sinorhizobium meliloti*. Bioresour Technol 83:145–151

Ben Rebah F, Prevost D, Yezza A et al (2007) Agro-industrial waste materials and wastewater sludge for rhizobial inoculant production: a review. Bioresour Technol 98:3535–3546

Berruti A, Lumini E, Balestrini R et al (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol 6:1559. https://doi.org/10.3389/fmicb.2015.01559

Bhardwaj D, Ansari MW, Sahoo RK et al (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13(1):1–9

Biederbeck VO, Geissler HJ (1993) Effect of storage temperatures on rhizobium meliloti survival in peat- and clay-based inoculants. Can J Plant Sci 73:101–110

Bissonnette N, Lalande R, Bordeleau LM (1986) Large-scale production of *Rhizobium meliloti* on Whey. Appl Environ Microbiol 52(4):838–841

Brimhall GH, Chadwick OA, Lewis CJ et al (1992) Deformational mass transport and invasive processes in soil evolution. Science 255:695–702

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154(2):275–304

Carnahan JE, Mortenson LE, Mower HF et al (1960) Nitrogen fixation in cell-free extracts of *Clostridium pasteurian*. Biochim Biophys Acta 44:520–535

Chanda S, Matai S, Chakrabatri S (1987) Deproteinized leaf juice as a medium for growth of *Rhizobium*. Indian J Exp Biol 25:573–575

Chang CH, Yang SS (2009) Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. Bioresour Technol 100:1648–1658

Coelho LF, de Lima CJB, Rodovalho CM et al (2011) Lactic acid production by new *Lactobacillus plantarum* LMISM6 grown in molasses: optimization of medium composition. Braz J Chem Eng 28(1):201–203

Cooper J (2004) Multiple responses of rhizobia to flavonoids during legume root infection. In: Callow JA (ed) Advances in botanical research incorporating advances in plant pathology. Academic, London, pp 1–62

Demain AL (2000) Microbial biotechnology. Trends Biotechnol 18(1):26–31

Dobereiner J, Day JM (1976) Associative symbioses in tropical grasses: characterization of microorganisms and dinitrogen fixing sites. In: Newton WE, Nymans CJ (eds) Proceedings of the first international symposium on nitrogen fixation. Washington State University Press, Pullman, pp 518–538

Dodd IC, Ruiz-Lozano JM (2012) Microbial enhancement of crop resource use efficiency. Curr Opin Biotechnol 23:236–242

Doroshenko EV, Bulygina ES, Spiridonova EM et al (2007) Isolation and characterization of nitrogen-fixing bacteria of the genus *Azospirillum* from the soil of a sphagnum peat bog. Mikrobiologiia 76(1):107–115

Douds DD, Nagahashi G, Pfeffer PE et al (2005) On-farm production and utilization of Arbuscular mycorrhizal fungus inoculum. Can J Plant Sci 85:15–21

Duraraj P, Maniarasan U, Nagarajan N (2016) Study of growth and yield of cluster bean in alkaline soil using organic manure and biofertilizers. Int J Pl An and Env Sci 6(2):22–27

Estrella MJ, Pieckenstain FL, Marina M et al (2004) Cheese whey: an alternative growth and protective medium for *Rhizobium loti* cells. J Ind Microbiol Biotechnol 31:122–126

FCO (1985) Biofertilizers and Organic Fertilizers in Fertilizer (Control) Order, 1985, National Centre of Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture, Govt of India. http://ncof.dacnet.nic.in/Training_manuals/Training_manuals_in_English/BF_and_OF_in_FCO.pdf. Accessed 2 Oct 2017

Fox AR, Soto G, Valverde C et al (2016) Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen-fixing bacterium *Pseudomonas protegens* Pf-5 X940. Environ Microbiol 18(10):3522–3534

Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321(1–2):35–59

Gandora V, Gupta RD, Bhardwaj KKR (1998) Abundance of *Azotobacter* in great soil groups of North-West Himalayas. J Indian Soc Soil Sci 46(3):379–383
Ghany TMA, Alawlaqi MM, Al Abboud MA (2013) Role of biofertilizers in agriculture: a brief review. Mycopath 11(2):95–101
Ghosh N (2004) Promoting bio-fertilizers in Indian agriculture. Econ Polit Wkly 39(52):5617–5625
Glick BR, Todorovic B, Czarny J et al (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242
Gomare KS, Mese M, Shetkar Y (2013) Isolation of *Azotobacter* and cost effective production of biofertilizer. Indian J Appl Res 3(5):54–56
Gurikar C, Naik MK, Sreenivasa MY (2016) *Azotobacter*: PGPR activities with special reference to effect of pesticides and biodegradation. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, vol 1. Springer, New Delhi, pp 229–244
Hameed S, Yasmin S, Malik S et al (2004) *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* strains isolated from cultivated legumes. Biol Fertil Soils 39(3):179–185
Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629
Hellriegel H, Wilfarth H (1888) Untersuchungenüber die Stickstoffnahrung der Gramineen und Leguminosen. Beilageheftzu der Zeitschrift des Vereinsfür die Rübenzucker-Industrie des DeutschenReiches, Bu'chdruckerei der "Post," Kayssler & Co, Berlin
Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97(20):8859–8873
Jaga PK, Upadhyay VB (2013) Effect of FYM, biofertilizer and chemical fertilizers on wheat. Asian J Soil Sci 8(1):185–188
Jain SK, Pathak DV, Sharma HR (2000) Alternate C substrate for mass production of *Rhizobium* inoculants. Haryana Agric Univ J Res 30:1–6
Javaid A (2009) Arbuscular mycorrhizal mediated nutrition in plants. J Plant Nutr 32(10):1595–1618
Jayaraj J, Yi H, Liang GH et al (2004) Foliar application of *Bacillus subtilis* AUBS 1 reduces sheath blight and triggers defense mechanisms in rice. J Plant Dis Protect 111(2):115–125
Kannaiyan S, Kumar K (2005) *Azolla* biofertilizers for sustainable rice production. Daya Publishing House, New Delhi
Kapulnic Y, Kigel J, Nur I et al (1981) Effect of *Azospirillum* inoculation on some growth parameters and n content of wheat, sorghum and panicum. Plant Soil 61:65–70
Kaushik BD (2014) Developments in Cyanobacterial biofertilizer. Proc Indian Nat Sci Acad 80(2):379–388
Kim KY, Jordan D, McDonald GA (1998) Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol Fertil Soils 26:79–87
Klironomos JN, Hart MM (2002) Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12:181–184
Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In Proceedings of the 4th international conference on plant pathogenic bacteria. Station de Pathologie Végétale et de Phytobactériologie, anger, 27 August−2 September
Kobiler D, Cohen-Sharon A, Tel-Or E (1981) Recognition between the N_2-fixing *Anabaena* and the water fern *Azolla*. FEBS Lett 133:157–160
Kukreja K, Suneja S, Goyal S et al (2004) Phytohormone production by *Azotobacter*-a review. Agric Rev 25(1):70–75
Kumar A, Kumari B, Mallick MA (2016) Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. Int J Pharm Pharm Sci 8(2):37–40
Kure AM, Patil SR, Jadhao VG (2016) Performance evaluation of developed lab scale fermenter. Int J Agric Engg 9(2):202–209
Lavania M, Nautiyal CS (2013) Solubilization of tricalcium phosphate by temperature and salt tolerant *Serratia marcescens* NBRI1213 isolated from alkaline soils. Afr J Microbiol Res 7(34):4403–4413. https://doi.org/10.5897/AJMR2013.5773

Lazcano C, Barrios-Masias FH, Jackson LE (2014) Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes. Soil Biol Biochem 74:184–192

Leo Daniel AE, Venkateswarlu B, Suseelendra D et al (2013) Effect of polymeric additives, adjuvants, surfactants on survival, stability and plant growth promoting ability of liquid bioinoculants. J Plant Physiol Pathol 01(02):1–5. https://doi.org/10.4172/2329-955X.1000105

Li XX, Liu Q, Liu XM et al (2016) Using synthetic biology to increase nitrogenase activity. Microb Cell Factories 15:43

Maier RJ, Moshiri F (2000) Role of the *Azotobacter vinelandii* nitrogenase-protective shethna protein in preventing oxygen mediated cell death. J Bacteriol 182(13):3854–3857

Malusa E, Sas-Paszt L, Ciesielska J (2012) Technologies for beneficial microorganisms inocula used as biofertilizers. Sci World J 2012:1. https://doi.org/10.1100/2012/491206

Mia MAB, Shamsuddin ZH (2010) *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. Afr J Biotech 9:6001–6009

Mishra DJ, Singh R, Mishra UK et al (2013) Role of bio-fertilizer in organic agriculture: a review. Res J Recent Sci 2(1):39–41

Moore D, Robson GD, Trinci APJ (2011) 21st century guidebook to fungi. Cambridge University Press, New York

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

Nagappan R (2013) Biopesticides and biofertilizers: ecofriendly sources for sustainable agriculture. J Biofertil Biopestici 4:1–2. https://doi.org/10.4172/2155-6202.1000e112

Nalawde AA, Bhalerao SA (2015) Comparative account of effect of biofertilizers on the growth and biochemical parameters of *Vigna mungo* (L. Hepper). Int J Adv Res Biol Sci 2(5):62–66

Nayak T, Patangray AJ (2015) Biofertilizer-beneficial for sustainable agriculture and improving soil fertility. Asia J Multidiscip Stud 3(2):189–194

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 7:540–556

Nobbe F, Hiltner L (1895) Inoculation of the soil for cultivating leguminous plants. US Patent 5,70,813, 3 November 1896

Okon Y, Albercht SL, Burris RH (1977) Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. Appl Environ Microbiol 33:85–88

Pandey A, Selvakumar P, Soccol CR et al (1999) Solid state fermentation for the production of industrial enzymes. Curr Sci 77(1):149–162

Parthiban K, Manikandan S, Ganesapandian S (2011) Production of cellulose I microfibrils from *Rhizobium* sp. and its wound healing activity on mice. Asian J Applied Sci 4:247–254

Pathak DV, Kumar M (2016) Microbial inoculants as biofertilizers and biopesticides. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, vol 1. Springer, New Delhi, pp 197–209

Patil N, Choudhri SD, Gawade SS et al (2013) Effect of different C sources on production and stability of biofertilizer. Int J of Adv Biotec and Res 4(1):85–95

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 48:1–7

Peng X, Börner RA, Nges IA et al (2014) Impact of bioaugmentation on biochemical methane potential for wheat straw with addition of *Clostridium cellulolyticum*. Bioresour Technol 152:567–571

Pikovskaya RI (1948) Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologia 17:362–370

Pindi PK, Satyanarayana SDV (2012) Liquid microbial consortium a potential tool for sustainable soil health. J Biofertil Biopestici 03:124. https://doi.org/10.4172/2155-6202.1000124

Podile AR, Kishore GK (2006) Plant growth promoting rhizobacteria. In: Gnanamanickam SS (ed) Plant associated bacteria. Springer, Dordrecht, pp 195–230

Ponmurugan P, Gopi C (2006) In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. Afr J Biotechnol 5:348–350
Prasad MP (2014) Optimization of fermentation conditions of phosphate solubilization bacteria – a potential bio-fertilizer. Merit Res J Microbiol Biol Sci 2(2):31–35
Prasanna R, Nain L, Pandey AK et al (2012) Microbial diversity and multidimensional interactions in the rice ecosystem. Arch Agron Soil Sci 58(7):723–744
Rafael A, Juan Cesar FO, Leonardo C (2017) Metabolic engineering of a diazotrophic bacterium improves ammonium release and biofertilization of plants and microalgae. Metab Eng 40:59. https://doi.org/10.1016/j.ymben.2017.01.002
Reddy KR, Reddy GB, Reddy MR et al (1977) Effects of *Azotobacter* inoculation and nitrogen levels on yield of sorghum. Indian J Agron 22(4):203–205
Richter DD, Markewitz D (1995) How deep is soil? Bio Sci 45:600–609
Rillig MC, Aguilar-Trigueros CA, Bergmann J et al (2015) Plant root and mycorrhizal fungal traits for understanding soil aggregation. New Phytol 205:1385–1388
Rodrigues PE, Rodregues LS, Oliveira ALM et al (2008) *Azospirillum amazonense* inoculation: effects on growth, yield and N_2-fixation of rice (*Oryza sativa* L.) Plant Soil 302:249–261
Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
Rosenberger RF, Elsden SR (1960) The yields of *Streptococcus faecalis* grown in continuous culture. J Gen Microbiol 22:726–739
Roychowdhury D, Paul M, Kumar Banerjee S (2015) Isolation, identification and characterization of phosphate solubilising bacteria from soil and the production of biofertilizer. Int J Curr Microbiol App Sci 4(11):808–815
Saithi S, Borg J, Nopharatana M et al (2016) Mathematical modelling of biomass and enzyme production kinetics by *Aspergillus niger* in solid-state fermentation at various temperatures and moisture contents. J Microb Biochem Technol 8:123–130
Secilia J, Bagyaraj DJ (1987) Bacteria and actinomycetes associated with pot cultures of vesicular arbuscular mycorrhizas. Can J of Microbiol 33:1069–1073
Senoo K, Kaneko M, Taguchi R et al (2002) Enhanced growth and nodule occupancy of red kidney bean and soybean inoculated with soil aggregate-based inoculants. Soil Sci Plant Nutr 48(2):251–259
Sethi SK, Adhikary SP (2012) Cost effective pilot scale production of biofertilizer using *Rhizobium* and *Azotobacter*. Afr J Biotechnol 11(70):13490–13493
Shanware A, Kalkar S, Trivedi M (2014) Potassium solublisers: occurrence, mechanism and their role as competent biofertilizers. Int J Curr Microbiol App Sc 3(9):622–629
Sharma S, Pant D, Singh S et al (2007) Mycorrhizae in Indian agriculture. In: Hamel C, Plenchette C (eds) Mycorrhizae in crop production. Haworth Press, Binghampton, pp 239–291
Sharma SB, Sayyed RZ, Trivedi MH et al (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2:587. https://doi.org/10.1186/2193-1801-2-587
Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353
Singh AK, Gauri C, Bhatt RP et al (2012) Comparative study of carrier based materials for rhizobium culture formulation. Indian J Agric Res 46(4):344–349
Singh S, Singh BK, Yadav SM et al (2014) Potential of biofertilizers in crop production in Indian agriculture. Amer J Plant Nutr Fertil Tech 4(2):33–40
Singh M, Dotaniya ML, Mishra A et al (2016a) Role of biofertilizers in conservation agriculture. In: Bisht JK, Meena VS, Mishra PK et al (eds) Conservation agriculture: an approach to combat climate change in Indian Himalaya. Springer, Singapore, pp 113–134
Singh JS, Kumar A, Rai AN et al (2016b) *Cyanobacteria*: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol 7:529. https://doi.org/10.3389/fmicb.2016.00529
Stewart WDP (1969) Biological and ecological aspects of nitrogen fixation by free-living microorganisms. Proc R Soc 172:367–388

Subba Rao NS (1977) Soil microorganisms and plant growth. Oxford/IBH Publishing Co, New Delhi
Subramaniyam R, Vimala R (2012) Solid state and submerged fermentation for the production of bioactive substances: a comparative study. Int J Sci Nat 3(3):480–486
Tao GC, Tian SJ, Cai MY et al (2008) Phosphate solubilizing and mineralizing abilities of bacteria isolated from soils. Pedosphere 18(4):515–523
Tarrand JJ, Krieg NR, Döbereiner J (1978) A taxonomic study of the *Spirillum lipoferum* group, with description of a new genus, *Azospirillum* gen. nov., and two species, *Azospirillum lipoferum* (*Beijerinck*) com nov. and *Azospirillum brasilense* sp. nov. Can J Microbiol 24:967–980
Tensingh Baliah N, Jeeva P (2016) Isolation, identification and characterization of phosphate solubilizing bacteria isolated from economically important tree species. Int J Sci Nat 7(4):870–876
Trivedi P, Pandey A (2007) Application of immobilized cells of *Pseudomonas putida* to solubilise insoluble phosphate in broth and soil conditions. J Plant Nutr Soil Sci 170:629–631
Trivedi M, Kalkar S, Shanware A (2016) Isolation, characterization & development of liquid formulations of potassium solubilizing fungi. Int J Adv Res 4(9):999–1003
Vaishampayan A, Sinha RP, Hader DP et al (2001) Cyanobacterial biofertilizers in rice agriculture. Bot Rev 67(4):453–516
van Rhijn P, Vanderleyden J (1995) The *Rhizobium*-plant symbiosis. Microbiol Rev 59(1):124–142
van Rossum DV, Schuurmans FP, Gillis M et al (1995) Genetic and phenotypic analysis of *Bradyrhizobium* strains nodulating Peanut (*Arachis hypogaea* L.) roots. Appl Environ Microbiol 61:1599–1609
Verma M, Sharma S, Prasad R (2011) Liquid biofertilizers: advantages over carrier- based biofertilizers for sustainable crop production. Newsl Intern Soc Environ Bot 17:2
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Waheed A, Afzal A, Sultan T et al (2014) Isolation and biochemical characterization of rhizobium from pea crop at Swabi. Int J Biosci 4(8):231–240
Wani SA, Chand S, Ali T (2013) Potential use of *Azotobacter chroococcum* in crop production: an overview. Curr Agri Res 1(1):35–38. 10.12944/CARJ.1.1.04
Wani SA, Chand S, Wani MA et al (2016) *Azotobacter chroococcum*–a potential biofertilizer in agriculture: an overview. In: Kakeem KR, Akhtar J, Sabir M (eds) Soil science: agricultural and environmental prospectives. Springer, Cham, pp 333–348
Wei GH (2002) *Rhizobium indigoferae* sp. nov. and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from *Indigofera* spp. and *Kummerowia stipulacea*. Int J Syst Evol Microbiol 52(6):2231–2223
Weir BS (2016) The current taxonomy of rhizobia http://www.rhizobia.co.nz/taxonomy/rhizobia.html. Accessed 4 Feb 2017
Yadav AK, Chandra K (2014) Mass production and quality control of microbial inoculants. Proc Indian Natn Sci Acad 80(2):483–489
Yehya M, Hamze M, Mallat H et al (2013) Prevalence and antibiotic susceptibility of *Bacteroides fragilis* group isolated from stool samples in North Lebanon. Braz J Microbio l44(3):807–812
Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63(4):968–89, table of contents
Zaiadan BK, Motorin DN, Baimakhanova GB et al (2014) Promising microbial consortia for producing biofertilizers for rice fields. Mikrobiologiia 83(4):467–474
Zohar-Perez C, Chet I, Nussinovitch A (2005) Mutual relationships between soils and biological carrier systems. Biotechnol Bioeng 92(1):54–60

Cyanobacteria: Source of Organic Fertilizers for Plant Growth

13

Y.K. Jhala, D.G. Panpatte, and R.V. Vyas

Abstract

Agriculturally beneficial microorganisms are an important tool for soil and plant health management. Cyanobacteria are best known for their ability to fix nitrogen, degrade organic waste and remediate heavy metals, agrochemicals and other pollutants. They are also involved in nutrient cycling and suppression of phytopathogens and also produce plant growth-promoting substances such as vitamins, hormones and enzymes. Cyanobacteria-based inoculants can improve the soil and plant health as well as minimize the cost of crop production. In this chapter, we are going to elaborate beneficial effects of cyanobacteria.

Keywords

Cyanobacteria • Organic waste • Phytopathogens • Nutrient cycling

13.1 Cyanobacteria Used as Biofertilizer

Besides increasing growth and production of crop, cyanobacteria play a vital role in conservation of soil fertility (Song et al. 2005). Benefits of algal biofertilizer include the following:

1. Due to filament-like structure and production of adhesives, it improves porosity of soil.

Y.K. Jhala (✉) • D.G. Panpatte • R.V. Vyas
Department of Agricultural Microbiology, B. A. College of Agriculture, Anand Agricultural University, Anand 388110, Gujarat, India
e-mail: ykjhala@aau.in

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_13

2. Enhancement of plant growth and development by secretion of plant growth-promoting substances, viz. hormones, vitamins, amino acids and other metabolites (Roger and Reynaud 1982; Rodriguez et al. 2006).
3. As it possesses jelly-like structure, it will enhance water holding capacity of soil (Roger and Reynaud 1982).
4. Decomposition of dead algae will lead to increase in soil (Saadatnia and Riahi 2009).
5. Reduces soil salinity (Saadatnia and Riahi 2009).
6. Prevents weeds growth (Saadatnia and Riahi 2009).
7. By production of organic acids, the availability of phosphorus to crops will be increased (Wilson 2006).

13.2 Cyanobacteria

Cyanobacteria comprise of Gram-negative photoautotrophic prokaryotes having large, heterogeneous and polyphyletic assembly of simple plants which perform oxygenic photosynthesis. Generally microalgae occur in water including freshwater and oceanic or salty water. They can also be found in harsh environments, e.g. hot springs (Anderson 2005), hypersaline waters, freezing zones and deserts (Singh 2014). From different agroecological regions, diazotrophic strains of cyanobacteria like *Nostoc linckia*, *Anabaena variabilis*, *Aulosira fertilissima*, *Calothrix* sp., *Tolypothrix* sp. and *Scytonema* sp. were isolated and efficiently utilized as biofertilizer in rice cultivation (Prasad and Prasad 2001). Nitrogen-fixing cyanobacteria comprise of heterocysts responsible for nitrogen fixation and vegetative cells which carry out photosynthesis and reproduction. Temperature range of 45–70° C (Castenholz 1978) and optimum 7.5–10 pH favour growth of cyanobacteria (Fogg 1956).

13.2.1 Cyanobacteria as Nitrogen-Fixing Biofertilizer

Cyanobacterial diazotrophy is carried out in two forms, i.e. free-living and symbiotic associations with water fern *Azolla*, cycads, *Gunnera*, etc. Cyanobacteria occur in both unicellular and multicellular filamentous form. Some cyanobacterial strains contain specialized thick-walled cells known as heterocyst containing nitrogenase enzyme being site of nitrogen fixation. All heterocystous cyanobacteria are aerobic photodiazotrophs and are considered to be significant common flora of arable lands. Nitrogen fixed by cyanobacteria is released either through secretion or upon degradation of cyanobacterial cells after death in the form of ammonia, polypeptides, free amino acids, vitamins and auxin-like substances (Subramanian and Sundaram 1986). Nitrogen contribution of cyanobacteria ranges from 20 to 30 kg N/ha and is thereby known to reduce significant amount of chemical nitrogenous fertilizers for crop production (Issa et al. 2014).

Favourable situations to switch over nitrogen fixation by cyanobacteria include unavailability of combined nitrogen and aerobic condition. Presence of less than

40 ppm ammoniacal-N cannot repress nitrogen fixation in soil-rice-algae system (Venkataraman 1979a, b); in the same way, at 30 ppm level of urea-nitrogen, cyanobacterial diazotrophy was not inhibited (Mekonnen et al. 2002). However, higher amount of combined nitrogen showed inhibition of cyanobacterial growth and nitrogen fixation ability.

Cyanobacterial strains like *Anabaena* and *Nostoc* can colonize soil and rocks and are having nitrogen fixation ability up to 20–25 kg/ha. Diazotrophic cyanobacterial strains, viz. *Nostoc*, *Anabaena*, *Tolypothrix* and *Aulosira*, are used as inoculants for rice crop. Water fern *Azolla* contains blue-green algae *Anabaena* which contributes up to 60 kg N/ha/season besides enriching soil with organic matter (Moore 1969) and considered to be leading cyanobacterial biofertilizer. Dry green algae contain high amount of macro- and micronutrients as well as amino acids (El-Fouly et al. 1992; Mahmoud 2001). Being adopted to aquatic habitat, blue-green algae can be easily multiplied on sewage and saline water. Kulk (1995) and Adam (1999) reported that the nitrogen-fixing cyanobacterium *Nostoc muscorum* can promote plant growth which corresponds to nitrogenase as well as nitrate reductase activities of cyanobacteria as well as amino acids and peptides produced by them.

Numerous cyanobacterial species like *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima* and *Tolypothrix tenuis* are considered to be efficient biofertilizers. In Asian countries like China, Vietnam, India, etc., cyanobacterial biofertilizers are popular as substitute of nitrogenous fertilizers in paddy cultivation (Venkataraman 1972; Lumpkin and Plucknett 1982). Paddy ecosystem provides favourable environment for the growth of cyanobacteria considering their requisite for light, water, temperature, humidity and nutrients.

Organic carbon content of soil is generally used as criteria to determine soil fertility. During green revolution, there was considerable increase in use of inorganic fertilizers without adding organic inputs which led to the exhaustion of soil carbon assets and results in unfertile soil. Photosynthetic microorganisms such as algae and cyanobacteria can add organic carbon in soil. De and Sulaiman (1950) reported build-up of organic matter by inoculation of cyanobacteria. Nekrasova and Aleksandrova (1982) established that algal biomass contributed considerably to humus formation, using 15^N study. Roger et al. (1987) showed that in favourable environment a good algal bloom in rice fields can add about 6–8 t of fresh biomass. Under laboratory conditions within 6 months, 0.03% (672 kg/ha) increase in soil organic carbon was observed by enrichment of native algae (Kaushik 1985), whereas inoculation of halotolerant cyanobacterial strains to sodic soils led to an addition of 5.3–7.6 t carbon/ha in a cropping season (Subhashini and Kaushik 1984). Microbial biomass carbon serves as an indicator of changing soil condition. The microbial biomass carbon was significantly increased in all the treatments who received cyanobacterial inoculation over uninoculated control (Albiach et al. 2000).

13.2.2 Cyanobacteria Improves Availability of Phosphorus

The second most important nutrient for plant growth is phosphorus. Unavailability of applied phosphorus is the major issue to be addressed as P gets fixed and thereby unavailable to the crops. Cyanobacteria can contribute to increase availability of phosphorus by solubilizing organic phosphorus through production of phosphatase enzymes. As far as inorganic forms of phosphorus like $(Ca)_3(PO_4)_2$, $FePO_4$, $AlPO_4$ and hydroxyapatite $[Ca_5(PO_4)_3OH]$ are concerned, cyanobacteria solubilize such compounds by wither producing chelators or by production of organic acids.

After death of cyanobacteria, phosphate present in the cyanobacterial cell PO_4 gets released in the soils which is easily available to plants and other organisms following mineralization. Fuller and Roger (1952) reported that, as compared to inorganic phosphorus, phosphate uptake was significantly higher when provided through algal inoculation and when both were delivered in equivalent quantities for longer time. Possible mechanisms for increased availability were proposed as cyanobacteria scavenge available phosphorus and incorporate it in to their cell biomass which is then made available to plants by its slow release through secretion, autolysis or microbial decomposition of dead cells.

Phosphate availability gets increased in soil, making it rich in organic matter, due to secretion of phosphatase enzymes as well as organic acids by cyanobacteria (Rother et al. 1988). Fuller and Roger (1952) reported increased uptake of phosphorus by plants from algal biomass as compared to inorganic phosphatic fertilizer when applied in same quantity. The reasons for improved uptake were more availability of phosphate from algal biomass for long time period, fixation of phosphate that does not occur when applied through algal material as well as incorporation of available phosphate in cellular biomass of algae.

13.2.3 Cyanobacteria Improve Physical Properties of Soil

Various compounds like polysaccharides, peptides and lipids are being released by cyanobacteria during their growth in soil which act as glue and hold soil particles together in the form of microaggregates. Moreover, fibre-like structures of polysaccharides can also trap clay particles and form microaggregates. These microaggregates when combined together form macroaggregates, which are larger soil aggregates. As algal filaments grow, they get intermingled which may also facilitate binding of soil particles with organic carbon. Due to its ability to enhance soil aggregation, cyanobacteria are known to improve soil quality of arid or semiarid regions. Kaushik 1998 showed that due to cyanobacterial inoculation, there were significant increases in soil aggregate stability as well as water holding capacity due to increase in the polysaccharide content of soils by algae (Roychoudhury et al. 1979; Singh 1961). Moreover, macroaggregates formed due to mucilaginous filaments of cyanobacteria can withstand wind- and water-mediated soil erosion, especially in light and sandy soils exposed to substantial cropping, and also favoured better seedling emergence of upland crops sown after the paddy harvest (Rogers and

Burns 1994). Rogers and Burns (1994) reported that inoculation of cyanobacteria improved the consistency of soil aggregate which in turn improved water holding capacity and aeration in soils that results in reduction of compactness of soils and supports below-ground biodiversity.

13.2.4 Reclamation of Saline Soil

As cyanobacteria are able to withstand extreme environments, they can be employed for improvement of the saline soils. Due to excessive amount of salts in upper layer, saline soils are less productive, firm and impermeable to water. Salt-affected soils are divided into alkaline or saline based on salt content. Alkaline soil is categorized by a high pH and transferrable sodium ions as well as detectable amounts of carbonates and can undergo extensive clay dispersal which results into low hydraulic conductivity and reduced soil aeration which makes soil sterile. On the other hand, saline soil is having high concentration of soluble salts that result in high osmotic tension to plant roots for water and nutrient absorption (Pandey et al. 1992). Cyanobacteria are known to produce oxalic acids which enable them to solubilize nutrients from insoluble carbonate (Singh 1961). Due to production of acids, cyanobacteria can lower down pH, electric conductivity and hydraulic conductivity of saline and alkaline soil which improves soil aggregation (Kaushik and Subhashini 1985). There are certain physiological benefits linked with cyanobacteria which empower them to survive under stress which includes restriction of sodium ion influx (Apte et al. 1987), concentrating inorganic (K^+ ion) or organic osmoregulators (Reed et al. 1984). Salt tolerance ranging from 7 to 15 g/L was observed in cyanobacterial strains such as *Anabaena oscillarioides*, *A. aphanizomenoides* and *Microcystis aeruginosa* (Moisander et al. 2002). They are also recognized for the production of exo-polysaccharides, which facilitates soil particle binding and thereby plays an important role in upgrading of soil moisture holding capacity. Flaibani et al. (1989) showed that exo-polysaccharides from cyanobacteria also contribute to remediation of desert soil.

13.2.5 Cyanobacteria as Plant Growth Promoters

Cyanobacteria produce plant growth hormones like gibberellins, cytokinin, auxin or abscisic acids, vitamins particularly vitamin B or amino acids, antibiotics and toxins. Majority of studies on the plant growth-promoting effects of cyanobacteria associated with rice crop have shown that cyanobacterial inoculation could enhance rice seed germination and root and shoot growth (Misra and Kaushik 1989a, b). Moreover, Gantar et al. (1995a, b) reported that inoculation of cyanobacteria enhanced root dry weight and chlorophyll content of wheat due to release of extracellular substances. Cyanobacteria are, on a wider scope, exploited as commercial bioinoculant for plant growth promotion, as they have wider biodiversity, can survive in variety of environments and have faster growth rate and simpler nutrient requirement (Ruffing 2011).

13.3 Crop Response

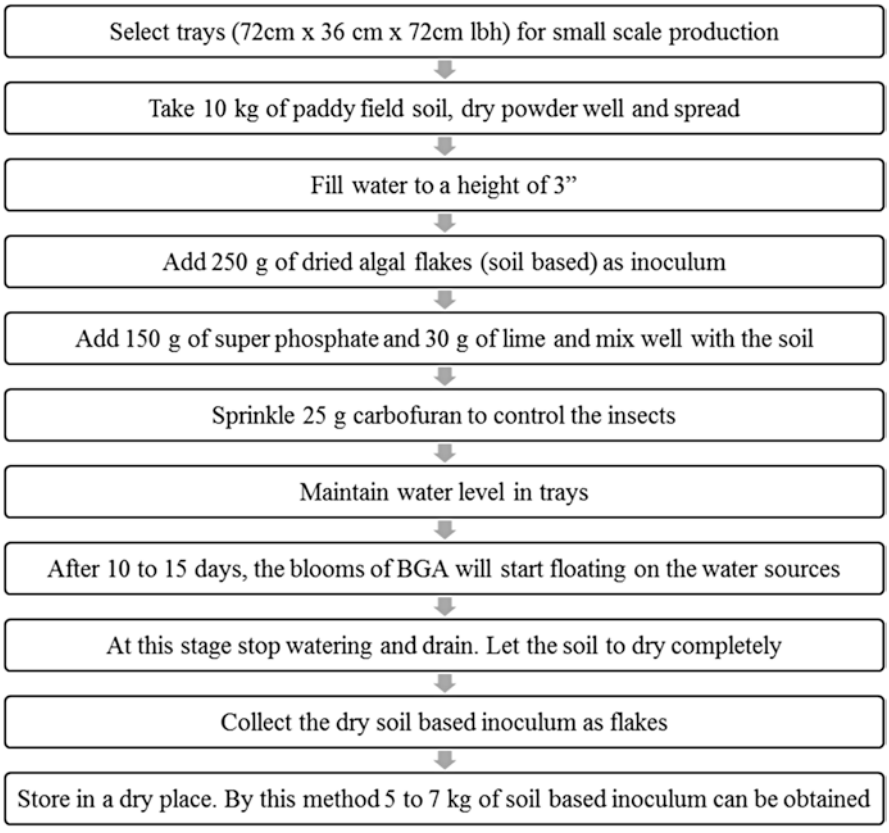
Singh et al. (1972) reported that, for rice grain yield, treatment receiving inoculation of algae and 60 kg/ ha urea was comparable with treatment receiving 120 kg nitrogen alone. From the results of field experiments done earlier, it seems that algal inoculation brings about 14% increases in paddy grain yield over the treatments and 16% over the control, corresponding to about 4.5 quintal grain per hectare per crop (Relwani 1965; Relwani and Subrahmanyan 1963; Sankaram et al. 1966). Aiyer et al. (1972) carried out an experiment comprising of 0, 50, 100 and 150 kg N as urea, wherein they observed statistically non-significant interaction between nitrogen and algalization, representing a constant positive influence of algal inoculation at various level of nitrogen. Results of farmers' field demonstrations carried out at Haryana showed 10–15% increase in rice yield in the presence of 150 kg/ha fertilizer nitrogen (Kaushik 1998). Generally, the response of algalization was positive at every level of nitrogenous fertilizers in the field, while the response is lower at a higher level of chemical fertilizers. Generally algal biofertilizers are recommended as a supplement of chemical nitrogenous fertilizers, and their effect remains visible even in the presence of high levels of fertilizer nitrogen (Venkataraman 1979a, b). Algal fertilization can also reduce sterility in rice from 16% in control to 11% in algalized series (Tahmida Begum et al. 1990). Results of farmers' field demonstration trials under All India Project on Algae showed that application of cyanobacterial biofertilizer in unfertilized fields gave 10–15% increase in rice grain yield, whereas in combination with low doses of chemical fertilizer, nitrogen yield equivalent to 25 kg N/ha could be obtained, and even at higher levels of fertilizer nitrogen, comparable benefits could be observed. Combined application of cyanobacterial biofertilizer along with other biofertilizers in consortia-phosphate-solubilizing *Pseudomonas striata* (PS) and mixed inoculum of vesicular arbuscular mycorrhiza (VAM) in rice followed by wheat showed the beneficial effects of inoculation in both the crops and also increased protein content of paddy and wheat to the extent of 9.18% and 10.25%, respectively (Manjunath et al. 2011). Similarly De and Mandal (1956) reported nitrogen fixation by cyanobacteria ranging from 13.8 to 44.4 kg/ha of nitrogen in cropped area in six rice-growing soils of West Bengal. In Japan, Watanabe (1951) estimated that addition of 20 kg/ha nitrogen is possible by application of *Tolypothrix tenuis*-based algal biofertilizer. Similarly, Watanabe (1951) confirmed addition of 18–45 kg N/ha by cyanobacteria using acetylene reduction technique. MacRae and Castro (1969) reported addition of 10–15 kg N/ha in rice fields due to cyanobacteria using 15 N technique. Henriksson (1971) reported that, in the field having abundant quantity of *Nostoc*, yearly nitrogen fixation rate was 15–51 kg N/ha/year. Metting (1981) has observed addition of up to 90 kg N/ha/year by algal inoculation. Soil physico-chemical properties and climate and biotic strains are the main factors limiting nitrogen fixation by cyanobacteria in paddy field. Application of phosphatic fertilizers was found to have a positive effect on establishment and growth of diazotrophic strains of cyanobacteria (Jha et al. 1965). Nitrogen fixed by cyanobacteria gets released into soil by either exudation or decomposition of cyanobacterial cell after death. Death of algal biomass is linked

with alternating cycles of desiccation and wetting during cultivation or finally after the harvest of the crop. Cyanobacterial inoculation results in gradual build-up of soil fertility with a residual effect on succeeding crop also. Nitrogen uptake studies indicated that 39% of the nitrogen from 15 N-labelled *Aulosira* sp. spread on soil and 51% from the algae incorporated into the soil was recovered in the rice crop. It shows that nitrogen fixed through cyanobacteria is readily available to rice (Wilson et al. 1980). Using 15 N, Reynault et al. (1975) have also shown that at least some of the nitrogen fixed and liberated by *Westiellopsis prolifica* is assimilated by rice plant.

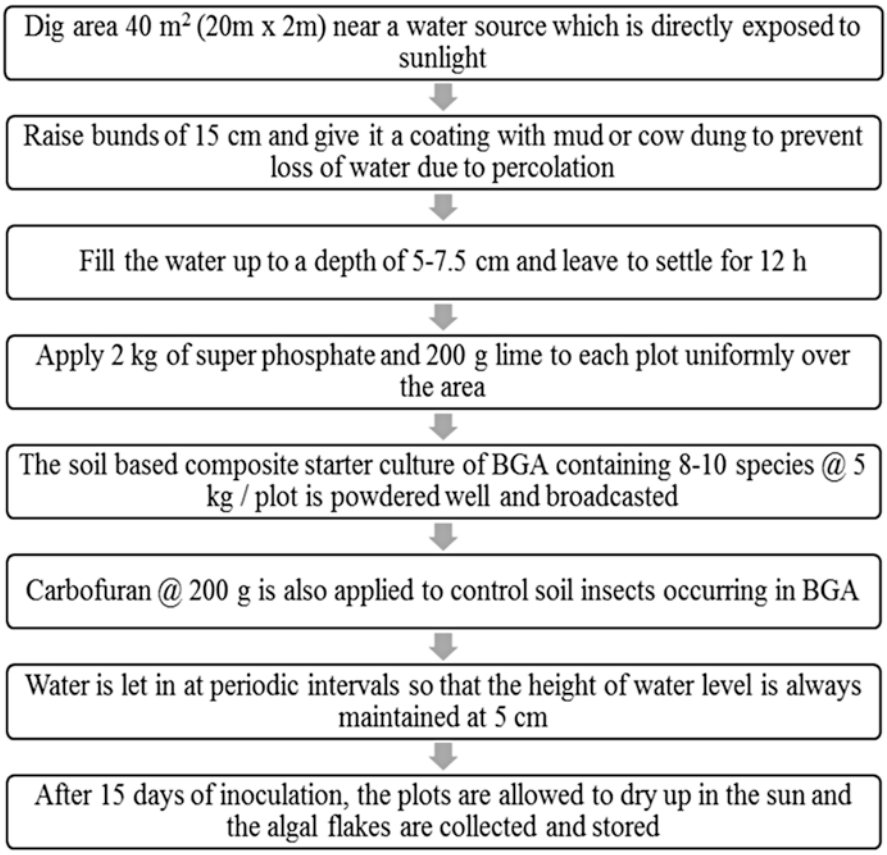
13.4 Mass Production of Cyanobacteria

Mass multiplication of cyanobacterial biofertilizer can be carried out in galvanized trays and also in field conditions. However, the large-scale production is advisable under field condition which is easily adopted by farmers.

13.4.1 Multiplication in Trays

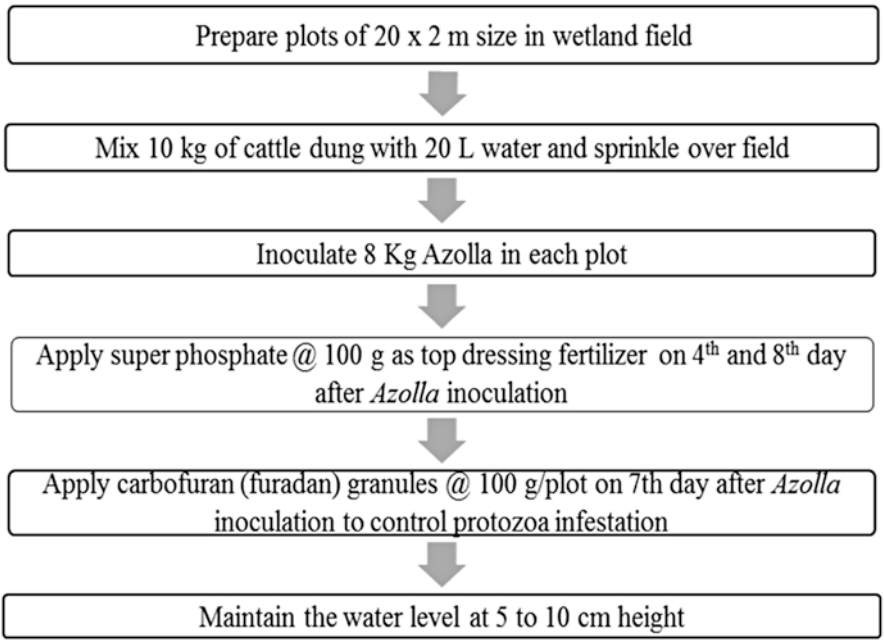


13.4.2 Multiplication Under Field Condition



13.4.3 Mass Production of Azolla

Azolla can be maintained in a nursery round the year, and from this azolla can be broadcasted in rice fields. A simple *Azolla* nursery method for mass multiplication of *Azolla* has been evolved for easy adoption by the farmers.



13.5 Methods of Application of BGA Biofertilizer

Mix 500 g quantity (recommended for one acre) of cyanobacterial biofertilizer with 4 kg dried and sieved farm soil, and broadcast this mixture after 3–6 days after transplanting in rice. If large quantity of cyanobacterial biofertilizer is applied, then it accelerates the reproduction and establishment of algae in the field. Care should be taken to maintain waterlogged condition for about 10–12 days after inoculation to allow good growth of algae**.** One third dose of recommended nitrogenous fertilizer can be supplemented with cyanobacteria-based biofertilizers. Routine farm management practices including pest control do not have any effect on establishment and activity of cyanobacteria in the field.

13.5.1 Precautions

When agrochemicals like fertilizer, pesticides, weedicides, insecticides, etc. are applied in the field, apply algae after 3–4 days of chemical application. If minute quantity of phosphatic fertilizer is added after application of cyanobacteria, its growth will be accelerated. Such blanket application should be applied as a part of total fertilizer dose to be incorporated for rice. One should apply cyanobacterial biofertilizer for at least four successive seasons to have collective effect. Repeated

application of cyanobacterial biofertilizer is not required as upon application; it will get established in the field and regrow once the environment becomes favourable.

References

Adam MS (1999) The promotive effect of cyanobacterium Nostoc muscorum on the growth of some crop plants. Acta Microbiol Pol 48(2):163–171

Aiyer RS, Salahudeen S, Venkataraman GS (1972) Long term algalization field trial with high yielding varieties of rice (Orvza sativa L.) Indian J Agric Sci 42:380–383

Albiach R, Canet R, Pomares F, Ingelmo F (2000) Microbiomass content and enzymatic after the application of organic amendments to a horticultural. Soil Bior Teach 75:43–48

Anderson RA (2005) Algal culturing techniques. Elsevier Academic press, Oxford

Apte SK, Reddy BR, Thomas J (1987) Relationship between sodium influx and salt tolerance of nitrogen-fixing cyanobacteria. Appl Environ Microbiol 53:1934–1939

Castenholz RW (1978) The biogeography of hot spring algal through enrichment cultures. Mitt Int Ver Limnol 21:296–315

De PK, Mandal LN (1956) Fixation of nitrogen by algae in rice soil. Soil Sci 81:453–458

De PK, Sulaiman M (1950) The influence of algal growth in the rice fields on the yield of crops. Indian J Agric Sci 20:327–342

El-Fouly MM, Abdalla FE, Shaaban MM (1992) Multipurpose large scale production of micro-algae biomass in Egypt. In: Abstracts of Proceedings on 1st Egyptian Etalian Symptoms on Biotechnology, Assiut, Egypt, 21–23, Nov. 1992

Flaibani A, Olsen Y, Painter TJ (1989) Polysaccharides in desert reclamation: compositions of exocellular proteoglycan complexes produced by filamentous blue-green and unicellular green edaphic algae. Carbohydr Res 190:235–248

Fogg GE (1956) The comparative physiology and biochemistry of the blue-green algae. Bacteriol Rev 20:148–165

Fuller WH, Roger RN (1952) Utilisation of the phosphorus of algal cells as measured by the Neubauer technique. Soil Sci 74:417–429

Gantar M, Kerby NW, Rowell P, Obreht Z, Scrimgeour R (1995a) Colonization of wheat (Triticum vulgare L.) by N2-fixing cyanobacteria. IV. Dark nitrogenase activity and effects of cyanobacteria on natural 15N abundance on plants. New Phytol 129:337–343

Gantar M, Rowell P, Kerby NW, Sutherland IW (1995b) Role of extracellular polysaccharide in the colonization of wheat (Triticum vulgare L.) roots by N2-fixing cyanobacteria. Biol Fertil Soils 19:41–48

Henriksson E (1971) Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil, Special Volume: 415

Issa AA, Abd-Alla MH, Ohyama T (2014) Nitrogen fixing Cyanobacteria: future prospect. In: Ohyama T (ed) Advances in biology and ecology of nitrogen fixation. INTECH publisher, Rijeka

Jha KK, Ali MA, Singh R, Bhattacharya PB (1965) Increasing rice production through the inoculation of Tolypothrix tenuis, a nitrogen fixing blue green algae. J Indian Soc Soil Sci 13:161–166

Kaushik BD (1985) Effect of native algal flora on nutritional and physico- chemical properties of sodic soils. Acta Bot Indica 13:143–147

Kaushik BD (1998) Cyanobacterial biotechnology G Subramanian. Science Publs Inc., Enfield, pp 211–222

Kaushik BD, Subhashini D (1985) Amelioration of salt affected soils with blue-green algae: improvements in soil properties. Proc Ind Natl Sci Acad 51:380–389

Kulk MM (1995) The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur J Plant Pathol 101(6):85–599

Lumpkin TA, Plucknett DL (1982) Azolla as a green manure: use and management in crop production: West view tropical agriculture series No.5. Westview Press, Boulder
MacRae IC, Castro TF (1969) Nitrogen fixation in some tropical soils. Soil Sci 103:277
Mahmoud MS (2001) Nutritional status and growth of maize plants as affected by green microalgae as soil additives. J Biol Sci 1:475–479
Manjunath M, Prasanna R, Sharma P, Nain L, Singh R (2011) Developing PGPR consortia using novel genera Providencia and Alcaligenes along with cyanobacteria for wheat. Arch Agron Soil Sci 57:873–883
Mekonnen AE, Prasanna R, Kaushik BD (2002) Response of Anabaena species to different nitrogen source. Acta Biol Hung 53:367–380
Metting B (1981) The systematic and ecology of soil algae. Bot Rev 147:195–312
Misra S, Kaushik BD (1989a) Growth promoting substances of cyanobacterial vitamins and their influence on rice plant. Proc Indian Sci Acad 55:295–300
Misra S, Kaushik BD (1989b) Growth promoting substances of cyanobacteria: detection of amino acids, sugars and auxins. Proc Ind Natl Sci Acad 6:499–504
Moisander PH, McClinton EIII, Paerl HW (2002) Salinity effects on growth, photosynthetic parameters and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecol 43:432–442
Moore AW (1969) Azolla: biology and agronomic significance. Botanical Rev 35:17–30
Nekrasova KA, Aleksandrove IV (1982) Participation of collembolas and earthworms in transformation of algal organic matter. Soviet Soil Sci 14(4):31–39
Pandey KD, Kashyap AK, Gupta RK (1992) Nitrogen fixation by cyanobacteria associated with moss communities in Schirmacher Oasis, Antarctica. Isr J Bot 41:187–198
Prasad RC, Prasad BN (2001) Cyanobacteria as a source biofertilizer for sustainable agriculture in Nepal. J Plant Sci Bot Orientalis:127–133
Reed RH, Richardson DL, Warr SRC, Stewart WDP (1984) Carbohydrate accumulation and osmotic stress in cyanobacteria. J Gen Microbiol 130:1–4
Relwani LL (1965) Studies of blue-green algae on paddy yield. Curr Sci 34:188–189
Relwani LL, Subrahmanyan R (1963) Role of bluegreen algae, chemical nutrients and partial soil sterilization on paddy yield. Curr Sci 32:441–433
Reynault J, Sasson A, Pearson HW, Stewart WDP (1975) Nitrogen fixation by free living microorganisms. Cambridge University Press, London
Rodriguez AA, Stella AA, Storni MM, Zulpa G, Zaccaro MC (2006) Effects of cyanobacterial extracelular products and gibberellic acid on salinity tolerance in Oryza sativa L. Saline Syst 2:7
Roger PA, Reynaud PA (1982) Free-living blue- green algae in tropical soils. Martinus Nijhoff Publisher, La Hague
Roger PA, Grant IF, Reddy PM (1987) Efficiency of nitrogen fertilizers for rice. IRRI, Manila
Rogers SL, Burns RG (1994) Changes in aggregate stability, nutrient status, indigenous microbial populations and seedling emergence following inoculation of soil with Nostoc muscorum. Biol Fertil Soils 18:209–215
Rother JA, Aziz A, Karim NH, Whitton BA (1988) Ecology of deepwater rice-fields in Bangladesh. 4. Nitrogen fixation by blue-green algal communities. Hydrobiologia 169:43–56
Roychoudhury P, Kaushik BD, GSR K, Venkataraman GS (1979) Effect of blue-green algae and Azolla application on aggregation status of soil. Curr Sci 48:454–455
Ruffing AM (2011) Engineered cyanobacteria: teaching an old bug new tricks. Bioeng Bugs 2:136–149
Saadatnia H, Riahi H (2009) Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plant. Plant Soil Environ 55(5):207–212
Sankaram A, Mudholkar MJ, Sahay MN (1966) Algae as aids in rice cultivation. Indian Farming 16:37–38
Singh RN (1961) Role of blue green algae in nitrogen economy of Indian agriculture. Indian Council of Agricultural Research, New Delhi

Singh JS (2014) Cyanobacteria: a vital bio-agent in eco-restoration of degraded lands and sustainable agriculture. Clim Change Environ Sustain 2:133–137
Singh DN, Venkataraman GS, Rao SBP, Bhattacharya A, Goyal SK (1972) Effect of algal inoculation on Jaya rice variety under field conditions. Labdev J Sci Technol 10-B(3/4):107–108
Song T, Martensson L, Eriksson T, Zheng W, Rasmussen U (2005) Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. FEMS Microbiol Ecol 54:131–140
Subhashini D, Kaushik BD (1984) Amelioration of salt affected soils with blue green algae. Phykos 23:273–277
Subramanian G, Sundaram SS (1986) Induced ammonia release by the nitrogen fixing cyanobacterium anabaena. FEMS Microbiol Lett 37:151–154
Tahmida Begum ZN, Mandal R, Akhter F, Das NC (1990) Perspectives in phycology. Today & Tomorrow's Printers & Publishers, New Delhi
Venkataraman GS (1972) Algal biofertilizer and rice cultivation. Today and Tomorrow's Publication, New Delhi
Venkataraman GS (1979a) Algal inoculation of rice fields. Nitrogen and rice. International Rice Research Institute. Los Banos, 311–321
Venkataraman GS (1979b) Nitrogen and Rice. International Rice Research Institute. Los Banos, pp 105–117
Watanabe A (1951) Production of cultural solution of some amino-acids by the atmospheric nitrogen fixing blue-green algae. Arch Biochem Biophys 34:50–55
Wilson LT (2006) Cyanobacteria: a potential nitrogen source in rice fields. Texas Rice 6:9–10
Wilson JT, Roger PA, Kulasooriya SA (1980) Blue green algae and rice. Los Banos, International Rice Research Institute

Application of Bioinoculants for Seed Quality Improvement

14

Caroline Fadeke Ajilogba, Oluwaseyi Samuel Olanrewaju, and Olubukola Oluranti Babalola

Abstract

The need for sustainable and organic agriculture, pesticide use reduction, greenhouse effect and ozone layer depletion have led to research on using microorganisms in planting. Seeds are in the heart of crop planting. The quality of the seeds determines the quality and quantity of the harvest. Different methods have been used to sanitize seeds to make them healthy and effective to attain optimal growth and achieve high crop yield. Both physical and biological methods have been used to attain effectiveness in crop production. Some of the biological methods discussed in this chapter include the use of bioinoculants as biopesticides, bioherbicides, biofungicides, biological resistance inducers and plant strengtheners.

Keywords

Bioinoculants • Disease • Plant growth-promoting rhizobacteria • Seed quality • Yield

14.1 Introduction

A major challenge that human population will be facing in the twenty-first century is the ability to carry out sustainable, environmentally sound crop production. Due to increase in population, this is necessary for food production, renewable energy and production of basic industrial compounds in the form of volatile organic

C.F. Ajilogba • O.S. Olanrewaju • O.O. Babalola (✉)
Food Security and Safety Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa
e-mail: olubukola.babalola@nwu.ac.za

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*, Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_14

compounds and secondary metabolites. Current production methods that are being used are dangerous to the health and the environment (Gunnell et al. 2007; Leach and Mumford 2008), as well as the never-ending reappearance of plant pathogens which militates against implementation of proper plant growth and general health. There is an increased demand for sound, bionomically compatible agricultural strategies for sustainable production.

Living things are classified as plants and animals. Plants constitute the primary producers and can make their foods by themselves. They are classified as vascular and nonvascular plants. The nonvascular plants comprise of mosses and liverworts, while the vascular plants were further classified as plants with and without seeds. Plants without seeds include ferns, "horse tails" and club mosses. Plants with seeds were further classified as angiosperm and gymnosperm. Gymnosperms include pine and fir trees. Angiosperms were further classified as monocots and dicots. Examples of monocots and dicots include grasses, palm trees and deciduous trees and vegetables, respectively. These seeds are quite important in reproduction in plants, and they eventually grow into plants. They are composed of the embryo which is the baby plant and the result of fertilization, the outer coat or testa that protects it and the endosperm that contains stored foods for the embryo (Powell 1998). Seeds can also be said to be propagating material such as tubers, rhizomes, bulbs, sets and diverse types of grafts, etc.

Basically, plants are made up of roots and shoots, the latter of which is composed of the stems, branches, leaves, seeds, flowers and fruits. Production of quality seeds will lead to production of quality yield (Santos 2013).

14.2 Seed

The quality of seed is a very crucial factor in agricultural production as poor seeds hamper the ability for more yield, thereby undermining the productivity of the farmer's labour. To determine a good seed, four basic parameters will be looked at:

- Physical qualities of the seed
- Physiological qualities
- Genetic qualities
- Seed health

These parameters are summarized in Table 14.1, and more explanations are given in other parts of the text.

The good qualities possessed by a seed determine its ability to efficiently produce good crops. This means that the seed must be able to withstand adverse conditions and agricultural practices, including soil fertility, rainfall and pest control ability. All these are very important and should never be undermined.

Table 14.1 Basic parameters considered in seed quality

Parameters	Explanations	References
Physical qualities	Less-damaged seed as these may not germinate; reduced weed seed and inert matter such as chaffs, stones, dirt etc.; reduction of infected seeds which are characterized by changes in colour; uniform size which says a lot about the seed vigour and viability All these can be detected by physically examining the seeds. This is the first step for farmers in understanding a good-quality seed, and necessary steps and precautions are taken before planting	Nanduri and Dakheel (2015)
Physiological	High germination rate and vigour. The ability of the seed to emerge from the soil after planting under normal conditions is used to classify its germination rate, while the vigour is its ability to withstand stressful conditions after germination. The fact that a seed germinates well does not mean that it cannot be low in vigour. It is only a viable seed that can fulfil its biological role	Nanduri and Dakheel (2015) and Bewley and Black (2012)
Genetic	Same variety of seeds which are generally referred to as cultivars. They have the same traits which are transferred from generation to generation. Genetic traits to look out for are as follows: It must have the right attributes and traits which are highly acceptable in the locality Pest and disease tolerance. The plant can coexist with pathogens and remain unaffected in terms of productivity High yielding ability. This includes nutrient use efficiency, adaptation to the immediate environmental conditions, plant architecture, pest and ability to contain diseases, etc. More explanations are given in other parts of the text	Hallauer et al. (2010) and Schröder and Prasse (2013)
Seed health	This signifies the presence or absence of pathogens. The pathogens include fungi, viruses, nematodes, animal and insect pests and bacteria. This attribute can be tested in the laboratory for proper checking because any disease present in the seeds may cause continuous disease development on the field; new diseases and pests that are not normally found can be imported into new regions	Ahmed et al. (2013) and (Agriquest)

14.3 Characteristics of Quality Seeds

Seed quality is the totality of all the factors that contribute to the performance of the seeds. This could be physiological, genetic and/or physical (Rickman et al. 2006). The quality of the seeds determines the value of the seeds; this invariably means that the higher the quality, the higher the value of the seeds and vice versa (IRRI 2009). This also determines the quality of crop production ability (Mbora et al. 2009) and the productivity of the farmer. A seed with high or good quality is also a healthy

seed, free from disease and disease inoculum and also produce healthy seedlings (Nguyen 2001). Others are listed below. Seed germination and vigour are very important for crops to be established (Mkandawire 2007). Imbibition damage is when a seed experiences death of its cell as a result of water intake into its cotyledon, leading to leakage of solute from the embryo and subsequent reduction in the transfer of food to the cotyledons. This eventually leads to decline in germination and overall growth rate. When a seed experiences imbibition damage, its vigour is reduced (Mkandawire 2007). Vigour is the quality of a seed that helps to ascertain its ability to perform well during germination and for it to be properly established in the soil and environment which in turn affects the overall crop yield (Santos 2013).

A quality seed must be genetically pure, that is, 100% of the genes of the crop not a mixture except where it is certified as hybrid (Brick 2014). It must be physically pure with about 98% purity for all crops. The seed must be free from weeds, other crop seeds and diseases and must be healthy (Brick 2014). Seed moisture is important as it helps to regulate the infestation of fungi. When the moisture content and temperature of a biological system or seed is high, it will lead to losses in vigour and viability that is irreversible (Francisco and Usberti 2008). Seed moisture increases with increase in humidity during storage, invariably leading to reduced or shorter shelf life (Santos 2013).

14.4 Importance of Quality Seeds

The output of a crop depends on the seed material used for sowing, so the seed is very vital in the production of crop if agriculture is to move forward (Santos 2013). The cost of seed used in planting is less compared with other materials needed in planting, and so is the amount of the seed needed to raise a crop (Agriquest n.d.). Quality seeds reduce loss to diseases and pests and also weed infestation. It increases yield, causes uniformity in maturity and enhances finished product performance, especially in terms of market value. Others are:

- Genetic purity
- Less disease infestation
- Increased yield
- Uniform population
- Less cost of production
- Vigorous etc.

14.5 How to Improve Quality of Seeds

Seed is crucial for crop production; therefore quality seed must be planted for increased productivity. Some of the factors affecting the quality of seeds are seed aging and imbibitions damage and also the interaction between these two. The quality of seeds has been improved using physical and biological methods.

14.5.1 Physical Method

It includes:

Vacuum-steam
Electron treatment
Thermal treatment in water and invigoration or seed priming which is discussed in this chapter.

Invigoration or seed priming is a hydration process whereby seeds are exposed to water that is not much as to allow germination to occur but will only allow the pregerminative processes involving the biochemical and physiological changes (Karthika and Vanangamudi 2013). It helps to improve the establishment of seeds which furthermore improves how the seeds perform by helping to increase the time of germination and how uniform this is for all seeds planted at that time (Warren and Bennet 1997; Halmer 2000). The process involves hydration, incubation and drying, and it helps to increase the ability of seeds to tolerate environmental stress.

The vigour of seeds is reduced as a result of aging, and when these seeds are sown, the timing of harvest is affected, thereby affecting the quality and yield of the harvest (Finch-Savage 1994):

- Drum priming is a seed hydration method where hydration takes place in a drum or a rotating cylinder (Rowse 1996). In this situation, a specified volume of water is added to the seeds to raise the moisture content.
- Solid matrix priming involves the use of carriers such as moist siliceous materials and clay or such with adjusted water potential that the seeds can be mixed into and allowed to sprout in. Sometimes the seeds are first treated with microbes before mixing them with solid matrix that contains enough water to facilitate the priming of the seeds (Taylor et al. 1988).
- Hydro-priming is the process of seed hydration whereby the seeds are pre-soaked in water for a while after which the seeds are exposed to 100% relative humidity (Warren and Bennet 1997). The challenges of this method are that it encourages microbial growth and the seeds are not uniformly hydrated (Van Pijlin et al. 1995).
- Aerated hydration (AH) is a process of hydrating seeds using aerated water in a column such that the water content is enough to facilitate radical protrusion. Immediately after the radical has protruded, the seed dies before sowing. This method was used for cauliflower and Brussels seeds in an 8 h treatment at 25 °C, and this resulted in increased seed vigour, growth of root and uniform rate of germination (Thornton and Powell 1992). Also Powell et al. (1993) observed that the quality of rapeseed increased after aerated hydration was applied.

14.5.2 Biological Methods

The increased demand for biological seed treatment has been speculated to be due to the opportunity it has been presented in the global seed treatment market linking it to at least 20% of the market (New Ag International 2015). Asia Pacific happens to be growing fast currently with expected 9% increase in demand between 2014 and 2020 (Mordor Intelligence LLP 2014). With this in mind, microbial seed inoculation for pest control at rhizospheric level has been attempted though soilborne pathogens are attracted by exudates released from the plants into the soil. Pest control by any defined treatment is not very feasible, but treatment of seeds happens to be a useful way of delivering biocontrol agents into the rhizosphere. The different treatments used may be dependent on the colonizing ability of the organism as seen in the case of the fungal entomopathogen *Metarhizium anisopliae* which has been discovered to be a very good rhizosphere colonizer (Pava-Ripoll et al. 2011). It has been used in the field of maize crops to protect them from loss caused by wireworm, *Agriotes obscurus* (Pilz et al. 2011). Spores of the entomopathogen were applied to seeds instead of soil, and this provided considerable protection against pathogens. The different biological methods employed in seed quality improvement include the following.

14.5.3 Plant Strengtheners

Biopriming or the use of microorganisms in seed priming is the process of hydrating seeds in microorganisms before drying and incubating them for 24 h and then eventually transferring them for planting (Callan et al. 1991, Mathre et al. 1995). It is also used along with biocontrol agent in seed priming process to control seed and soilborne diseases (Reddy 2012). This was experimented using sweet corn and chickpea where biopriming with *P. fluorescens* served as biocontrol agent against *Pythium* spp. Fungi and antagonistic bacteria have been used in biopriming; the use of *Trichoderma* to increase root growth and invariably plant growth has also been included (Vinale et al. 2008). Also, wheat plants whose seeds were bioprimed with drought-resistant *T. harzianum* experienced reduced stress and increased vigour (Shukla et al. 2015).

14.5.4 Plant Extracts

It is important to search for cheap pathogen control measures that are at the same time ecologically sound and environmentally safe. Plant extracts are rich in phytochemicals which are able to suppress or eliminate pathogens. Although chemical treatments have been prioritized, due to concerns over safety and cost, there is a gradual shift to nonchemical measures for seed treatments. Plant extracts such as flavonoids and phenolic compounds have all been proven to affect fungal development both in vitro and in vivo, sometimes by inhibiting germination of affected

Table 14.2 Plant extracts and their effects on seeds and plants

Plants treated	Source of extract	Effect on seed/plant	References
Maize	*Moringa oleifera*	Growth promotion	Basra et al. (2011)
Green gram	Seaweed	Growth promotion	Zodape et al. (2010)
	S. officinalis and *R. officinalis*	Biocontrol against *Alternaria* spp.	Dellavalle et al. (2011)
Chilli	Zimmu	Biocontrol against *Pythium aphanidermatum*	Muthukumar et al. (2010)
Lupine	*Nerium oleander*, *Ocimum basilicum, Eugenia jambolana*, *Ambrosia maritima*, *Calotropis procera*, *Acacia nilotica* and *Citrullus colocynthis*	Biocontrol against *Fusarium oxysporum* f. sp. *lupini* and *Fusarium. oxysporum* f. sp.	Abdel-Monaim et al. (2011)
Tomato	*Eucalyptus camaldulensis* (eucalyptus), *Ocimum basilicum* (sweet basil), *Nerium oleander* (oleander), *Azadirachta indica* (neem), *Datura stramonium* (jimsonweed) and *Allium sativum* (garlic)	Biocontrol against *Alternaria solani*	Nashwa and Abo-Elyousr (2012)

seeds, mycelial growth of pathogens, seed infection and formation of spores. Plant extracts have been significant in the improvement of seed quality and field emergence (Ur Rehman et al. 2014). Pull out of some extracts on seed and plant growth is represented in Table 14.2.

14.5.5 Biological Resistance Inducers

Priming plants with bioinoculants allow them to conserve energy and reduce the time needed for defence reactions during an attack by a pathogen. It causes the induction of a faster defence reaction of the plants toward pathogens and biotic and abiotic stresses in the environment (Van Loon 2007; Yang et al. 2009). Inducers closely associated to plants cause induced systemic resistance (ISR), usually plant growth-promoting rhizobacteria (PGPR), leading to resistance in roots and other plant areas (Vacheron et al. 2013). ISR is majorly underlined by the jasmonic acid and ethylene-dependent mechanisms (Beneduzi et al. 2012). Resistance by induction is one of the strategies that PGPR use in boosting plant defence mechanisms against pathogens (Van Der Ent et al. 2009). When attacked, plants activate their immune systems for defence which is initiated upon recognition of any invasion through the microbe-associated molecular patterns and/or other molecules (Burketova et al. 2015).

14.5.6 Bioinoculants

Bioinoculants are microorganisms such as bacteria, fungi and algae that are alive and are able to promote growth by facilitating nutrient, phosphorus and nitrogen uptake and suppressing growth of plant pathogens (IIRR 1996). They are microbial or soil inoculants that are prepared to promote plant growth and suppress diseases by using different mechanisms (Ajilogba and Babalola 2013). They are able to cater for the different needs of the plant, especially in the rhizosphere (Raja et al. 2006). Bioinoculants also referred to as microbial inoculants (Berg 2009; Ajilogba and Babalola 2013) have also been used interchangeably to refer to microorganisms used as biofertilizers (Vessey 2003; Muraleedharan et al. 2010), a contraction for biological fertilizer (Rodríguez-Navarro et al. 2011). Bioinoculants are effective in plant growth, either as individual inoculants or in combination with other inoculants. Owing to their function, we will be looking at them as biofertilizers, biopesticides, biofungicides and bioherbicides.

14.5.6.1 Biofertilizers

The continuous use and reliability on chemical fertilizers for crop yield improvements are likely to cause further losses in fertility and serious effect on the community and activities in the microbiota of the soil. Uncontrolled use of these chemical fertilizers has been reported to show negative impact on productivity, contamination and disease susceptibility, ultimately leading to loss in the economy (Zaidi et al. 2015; Insam et al. 2015). The advent of biofertilizer has shown greater promise in alleviating these problems (Kumar et al. 2016). They are microbial or soil inoculants that contain living organisms and are actives in growth promotion by enhancing nutrient uptake of plants. Biofertilizers can be directly applied to the soil, coated on the seeds or applied on the surfaces of plants. They colonize the rhizosphere or the interior of the plant roots. These biofertilizers include liquid forms and are carrier based (Rivera-Cruz et al. 2008). They can be bacteria like *Bacillus sp.* (Jacobsen et al. 2004), *Pseudomonas sp.* (Loper et al. 2007) and *Rhizobium sp.* (Long 2001); fungal like *Coniothyrium*, *Ampelomyces* and *Trichoderma* (Harman et al. 2004); or algae like blue-green algae and *Azolla* (IIRR 1996). Many of the PGPR are also biocontrol agents making use of a range of mechanisms that have been extensively discussed in different works (Berg 2009; Ahemad and Kibret 2014; Babalola 2010). Microbial inoculation of seed has been proven to be an efficient delivery means of introducing PGPR, majorly where plant response is determined by rhizosphere colonization.

The technology of biofertilizer production is comparatively new and still in developmental process, and some challenges are still experienced in producing it. These include:

- Obtaining competent PGPR strains: efficiency in terms of region, colonization ability and establishments. Due to the different abilities of the different strains, getting a suitable PGPR strain for bioinoculant production is not very easy (Herrmann and Lesueur 2013).

- Unavailability and jejune life span of suitable carriers.
- Tolerance toward unpredictable field temperature, instability and chances of contamination.
- Possible genotypic changes resulting from selected PGPR interacting with unsought organisms which may alter their significant traits. There are likely possibilities that the selected strains may be mutated in fermentation, causing partial inefficiency and loss of viability. This may result in economical loss and increase in the cost of production (Srivastava et al. 2016).
- Shortage of well-equipped storage facility.
- Shortage or unavailability of transport system.
- Poor inconsistent demand and limited marketing opportunities which might be a result of proper awareness programs for the farmers concerning the importance of biofertilizers. The emergence of genomic technologies has brought hope to the production of biofertilizers with more predictable and consistent effects on crop yield.

14.5.6.2 Biopesticides

They are microbial or soil inoculants that are involved in suppressing and safely controlling insect pests, for example, *B. thuringiensis* (Poopathi and Abidha 2009), and they also contain biocontrol agents (BCA) (Berg 2009). They are also microorganisms that help to stimulate plant growth by controlling deleterious organisms like destructive insects (Vessey 2003).

14.5.6.3 Bioherbicides

They are phytotoxins produced by microorganisms that are important in biological control of weeds (Boyetchko and Peng 2004). They are also plant pathogens that are developed in such a way that their mode of operation is like that of chemical herbicides (Charudattan 1991), for example, using white smut fungus (*Entyloma ageratinae* sp. nov.) for the control of mist flower (*Ageratina riparia*), an exotic weed that was destroying Hawaiian forest (Trujillo 2005).

14.5.6.4 Biofungicides

They are biological fungicides that are beneficial and living microorganisms used to control fungal plant pathogens. They consist of both beneficial fungi and bacteria; examples include *Trichoderma harzianum* that are used to control pathogens like *Pythium*, *Thielaviopsis, Rhizoctonia*, *Sclerotinia* and *Fusarium*. They do this through direct competition, antibiosis, induced resistance and parasitism and predation (Thomas 2009). They can also be naturally occurring substances that are used in disease control especially of fungi origin (Francis and Keinath 2010). Fungicidal seed treatment is important to avoid attack by soilborne pathogens as it introduces the inoculum directly into the rhizosphere where pathogenic effects are really felt on crops (Berg 2009). Different fungal antagonists have been developed, but they have not been used more frequently (Abo-Elyousr et al. 2009). When tested, they have been used by direct immersing of seed in liquid suspensions of cells. *Bacillus* spp. have been tested to be good antifungal agents majorly because of their ability

to produce heat-resistant endospores, and they have been widely used as fungal antagonists in various works (Ajilogba et al. 2013; Dinesh et al. 2015; Gholami et al. 2014). *Bacillus subtilis* is currently marketed as Kodiak (O'callaghan 2016); it can be introduced as slurry in consortia with other recognized fungicides. Generally, microbial antagonists provide plant protection where there are no chemical treatments, as seen in oilseed rape, delivery of *Serratia plymuthica* for the suppression of *Verticillium dahlia* (Müller and Berg 2008).

14.5.6.5 Effect of Bioinoculants

Chemicals have been used in time past to combat the issue of pests and pathogens of plants as they reduce quality and quantity of yields. Chemical control though effective to some degree also created problems for the microbial communities by creating imbalance in such communities which may adversely affect beneficial microbes and lead to pathogen strains that become resistant to chemicals (Asaka and Shoda 1996). Owing to the adverse effect of chemical fertilizers on human health and the environment (Gerhardson 2002), excessive use which can lead to cancer and other forms of abnormality in humans (Ajilogba and Babalola 2013; Pathak et al. 2013), the use of microorganisms to suppress pathogens and diseases and promote growth has been emphasized.

Pathak et al. (2013) observed that in the presence of a combination of *Azotobacter chroococcum* and farmyard manure, plant growth was the highest with about 51.1% compared with other combination and control. Also, Joolka et al. (2004) reported increase in plant growth of pecan seedlings, which is the effect of inoculation with combined biofertilizers. Bioinoculants could be prepared from bacteria, fungal or algae or combination of some or all. Depending on their functions, no matter the composition of their preparation, their different names are formed. The best example is that of legume seeds which maximize yield through the help of viable rhizobia strains which are introduced into the rhizosphere for massive, rapid colonization, nitrogen fixation and nodule formation. The practice of mixing naturally inoculated soil with seeds was recommended for legumes in the USA, the first patent being Nitragen, established in 1896 (Bashan 1998).

Ajilogba et al. (2013) reported that four *Bacillus* species were able to promote growth of tomato plants and also suppress plant disease (*Fusarium* wilt). In a study by Anandaraj et al. (2010), it was observed that the combination of mixed inoculants of *Rhizobium sp.*, *Pseudomonas fluorescence* and *Bacillus megaterium* increased growth of gram seeds and also increased yield. There was an increase in groundnut growth, yield and its nodulation after being inoculated with a combination of *Bacillus sp.* and *Pseudomonas sp.* (Pan et al. 1999).

14.5.6.6 Plant Microbe Interactions

Plant-associated microbes carry out significant functions in plant growth and health. The significant functions are carried out directly or indirectly. Direct interactions involve stimulation of hormones, improvement of nutrient acquisition and secreted exudates. Various mechanisms are involved in pathogen biocontrol, which is often

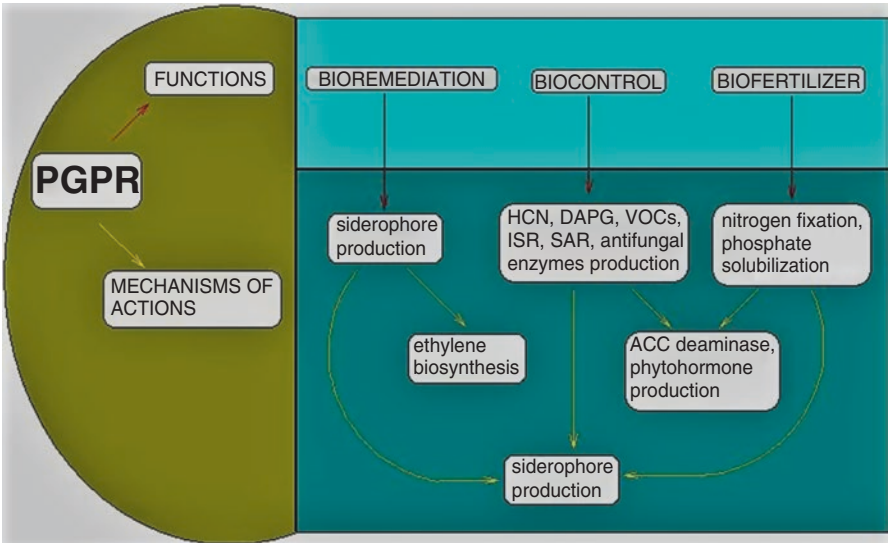


Fig. 14.1 Different mechanisms employed by PGPR in promoting plant growth and health

indirectly associated with plant growth (Fig. 14.1). Members of the bacterial genera *Azospirillum* and *Rhizobium* are established plant growth promoters (Bashan and De-Bashan 2010), while *Pseudomonas*, *Stenotrophomonas*, *Serratia*, *Bacillus* and *Streptomyces* and the fungal genera *Trichoderma*, *Coniothyrium* and *Ampelomyces* are reference organisms to demonstrate influence on plant health and as biocontrol agents (Ajilogba et al. 2013; Anandaraj et al. 2010; Dinesh et al. 2015; Müller and Berg 2008). Based on these interactions, it is possible to develop bioinoculants for use in agricultural biotechnology. Diverse mechanisms are involved in plant–microbe interactions as depicted in Fig. 14.1 and indicated in many works such as Compant et al. (2005), Hayat et al. (2010) and Ahemad and Kibret (2014).

The intensity, duration and outcome of plant and microbe interactions are influenced by the abidance of adherent microbial populations (Lau and Lennon 2011). Interaction between plants and microbes is mutual as both sides are affected. Exudates released by plants into the rhizosphere attract microbes, thereby determining the *Rhizobium* of the plants (Haichar et al. 2014). This makes the rhizosphere zone of intense microbial activity. *Fusarium oxysporum* and *P. fluorescens* WCS365 influence the organic acid and sugars in tomato root exudates as shown by Kamilova et al. (2006). Many organisms that are beneficial are good rhizosphere colonizers (Babalola 2010). Lots of reviews have in-depth discussion on plant–microbe interactions; they can be accessed for more information regarding the topic.

14.6 Conclusion, Future Prospects and Recommendations

It has been established that bioinoculants applied individually or in consortia have effect on plant growth and seed health. Getting the right strains for efficient colonization and the right combination of strains are important. Molecular biology techniques and screening genotypes may help to identify and inadvertently develop more effective PGPR inoculant strains. Seed quality test carried out before planting will also aid the effect of the inoculum as there is less competition for nutrients. Exploitation of microorganisms through their application in beneficial plant–microbe interactions offers promising and environmentally friendly strategies to improve conventional and organic agriculture worldwide.

References

Abdel-Monaim M, Abo-Elyousr K, Morsy K (2011) Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine (Lupinus termis Forsik). Crop Prot 30:185–191

Abo-Elyousr KAM, Hashem M, Ali EH (2009) Integrated control of cotton root rot disease by mixing fungal biocontrol agents and resistance inducers. Crop Prot 28:295–301

Agriquest. Quality seed and its importance in agriculture [Online]. Available: http://agriquest.info/index.php/quality-seed-and-its-importance-in-agriculture (Accessed)

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ahmed M, Hossain M, Hassan K, Dash CK (2013) Seed health and quality test of three rice varieties for the detection of fungi associated with seed sample. Univers J Plant Sci 1:37–42

Ajilogba CF, Babalola OO (2013) Integrated management strategies for tomato Fusarium wilt. Biocontrol Sci 18:117–127

Ajilogba CF, Babalola OO, Ahmad F (2013) Antagonistic effects of *Bacillus* species in biocontrol of tomato Fusarium wilt. Stud Ethno Med 7:205–216

Anandaraj B, Leema R, Delapierre A (2010) Studies on influence of bioinoculants (*Pseudomonas fluorescens, Rhizobium sp*., *Bacillus megaterium*) in green gram. J Biosci Technol 1:95–99

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

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

Bashan Y, De-Bashan LE (2010) Chapter two – how the plant growth-promoting bacterium Azospirillum promotes plant growth—a critical assessment. In: Donald LS (ed) Advances in agronomy. Academic Press, New York

Basra S, Iftikhar M, Afzal I (2011) Potential of Moringa (Moringa oleifera) leaf extract as priming agent for hybrid maize seeds. Seeds 13:1006–1010

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051

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

Bewley JD, Black M (2012) Physiology and biochemistry of seeds in relation to germination: volume 2: viability, dormancy, and environmental control. Springer Science & Business Media, Berlin

Boyetchko SM, Peng G (2004) Challenges and strategies for development of mycoherbicides. In: Arora DK (ed) Fungal biotechnology in agricultural, food, and environmental applications. Marcel Dekker, New York

Brick MA (2014) Improve yield with high quality seed. In: University, C. S (ed) Colorado State University extension. Colorado State University, Fort Collins
Burketova L, Trda L, Ott PG, Valentova O (2015) Bio-based resistance inducers for sustainable plant protection against pathogens. Biotechnol Adv 33:994–1004
Callan NW, Mathre DE, Miller JB (1991) Field performance of sweet corn seed bio-primed and coated with Pseudomonas fluorescens AB254. Hort Science 26:1163–1165
Charudattan R (1991) The mycoherbicide approach with plant pathogens. In: Tebeest DO (ed) Microbial control of weeds. Chapman and Hall, New York
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
Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Dalla Rizza M (2011) Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria spp. Chilean J Agric Res 71:231
Dinesh R, Anandaraj M, Kumar A, Bini YK, Subila KP, Aravind R (2015) Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. Microbiol Res 173:34–43
Finch-Savage WE (1994) Influence of seed quality on crop establishment, growth and yield. In: Basra AS (ed) Seed quality: basic mechanisms and agricultural implications. Food Products Press, New York
Francis R, Keinath A. (2010) Biofungicides and chemicals for managing diseases in organic vegetable production *Information leaflet 88* [Online]
Francisco FG, Usberti R (2008) Seed health of common bean stored at constant moisture and temperature. Sci Agric (Piracicaba, Braz) 65:613–619
Gerhardson B (2002) Biological substitutes for pesticides. Trends Biotechnol 20:338–343
Gholami M, Khakvar R, Niknam G (2014) Introduction of some new endophytic bacteria from *Bacillus* and *Streptomyces* genera as successful biocontrol agents against *Sclerotium rolfsii*. Arch Phytopathol Plant Protect 47:122–130
Gunnell D, Eddleston M, Phillips MR, Konradsen F (2007) The global distribution of fatal pesticide self-poisoning: Systematic review. BMC Public Health 7(1)
Haichar FEZ, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground. Soil Biol Biochem 77:69–80
Hallauer AR, Carena MJ, Miranda Filho JD (2010) Quantitative genetics in maize breeding. Springer Science & Business Media, New York
Halmer P (2000) Commercial seed treatment technology Seed Technology and Its Biological Basis. Sheffield Academic Press, Sheffield, pp 257–286
Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) Trichoderma species--opportunistic, avirulent plant symbionts. Nature Rev Microbiol 2:43
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 60:579–598
Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97:8859–8873
IIRR (1996) Environmentally sound technologies for women in agriculture. IRR, New Delhi
Insam H, Gómez-Brandón M, Ascher J (2015) Manure-based biogas fermentation residues–friend or foe of soil fertility? Soil Biol Biochem 84:1–14
IRRI (2009) Rice ecosystems. http://www.ppi-ppic.org/ppiweb/filelib.nsf/0/6191D544DF714DEF48257074002E78E6/$file/Rice%20HB%20p2-5.pdf
Jacobsen BJ, Zidack NK, Larson BJ (2004) The role of *Bacillus*-based biological control agents in integrated pest management systems: plant diseases. In: Symposium- The nature and application of biocontrol microbes: *Bacillus* sp. Phytopathology 94: 1272–1275
Joolka NK, Singh RR, Sharma MM (2004) Influence of biofertilizers, GA3 and their combinations on growth of pecan seedlings. Ind J Hort 61:226–228

Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B (2006) Effects of the tomato pathogen *Fusarium oxysporum* f. sp. radicis-lycopersici and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudate. Mol Plant-Microbe Interact 19:1121–1126

Karthika C, Vanangamudi K (2013) Biopriming of maize hybrid COH (M) 5 seed with liquid biofertilizers for enhanced germination and vigour. Afr J Agric 8:3310–3317

Kumar A, Singh M, Singh PP, Singh SK, Singh PK, Pandey KD (2016) Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. Biocatal Agric Biotechnol 8:1–7

Lau JA, Lennon JT (2011) Evolutionary ecology of plant-microbe interactions: soil microbial structure alters selection on plant traits. New Phytol 192:215–224

Leach AW, Mumford JD (2008) Pesticide Environmental Accounting: A method for assessing the external costs of individual pesticide applications. Environ Pollut 151(1):139–147

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

Loper JE, Kobayashi DY, Paulsen IT (2007) The Genomic Sequence of *Pseudomonas fluorescens* Pf-5: Insights Into Biological Control. Phytopathology 97(2):233–238

Mathre DE (1995) Combined Biological and Chemical Seed Treatments for Control of Two Seedling Diseases of Sweet Corn. Plant Dis 79(11):1145

Mbora A, Schmidt L, Angaine P, Meso M, Omondi W, Ahenda J, Barnekov Lilleso JP Mwanza J, Mutua WR, Mutua NA, and Jamnadass R (2009). Tree Seed Quality Guide. Available on line at http://www.worldagroforestry.org

Mkandawire CH (2007) Review of bambara groundnut (*Vigna subterranea* (L.) Verdc.) production in Sub-Sahara Africa. Agric J 2:464–470

Mordor Intelligence LLP (2014) Asia Pacific Biological Seed Treatment Market 2014–2019. In: Market Shares, Forecasts and Trends. Modorintelligence.com

Müller H, Berg G (2008) Impact of formulation procedures on the effect of the biocontrol agent *Serratia plymuthica* HRO-C48 on *Verticillium* wilt in oilseed rape. BioControl 53:905–916

Muraleedharan H, Seshadri S, Perumal K (2010) Biofertilizer (Phosphobacteria). Shri AMM Murugappa Chettiar Research Centre, Taramani

Muthukumar A, Eswaran A, Nakkeeran S, Sangeetha G (2010) Efficacy of plant extracts and biocontrol agents against Pythium aphanidermatum inciting chilli damping-off. Crop Prot 29:1483–1488

Nanduri KR, Dakheel AJ (2015) Manual of seed production of salt tolerant crops. International Center for Biosaline Agriculture, Dubai

Nashwa S, Abo-Elyousr KA (2012) Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Prot Sci 48:74–79

New Ag International (2015) Biocontrol in Asia: Gaining momentum! Seed treatments with biologicals: a new and promising frontier. http://dunhamtrimmer.com/wp-content/uploads/2012/07/Biocontrol-in-Asia-Gaining-Momentum.pdf

Nguyen VN (2001) Rice Production, Consumption and Nutrition. FAO, Rome

O'callaghan M (2016) Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl Microbiol Biotechnol 100:5729–5746

Pan B, Bai YM, Leibovitch S, Smith DL (1999) Plant-growth-promoting rhizobacteria and kinetin as ways to promote corn growth and yield in a shortgrowing-season area. Eur J Agron 11:179–186

Pathak DV, Singh S, Saini RS (2013) Impact of bio-inoculants on seed germination and plant growth of guava (*Psidium guajava*). Glob J Wood Sci For Wildl 1:15–17

Pava-Ripoll M, Angelini C, Fang W, Wang S, Posada FJ, St Leger R (2011) The rhizosphere-competent entomopathogen Metarhizium anisopliae expresses a specific subset of genes in plant root exudate. Microbiology 157:47–55

Pilz C, Enkerli J, Wegensteiner R, Keller S (2011) Establishment and persistence of the entomopathogenic fungus *Metarhizium anisopliae* in maize fields. J Appl Entomol 135:393–403

Poopathi S, Abidha S (2009) A medium for the production of biopesticides (*Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis*) in mosquito control. J Econ Entomol 102:1423–1430
Powell AA (1998) Seed improvement by selection and invigoration. Sci Agric 55:126–133
Powell AA, Thornton JM, Matthews S, Yule L (1993) Invigoration of oilseed rape (*Brassica napus*) by aerated hydration. Seed Res Special volume:728–733
Raja P, Uma S, Gopal H, Govindarajan K (2006) Impact of bioinoculants consortium on rice root: biological nitrogen fixation and plant growth. J Biol Sci 6:815–823
Reddy PP (2012) Bio-priming of seeds. In: Recent advances in crop protection. Springer, pp 83–90
Rickman JF, Bell M, and Shires D (2006). Seed Quality. Available at http://www.knowledgebank.irri.org
Rivera-Cruz MDC, Trujillo Narcía A, Córdova Ballona G, Kohler J, Caravaca F, Roldán A (2008) Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops. Soil Biol Biochem 40:3092–3095
Rodríguez-Navarro DN, Oliver M, Contreras MA, Ruiz-Sainz JE (2011) Soybean interactions with soil microbes, agronomical and molecular aspects. Agron Sustain Dev 31:173–119
Rowse HR (1996) Drum priming – a non-osmotic method of priming seeds. Seed Sci Technol 24:281–294
Santos BM (2013) Seed quality and seeding technology. In: Olson SM, Simonne EH (eds) The vegetable production handbook for Florida. Horticultural Sciences Department, UF/IFAS Extension, Gainesville
Schröder R, Prasse R (2013) From nursery into nature: a study on performance of cultivated varieties of native plants used in re-vegetation, their wild relatives and evolving wild× cultivar hybrids. Ecol Eng 60:428–437
Shukla N, Awasthi R, Rawat L, Kumar J (2015) Seed biopriming with drought tolerant isolates of *Trichoderma harzianum* promote growth and drought tolerance in *Triticum aestivum*. Ann Appl Biol 166:171–182
Srivastava V, Chauhan PS, Misra S (2016) Bioprospecting for improving soil health and crop productivity: Indian patent landscape. Intellect Prop Issues Biotechnol 58
Taylor AG, Klien DE, Whitlow TH (1988) SMP: solid matrix priming of seeds. Sci Hortic 37:1–11
Thomas C (2009) Managing plant diseases with biofungicides. In: Agriculture, P. D. O (ed) Integrated Pest management program. Pennsylvania Department of Agriculture, Harrisburg
Thornton JM, Powell AA (1992) Short term aerated hydration for the improvement of seed quality in *Brassica oleracea* L. Seed Sci Res 2:41–49
Trujillo EE (2005) History and success of plant pathogens for biological control of introduced weeds in Hawaii. Biol Control 33:113–122
Ur Rehman H, Nawaz Q, Basra SMA, Afzal I, Yasmeen A (2014) Seed priming influence on early crop growth, phenological development and yield performance of linola (Linum usitatissimum L.) J Integr Agric 13:990–996
Vacheron J, Desbrosses G, Bouffaud M-L, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C (2013) Plant growth promoting rhizobacteria and root system functioning. Front Plant Sci 4:356
Van Der Ent S, Van Wees SCM, Pieterse CMJ (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70:1581–1588
Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254
Van Pijlin JG, Groot SPC, Kraak HL, Bergervoet JHW (1995) Effects of pre-storage hydration on germination performance, moisture content, DNA synthesis and controlled deterioration of tomato (*Lycopersicon esculentum* Mill.) seeds. Seed Sci Res 6:57–63
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M (2008) A novel role for Trichoderma secondary metabolites in the interactions with plants. Physiol Mol Plant Pathol 72(1–3):80–86

Warren JE, Bennett MA (1997) Seed hydration using the drum priming system. Hort Science 32:1220–1221

Yang J, Kloepper JW, Ryu C-M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Zaidi A, Ahmad E, Khan MS, Saif S, Rizvi A (2015) Role of plant growth promoting rhizobacteria in sustainable production of vegetables: current perspective. Sci Hortic 193:231–239

Zodape S, Mukhopadhyay S, Eswaran K, Reddy M, Chikara J (2010) Enhanced yield and nutritional quality in green gram (*Phaseolus radiatus* L) treated with seaweed (*Kappaphycus alvarezii*) extract. J Sci Ind Res 69:468–471

Role of Biofertilizers in Sustainable Agriculture Under Abiotic Stresses

15

Sh.M. Selim and Mona S. Zayed

Abstract

Sustainable agriculture development is a very important challenge that encounters the world nowadays as it requires increasing the productivity of plants with minimal disturbance of the environment. Plant growth is very susceptible to different conditions that affect its productivity and yield. These conditions could be divided into biotic (living) and abiotic (nonliving) stresses. Biotic stress includes interference from pathogenic microorganisms, insects, and higher animals, which include humans, while abiotic stress includes soil salinity, waterlogging, drought, high and low temperatures, wind, intense light, heavy metals, and inadequate or excessive mineral nutrients. Most of the abiotic stress factors could be attributed to different climatic changes which are considered the major reasons for regression of principal crop productivity. Plant species are surrounded by diverse beneficial microorganisms that dominate in their rhizosphere and have the ability to stimulate plant growth and protect them against different stress conditions. Different microbial activities have the ability to improve plant tolerance to biotic and abiotic stress conditions. The role of alleviation depends on the plant genus, the stress type, the microbial species, and the type of relationship between microorganisms and the plant. Microorganisms could enhance plant survival, growth, performance, and yield by several functions such as stimulating root growth by production of phytohormones, enabling water uptake to roots by production of polysaccharides in the root hair zone, improving plant nutrition by increasing nutrients through solubilization of phosphate, secreting siderophores for iron, and fixing dinitrogen, which is either associative or nonassociative. Using microbial inoculants is considered an important task in the next decades to counter abiotic stress in different regions.

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

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_15

Keywords
Plant growth • Stress • Rhizosphere • Microbial inoculants

15.1 Introduction

Agriculture is one of the hoariest profitable sectors in the world that is affected by different factors, at which it always depends on soil fertility and climatic conditions.

At the beginning of the twenty-first century, the increasing human population became a serious problem since it, alongside the abundance of different biotic and abiotic stresses as well as reduction in land availability for cultivation, is considered a vital threat to sustainable development (Shahbaz and Ashraf 2013). The development of sustainable agriculture requires increasing the productivity of plants and animals, as well as ensuring minimal disturbance of the environment, which required substitution of different hazardous materials, like mineral fertilizers and pesticides that are frequently used in agriculture, by environment-friendly biofertilizers, which could improve the nutrition of crops, as well as protect plants against biotic stresses such as "pathogens and pests" and abiotic stresses such as "pollution and different climatic changes" (Noble and Ruaysoongnern 2010; Yang et al. 2009). The agricultural sector is very susceptible to climatic changes particularly in tropical regions that face increases in different stress factors which are considered major reasons for regression of principal crop productivity (Grover et al. 2011).

Recent studies showed that numerous plant species are surrounded by diverse beneficial microorganisms that stimulate plant growth and protect them against different biotic and abiotic stresses (Lugtenberg and Kamilova 2009). These microorganisms dominate in the rhizosphere which are often beneficial to plants and can improve their survival and performance under stress conditions. They enhance plant growth and yield by several functions such as stimulating root growth by production of phytohormones, enabling water uptake to roots by production of polysaccharides in the root hair zone, improving plant nutrition by increasing nutrients through solubilization of phosphate, secreting siderophores for iron, and fixing dinitrogen, which is either associative or nonassociative (Dimkpa et al. 2009).

The present chapter discusses the newest work on the role of biofertilizers in assisting crops to cope various abiotic stresses like heat, chilling, drought, waterlogging, and salt stress, which are considered the most common stresses caused by climatic changes.

15.2 Plant-Growth-Promoting Microorganisms (PGPMs)

The term "PGPM" encompasses a wide variety of bacteria and fungi whose functions and properties favor plant growth and survival. These microorganisms live in close contact to the plant root zone that is defined as the rhizosphere at which the

roots are thought to be a major source of nutrients for them (Carmen and Roberto 2011). These microorganisms could be characterized into two groups, plant-growth-promoting fungi (PGPF) and plant-growth-promoting bacteria (PGPB) which are also called plant-growth-promoting rhizobacteria or "PGPR" (Lugtenberg and Kamilova 2009).

PGPMs are known by their ability to improve the growth of vegetables and crops subjected to abiotic stress conditions which are considered important applications for sustainable agriculture developments (Egamberdieva and Kucharova 2009).

PGPRs belong to different bacterial genera including *Bacillus*, *Pseudomonas*, *Burkholderia*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Rhizobium*, *Frankia*, *Enterobacter*, *Streptomyces*, *Erwinia*, *Caulobacter*, *Serratia*, *Micrococcus*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Hyphomicrobium*, and *Ochrobactrum* (Dimkpa et al. 2008; Gray and Smith 2005; Grover et al. 2011; Tokala et al. 2002), while PGPFs include endomycorrhizae and *Trichoderma* (Chakraborty et al. 2015; de Zelicourt et al. 2013; Vitti et al. 2015).

15.3 Types of Abiotic Stresses

Stress could be defined as any unfavorable condition or substance that affects or blocks plant's metabolism, growth, or development and leads to substantial crop losses worldwide (Lichtenthaler 1996, 1998). Stress factors could be divided into biotic (living) and abiotic (nonliving) stresses. Biotic stress includes interference from pathogenic microorganisms, insects, and higher animals, which include humans, while abiotic stress includes soil salinity, waterlogging, drought, high and low temperatures, wind, intense light, heavy metals, and inadequate or excessive mineral nutrients (Mittler 2006; Vinocur and Altman 2005; Wahid et al. 2007).

15.3.1 Salinity

Salinity is one of the environmental stress factors that limit the productivity of agricultural crops, as it has adverse effects on seed germination and leaf development in addition to plant growth and yield (Carmen and Roberto 2011; Munns and Tester 2008; Paul and Lade 2014).

The term salinity in agriculture refers to the presence of a high concentration of soluble salts around the root zone; a concentration that is over its normal limit causes high osmotic pressures and affects plant growth by restricting the uptake of water as well as affecting the absorption balance of essential nutritional ions of the roots.

Saline soils usually contain a mixture of salt constituents such as chlorides, sulfates, carbonates, and bicarbonates of sodium, calcium, and magnesium. The proportions between these ions vary widely from place to place depending on the source of salts (Carmen and Roberto 2011; Tester and Davenport 2003). Salinity impedes photosynthesis and increases photorespiration, altering the normal ion

homeostasis of cells by causing nutrient imbalance, which is caused by loss of the plant's ability to control nutrient uptake and/or transport from root to shoot leading to ion deficiencies (Munns 2002). The main reason for these nutrient deficiencies could be related to the abundant presence of ions like Na^+ and Cl^- in the soil solution that could cause a decrease in the activity of other essential elements in the soil and lead to the reduction in the uptake and accessibility of some elements by the plants (Bianco and Defez 2009).

Salinity affects plants in different ways such as osmotic effects, specific-ion toxicity, and/or nutritional disorders (Läuchli and Epstein 1990). Several workers have reported the ability of some PGPMs to induce salt tolerance in plants (de Zelicourt et al. 2013; Miliute et al. 2015).

15.3.2 Temperature Stress

Temperature stress could be defined as the increase or decrease in the temperature more than the critical edge, for a period of time, that is adequate enough to cause irretrievable damage to plant growth and development (de Zelicourt et al. 2013; Hasanuzzaman et al. 2013).

Temperature stresses either high or low are considered to be the major abiotic stresses that restrict crop production.

- **High temperature**

Due to global warming, the Earth is now facing numerous extreme temperature conditions from high to very high temperature which cause a major regression in sustainable agricultural developments particularly in tropical regions. High temperature has detrimental effects on plant metabolism. It affects plant growth and productivity which lead to substantial crop losses as it induces different cellular changes particularly the accumulation of reactive oxygen species (ROS) which cause oxidative stress in plant cells (Hasanuzzaman et al. 2013). Several workers have reported the ability of heat-resistant bacteria to induce high-temperature tolerance in plants (Chakraborty et al. 2015).

- **Low temperature**

Low temperature or cold stress is one of the major environmental factors that frequently affect cell division, photosynthesis, water transport, plant growth, and crop productivity, particularly in arctic regions. Low temperature ranges from chilling stress (0–15 °C) that results from low temperatures and produces damage without forming ice crystals in plant tissues to freezing stress (<0 °C) that forms ice within plant tissues (Hasanuzzaman et al. 2013).

Several workers have reported the ability of cold-tolerant bacteria to induce cold tolerance in plants (Barka et al. 2006; Chang et al. 2007; Selvakumar et al. 2008a, b). Attempts are currently being made to identify bacteria from the phyllosphere,

which have low ice nucleating activity, and use them as foliar spray, because ice nucleation has been recognized as the major cause of plant damage in low temperature (Selvakumar et al. 2012).

15.3.3 Soil pH

Soil pH is a measure of acidity and alkalinity. The optimal pH for most of the plants ranges between 5.5 and 7.0; however, many plants can survive and grow at pH values outside this range. Soil pH controls many processes that take place in the soil, particularly, nutrient availability and soil's physical, chemical, and biological properties and their processes, thus affecting plant growth. Therefore, it is of vital importance to maintain proper pH level to get full yield potential from plants (Kajlaa et al. 2015). There are two types of extreme soil pH, soil acidity and alkalinity.

- **Soil acidity**

Soils become acidic when basic elements such as calcium, magnesium, sodium, and potassium that are held by soil colloids are replaced by hydrogen ions. Plants growing in acidic soils (pH <5.5) suffer from numerous deleterious factors such as aluminum (Al) toxicities and nutrient deficiencies like phosphorus (P), boron (B), and molybdenum (Mo) (Mora et al. 2007; Poschenrieder et al. 2008). In addition, acidic soils have low water-holding capacity and are subject to water erosion (Fageria and Baligar 2003), which cause low activities of beneficial microorganisms and reduced plant root growth that limits absorption of nutrients and water which finally lead to very low productivity of plants (Kajlaa et al. 2015).

- **Soil alkalinity**

Soils that contain pH more than 7 are considered as alkaline. Soils with pH higher than > 8.5 have an indigent structure and a low infiltration capacity (Bolt 1980). It often has a rigid calcareous layer at the depth of 0.5 to 1 meter from the surface. Also, alkaline soil has inconvenient physicochemical properties, mostly due to the dominating existence of sodium carbonate that causes swelling of the soil. All previous properties lead to the reduction of plant growth and productivity.

15.3.4 Water Stress

Water is a very important factor for agricultural development not only in arid and semiarid areas but also in regions with abundant rainfall. There are two types of water stresses that affect plant growth and productivity: waterlogging and drought.

- **Waterlogging**

Waterlogging is a condition at which soil structure is saturated with water, which consequentially causes inadequate oxygen in soil pore spaces that affects respiration of plant roots. Under this circumstance, different gases accumulate in the root zone such as carbon dioxide and ethylene which causes leaf senescence and affects the growth and development of plants (Dong et al. 1983).

- **Drought stress**

Plant water deficit is recognized when rate of transpiration exceeds water uptake, which causes a reduction in the cell's relative water content and volume and swelling (Lawlor and Cornic 2002). Cellular water deficit is a common phenomenon that occurs from diverse stresses such as drought, salinity, as well as low and high temperature (Song et al. 2009). Drought affects all growth stages of the plant through reducing seed germination and seedling development as well as different morphological and molecular changes (Farooq et al. 2009; Kaya et al. 2006; Nezhadahmadi et al. 2013). Also, it affects different physiological processes such as photosynthesis efficiency, relative water content, leaf water potential, transpiration rate, leaf temperature, and stomatal conductance (Machado and Paulsen 2001).

15.4 Roles of Alleviating Abiotic Stresses

Different abiotic stresses cause major losses in all sectors of agricultural production worldwide (Bray et al. 2000; Suzuki et al. 2014). The sector of plant production could be able to overcome these stresses to a great extent by using different strategies such as the following:

15.4.1 Using Chemical Fertilizers

Chemical fertilizers are used to improve plant growth and to overcome different abiotic stresses. Because they cause health hazards and environmental pollution, they are not preferable for sustainable development of agriculture.

15.4.2 Plant Breeding

This process aims to select a plant variety that is resistant to stresses. This strategy is not preferable, as it is time consuming and requires availability of many resistant varieties.

15.4.3 Using Different Microbial Inoculants

Inoculation of plants with microbial inoculants that adapted to adverse abiotic stress conditions is considered as an environment-friendly strategy that could stimulate the growth of plants and protect them against the harmful effects of various abiotic stresses, by strengthening plants' natural defense "resistance inducers" (Conrath et al. 2015; Marulanda et al. 2007, 2009).

15.5 Role of Plant-Growth-Promoting Microorganisms in Alleviation of Abiotic Stresses

Different microbial activities often result in improving plant tolerance to abiotic stress conditions. The role of alleviation depends on the plant genus, the stress type, the microbial species, and the type of relationship between the microorganism and plant. Microorganisms have different mitigation methods that range from producing protective metabolites to inducing plants to produce mitigation compounds, which improve plant tolerance to abiotic stresses that is called "induced systemic tolerance" (IST). This term has been proposed for PGPMs which prompt different physical and chemical changes in plants that subsequently enhance plant tolerance to abiotic stresses (Barea 2015; Grover et al. 2011; Shrivastava and Kumar 2015), such as the following:

15.5.1 Production of Phytohormones

Most PGPMs are able to produce different phytohormones as well as induce plants to produce hormones such as indole acetic acid, cytokinins, gibberellins, and abscisic acid as well as some other growth regulators (Hayat et al. 2010; Spaepen et al. 2008). These phytohormones are believed to play a key role in the adaptation of mechanisms of plants that are exposed to environmental stresses in the form of changing root morphology; increasing root growth, length, and surface area; as well as enhancing the formation of lateral roots and root hairs (Potters et al. 2007; Spaepen et al. 2008; Spaepen and Vanderleyden 2011). All previous changes help in improving water acquisition and nutrient uptake which are expected to cause alleviation of abiotic stress effects on the plant (Diby et al. 2005; Egamberdieva and Kucharova 2009; Paul and Lade 2014). A wide range of PGPMs has been reported as a producer of different phytohormones and/or inducer for plants to produce phytohormones such as *Pseudomonas fluorescens*, *Azotobacter chiroococcum*, and *Azospirillum brasilense* (El-Fattah et al. 2013; Manaf and Zayed 2015; Zayed 2012).

15.5.2 Production of ACC Deaminase

Ethylene is a gaseous growth factor that is produced naturally in plants. It participates in various cellular processes and plant development (Dolan 1997). Also, it regulates root and shoot growth (Miliute et al. 2015). It is known as stress hormone because most abiotic stresses inducing its production dramatically, whereas elevated concentrations of ethylene causes leaf senescence, chlorosis, flower wilting, etc. which have detrimental effects on plant growth and health (Czarny et al. 2006; Etesami et al. 2015; Hermosa et al. 2012; Jha and Saraf 2015).

Ethylene is synthesized in plants by converting S-adenosylmethionine (S-AdoMet) by 1-aminocyclopropane-1-carboxylate synthase (ACS) (that is also called ACC oxidase) to 1-aminocyclopropane-1-carboxylate (ACC), which is the immediate precursor of ethylene production (Bleecker and Kende 2000; Grover et al. 2011; Miliute et al. 2015). Recently, it was discovered that many plant-growth-promoting rhizobacteria (PGPR) contain an enzyme termed 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase that has the ability to cleave the ethylene precursor ACC to α-ketobutyrate and ammonia. The consequence of this degradation is the reduction of ethylene produced by plants as well as the regulation of its level which consequently prevents the growth inhibition caused by its high levels in the plants subjected to stress (Saleem et al. 2007; Singh et al. 2011). Therefore, plants treated with microorganisms containing ACC deaminase may have a comparatively extensive root growth due to decreasing ethylene level (Glick 2014; Hermosa et al. 2012; Safronova et al. 2006; Shaharoona et al. 2006a, b). The consequences of decreasing the ethylene level in plants by PGPMs that produce ACC deaminase are to increase their tolerance toward various stresses and protect them from the deleterious effects of numerous environmental stresses, such as flooding (Grichko and Glick 2001), metals (Burd et al. 2000), drought (Mayak et al. 2004a) and salinity stress (Mayak et al. 2004b).

A wide range of PGPMs have been reported as producers for the enzyme 1-ami nocyclopropane-1-carboxylate deaminase, such as the genera *Bacillus*, *Pseudomonas*, *Klebsiella*, *Serratia*, *Arthrobacter*, *Streptomyces*, *Microbacterium*, *Achromobacter*, *Acidovorax*, *Alcaligenes*, *Enterobacter*, *Agrobacterium genomovars*, *Burkholderia*, *Methylobacterium fujisawaense*, *Rhodococcus*, *Azospirillum lipoferum*, *Rhizobium*, *Sinorhizobium meliloti*, *and Variovorax paradoxus* (Belimov et al. 2001; Blaha et al. 2006; Esquivel-Cote et al. 2010; Hontzeas et al. 2004; Ma et al. 2003; Pandey et al. 2005; Penrose and Glick 2001).

15.5.3 Conserving Ion Homeostasis

Homeostasis literally means “same state”; thus, ion homeostasis could be defined as “the ability of internal systems of the plant or cell to maintain the concentrations of its internal ions stable to remain very nearly constant, even in the presence of any environmental stresses” (Niu et al. 1995). Maintenance of intracellular ionic homeostasis is very important to all physiological functions of living cell.

Potassium and calcium are necessary for regulating many metabolic processes. For instance, potassium "K^{+}" is essential for stomatal movements and protein synthesis, as it is essential for the binding of tRNA to ribosomes (Caravaca et al. 2004; Chakraborty and Chakraborty 2015), while calcium "Ca_2^{+}" is constitutional for ionic balance, gene expression, cell growth, cell division, cell development, as well as metabolism of lipids, proteins, and carbohydrates (Tuteja 2007). Many external stress factors such as light, salinity, drought, and high temperature could cause changes in cellular K^{+} and Ca_2^{+} levels, which affect plant growth and development (Mahajan and Tuteja 2005). For instance, salinity is considered as mainly responsible for causing changes in the ratio of ion homeostasis in the plant system as it causes excessive uptake of Na^{+} and reduction of K^{+} and Ca_2^{+} uptake, mobility, and transport to the growing parts of the plant (Giri et al. 2007; Paul and Lade 2014).

Different PGPRs have the ability to reduce the salt toxicity in several plants by decreasing the Na^{+} concentration and increasing the K^{+} and Ca_2^{+} concentration in the cells either by altering host physiology or directly by reducing foliar accumulation of toxic ions (Na^{+} and Cl^{-}) as well as improving the nutritional status of both macro- (N, P, and K) and micronutrients (Zn, Fe, Cu, and Mn) (Bano and Fatima 2009; Hamdia et al. 2004; Kohler et al. 2009). These processes could relieve the deleterious effect of salinity on plant growth and yield (Giri et al. 2007). Different PGPRs were reported as ion homeostasis conserver such as *Pseudomonas* with eggplants (Fu et al. 2010), *Azospirillum* with maize (Ashraf et al. 2004), and *Bacillus subtilis* GB03 with *Arabidopsis* (Zhang et al. 2008).

15.5.4 Accumulation of Osmolytes

Osmolytes are compounds that play essential roles in the adaptation of cells to various abiotic stresses conditions by assisting in the regulation of osmotic pressure in the cytoplasm as well as stabilizing proteins and cell membranes when water, salt, or temperatures are unfavorable for plant growth or survival (McNeil et al. 1999; Tiwari et al. 2010).

Different researches reported that osmotic adjustment in plants subjected to salt, drought, and/or high-temperature stress occurs through accumulation of high concentrations from osmotically active compounds known as osmolytes. These compounds are dissimilar in their composition. It could be characterized as low molecular weight compounds (sugar and sugar alcohols), methylated tertiary N compounds (glycine betaine), and amino acids (proline and glutamate) as well as other low molecular weight metabolites (Parida and Das 2005; Rahnama and Ebrahimzadeh 2004; Shukla et al. 2012).

PGPRs have been demonstrated to enhance plant stress tolerance by contributing in the accumulation of osmolytes in plants.

- **Proline**

Proline is an important amino acid. It accumulates in plant tissues under several abiotic stresses such as drought, salt stress, temperature, as well as other stresses in plants. It helps substantially in the adjustment of cytoplasmic osmotic. It is usually defined as stress marker molecule (Goswami et al. 2016; Kohler et al. 2009; Verbruggen and Hermans 2008).

It has been proved that proline has the ability to stabilize the subcellular structures through stabilizing cell membranes as it interacts with phospholipids and protecting protein structures against denaturation (Ashraf and Foolad 2007). It is also suggested that proline has ROS scavenging activity (Matysik et al. 2002). In addition to the abovementioned properties, accumulation of proline buffers cellular redox potential (Jain et al. 2001; Wahid and Close 2007), as well as enhances the activity of different enzymes in the cell subjected to environmental stresses (Kishor et al. 2005; Verbruggen and Hermans 2008).

Several studies correlated the increment of proline biosynthesis in various plant species subjected to different abiotic stresses with their inoculation by different PGPMs (Jha and Saraf 2015; Kohler et al. 2009; Manaf and Zayed 2015; Paul and Lade 2014; Sandhya et al. 2010; Vardharajula et al. 2011; Zarea et al. 2012). *Zea mays* plants subjected to salt stress showed increment in proline production upon inoculation with *Rhizobium* sp. and *Pseudomonas* sp. (Bano and Fatima 2009; Grover et al. 2011). Endomycorrhizal fungi have also been reported to induce high proline accumulation in plants subjected to abiotic stresses (Chakraborty and Chakraborty 2015; Manaf and Zayed 2015).

- **Glycine betaine (GB)**

Glycine betaine is a quaternary ammonium compound which is also a N-methylated amino acid derivative. It is normally accumulated in different plant species subjected to diverse abiotic stresses, such as salt, drought, and extreme temperature (Ashraf and Foolad 2007; Chen and Murata 2008, 2011). It can stabilize the structures and the activities of enzymes in the cells. Also, it maintains the integrity of cell membranes and prevents protein denaturation (Gorham 1995). Interestingly, it was reported that endomycorrhizal fungi induce the biosynthesis of glycine betaine approximately twofold in their host plants subjected to salt stress when compared to non-inoculated plants (Al-Garni 2006).

- **Soluble sugars**

The function of sugars in the cell is not confined to be osmoprotectants during stress but also, they acts as substrates for plant growth as well as regulators for gene expression (Keunen et al. 2013; Koch 1996). There are different types of sugars that act as osmolyte such as sucrose, fructose, maltose, rhamnose, and trehalose (Ranganayakulu et al. 2013).

Trehalose is the most popular sugar that has been reported as an osmoprotectant, which can offer protection to plants against different abiotic stresses including drought, high salt, and extreme temperature (Chakraborty and Chakraborty 2015; Glick 2012). It forms a gel phase to overcome cell dehydration during drought and salinity stresses. In addition, it can prevent the degradation and aggregation of some proteins that frequently occur during both high- and low-temperature stresses (Glick 2012).

Different microorganisms have the ability to support accumulation of trehalose in plants subjected to abiotic stresses, such as endomycorrhizal fungi, symbiotic bacteria such as *Rhizobium* spp., and free-living PGPRs (Grover et al. 2011; Suárez et al. 2008). Also, trehalose is considered one of the main storage carbohydrates in endomycorrhizal fungi. It is present in the extraradical mycelium as well as in the spores (Bécard et al. 1991). Plants inoculated with endomycorrhizal fungi showed high accumulation of trehalose in plants which is suggested to have an important role in protecting plants from abiotic stresses (Hoekstra et al. 1992; Schubert et al. 1992).

Also, some reports confirmed that PGPRs could be genetically engineered to overproduce trehalose to be used as a biofertilizer to overcome some abiotic stresses such as *Rhizobium etli* with bean plants which showed resistance to drought stress when compared to plants inoculated with wild type (Suárez et al. 2008). Similarly, maize plants inoculated with *Azospirillum brasilense* that had previously been genetically engineered to overproduce trehalose were more resistant to drought and produced more biomass than plants treated with wild type (Rodríguez-Salazar et al. 2009).

15.5.5 Production of Microbial Exopolysaccharides

Exopolysaccharides are extracellular polymeric substances produced by PGPRs inhabiting the rhizosphere such as *Pseudomonas* spp. (Grover et al. 2011), *Bacillus* spp., *Sinorhizobium* spp., *Escherichia* spp., *Acetobacter* spp., *Halomonas* spp., *Geobacillus thermodenitrificans*, *Bacillus licheniformis*, *Halococcus* sp., and *Halobacterium* sp. (LaPaglia and Hartzell 1997; Singha 2012).

The exopolysaccharides form a sheath or biofilm between roots and soil to act as an interface between root cells and the surrounding environment. It acts as a protective barrier against desiccation, salt stress, and UV radiations (Chen and Murata 2008). As well, it binds soil particles to form microaggregates and macroaggregates that improve soil's macropores and structure in the rhizosphere which results in improved water availability to inoculated plants (Grover et al. 2011; Paul and Sarma 2006; Sandhya et al. 2009; Upadhyay et al. 2011).

Also, exopolysaccharides have the ability to bind different cations, such as Na^+, which cause a decrease in the availability of Na^+ in the soil, thus supporting alleviation of salt stress for plants subjected to salinity stress (Hassen et al. 2016; Nadeem et al. 2010).

Roberson and Firestone (1992) reported that microbial exopolysaccharides have high water-holding capacity which gives it the potentiality to regulate the flow of nutrients and water to plant roots through it. *Pseudomonas putida* GAP-P45 is known as EPS-producing strain; it has the ability to produce exopolysaccharides

which form a biofilm on the root surface of sunflower seedlings that imparts tolerance to plants against drought stress (Chakraborty and Chakraborty 2015).

15.5.6 Biosynthesis of Antioxidative Enzymes

The normal plant cellular metabolisms such as respiration and photosynthesis release reactive oxygen species (ROS) in small quantities as by-products such as superoxide O_2^-, hydrogen peroxide H_2O_2, hydroxyl radical OH^-, nitric oxide NO^-, hydroperoxy radical HOO^-, lipid peroxide radical ROO^-, peroxynitrite $ONOO^-$, and singlet oxygen $^1O_2$, of which each one of them has definite signaling roles during growth and development (Aruoma 1994; Goswami et al. 2016; Kunwar and Priyadarsini 2011). The concentration of reactive oxygen species increases during various abiotic stresses especially salt stress which make them toxic to plant cells and inhibitor to cellular metabolism as it causes damage to cell components such as lipids, proteins, and nucleic acid (Azooz et al. 2011; Goswami et al. 2016).

To decrease the effect of reactive oxygen species, plants have evolved different efficient antioxidant systems called antioxidative enzymes or ROS scavenging enzymes that can protect them from damages (Azooz et al. 2011). The ROS scavenging enzymes include catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Apel and Hirt 2004; Koyro et al. 2012), which are present in different cellular organs especially in chloroplast and mitochondria (Apel and Hirt 2004).

Different researches have reported that PGPMs induce significant increase of antioxidative enzymes in plants subjected to different abiotic stresses, and these PGPMs are believed to be subsidizing in the tolerance of plants to different abiotic stresses especially salt stress (Chakraborty and Chakraborty 2015; Nautiyal et al. 2008; Zhang et al. 2008).

For instance, *Medicago* plants inoculated with *Sinorhizobium meliloti* strain that has the ability to produce IAA showed less oxidative damage in the form of reduced chlorosis, necrosis, and drying compared to uninoculated plants. These results could be attributed to high antioxidant enzyme activity that is induced by *Sinorhizobium meliloti* which subsidized in enhancing plant tolerance against salt stress (Bianco and Defez 2009). Also, lettuce plants inoculated with endomycorrhizal fungi as well as *Pseudomonas mendocina* and subjected to salt stress showed increases in plant growth compared to uninoculated plants as a result of high antioxidant enzyme activity induced by both microorganisms (Kohler et al. 2010).

15.5.7 Enhancement of Plants Nutritional Status

The nutritional statue of plants prominently affects their ability to adapt adverse environmental stress conditions since nutritional imbalance impedes plant growth, development, and yield. The imbalances on nutrient availability practically result from the effect of adverse abiotic stress especially salinity (Paul and Lade 2014).

For instance, crop performance may be adversely affected by salinity in the form of nutritional disorders such as reduction of uptake and accumulation of nitrogenous compounds (Feigin 1985), phosphate (Sharpley et al. 1992), as well as potassium (Botella et al. 1997).

PGPRs have been proved to be essential in the circulation of nutrients in soil to be available for plants. Nitrogen-fixing bacteria either associative like *Rhizobium* sp. and *Frankia* or nonassociative like *Azotobacter* sp. and *Azospirillum* sp. provide plants with nitrogenous compounds. Many strains of PGPMs (PGPRs and PGPFs) can solubilize inorganic phosphate and/or mineralizing organic phosphate, thus reducing the need for chemical fertilizers such as endomycorrhizae and *Bacillus megaterium* (Ogut et al. 2010; Spaepen et al. 2008).

Azospirillum brasilense have been reported to reduce the damaging effects of NaCl on wheat seedlings (Creus et al. 1997). Also lettuce seeds inoculated by *Azospirillum* sp. and subjected to NaCl recorded better seed germination and vegetative growth compared to non-inoculated plants (Barassi et al. 2006). Similarly, *Sinorhizobium meliloti* RD64 strain which has the ability to overproduce IAA and high phosphatase activity has proved its ability to protect plants against salinity stress as a result of improving nutrient contents in plants especially phosphate (Bianco and Defez 2010). Correspondingly, endomycorrhizal fungi have the ability to improve phosphorus concentration, nitrogen fixation, nodulation, as well as higher antioxidant activity in *Trifolium alexandrinum* plants co-inoculated with AM fungi and *Rhizobium* sp. that result in tolerance of plants to salinity stress (Garg and Manchanda 2008; Shokri and Maadi 2009).

15.6 Challenges of Future Perspectives

Using of microbial inoculants as inputs for sustainable agriculture development is considered an important challenge during the next decades as it requires starting a wide strategic plan to maximize the benefits from application of PGPMs to plants and soil. This plan requires different stipulations to ensure its efficiency. These stipulations could be summarized as follows:

- Selection of rhizosphere-competent microorganisms which have plant-growth-promoting attributes and high competition properties (Hynes et al. 2008) because competition for limited nutrients is critical. The most prominent beneficial effect of inoculation with potential PGPMs is durability in poor soils Dimkpa et al. 2009; Ramos Solano et al. 2007). Therefore, even though there are huge numbers of associative and entophytic microorganisms that revealed plant-growth-promoting properties in research laboratory and greenhouse levels, most of them frequently fail to exhibit reliable performance in natural conditions as a result of different environmental factors such as soil type, nutritional status of soil, host plant genotype, and age as well as climatic conditions which affect their survival that subsequently reduce their benefit to plant (Bhattacharyya and Jha 2012).

- Selection of good carrier and using a suitable formulation process to produce efficient microbial inoculant.
- Carriers are abiotic substrates that are used in the formulation process of microbial inoculants. It should be able to deliver the right number of viable cells in good physiological condition and at the right time. It has been shown that carriers could improve the survival and effectiveness of microbial inoculants by physically protecting the microbial culture from biotic and abiotic stresses (Zayed 2016).
- Co-inoculation of plants with different microbial strains that have the ability to contribute together to relieve different abiotic stresses.
- Co-inoculation of different PGPRs is considered a fruitful strategy to alleviate negative effects of abiotic stress in plants, such as dual inoculation of *Rhizobium* sp. and *Azospirillum* sp. to legumes which caused an increase in the total number of nodules, acetylene reduction activities, and the total N content compared to legumes inoculated with *Rhizobium* sp. alone (Dardanelli et al. 2008; Remans et al. 2007, 2008).
- Also, *Azospirillum* sp. is considered a helper for *Rhizobium* sp. that stimulates nodulation, nodule function, as well as plant metabolism (Molla et al. 2001; Verma et al. 2010). Similarly,
- *Zea mays* plants co-inoculated with *Rhizobium* sp. and *Pseudomonas* sp. recorded increased proline concentration, relative water content of leaves, selective uptake of K^+ ions, and decreased electrolyte leakage that resulted in additional salt tolerance compared to plants inoculated with *Rhizobium* sp. alone (Bano and Fatima 2009).
- Developing new soil management practices which favor the diversity, development, and activity of PGPMs that inhabit the soil.
- Traditional agricultural practices have limited effectiveness in improving agricultural productivity, while some agricultural management methods such as soil tillage and irrigation have a great effect on soil characteristics as well as altering the quantity, the survival, and the effectiveness of microbial populations in soil (García-Orenes et al. 2013; Jangid et al. 2008).
- Proposing a computer simulation system that shows the survival rate of introduced microorganisms in any given microbial community, to expect the proportion of its effectiveness on plant-growth stimulation (Strigul and Kravchenko 2006).

Reference

Al-Garni SMS (2006) Increasing NaCl-salt tolerance of a halophytic plant Phragmites Australis by mycorrhizal symbiosis. Am-Eurasian J Agric Environ Sci 1(2):119–126

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Aruoma O (1994) Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxicol 32(7):671–683

Ashraf M, Foolad M (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59(2):206–216

Ashraf M, Hasnain S, Berge O, Mahmood T (2004) Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40(3):157–162

Azooz MM, Youssef AM, Ahmad P (2011) Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. Int J Plant Physiol Biochem 3(14):253–264

Bano A, Fatima M (2009) Salt tolerance in Zea mays (L). Following inoculation with Rhizobium and Pseudomonas. Biol Fertil Soils 45(4):405–413

Barassi C, Ayrault G, Creus C, Sueldo R, Sobrero M (2006) Seed inoculation with Azospirillum mitigates NaCl effects on lettuce. Sci Hortic 109(1):8–14

Barea J (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(2):261–282

Barka EA, Nowak J, Clément 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

Bécard G, Doner L, Rolin D, Douds D, Pfeffer P (1991) Identification and quantification of trehalose in vesicular-arbuscular mycorrhizal fungi by in vivo13C NMR and HPLC analyses. New Phytol 118(4):547–552

Belimov AA, Safronova VI, Sergeyeva TA, Egorova TN, Matveyeva VA, Tsyganov VE, Borisov AY, Tikhonovich IA, Kluge C, Preisfeld A (2001) Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 47(7):642–652

Bhattacharyya P, Jha D (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(4):1327–1350

Bianco C, Defez R (2009) Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing Sinorhizobium meliloti strain. J Exp Bot 60(11):3097–3107

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(14):4626–4632

Blaha D, Prigent-Combaret C, Mirza MS, Moënne-Loccoz Y (2006) Phylogeny of the 1-aminocyc lopropane-1-carboxylic acid deaminase-encoding gene acdS in phytobeneficial and pathogenic Proteobacteria and relation with strain biogeography. FEMS Microbiol Ecol 56(3):455–470

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16(1):1–18

Bolt GH (ed) (1980) Soil chemistry. Part B, Physico-chemical models (Developments in soil science, 5b.). Elsevier, Amsterdam

Botella M, Martinez V, Pardines J, Cerda A (1997) Salinity induced potassium deficiency in maize plants. J Plant Physiol 150(1–2):200–205

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. Biochem Mol Biol Plants 1158:e1203

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46(3):237–245

Caravaca F, Figueroa D, Barea J, Azcon-Aguilar C, Roldan A (2004) Effect of mycorrhizal inoculation on nutrient acquisition, gas exchange, and nitrate reductase activity of two Mediterranean-autochthonous shrub species under drought stress. J Plant Nutr 27(1):57–74

Carmen B, Roberto D (2011) Soil bacteria support and protect plants against abiotic stresses. In: A Shanker Abiotic stress in plantsmechanisms and adaptations, Pub InTech:143–170

Chakraborty U, Chakraborty B (2015) Abiotic stresses in crop plants. CABI, Wallingford

Chakraborty U, Chakraborty B, Dey P, Chakraborty AP (2015) Role of microorganisms in alleviation of abiotic stresses for sustainable agriculture. Abiotic stresses in crop plants. CABI, Wallingford, pp 232–253

Chang W-S, van de Mortel M, Nielsen L, de Guzman GN, Li X, Halverson LJ (2007) Alginate production by pseudomonas putida creates a hydrated microenvironment and contributes

to biofilm architecture and stress tolerance under water-limiting conditions. J Bacteriol 189(22):8290–8299
Chen TH, Murata N (2008) Glycinebetaine: an effective protectant against abiotic stress in plants. Trends Plant Sci 13(9):499–505
Chen TH, Murata N (2011) Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant Cell Environ 34(1):1–20
Conrath U, Beckers GJ, Langenbach CJ, Jaskiewicz MR (2015) Priming for enhanced defense. Annu Rev Phytopathol 53:97–119
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 (Paris) 35:939–944
Czarny JC, Grichko VP, Glick BR (2006) Genetic modulation of ethylene biosynthesis and signaling in plants. Biotechnol Adv 24(4):410–419
Dardanelli MS, de Cordoba FJF, Espuny MR, Carvajal MAR, Díaz MES, Serrano AMG, Okon Y, Megías M (2008) Effect of Azospirillum brasilense coinoculated with Rhizobium on Phaseolus vulgaris flavonoids and Nod factor production under salt stress. Soil Biol Biochem 40(11):2713–2721
de Zelicourt A, Al-Yousif M, Hirt H (2013) Rhizosphere microbes as essential partners for plant stress tolerance. Mol Plant 6(2):242–245
Diby P, Sarma YR, Srinivasan V, Anandaraj M (2005) Pseudomonas Fluorescens mediated vigour in black pepper (Piper Nigrum L.) under green house cultivation. Ann Microbiol 55(3):171
Dimkpa C, Svatoš A, Merten D, Büchel G, Kothe E (2008) Hydroxamate siderophores produced by Streptomyces acidiscabies E13 bind nickel and promote growth in cowpea (Vigna unguiculata L.) under nickel stress. Can J Microbiol 54(3):163–172
Dimkpa C, Weinand T, Asch F (2009) Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32(12):1682–1694
Dolan L (1997) The role of ethylene in the development of plant form. J Exp Bot 48(2):201–210
Dong J, Yu Z, Yu S (1983) Effect of increased ethylene production during different periods on the resistance of wheat plants to waterlogging. Acta Phys Sin 9(4):383–389
Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol Fertil Soils 45(6):563–571
El-Fattah DAA, Eweda WE, Zayed MS, Hassanein MK (2013) Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of Azotobacter chroococcum inoculant. Ann Agric Sci 58(2):111–118
Esquivel-Cote R, Ramírez-Gama RM, Tsuzuki-Reyes G, Orozco-Segovia A, Huante P (2010) Azospirillum lipoferum strain AZm5 containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. Plant Soil 337(1–2):65–75
Etesami H, Alikhani HA, Hosseini HM (2015) Indole-3-acetic acid and 1-aminocyclopropane-1-carboxylate deaminase: bacterial traits required in rhizosphere, rhizoplane and/or endophytic competence by beneficial bacteria. In: Maheshwari DK (ed) Bacterial metabolites in sustainable agroecosystem. Springer, Cham, pp 183–258
Fageria N, Baligar V (2003) Methodology for evaluation of lowland rice genotypes for nitrogen use efficiency. J Plant Nutr 26(6):1315–1333
Farooq M, Wahid A, Kobayashi N, Fujita D, Basra S (2009) Plant drought stress: effects, mechanisms and management. In: Sustainable agriculture. Springer, pp 153–188
Feigin A (1985) Fertilization management of crops irrigated with saline water. In: Biosalinity in Action: Bioproduction with Saline Water. Springer, pp 285–299
Fu Q, Liu C, Ding N, Lin Y, Guo B (2010) Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium Pseudomonas sp. DW1 on growth of eggplant (Solanum melongena L.) seedlings under salt stress. Agric Water Manag 97(12):1994–2000
García-Orenes F, Morugán-Coronado A, Zornoza R, Scow K (2013) Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem. PLoS One 8(11):e80522

Garg N, Manchanda G (2008) Effect of arbuscular mycorrhizal inoculation on salt-induced nodule senescence in Cajanus cajan (pigeonpea). J Plant Growth Regul 27(2):115
Giri B, Kapoor R, Mukerji K (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(4):753–760
Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012(5):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
Gorham J (1995) Betaines in higher plants–biosynthesis and role in stress metabolism. Semin Ser 56:173
Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric 2(1):1127500
Gray E, Smith D (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol Biochem 37(3):395–412
Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39(1):11–17
Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27(5):1231–1240
Hamdia MAE-S, Shaddad M, Doaa MM (2004) Mechanisms of salt tolerance and interactive effects of Azospirillum brasilense inoculation on maize cultivars grown under salt stress conditions. Plant Growth Regul 44(2):165–174
Hasanuzzaman M, Nahar K, Fujita M (2013) Extreme temperature responses, oxidative stress and antioxidant defense in plants. INTECH Open Access Publisher
Hassen AI, Bopape F, Sanger L (2016) Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants. In: Microbial inoculants in sustainable agricultural productivity. Springer, India, pp 23–36
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
Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of Trichoderma and of its genes. Microbiology 158(1):17–25
Hoekstra F, Crowe J, Crowe L, Tv R, Vermeer E (1992) Do phospholipids and sucrose determine membrane phase transitions in dehydrating pollen species? Plant Cell Environ 15(5):601–606
Hontzeas N, Saleh SS, Glick BR (2004) Changes in gene expression in canola roots induced by ACC-deaminase-containing plant-growth-promoting bacteria. Mol Plant-Microbe Interact 17(8):865–871
Hynes RK, Leung GC, Hirkala DL, Nelson LM (2008) Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada. Can J Microbiol 54(4):248–258
Jain M, Mathur G, Koul S, Sarin N (2001) Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (Arachis hypogaea L.) Plant Cell Rep 20(5):463–468
Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS, Reeves JH, Jenkins MB, Endale DM, Coleman DC, Whitman WB (2008) Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biol Biochem 40(11):2843–2853
Jha CK, Saraf M (2015) Plant growth promoting rhizobacteria (PGPR): a review. E3 J Agric Res Dev 5:108–119
Kajlaa M, Yadava VK, Khokharc J, Singhb S, Chhokara R, Meenaa RP, Sharmaa R (2015) Increase in wheat production through management of abiotic stresses: a review. J Appl Nat Sci 7(2):1070–1080
Kaya MD, Okçu G, Atak M, Çıkılı Y, Kolsarıcı Ö (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (Helianthus annuus L.) Eur J Agron 24(4):291–295

Keunen E, Peshev D, Vangronsveld J, Van Den Ende W, Cuypers A (2013) Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. Plant Cell Environ 36(7):1242–1255

Kishor PK, Sangam S, Amrutha R, Laxmi PS, Naidu K, Rao K, Rao S, Reddy K, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci 88(3):424–438

Koch K (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Biol 47(1):509–540

Kohler J, Hernández JA, Caravaca F, Roldán A (2009) Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. Environ Exp Bot 65(2):245–252

Kohler J, Caravaca F, Roldán 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(3):429–434

Koyro H-W, Ahmad P, Geissler N (2012) Abiotic stress responses in plants: an overview. In: Environmental adaptations and stress tolerance of plants in the era of climate change. Springer, New York, pp 1–28

Kunwar A, Priyadarsini K (2011) Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci 1(2):53

LaPaglia C, Hartzell PL (1997) Stress-induced production of biofilm in the hyperthermophile Archaeoglobus fulgidus. Appl Environ Microbiol 63(8):3158–3163

Läuchli A, Epstein E (1990) Plant responses to saline and sodic conditions. Agric Salinity Assess Manag 71:113–137

Lawlor D, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25(2):275–294

Lichtenthaler HK (1996) Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol 148(1–2):4–14

Lichtenthaler HK (1998) The stress concept in plants: an introduction. Ann N Y Acad Sci 851(1):187–198

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Ma W, Sebestianova SB, Sebestian J, Burd GI, Guinel FC, Glick BR (2003) Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in Rhizobium spp. Antonie Van Leeuwenhoek 83(3):285–291

Machado S, Paulsen GM (2001) Combined effects of drought and high temperature on water relations of wheat and sorghum. Plant Soil 233(2):179–187

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444(2):139–158

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

Marulanda A, Porcel R, Barea J, Azcón R (2007) Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive Glomus species. Microb Ecol 54(3):543

Marulanda A, Barea J-M, Azcón R (2009) Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. J Plant Growth Regul 28(2):115–124

Matysik J, Bhalu B, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr Sci 82(5):525–532

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

Mayak S, Tirosh T, Glick BR (2004b) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166(2):525–530

McNeil SD, Nuccio ML, Hanson AD (1999) Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol 120(4):945–949

Miliute I, Buzaite O, Baniulis D, Stanys V (2015) Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste-Agriculture 4(102):465–478
Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11(1):15–19
Molla AH, Shamsuddin Z, Halimi M, Morziah M, Puteh A (2001) Potential for enhancement of root growth and nodulation of soybean co-inoculated with Azospirillum and Bradyrhizobium in laboratory systems. Soil Biol Biochem 33(4):457–463
Mora M, Cartes P, Núñez P, Salazar M, Demanet R (2007) Movement of NO3–N and NH4+−N in an Andisol and its influence on ryegrass production in a short term study. J Soil Sci Plant Nutr 7:46–63
Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25(2):239–250
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
Nadeem SM, Zahir ZA, Naveed M, Asghar HN, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Soc Am J 74(2):533–542
Nautiyal CS, Govindarajan R, Lavania M, Pushpangadan P (2008) Novel mechanism of modulating natural antioxidants in functional foods: involvement of plant growth promoting rhizobacteria NRRL B-30488. J Agric Food Chem 56(12):4474–4481
Nezhadahmadi A, Prodhan ZH, Faruq G (2013) Drought tolerance in wheat. Sci World J 2013:1–12
Niu X, Bressan RA, Hasegawa PM, Pardo JM (1995) Ion homeostasis in NaCl stress environments. Plant Physiol 109(3):735
Noble AD, Ruaysoongnern S (2010) The nature of sustainable agriculture. In: Soil microbiology and sustainable crop production. Springer, Berlin, pp 1–25
Ogut M, Er F, Kandemir N (2010) Phosphate solubilization potentials of soil Acinetobacter strains. Biol Fertil Soils 46(7):707–715
Pandey P, Kang S, Maheshwari D (2005) Isolation of endophytic plant growth promoting Burkholderia sp. MSSP from root nodules of Mimosa pudica. Curr Sci 89(1):177–180
Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60(3):324–349
Paul D, Lade H (2014) Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. Agron Sustain Dev 34(4):737–752
Paul D, Sarma Y (2006) Plant growth promoting rhizhobacteria (PGPR)-mediated root proliferation in black pepper (Piper nigrum L.) as evidenced through GS root software. Arch Phytopathol Plant Protect 39(4):311–314
Penrose D, Glick B (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(4):368–372
Poschenrieder C, Gunsé B, Corrales I, Barceló J (2008) A glance into aluminum toxicity and resistance in plants. Sci Total Environ 400(1):356–368
Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MA (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12(3):98–105
Rahnama H, Ebrahimzadeh H (2004) The effect of NaCl on proline accumulation in potato seedlings and calli. Acta Physiol Plant 26(3):263–270
Ramos Solano B, Pereyra de la Iglesia M, Probanza A, Lucas García J, Megías M, Gutierrez Mañero F (2007) Screening for PGPR to improve growth of Cistus ladanifer seedlings for reforestation of degraded mediterranean ecosystems. Plant Soil 289:59–68
Ranganayakulu G, Veeranagamallaiah G, Sudhakar C (2013) Effect of salt stress on osmolyte accumulation in two groundnut cultivars (Arachis hypogaea L.) with contrasting salt tolerance. African J Plant Sci 7(12):586–592
Remans R, Croonenborghs A, Gutierrez RT, Michiels J, Vanderleyden J (2007) Effects of plant growth-promoting rhizobacteria on nodulation of Phaseolus vulgaris L. are dependent on plant P nutrition. Eur J Plant Pathol 119(3):341–351
Remans R, Ramaekers L, Schelkens S, Hernandez G, Garcia A, Reyes JL, Mendez N, Toscano V, Mulling M, Galvez L (2008) Effect of Rhizobium–Azospirillum coinoculation on nitrogen fixation and yield of two contrasting Phaseolus vulgaris L. genotypes cultivated across different environments in Cuba. Plant Soil 312(1–2):25–37

Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in a soil Pseudomonas sp. Appl Environ Microbiol 58(4):1284–1291

Rodríguez-Salazar J, Suárez R, Caballero-Mellado J, Iturriaga G (2009) Trehalose accumulation in Azospirillum brasilense improves drought tolerance and biomass in maize plants. FEMS Microbiol Lett 296(1):52–59

Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42(3):267–272

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(10):635–648

Sandhya V, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing Pseudomonas putida strain GAP-P45. Biol Fertil Soils 46(1):17–26

Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswarlu B (2010) Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Plant Growth Regul 62(1):21–30

Schubert A, Wyss P, Wiemken A (1992) Occurrence of trehalose in vesicular-arbuscular mycorrhizal fungi and in mycorrhizal roots. J Plant Physiol 140(1):41–45

Selvakumar G, Kundu S, Joshi P, Nazim S, Gupta A, Mishra P, Gupta H (2008a) Characterization of a cold-tolerant plant growth-promoting bacterium Pantoea dispersa 1A isolated from a subalpine soil in the north western Indian Himalayas. World J Microbiol Biotechnol 24(7):955–960

Selvakumar G, Mohan M, Kundu S, Gupta A, Joshi P, Nazim S, Gupta H (2008b) Cold tolerance and plant growth promotion potential of Serratia marcescens strain SRM (MTCC 8708) isolated from flowers of summer squash (Cucurbita pepo). Lett Appl Microbiol 46(2):171–175

Selvakumar G, Panneerselvam P, Ganeshamurthy AN (2012) Bacterial mediated alleviation of abiotic stress in crops. In: Bacteria in agrobiology: stress management. Springer, Berlin, pp 205–224

Shaharoona B, Arshad M, Zahir Z (2006a) Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (Zea mays L.) growth under axenic conditions and on nodulation in mung bean (Vigna radiata L.) Lett Appl Microbiol 42(2):155–159

Shaharoona B, Arshad M, Zahir ZA, Khalid A (2006b) Performance of Pseudomonas spp. containing ACC-deaminase for improving growth and yield of maize (Zea mays L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38(9):2971–2975

Shahbaz M, Ashraf M (2013) Improving salinity tolerance in cereals. Crit Rev Plant Sci 32(4):237–249

Sharpley A, Meisinger J, Power J, Suarez D (1992) Root extraction of nutrients associated with long-term soil management. In: Limitations to plant root growth. Springer, New York, pp 151–217

Shokri S, Maadi B (2009) Effects of arbuscular mycorrhizal fungus on the mineral nutrition and yield of Trifolium alexandrinum plants under salinity stress. J Agron 8(2):79–83

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(2):123–131

Shukla PS, Agarwal PK, Jha B (2012) Improved salinity tolerance of Arachishypogaea (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. J Plant Growth Regul 31(2):195–206

Singh JS, Pandey VC, Singh D (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140(3):339–353

Singha TK (2012) Microbial extracellular polymeric substances: production, isolation and applications. IOSR J Pharm 2(2):271–281

Song L, Li F, Fan X, Xiong Y, Wang W, Wu X, Turner N (2009) Soil water availability and plant competition affect the yield of spring wheat. Eur J Agron 31(1):51–60

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3(4):a001438

Spaepen S, Dobbelaere S, Croonenborghs A, Vanderleyden J (2008) Effects of Azospirillum brasilense indole-3-acetic acid production on inoculated wheat plants. Plant Soil 312(1–2):15–23

Strigul NS, Kravchenko LV (2006) Mathematical modeling of PGPR inoculation into the rhizosphere. Environ Model Softw 21(8):1158–1171

Suárez R, Wong A, Ramírez M, Barraza A, Orozco MC, Cevallos MA, Lara M, Hernández G, Iturriaga G (2008) Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. Mol Plant-Microbe Interact 21(7):958–966

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. New Phytol 203(1):32–43

Tester M, Davenport R (2003) Na+ tolerance and Na+ transport in higher plants. Ann Bot 91(5):503–527

Tiwari JK, Munshi AD, Kumar R, Pandey RN, Arora A, Bhat JS, Sureja AK (2010) Effect of salt stress on cucumber: Na+−K+ ratio, osmolyte concentration, phenols and chlorophyll content. Acta Physiol Plant 32(1):103–114

Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra M (2002) Novel plant-microbe rhizosphere interaction involving Streptomyces lydicus WYEC108 and the pea plant (Pisum sativum). Appl Environ Microbiol 68(5):2161–2171

Tuteja N (2007) Abscisic acid and abiotic stress signaling. Plant Signal Behav 2(3):135–138

Upadhyay S, Singh J, Singh D (2011) Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. Pedosphere 21(2):214–222

Vardharajula S, Zulfikar Ali S, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting Bacillus spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6(1):1–14

Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. Amino Acids 35(4):753–759

Verma JP, Yadav J, Tiwari K, Lavakush SV (2010) Impact of plant growth promoting rhizobacteria on crop production. Int J Agric Res 5(11):954–983

Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol 16(2):123–132

Vitti A, Nuzzaci M, Scopa A, Sofo A (2015) Indirect and direct benefits of the use of Trichoderma harzianum Strain T-22 in Agronomic plants subjected to abiotic and biotic stresses. In: Chakraborty U, Chakraborty B (eds) Abiotic stresses in crop plants. CABI, Wallingford, p 222

Wahid A, Close T (2007) Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biol Plant 51(1):104–109

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61(3):199–223

Yang J, Kloepper JW, Ryu C-M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4

Zarea M, Hajinia S, Karimi N, Goltapeh EM, Rejali F, Varma A (2012) Effect of Piriformospora indica and Azospirillum strains from saline or non-saline soil on mitigation of the effects of NaCl. Soil Biol Biochem 45:139–146

Zayed MS (2012) Improvement of growth and nutritional quality of Moringa oleifera using different biofertilizers. Ann Agric Sci 57(1):53–62

Zayed MS (2016) Advances in formulation development technologies. In: Microbial inoculants in sustainable agricultural productivity. Springer, pp 219–237

Zhang H, Kim M-S, Sun Y, Dowd SE, Shi H, Paré PW (2008) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mol Plant-Microbe Interact 21(6):737–744

Endophyte Microbes: A Weapon for Plant Health Management

16

Rajesh Ramdas Waghunde, Rahul Mahadev Shelake, Manisha S. Shinde, and Hidenori Hayashi

Abstract

Endophytes are symptomless fungal or bacterial microorganisms found in almost all living plant species reported so far. Most of the endophytes form a symbiotic association with their host plants by colonizing the internal tissues, which has made them valuable for agriculture as a tool in improving crop health. Bacterial and fungal endophytes are also a valuable source of several key components such as phytohormones (auxins and gibberellins) that help in growth and development of the host plant. Some of the chemicals produced by endophytic microbes have antifungal, antibacterial, and insecticidal properties, which strongly inhibit the growth of other organisms, including phytopathogens. Natural compounds that have been isolated from endophytes can be used as an alternative source with direct application in diverse fields ranging from crop protection to human welfare. They also help the host plants to tolerate various biotic and abiotic stress conditions resulting in better growth and higher yield. Also, endophytic fungi have been emerging as an ideal tool in biotechnology and crop protection research. In this chapter, the historical development of the term endophyte,

R.R. Waghunde (✉)
Department of Plant Pathology, College of Agriculture, Navsari Agricultural University, Bharuch, Gujarat, India
e-mail: rajeshpathology191@gmail.com

R.M. Shelake • H. Hayashi
Proteo-Science Center, Ehime University, Matsuyama, Japan
e-mail: rahultnau@gmail.com; hayashi.hidenori.mj@ehime-u.ac.jp

M.S. Shinde
Department of Plant Pathology, Polytechnic in Agriculture, S.K. Nagar Dantiwada Agricultural University, Deesa, Gujarat, India
e-mail: manisha5476@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_16

isolation, and identification techniques, colonization pathways, host-endophyte interactions, and recent advances in the utilization of endophytes in plant health management are discussed.

Keywords
Endophytes • Colonization • 16S rRNA gene sequencing • Plant-microbe interactions • Phytohormones • Biotic and abiotic stress • Biocontrol

16.1 Introduction

The higher organisms such as plants and animals including humans have a direct or indirect association with a diverse microbial world. This association could be mutual, parasitic, or beneficial to one partner with no effect on the other one, i.e., commensalistic (Rosenblueth and Martínez-Romero 2006). Therefore, the concept of "plant microbiome" or "plant microbiota" has evolved to study plants together with microbes living in close association with the host plants. The associated-microbial species consist of not only bacteria or fungi; it also includes some archaea and protists. Among them, microbes living inside plant tissues for at least some part of their life without causing apparent pathogenic symptoms, popularly known as endophytes, play a crucial role in the growth and development of plants (Hardoim et al. 2015). Recent reports have shown their immense potential in agriculture as growth-promoting, biofertilizer and biocontrol agents. Endophytes isolated from different plants produce several industrial enzymes such as hydrolases, chitinases laminarinases, and glucanases (Lu et al. 2007). Endophytes are a source of antimicrobial agents (Phongpaichit et al. 2006; Verma et al. 2009) and secondary metabolites (Wang et al. 2014a) and in the production of medicinal products such as antibiotics (Gangadevi and Muthumary 2008; Kour et al. 2008; Lin et al. 2010).

In this chapter, we address the historical development of the term "endophyte," interactions between plant-endophyte species, strategies used for the colonization of host species, and the use of beneficial effects of endophytes in agriculture for healthy plant development leading to higher crop yields. Literal meaning and actual use of the term endophyte is deeply discussed to differentiate endophyte microbes in comparison with pathogenic microbes. After getting an entry in plant tissues, how do they establish themselves and how do endophyte manages tackle with the plant defense systems? In this section, we have focused on bacterial and fungal species. Furthermore, we have summarized recent advances describing isolation, identification, and functional aspects of endophytes from different plants.

16.2 Historical Development of the Term "Endophyte"

The fossil studies suggest that microbial association with plants has been dated back to more than 400 million years, during early establishment of terrestrial life, thus contributing a significant role in the transition of life from water to land (Krings

et al. 2007; Rodriguez et al. 2009). The word endophyte is derived from the combination of two Greek words, *endon* and *phyton*, that literally mean within and plant, respectively. The term endophyte was first coined as "Entophytae" for partially parasitic fungi by German scientist Heinrich Friedrich Link in 1809 (Link 1809). Mostly pathogenic or parasitic fungi were described as endophytic species, and nothing was known about bacterial endophytes (Nees von Esenbeck 1817; Unger 1833; De Bary 1866). In the nineteenth century, well-known scientists like Pasteur believed that the plants are sterile and free of any microbes (Compant et al. 2012). Galippe (1887a, b) reported for the first time that different microbes including bacteria can grow inside vegetable plants such as carrot, onion, potato, celery, turnip, sugar beet, lettuce, cabbage, salsify, and radish. Fernbach (1888) repeated the same experiments as Galippe but believed that the microbial contamination might be the main source. In the following year, Bernheim (1888) confirmed the occurrence of beneficial microorganisms within plants. At the same time, Beijerinck (1888) discovered that the bacteria from the root nodule of leguminous plants were capable of fixing atmospheric nitrogen. Only fungal association with plant was described as endophytic during the early years of the twentieth century, but the discovery of bacterial association with the plant established the concept of bacterial endophyte (Chanway 1996; Hallmann et al. 1997). Freeman (1904) described an entire life history of a fungal endophyte in the seeds of darnel (*Lolium temulentum*). Endophytes are reported in a wide range of host plants including lower to higher plant species (Stone et al. 2000).

Endophytes are defined as "those organisms that spend at least part of their lifecycle within the plant species without harm, and host plant does not show any obvious symptoms" (Hardoim et al. 2015). It could be any part of the host plant that can be inhabited by endophytic microbes. It is important to consider the nature and type of microbial species while defining it as an endophyte because many pathogens are latent at some stage of their lifecycle and host plants do not show any visual symptoms (Petrini 1991). Thus, an inclusion of terminology, i.e., "an absence of morphological symptoms," make a distinction between endophyte and pathogenic microbes (Schulz 2006).

16.3 Identification and Isolation Techniques

16.3.1 Morphological Characteristics and Microscopy

Isolation of new endophytic species is the first step toward the identification and in-depth characterization of various parameters such as population dynamics, species diversity, or potential source to improve plant health or screening for the production of other useful chemicals such as secondary metabolites (Tejesvi et al. 2011). The living plant parts are surface sterilized to remove all microbes from the plant surface. The methods used for surface sterilization generally include several steps depending on the tissue type. Only internal (intra- and intercellular) microbes can be isolated by incubating the plant tissues onto nutrient agar plates (Sun and Guo 2012).

Methods used for the identification of endophyte evolved with time. The traditional method for identifying unknown species includes the comparative analysis of morphological and phenotypic characteristics of the already known species. Scientists are referring standard sources such as *Bergey's Manual of Systematic Bacteriology* or previous well-characterized data for typical strains (Clarridge and Alerts 2004; Janda and Abbott 2007). Also, traditional methods of fungal classification are reliant on reproductive structures; hence, several non-sporulating fungi cannot be assigned with taxonomic names (Sun and Guo 2012).

16.3.2 Molecular Techniques

Recently, the 16S rRNA gene sequencing has been effectively applied to identify endophytic isolates and further applied to study their evolutionary relationship by phylogenetic analysis (Petrosino et al. 2009; Bredow et al. 2015). The common use of 16S rRNA gene sequences for confirming a new isolate is due to a number of factors. These factors comprise the presence of 16S rRNA genes in all bacterial and fungal species; the 16S rRNA genes have not shown big nucleotide variations over time, suggesting highly conserved functional features. Additionally, the sequence length up to 1500 bp is large enough for several bioinformatic investigations (Janda and Abbott 2007). Some examples of endophytes (recent 10 years) isolated from different host plants are summarized in Table 16.1. The host tissue, culture medium, and identification method used for investigation of unreported species varies and depends on the endophyte type, plant part, and nutrient requirement.

DNA barcoding is one of the most precise techniques in identification of fungal endophytes and will play a vital role in the future (Sun and Guo 2012). It employs an identification of species by sequencing a higher conserved standardized gene region (Hebert et al. 2003). The intraspecific distance of the DNA barcode region should not surpass the interspecific distance. Identification is simple when a DNA sequence is highly conserved and unique to a single microbial species (Hebert et al. 2003; Letourneau et al. 2010). A successful example of DNA barcode region in the classification of several species in the animal kingdom includes the 648-bp region of *cytochrome C oxidase 1* gene (Hebert et al. 2003; Hajibabaei et al. 2006).

16.4 Colonization of Plants by Endophytic Microbes

16.4.1 Colonization by Fungi

Colonization of specific plant by endophytes can be influenced by several factors, including plant phenotype and genotype, the microbial species and strain type, environmental conditions, and type of tissue targeted by microbes to colonize the plant. Fungi are eukaryotes and bacteria are prokaryotes, but their modes of colonization are not completely different (Rosenblueth and Martínez-Romero 2006). For example, both of them can enter inside the plant cell and grow either in an intracellular or

Table 16.1 Methods used for isolation and identification of endophyte in recent ten years

Parameters	Host plant	Tissue	Characteristics		References
			Medium	Method	
Fungal species					
Phomopsis sp., *Botryosphaeria* sp.	Garcinia	Leaf, shoot	PDA	rRNA gene sequencing	Phongpaichit et al. (2006)
131 endophytic isolates	*Annona squamosa*	Whole plant	PDA	ITS rDNA assay	Lin et al. (2007)
174 endophytic fungal species	*Camptotheca acuminate*	Twig, bark, root	PDA	ITS rDNA assay	Lin et al. (2007)
Bartalinia robillardoides	*Aegle marmelos Correa*	Leaf	PDA	Morphology	Gangadevi and Muthumary (2008)
Fusarium oxysporum, *Juniperus recurva*	*Juniperus recurva*	Whole plant	PDA	Morphological and rRNA gene sequencing	Kour et al. (2008)
Thirty-four fungal isolates	Artemisia species	NA	PDA		Sun (2009)
Fusarium sp., *Epulorhiza* sp.	*Holcoglossum*	Root	PDA	rDNA ITS sequencing	Tan et al. (2012)
127 endophytic fungal species	*Dendrobium*	Seed, root	PDA	Morphological and rRNA gene sequencing	Chen et al. (2012)
Curvularia sp., *Fusarium* sp., *Geotrichum* sp., *Aspergillus* sp., *Gliocladium* sp., *Colletotrichum* sp.	Cocoa	Branch, leaf, shoots	PDA	Morphology and microscopic properties	Amin et al. (2014)
Pezicula sp.	*Forsythia viridissima*	NA	PDA	ITS rDNA assay	Wang et al. (2014b)
Beauveria sp., *Fusarium* sp.	*Dendrobium*	Seeds	PDA	In situ seed baiting	Khamchatra et al. (2016)
Emericella qaudrilineata	*Pteris pellucida*	NA	PDA	Morphology	Goutam (2016)
Colletotrichum sp., *Fusarium* sp., *Guignardia* sp., *Phomopsis* sp., *Phoma* sp., *Microdochium* sp.	*Rhoeo spathacea*	Leaf, root	PDA	Morphology and rRNA sequencing	Alvin et al. (2016)
174 endophytic fungal species	*Camptotheca acuminate*	Twig, bark, root	PDA	ITS rDNA assay	Lin et al. (2007)

(continued)

Table 16.1 (continued)

Parameters	Host plant	Tissue	Characteristics		References
			Medium	Method	
Bacterial species					
Three rhizobial strains (RRE3, RRE5, and RRE6)	*Oryza sativa* L.	Roots	Fahraeus medium (N-free)	16S rRNA gene sequencing	Singh et al. (2006)
Bacillus amyloliquefaciens	*Scutellaria*	Roots	PDA	16S rRNA gene sequencing	Sun et al. (2006)
Paenibacillus polymyxa	*Stemona japonica*	Roots		Morphology and 16S rRNA gene sequencing	Lu et al. (2007)
Streptomyces sp., *Streptosporangium* sp., *Microbispora* sp., and three more isolates	*Azadirachta indica* A. Juss.	Leaf, stem, root	S-agar medium	Morphological characteristics	Verma et al. (2009)
Gammaproteobacteria and *Firmicutes*	Grapevine	Flowers	R2A medium	16S rRNA gene sequencing	Compant et al. (2011)
174 unique bacterial endophytes	Tomato	Leaf, stem, root	Tryptic soy agar	16S rRNA gene sequencing	Rashid et al. (2012)
Stenotrophomonas maltophilia, *Ochrobactrum* spp., and seven other genera	Rice	Seeds	R2A medium	16S rRNA gene sequencing, PCR-based denaturing gradient gel electrophoresis	Hardoim et al. (2012)
Arthrobacter sp., *Brevibacterium* sp., *Corynebacterium* sp., and eight more isolates	Banana cv. Grand Naine	Shoot tips	Tryptic soy agar	16S rRNA sequencing	Sekhar and Thomas (2015)

PDA potato dextrose agar, *ITS* internal transcribed spacer, *NA* data not available

intercellular environment. However, there are certain differences in the pathways pursued by bacteria or fungi.

Unlike mycorrhizal fungi that are limited to rhizosphere and grow in plant root zone, fungal endophytes can be distinguished based on their ability to grow in other parts of the plant such as stems or leaves (Stone et al. 2004). In general, endophytic fungi have been divided into four categories depending on their host specificity, taxonomy, and evolutionary aspects (Rodriguez et al. 2009). The clavicipitaceous endophytes (C endophytes) were categorized as class 1 that colonizes shoot or rhizome, and mode of transmission is primarily vertical. The non-clavicipitaceous endophytes (NC endophytes) have been divided into three categories. The first group of NC endophytes is recognized as class 2 that colonizes the shoot, root, and/or rhizome and may grow in both above- and belowground tissues. Class 2 endophytes have a unique ability to help the colonizing host plant in habitat-dependent stress tolerance (Rodriguez et al. 2008). The next category includes class 3 NC endophytes that colonize mainly the shoot and contain mostly Dikaryomycota members that transmit horizontally. Class 4 members are restricted to the root and comprise dark, septate mycorrhiza-like endophytes (Hardoim et al. 2015).

16.4.2 Colonization by Bacteria

The most preferred plant part by bacterial species to make an entry into the plant is the root which attracts microbes attributable to rhizodeposits and root exudates (Compant et al. 2010). Specifically, active penetration can be achieved in the apical zone with the thin-walled surface layer or through wounds (Fig. 16.1). Some reported cases include *Paenibacillus polymyxa* in *Arabidopsis* (Timmusk et al. 2005), *Azoarcus* sp. strain BH72 in rice (Reinhold-Hurek et al. 2006), *Pantoea* sp. in maize (Ikeda et al. 2013), and *Lysinibacillus* sp. in banana (Andrade et al. 2014). The root hairs, particularly the area around the emerging lateral roots, are a soft target for passive penetration (Malfanova et al. 2013; Kobayashi and Palumbo 2000). Even though root tissues are primary targets for penetration, population densities of endophytic microbes in the rhizosphere are generally not as high as pathogenic microbes. This is due to the fact that root tissues are the primary site of infection (Kobayashi and Palumbo 2000).

The primary site of bacterial colonization is intercellular spaces in plant tissue, e.g., *Acetobacter diazotrophicus* in Brazilian sugarcane (James et al. 1994) and *Herbaspirillum seropedicae* in root exudation sites of maize, sorghum, wheat, and rice plants (Roncato-Maccari et al. 2003). Intracellular species are also reported in the nature, e.g., *Azoarcus* sp. in grasses (Hurek et al. 1994) and *Gluconacetobacter diazotrophicus* in *Arabidopsis* (Cocking et al. 2006). Some bacterial endophytes have been shown to colonize inter- as well as intracellular spaces and systemically spread from root surface to aerial tissues, e.g., *Burkholderia* sp. strain PsJN in grapevines (Compant et al. 2005).

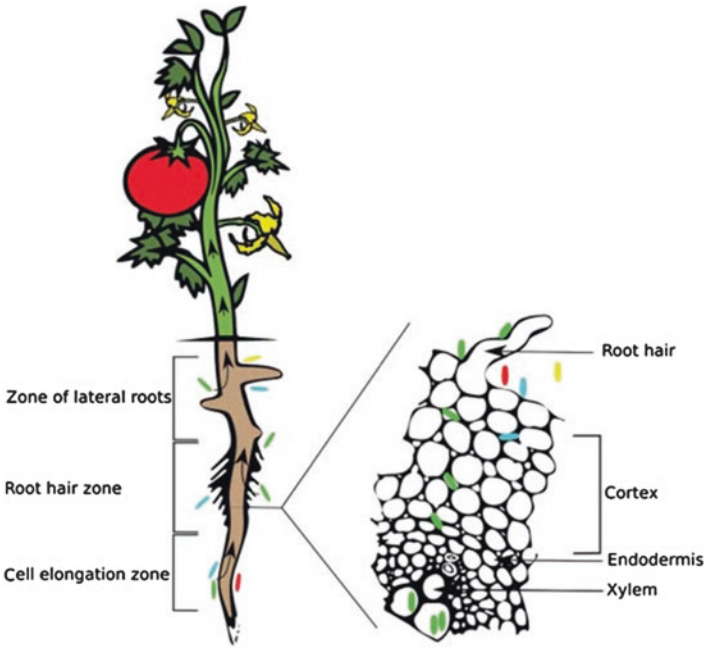


Fig. 16.1 The main colonization pathways used by endophytic bacteria. Endophytes can enter a host plant through several points in root zones as shown in the diagram above. Endophytes can either colonize the plant tissues at the site of entry (shown in *blue*) or move deeper inside or occupy the intercellular space of the cortex and xylem vessels (shown in *green*). *Red* and *yellow* represent rhizospheric bacteria which are unable to colonize inner plant tissues. (This is reused with permission from Malfanova et al. 2013)

Aerial shoot portion of the plant also excretes some amount of exudates on its surface that attracts microbes (Compant et al. 2010). However, only certain bacterial species can enter through stomata or hydathodes or damaged tissues. This is because shoot surface is exposed to sunlight and is prone to desiccation and lacks nutrient availability unless bacteria enter inside the plant tissue by penetrating the endodermis layer (Malfanova et al. 2013). Only certain bacterial species can sustain and able to colonize aerial tissues (Gasser et al. 2011; Hardoim et al. 2015). Some endophytes can colonize reproductive organs of the plants (seeds, flowers, and fruits), e.g., *Bacillus* and *Pseudomonas* spp. in *Cucurbita pepo* L. confirmed by single-stranded conformation polymorphism of 16S rRNA genes (Furnkranz et al. 2012).

16.5 Host-Endophyte Interactions

The host-endophyte relationship is supposed to be more complex than plant-plant or that of within the microbial world themselves. Also, it may differ depending on the degree of intimacy between the microbe and host plant. Some microbes spend their

whole life inside the host except for plant-to-insect-to-plant and plant-to-plant transmission, commonly regarded as "obligate" (Hardoim et al. 2008). Examples of this category consist of several fungi like *Epichloe* sp. and *Neotyphodium* sp. from Clavicipitaceae family in several grasses (Schardl et al. 2004). Some endophytes spend part of their life outside the plant parts (known as epiphytes) and make an entry as soon as favorable conditions are available, called as "opportunistic" such as fungal genus *Trichoderma* (Waghunde et al. 2016) and bacterial genus *Azospirillum* (Steenhoudt and Vanderleyden 2000). These opportunistic endophytes enter the plant endosphere and benefit for protection and nutrient availability inside the plant microenvironment.

Some endophytes are "facultative" that consume nutrients from the host plants. However, this commensalistic interaction of endophytes with plants is a matter of debate because nutrient consumption by endophytes could be "burden" on host plant (Hardoim et al. 2012). The primary site of penetration of facultative endophytes is the emerging lateral roots or the wounds caused by phytopathogens.

16.6 Endophytes in Plant Health Management

Majority of the farming community uses agrochemicals as a sole method to control insect pest and plant diseases. An excessive use of these chemicals has resulted in the development of resistance in pest and diseases not only in traditional plant varieties but also in transgenic plants. Prevalence of these chemicals can cause severe health issues to the farmers, livestock, and consumers. Also, these agrochemicals cannot be degraded by biological means, and it causes environmental pollution. Therefore, application of naturally available microbes is a safe alternative and also a complementary way to tackle the pests and phytopathogens. Endophytic microbes have a huge potential as an alternative source to agrochemicals and are now gaining attention from plant scientists and microbiologists. The phase of 1981 to 1985 can be regarded as the Golden Era of endophyte research, as an investigation of endophytic microbes demonstrated their ability to protect host plant against insect pests. Webber (1981) provided evidence for the first time that bark endophyte *Phomopsis oblonga* plays a role in the protection against fungal pathogen *Ceratocystis ulmi*, responsible for Dutch elm disease. The biological control was achieved by preventing the breeding of beetle *Physocnemum brevilineum* that acts as vectors of *C. ulmi*. This discovery prompted several others to study endophytes as biocontrol agents.

Recent studies have revealed endophytes isolated from different parts of the plants that produce several antimicrobial compounds (Strobel 2003; Zhang et al. 2006; Kharwar et al. 2009). Interestingly, microbial endophytes associated with the medicinal plants are reported to produce a higher number of bioactive compounds (Gond et al. 2007; Kharwar et al. 2008; Verma et al. 2009). Also, recent studies have suggested the potential use of endophytes in biotechnological research and their application in the laboratory plus field level (Araujo et al. 2008). There are two big advantages while applying endophytes in agriculture. At first, endophytes that are derived from the host plants can easily escape and survive against the defense

mechanisms of host plants. Secondly, they are less vulnerable to external stress (biotic and abiotic) and grow normally inside the host plant.

A variety of plant growth promotion (PGP) mechanisms through different pathways has been proposed for endophytic microbes. Nevertheless, only a small number of pathways have been verified in the host plant (Fig. 16.2). These pathways may include either of the following mechanisms: growth stimulation of host plant by producing hormones, biofertilization by solubilizing minerals or fixing atmospheric nitrogen or in iron homeostasis, or stress tolerance induced by ethylene or though production of the ACC deaminase enzyme and by protecting the host plant from environmental pollutants through rhizoremediation (summarized in Fig. 16.2). In this section, we have discussed the role of endophytes in PGP activity and their application in biotic and abiotic stresses.

16.6.1 Plant Growth Promotion Activity

16.6.1.1 Phytohormones Production

Endophytes secrete various hormones that may enhance plant growth (Gimenez et al. 2007). The PGP activity induced by endophytes protects the host plant and prevents from a variety of abiotic and biotic stresses, reflecting plant vigor or persistence. Many studies demonstrated that the plants colonized by endophytes obtain growth promotion, resistance to drought stress, and tolerance to unsuitable soil conditions (Malinowski et al. 2004). The production of phytohormones by endophyte microbes, thereby inducing better growth of the host plant, is one of the most studied mechanisms of PGP activity (Hardoim et al. 2015). Mostly, root-associated endophytes produce phytohormones, especially gibberellins and auxins. Endophytes can produce several other plant hormones including abscisic acid, ethylene, and cytokinins (Lugtenberg et al. 2013; Pliego et al. 2011; Garcia de Salome et al. 2001, 2006; Spaepen et al. 2009). Plant defense mechanisms are dependent on higher demands of energy, and enhanced growth induced by hormones helps the host to fight against biotic and abiotic stresses.

An endophytic fungus, *Colletotrichum* sp. in *Artemisia annua*, produces substances like indole-3-acetic acid (IAA), a hormone that belongs to the auxin group that helps in colonization and regulates plant processes (Lu et al. 2000). The PGP activity has been reported for several endophytic bacteria (Gasser et al. 2011; Malfanova et al. 2011). It has been anticipated that about 80% of the endophytic rhizobacteria can produce auxins and help in PGP by influencing several plant processes (Garcia de Salome et al. 2001; Spaepen et al. 2009). Vendan et al. (2010) reported auxin production in ginseng (*Panax ginseng* C.A. Meyer) and Shcherbakov et al. (2013) in *Sphagnum* mosses. Many endophytic rhizobacteria also secrete gibberellins in the rhizosphere which is known to participate in several biological processes like cell division and cell elongation in meristematic tissues and also in seed germination.

The PGP activity exerted by the combination of bacterial genera has been accounted for many plants, but the specific role of each partner in the association to

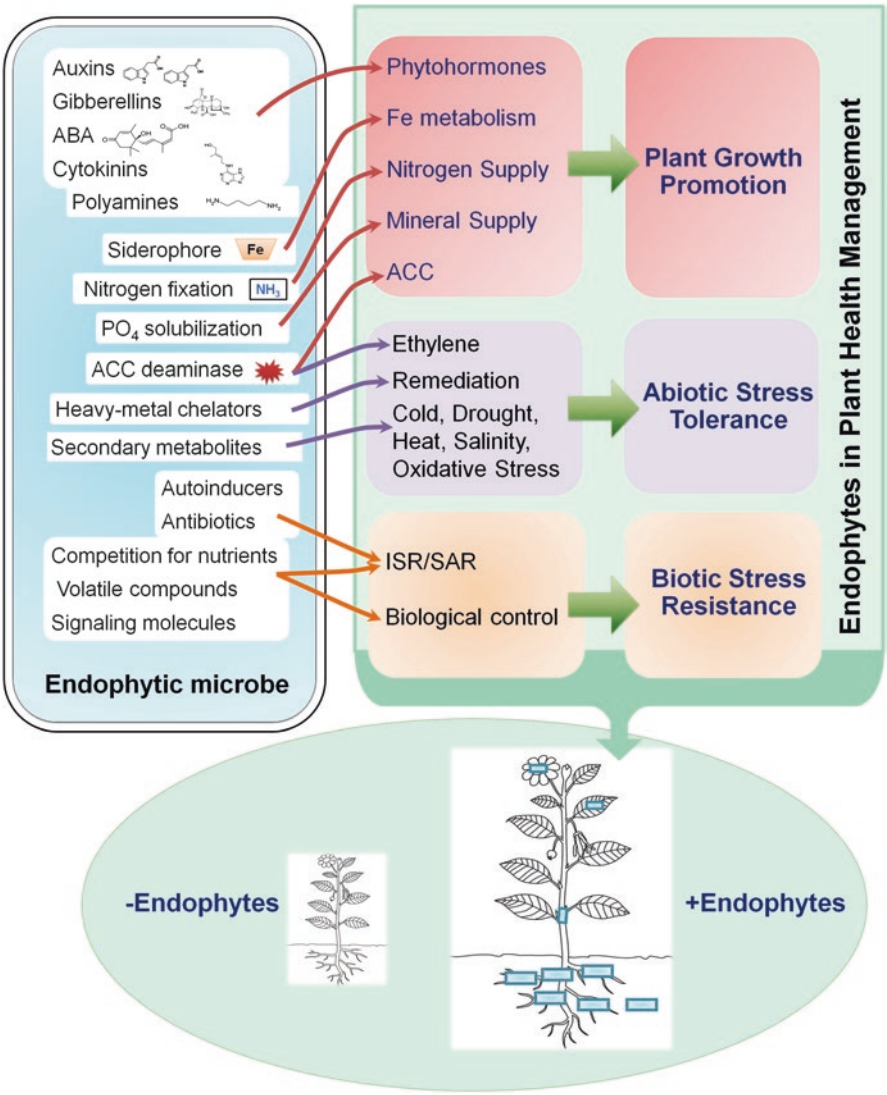


Fig. 16.2 Diagrammatic representation of the plant growth promotion (PGP), mechanisms for abiotic and biotic stress tolerance mediated by endophytic microbes is drawn. The various chemicals/effects produced by endophytic microbes are mentioned inside the cell (*top-left panel*). The *top-right panel* (marked with *green*) depicts the various mechanisms positively regulated by endophytes for the host plant. At the *bottom, left panel* (−endophytes) shows the plant growth without the use of endophyte microbes, and in the *right panel* (+endophytes), improved plant growth occurs due to the beneficial effects of applied endophytes. Abbreviations used: ACC, 1-aminocycl opropane-1-carboxylate; ABA, abscisic acid; ISR/SAR, induced systemic resistance/systemic acquired resistance

PGP has not been studied so far. For example, *Brassica napus* L., *Solanum lycopersicum* Mill (Nejad and Johnson 2000), *Oryza sativa* L. (Adhikari et al. 2001), *Glycine max* L. (Kraus and Loper 1992), and spontaneous legumes (Zakhia et al. 2006) have shown improved growth when consortia of endophytic species were applied. The *Bacillus subtilis* strain HC8 significantly promotes the growth of radish. Moreover, it also produces gibberellins that enhance plant growth (Malfanova et al. 2011). The IAA-producing endophytic bacteria *P. putida* CR3 and *Rahnella aquatilis* HC2 found to stimulate the growth of some cereals and of radish (Malfanova et al. 2013).

16.6.1.2 Iron Metabolism

Siderophores are small chelating compounds with high affinity for iron (Fe^{3+}). Some endophytes produce dramatically a high number of siderophores under iron stress conditions and acquire iron from the environment (Kajula et al. 2010; Johnson et al. 2013). Recently completed genomes of endophytic microbes consist of high number of genes encoding for proteins involved in iron homeostasis. For example, *Enterobacter* sp. 638 consists of nine transporter genes for siderophores (Taghavi et al. 2010). On the other hand, proteobacterial endophytes lacking the siderophore-production ability have shown to possess membrane receptor proteins for the uptake of siderophore-iron complexes produced by other endophytes (Mitter et al. 2013). The exact role of siderophores in the process of colonization by endophytes is poorly studied, but some reports have suggested that they induce the ISR (van Loon et al. 2008), supply iron to the host plant (*Epichloe festucae* association with ryegrass, Johnson et al. 2013), or suppress the growth of phytopathogens (inhibition of *Xylella fastidiosa* by *Methylobacterium* sp. in Citrus, Araujo et al. 2008).

16.6.1.3 Nitrogen Fixation and Phosphate Solubilization

Nitrogen is a major micronutrient for plant growth, and farmers are using chemical N_2 fertilizers to get higher crop yield. An environmental-friendly and financially beneficial alternative to chemical fertilizers is the use of biological N_2-fixing microbes. Biological N_2 fixation by endophytic microbes and its supply to the host plant is a major PGP mechanism through the application of endophytes. It is also a well-studied mechanism involving symbiotic association of free-living microbes as well as endophytes with the plants. Methods followed for investigation include measuring nitrogenase gene expression and nitrogen isotope analysis (Malfanova et al. 2013).

Many root endophytes are involved in the process of N_2 fixation (e.g., *Azoarcus* sp., *Acetobacter* sp., and *Herbaspirillum*, etc.). An endophytic diazotroph, *Paenibacillus polymyxa* P2b-2R, isolated from lodgepole pine is capable of fixing nitrogen (Puri et al. 2016). This endophytic species is also capable of fixing nitrogen in other host plants like canola (*Brassica napus* L.) thereby promoting growth and indicating its broad range host capability. Recently, Moyes et al. (2016) provided evidence for foliar N_2 fixation by endophytic acetic acid bacteria from forest limber

pine (*Pinus flexilis*). They incubated foliage twigs of *P. flexilis* with isotopic nitrogen ($^{13}N_2$)-enriched air and recorded isotopic distribution along with nitrogenase activity. Foliar endophytes can be exploited to develop a low-cost N_2-fixing strategy for long-lived conifers.

Burkholderia phytofirmans strain PsJN is a well-studied bacterial endophyte that involved in PGP mechanism across a broad range of hosts from monocots to dicots including potato, tomato, cucumber, sweet pepper, watermelon, cantaloupe, grapevine, *Arabidopsis*, switchgrass, maize, and wheat (reviewed in Lowman et al. 2016). Strain PsJN is a suitable candidate for genetic engineering studies because of the detailed analysis of completely sequenced genome (Mitter et al. 2013). The strain PsJN transformed with genes of *nif* operon from free-living N_2-fixing bacterium *Burkholderia phymatum* STM 815 and *Sphingomonas* sp. strain NSL was inoculated in switchgrass seed, and seedlings were grown under limited N_2 supply. The transformed PsJN strain was able to promote the growth of switchgrass. Such approach explores the novel way of applying N_2-fixing endophytes as an alternative to decrease the use of chemical fertilizers.

Besides nitrogen and potassium, phosphorus is one of three major nutrients limiting growth of crop plants in natural soils. Although natural soil is rich in phosphorus, plant roots can take up only inorganic form. Plant-associated endophytes convert unavailable phosphorus into a bioavailable inorganic form. For example, an endophytic fungus, *Piriformospora indica*, colonizes the roots of a wide range of plant species including *Arabidopsis*, maize, tobacco, and barley. *P. indica* expresses phosphate transporter and promotes plant growth through higher phosphate uptake, in a mode-like mycorrhizal fungi (Shahollari et al. 2005; Yadav et al. 2010). Asymptomatic fungus *Colletotrichum tofieldiae*, isolated from *Arabidopsis* plants from central Spain, colonizes root and shoot, transfers phosphorus to shoots, and enhances growth plus fertility only under phosphorus-deficient environment (Hiruma et al. 2016).

16.6.1.4 Role of ACC Deaminase, Polyamines, and Other Compounds

The plants produce ethylene as a stress signal under various biotic and abiotic stress conditions. Its precursor, 1-aminocyclopropane-1-carboxylate (ACC), can be degraded by the enzyme ACC deaminase, which converts ACC into two products as α-ketobutyrate and ammonia. The ACC deaminase-producing rhizobacteria can alleviate ethylene-induced stress because of salinity, flooding, heavy metals, drought, toxic chemicals, and phytopathogens (Hardoim et al. 2008; Glick et al. 2007). Some volatile compounds have plant growth-promoting activities, such as acetoin and 2,3-butanediol (Ryu et al. 2003). Also, some polyamines synthesized by the bacterium *Azospirillum brasilense* positively regulate the plant growth and development (Perrig et al. 2007).

16.6.2 Endophytes in Biotic Stress

16.6.2.1 Plant Disease Resistance

The plant resistance mechanisms are divided into two types, SAR and ISR. SAR pathway is generally induced by the pathogen attack, mediated by salicylic acid, and associated with the accumulation of pathogenesis-related proteins (PRP). Conversely, ISR is induced by some nonpathogenic activities and mediated by jasmonic acid or ethylene. ISR is not associated with the PRP accumulation (Tripathi et al. 2008). These PRPs contain a variety of enzymes, some of which may act directly to lyse the pathogenic microbes, including chitinases and β-1,3-glucanases (Fukuda and Shinshi 1994), reinforce cell-wall boundaries to resist infections, or induce the localized cell death. Fungal endophytes induce ISR that may also associate with the expression of pathogenesis-related genes. *F. solani*, isolated from the root tissues of tomato, elicited ISR against the tomato foliar pathogen, *Septoria lycopersici*, and triggered the expression of PR genes, particularly PR5 and PR7 expression in the roots (Kavroulakis et al. 2007).

The ISR induced by endophytic genera *Bacillus*, *Pseudomonas*, and *Serratia* in different plant-pathogen systems and signaling mechanisms involved in the defense priming are reviewed in several reports (Kloepper and Ryu 2006; Pieterse et al. 2014). The plant defense mechanism "remembers" signals induced by ISR and protect non-exposed plant parts against pathogens and pest in the future. Although several endophytic bacteria have been reported to induce a salicylic acid-mediated-ISR, the plant hormones (especially jasmonic acid and ethylene) also play a vital role in induction of ISR (Pieterse et al. 2012). Meanwhile the detailed mechanism of the defense priming during ISR is not yet entirely known; the proof for role of transcription co-regulator NPR1 in the jasmonic acid/ethylene-dependent ISR has been provided; and the cytosol-specific function of the NPR1, different from the function involved in SAR induced by pathogen attack, has been known (Spoel et al. 2003; Stein et al. 2008). Further, the role of transcription factors MYB72 and MYC2 in the establishment of the ISR induced by rhizobacteria and priming of jasmonic acid/ethylene-dependent defense genes has been established (Pozo et al. 2008; Van Der Ent et al. 2008).

16.6.2.2 Biocontrol

Biocontrol can be defined as the reduction of inoculums or disease-causing activity of phytopathogens through the application of microorganisms (Cook and Baker 1983). Plants develop several mechanisms against unfavorable environments such as drought, cold, salt stress, or pathogens. Morphological and biochemical changes, including cellular necrosis, hypersensitive response, and phytoalexin production, respond to the various stresses rapidly. Since fungal endophytes may evolve from the plant pathogenic fungi, plant defense could be triggered by fungal endophytes such as pathogens. The plant defense mechanisms associated with endophytes can be enhanced through production of secondary metabolites. In the endophytic niche, endophytes obtain a reliable source of nutrition from the plant fragment, exudates, and leachates, and in exchange, they protect the host against other microorganisms

(Gao et al. 2010). In another strategy of mutualism, fungal endophytes protect the host plant by competition for nutrients with the pathogens. They rapidly colonize plant tissues draining the available substrates and thereby inhibiting the pathogen growth due to starvation, so that none would be available for pathogens to grow (Pal and Gardener 2006). Furthermore, the plants produce lignin and other cell-wall deposits to limit the growth of endophytes and cause it to be virulent (Harman et al. 2004). As a result, the cell wall becomes reinforced after endophytic colonization; thus, it becomes difficult for pathogens to infest.

Hyperparasitism is another ecological approach that endophytes offer to defend the host plant. In hyperparasitism, the pathogenic species are directly attacked by endophytic microbes that kill it or its propagules. Fungal endophytes parasitize around the hyphae of pathogenic species by different means, for instance, twisting, coiling, penetrating the pathogen hyphae, and secreting lyase to decompose the cell wall. *Trichoderma* species are known to produce a range of enzymes that are directly used against the cell wall degradation of pathogenic fungi to utilize the fragment of pathogens (Gao et al. 2010; Waghunde et al. 2016).

The endophytic fungus *Penicillium commune* isolated from the host plant *Olea europaea* Cv. Cobrançosa has been shown the ability to suppress the growth of the phytopathogen *Colletotrichum acutatum*, which caused anthracnose, one of the major olive diseases (Martins et al. 2013). The establishment of interaction between endophytic fungus *Beauveria bassiana* and one of the most important grapevine pathogens, *Plasmopara viticola*, was first studied by Jaber et al. (2013). Rhizobacterial strains *Pseudomonas aeruginosa* 231–1 were isolated from the roots of watermelon plants grown in the Mekong Delta of Vietnam, which protect watermelon plants from infection by *Didymella bryoniae*, the cause of gummy stem blight.

It has been demonstrated that *P. indica* isolate has plant-promoting properties in numerous plants species and induces resistance against root and shoot pathogens in barley, wheat, *Arabidopsis*, and several other crops. *P. indica* can inhibit the colonization of the most damaging barley pathogens, including *Gaeumannomyces graminis* (take all), *Blumeria graminis* (powdery mildew), *Fusarium graminearum* (head blight), and *Pyrenophora teres* (“net blotch”) (Khaosaad et al. 2007; Macia-Vicente et al. 2008; Baltruschat et al. 2008; Guo et al. 2013). Beneficial effect of *P. indica* in barley has been demonstrated to induce ISR due to elevated antioxidant activity through glutathione-ascorbate cycle (Waller et al. 2005).

Hassan and Hossein (2016) isolated *Stenotrophomonas maltophilia* from rice seeds and tested for the production of volatile and diffusible antibiotics against *M. grisea*, for PGP traits. Soil application of *S. maltophilia* showed better growth and suppressed blast disease in rice seedlings suggesting its potential role as biocontrol agents of *M. grisea* or biofertilizer. Two endophytic microbes, *Alcaligenes faecalis* S18 and *Bacillus cereus* S42, isolated from *Nicotiana glauca* suppressed the *Fusarium* wilt disease and enhanced plant growth of tomato (Abdallah et al. 2016). Selim and coworkers (2017) studied the antifungal potential of three endophytic bacterial strains, *Stenotrophomonas maltophilia* H8, *Pseudomonas aeruginosa* H40, and *Bacillus subtilis* H18.

16.6.3 Endophytes in Abiotic Stress

There is an increasing interest in developing the potential biotechnological applications of endophytic microbes for improving plant stress tolerance and sustainable food production. In addition to enhanced growth properties, modulation of plant metabolism and phytohormone signaling by the endophytic bacteria enhances adaptation to environmental abiotic or biotic stress. Endophytic bacteria present a special interest for improved crop adaptation to stress as they have the advantage of being relatively protected from the harsh environment of the soil under drought, high salt, or other stress conditions. Some examples of endophytes conferring abiotic stress tolerance to host plants are discussed in this subsection.

The protection of cucumber plants against cucumber anthracnose induced by *Pseudomonas fluorescens* strain 89B-61 demonstrated that endophytic bacteria could elicit ISR in plants (Wei et al. 1991; Kloepper and Ryu 2006). Subsequent studies established that the ISR was induced by endophytic bacteria of genus *Bacillus*, *Pseudomonas*, and *Serratia* in different plant-pathogen systems (Kloepper and Ryu 2006; Pieterse et al. 2014). Bacterial endophyte *Burkholderia phytofirmans* PsJN enhances cold tolerance of grapevine plants by altering photosynthetic activity and metabolism of carbohydrates involved in cold stress tolerance (Ait et al. 2006; Fernandez et al. 2012; Theocharis et al. 2012). The bacterium presence in the plant helped in adaptation to chilling temperatures. Similar positive effect on metabolic balance and reduced effect of drought stress was demonstrated in wheat plants grown under reduced irrigation conditions (Naveed et al. 2014).

Cohen et al. (2009) demonstrated that water stress tolerance in maize plants was alleviated by accumulation of the ABA produced by *Azospirillum* spp., and the PGP effect was further enhanced by hormones which may regulate osmotic stress tolerance and water balance in plant (Tuteja 2007). *Pseudomonas pseudoalcaligenes* induces accumulation of a higher amount of glycine betaine-like compounds leading to improved salinity stress tolerance in rice (Jha et al. 2011). In another example, ACC deaminase-producing *Pantoea agglomerans* JP3-3 and *Achromobacter xylosoxidans* strain AX 10 were shown to alleviate the stress of *Brassica* sp. plants grown in copper-contaminated soils and improved copper uptake by the plants (Ma et al. 2009; Zhang et al. 2011).

Chemical and modern biotechnological approaches (e.g., transgenics, nanoparticles) used for bioremediation are either costly or their long-term effects on the living organisms or on the environment are unpredictable. Therefore, in recent years, plant-microbe-metal ion interactions are being studied pertaining to the use of naturally capable microbes in heavy-metal remediation or to confer heavy-metal ion tolerance or higher uptake of essential metal ions by plants (Rajkumar et al. 2012). Inorganic and organic chemicals secreted by root-colonizing endophytes can alter the bioavailable metal levels in rhizosphere through diverse biogeochemical mechanisms like chelation by chemical compounds, immobilizing toxic metal ions, solubilization of unavailable forms into bioavailable one, transformation, translocation, precipitation, and volatilization (Rajkumar et al. 2012). Such processes help the host plants in metal uptake directly conferring tolerance against metal stress.

Also, altered metal uptake in plant tissues improves overall biomass production indirectly. Siderophores have high affinity for iron, but they can also bind to other metals such as Zn Cd, Ga, Al, Cu, and Pb (Schalk et al. 2011). An example of plant metal uptake induced by endophytes includes Mn-resistant endophytic bacteria isolated from a Mn-hyperaccumulator species *Phytolacca Americana* (Zhang et al. 2015). Higher Mn uptake and biomass production was observed in *P. Americana* inoculated with Mn-resistant bacterial strains.

16.7 Concluding Remarks

In brief, numerous reports have revealed a range of beneficial features of endophytes for better plant health management. Nevertheless, there is a great scope to explore the novel functions exerted by endophytic microbes and their utilization for enhancing plant growth and development. Interdisciplinary approach involving a combination of traditional and modern biotechnological methods will help in advancement toward improved plant health and sustainable food production. Discovery of new ways for using endophytic microbes in agriculture will be a step toward safeguarding our environment and ultimately helping to achieve the food security.

References

Abdallah RA, Mokni-Tlili S, Nefzi A et al (2016) Biocontrol of Fusarium wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Nicotiana glauca* organs. Biol Control 97:80–88

Adhikari TB, Joseph CM, Yang GP et al (2001) Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. Can J Microbiol 47:916–924

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 72:7246–7252

Alvin A, Kalaitzis JA, Sasia B, Neilan BA (2016) Combined genetic and bioactivity-based prioritization leads to the isolation of an endophyte-derived antimycobacterial compound. J Appl Microbiol 120:1229–1239

Amin N, Salam M, Junaid M, Baco MS (2014) Isolation and identification of endophytic fungi from cocoa plant resistante VSD M.05 and cocoa plant susceptible VSD M.01 in South Sulawesi, Indonesia. Int J Curr Microbiol Appl Sci 3:459–467

Andrade LF, De Souza GL, Nietsche S et al (2014) Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. J Microbiol 52:27–34

Araujo WL, Lacava PT, Andreote FD, Azevedo JL (2008) Interaction between endophytes and plant host: biotechnological aspects. In: Barka EA, Clément C (eds) Plant-microbe interactions. Research Signpost, Trivandrum, pp 95–115

Baltruschat H, Fodor J, Harrach BD, Niemczyk E 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

Beijerinck MW (1888) Cultur des bacillus radicola aus den Knöllchen. Bot Ztg 46:740–750

Bernheim H (1888) Die parasitären Bakterien der Ceralien. Chemiker-Zeitung 12:1321

Bredow C, Azevedo JL, Pamphile JA et al (2015) In silico analysis of the 16S rRNA gene of endophytic bacteria, isolated from the aerial parts and seeds of important agricultural crops. Genet Mol Res 14:9703–9721

Chanway CP (1996) Endophytes: they're not just fungi! Can J Bot 74:321–322

Chen J, Wang H, Guo SX (2012) Isolation and identification of endophytic and mycorrhizal fungi from seeds and roots of *Dendrobium* (Orchidaceae). Mycorrhiza 22:297–307

Clarridge JE, Alerts C (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev 17:840–862. https://doi.org/10.1128/CMR.17.4.840

Cocking EC, Stone PJ, Davey MR (2006) Intracellular colonization of roots of Arabidopsis and crop plants by *Gluconacetobacter diazotrophicus*. In Vitr Cell Dev Biol – Plant 42:74–82

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, Nowak J et al (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth- promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71:1685–1693

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 et al (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, Sessitsch A, Mathieu F (2012) The 125th anniversary of the first postulation of the soil origin of endophytic bacteria- a tribute to M.L.V. Galippe. Plant Soil 356:299–301

Cook RJ, Baker KF (1983) The nature and practice of biological control of plant pathogens. American Phytopathological Society, University of Michigan, Ann Arbor, p 539

De Bary A (1866) Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. Verlag von Wilhelm Engelmann, Leipzig

Fernandez O, Theocharis A, Bordiec S et al (2012) *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. Mol Plant-Microbe Interact 25:496–504

Fernbach A (1888) De l'absence des microbes dans les tissus végétaux. Ann Inst Pasteur 2:567–570

Freeman EM (1904) The seed fungus of *Lolium temulentum* L., the darnel. Philos Trans R Soc London B 196:1–27

Fukuda Y, Shinshi H (1994) Characterization of a novel cis-acting element that is responsive to fungal elicitor in the promoter of a tobacco class I chitinases gene. Plant Mol Biol 24:485–493

Fürnkranz M, Lukesch B, Müller H et al (2012) Microbial diversity inside pumpkins: microhabitat-specific communities display a high antagonistic potential against phytopathogens. Microb Ecol 63:418–428

Galippe V (1887a) Note sur la présence de micro-organismes dans les tissus végétaux. C R Seances Soc Biol Fil 39:410–416

Galippe V (1887b) Note sur la présence de micro-organismes dans les tissus végétaux (deuxième note). C R Seances Soc Biol Fil 39:557–560

Gangadevi V, Muthumary J (2008) Taxol, an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides* Tassi, isolated from a medicinal plant, *Aegle marmelos Correa* ex Roxb. World J Microbiol Biotechnol 24:717–724

Gao FK, Dai CC, Liu XZ (2010) Mechanism of fungal endophytes in plant protection against pathogen. Afr J Microbiol Res 4:1346–1351

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

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

Gasser I, Cardinale M, Muller H et al (2011) Analysis of the endophytic lifestyle and plant growth promotion of *Burkholderia terricola* ZR2-12. Plant Soil 347:125–136
Gimenez C, Cabrera R, Reina M, Coloma A (2007) Fungal endophytes and their role in plant protection. Curr Org Chem 11:707–720
Glick BR, Todorovic B, Czarny J et al (2007) Promotion of plant growth by bacterial ACC deaminase. Cr Rev Plant Sci 26:227–242
Gond SK, Verma VC, Kumar A et al (2007) Study of endophytic fungal community from different parts of *Aegle marmelos* Correae (Rutaceae) from Varanasi (India). World J Microbiol Biotechnol 23:1371–1375
Goutam J (2016) Isolation and identification of antibacterial compounds isolated from endophytic fungus *Emericella qaudrilineata*. Nat Prod Chem Res 4:205
Guo J, Grosch R, Schwarz D (2013) Impact of *Piriformospora indica* on the growth of different tropical vegetables and on *Fusarium oxysporum* f. sp. niveum. In: Proceedings of the 5th International Symposium on plant protection and plant health in Europe, p 184
Hajibabaei M, Janzen DH, Burns JM et al (2006) DNA barcodes distinguish species of tropical Lepidoptera. Proc Natl Acad Sci U S A 103:968–971
Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914
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
Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD (2012) Dynamics of seed-borne rice endophytes on early plant growth stages. PLoS One 7:e30438
Hardoim PR, van OLS, Berg G et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320
Harman GE, Howell CR, Viterbo A et al (2004) Trichoderma species opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56
Hassan E, Hossein AA (2016) Suppression of the fungal pathogen Magnaporthe grisea by Stenotrophomonas maltophilia, a seed-borne rice (Oryza sativa L.) endophytic bacterium. Arch Agron Soil Sci 62:1271–1284
Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond B 270:313–321
Hiruma K, Gerlach N, Sacristán S et al (2016) Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. Cell 165:464–474
Hurek T, Reinhold-Hurek B, Van Montagu M, Kellenberger E (1994) Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. J Bacteriol 176:1913–1923
Ikeda AC, Bassani LL, Adamoski D et al (2013) Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. Microb Ecol 65:154–160
Jaber LR, Vidal S, Pertot I (2013) Can endophytic *Beauveria bassiana* protect grapevine against *Plasmopara viticola*? In: Proceedings of endophytes for plant protection: the state of the art, p 169
James E, Reis V, Olivares F et al (1994) Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. J Exp Bot 45:757–766
Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol 45:2761–2764
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
Johnson LJ, Koulman A, Christensen M et al (2013) An extracellular siderophore is required to maintain the mutualistic interaction of *Epichloe festucae* with *Lolium perenne*. PLoS Pathog 9:e1003332
Kajula M, Tejesvi MV, Kolehmainen S et al (2010) The siderophore ferricrocin produced by specific foliar endophytic fungi *in vitro*. Fungal Biol 114:248–254

Kavroulakis NS, Zervakis GI, Ehaliotis C et al (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

Khamchatra NM, Dixon K, Chayamarit K et al (2016) Using in situ seed baiting technique to isolate and identify endophytic and mycorrhizal fungi from seeds of a threatened epiphytic orchid, *Dendrobium friedericksianum* Rchb.f. (Orchidaceae). Agric Nat Resour 50:8–13

Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39:727–734

Kharwar RN, Verma VC, Strobel G, Ezra D (2008) The endophytic fungal complex of *Catharanthus roseus* (L.) G. Don. Curr Sci 95:228–233

Kharwar RN, Verma VC, Kumar A (2009) Javanicin, an antibacterial naphthaquinone from an endophytic fungus of neem – *Chloridium* sp. Curr Microbiol 58:233–238

Kloepper JW, Ryu CM (2006) Bacterial endophytes as elicitors of induced systemic resistance. In: Microbial root endophytes. Springer, Berlin/Heidelberg, pp 33–52

Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon CW, White JF (eds) Microbial endophytes. Dekker, New York, pp 199–236

Kour A, Shawl AS, Rehman S et al (2008) Isolation and identification of an endophytic strain of *Fusarium oxysporum* producing podophyllotoxin from *Juniperus recurva*. World J Microbiol Biotechnol 24:1115–1121

Kraus J, Loper JE (1992) Lack of evidence for a role of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5 in biological control of Pythium damping-off of cucumber. Phytopathology 82:264–271

Krings M, Taylor TN, Hass H et al (2007) Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. New Phytol 174:648–657

Letourneau A, Seena S, Marvanová L, Bärlocher F (2010) Potential use of barcoding to identify aquatic hyphomycetes. Fungal Divers 40:51–64

Lin X, Lu C, Huang Y et al (2007) Endophytic fungi from a pharmaceutical plant, *Camptotheca acuminata*: isolation, identification and bioactivity. World J Microbiol Biotechnol 23:1037–1040

Lin X, Huang YJ, Zheng ZH et al (2010) Endophytes from the pharmaceutical plant, *Annona squamosa*: isolation, bioactivity, identification and diversity of its polyketide synthase gene. Fungal Divers 41:41–51

Link HF (1809) Observationes in ordines plantarum naturales, dissertation prima, complectens anandrarum ordines Epiphytas, Mucedines, Gastromycos et Fungos. Der Gesellschaft Naturforschender Freunde zu Berlin, Berlin

Lowman S, Kim-Dura S, Mei C, Nowak J (2016) Strategies for enhancement of switchgrass (*Panicum virgatum* L.) performance under limited nitrogen supply based on utilization of N-fixing bacterial endophytes. Plant Soil 405:47–63

Lu H, Zou WX, Meng JC et al (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Plant Sci 151:67–73

Lu F, Sun L, Lu Z et al (2007) Isolation and identification of an endophytic strain EJS-3 producing novel fibrinolytic enzymes. Curr Microbiol 54:435–439

Lugtenberg B, Malfanova N, Kamilova F, Berg G (2013) Plant growth promotion by microbes. In: Molecular microbial ecology of the rhizosphere. Wiley-Blackwell, Hoboken, pp 561–073

Ma Y, Rajkumar M, Freitas H (2009) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manag 90:831–837

Macia-Vicente JG, Jansson HB, Mendgen K, Lopez-Llorca LV (2008) Colonization of barley roots by endophytic fungi and their reduction of take-all caused by *Gaeumannomyces graminis* var. tritici. Can J Microbiol 54:600–609

Malfanova N, Lugtenberg B, Berg G (2013) Bacterial Endophytes: who and where and what are they doing there? In: de Bruijn FJ (ed) Molecular microbial ecology of the Rhizosphere. Wiley-Blackwell, Hoboken, pp 15–37

Malfanova N, Kamilova F, Validov et al (2011) Characterization of *Bacillus subtilis* HC8, a novel plant-beneficial endophytic strain from giant hogweed. Microb Biotech 4:523–532

Malinowski DP, Zuo H, Belesky DP, Alloush GA (2004) Evidence for copper binding by extracellular root exudates of tall fescue but not perennial ryegrass infected with *Neotyphodium* spp. endophytes. Plant Soil 267:1–12

Martins F, Pereira JA, Bento A, Baptista P (2013) Plant-mediated effects on antagonistic activity of endophytic fungi towards olive fungal diseases. In: Schneider C, Leifert C, Feldmann F (eds) Endophytes for plant protection: the state of the art, pp 127–128

Mitter B, Petric A, Shin MW et al (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

Moyes AB, Kueppers LM, Pett-Ridge J et al (2016) Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. New Phytol 210:657–668

Naveed M, Hussain MB, Zahir ZA et al (2014) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. J Plant Growth Regul 73:121–131

Nees von Esenbeck CG (1817) Das system der Pilze und Schwämme. Stahelschen Buchhandlung, Würzburg

Nejad P, Johnson PA (2000) Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. Biol Control 18:208–215

Pal KK, Gardener BM (2006) Biological control of plant pathogens. The plant health Instructor 2:1117–1142

Perrig D, Boiero ML, Masciarelli OA et al (2007) Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. Appl Microbiol Biotechnol 75:1143–1150

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer-Verlag, New York, pp 179–197

Petrosino JF, Highlander S, Luna RA et al (2009) Metagenomic pyrosequencing and microbial identification. Clin Chem 55:856–866

Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunol Med Microbiol 48:367–372

Pieterse CM, Van der Does D, Zamioudis C et al (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Pieterse CM, Zamioudis C, Berendsen RL et al (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375

Pliego C, Kamilova F, Lugtenberg B (2011) Plant growth-promoting bacteria: fundamentals and exploitation. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer, Berlin, pp 295–343

Pozo MJ, Van Der Ent S, Van Loon LC, Pieterse CM (2008) Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. New Phytol 180:511–523

Puri A, Padda KP, Chanway CP (2016) Evidence of nitrogen fixation and growth promotion in canola (*Brassica napus* L.) by an endophytic diazotroph *Paenibacillus polymyxa* P2b-2R. Biol Fertil Soils 52:119–125

Rajkumar M, Sandhya S, Prasad MN, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30:1562–1574

Rashid S, Charles TC, Glick BR (2012) Isolation and characterization of new plant growth-promoting bacterial endophytes. Appl Soil Ecol 61:217–224

Reinhold-Hurek B, Maes T, Gemmer S et al (2006) An endoglucanase is involved in infection of rice roots by the not-cellulose-metabolizing endophyte *Azoarcus* sp. strain BH72. Mol Plant-Microbe Interact 19:181–188

Rodriguez RJ, Henson J, Van Volkenburgh E et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Roncato-Maccari LDB, Ramos HJO, Pedrosa FO et al (2003) Endophytic *Herbaspirillum seropedicae* expresses *nif* genes in gramineous plants. FEMS Microbiol Ecol 45:39–47

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19:827–837

Ryu CM, Farag MA, CH H (2003) Bacterial volatiles promote growth of Arabidopsis. Proc Natl Acad Sci U S A 100:4927–4932

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340

Schulz B (2006) What are endophytes? In: Schulz B, Boyle CJ, Sieber TN (eds) Microbial root endophytes, vol 9. Springer, Berlin/Heidelberg, pp 1–13

Sekhar AC, Thomas P (2015) Isolation and identification of shoot-tip associated endophytic bacteria from banana cv. Grand Naine and testing for antagonistic activity against *Fusarium oxysporum* f. sp. Cubense. Am J Plant Sci 6:943–954

Selim HMM, Gomaa NM, Essa AMM (2017) Application of endophytic bacteria for the biocontrol of *Rhizoctonia solani* (Cantharellales: ceratobasidiaceae) damping-off disease in cotton seedlings. Biocontrol Sci Technol 27:81–95

Shahollari B, Varma A, Oelmuller R (2005) Expression of a receptor kinase in Arabidopsis roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in triton X-100 insoluble plasma membrane microdomains. J Plant Physiol 162:945–958

Shcherbakov AV, Bragina AV, Kuzmina EY et al (2013) Endophytic bacteria of sphagnum mosses as promising objects of agricultural microbiology. Microbiology 82:306–315

Singh RK, Mishra RPN, Jaiswal HK et al (2006) Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. Curr Microbiol 52:345–349

Spaepen S, Vanderleyden J, Okon Y (2009) Plant growth-promoting actions of rhizobacteria. Ann Botan Res 51:283–320

Spoel SH, Koornneef A, Claessens SM (2003) NPR1 Modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15:760–770

Steenhoudt O, Vandereyden J (2000) Azospirillum, free-living nitrogen fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506

Stein E, Molitor A, Kogel KH, Waller F (2008) Systemic resistance in *Arabidopsis* conferred by the mycorrhizal fungus *Piriformospora indica* requires jasmonic acid signaling and the cytoplasmic function of NPR1. Plant Cell Physiol 49:1747–1751

Stone JA, Bacon CW, White JFJ (2000) An overview of endophytic microbes: endophytism defined. In: Microbial endophytes. Dekker, New York, pp 3–30

Stone JK, Polishook JD, White JRJ (2004) Endophytic fungi. In: Mueller G, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Burlington, pp 241–270

Strobel GA (2003) Endophytes as sources of bioactive products. Microb Infect 5:535–544

Sun M (2009) Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species. Fungal Divers 36:69–88

Sun X, Guo LD (2012) Endophytic fungal diversity: review of traditional and molecular techniques. Mycology 1:65–76

Sun L, Lu Z, Bie X et al (2006) Isolation and characterization of a co-producer of fengycins and surfactins, endophytic *Bacillus amyloliquefaciens* ES-2, from *Scutellaria baicalensis* Georgi. World J Microbiol Biotechnol 22:1259–1266

Taghavi S, van der Lelie D, Hoffman A et al (2010) Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. PLoS Genet 6:e1000943

Tan XM, Chen XM, Wang CL et al (2012) Isolation and identification of endophytic fungi in roots of nine *Holcoglossum* plants (Orchidaceae) collected from Yunnan, Guangxi, and Hainan provinces of China. Curr Microbiol 64:140–147

Tejesvi MV, Kajula M, Mattila S, Pirttilä AM (2011) Bioactivity and genetic diversity of endophytic fungi in *Rhododendron tomentosum* Harmaja. Fungal Divers 47:97–107

Theocharis A, Bordiec S, Fernandez O (2012) *Burkholderia phytofirmans* PsJN primes *Vitis vinifera* L. and confers a better tolerance to low nonfreezing temperatures. Mol Plant-Microbe Interact 25:241–249

Timmusk S, Grantcharova N, Wagner EG (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl Environ Microbiol 71:7292–7300

Tripathi S, Kamal S, Sheramati I et al (2008) Mycorrhizal fungi and other root endophytes as biocontrol agents against root pathogens. Mycorrhiza 3:281–306

Tuteja N (2007) Abscisic acid and abiotic stress signaling. Plant Signal Behav 2:135–138

Unger F (1833) Die Exantheme der Pflanzen und einige mit diesen verwandten Krankheiten der Gewächse: pathogenetisch und nosographisch dargestellt. Verlag Carl Gerold, Vienna. manusal

Van Der Ent S, Verhagen BW, Van Doorn R (2008) MYB72 is required in early signaling steps of rhizobacteria-induced systemic resistance in *Arabidopsis*. Plant Physiol 146:1293–1304

van Loon LC, Bakker P, Van der Heijdt WHW et al (2008) Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance. Mol Plant-Microbe Interact 21:1609–1621

Vendan RT, YJ Y, Lee SH, Rhee YH (2010) Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. J Microbiol 48:559–565

Verma VC, Gond SK, Kumar A et al (2009) Endophytic actinomycetes from *Azadirachta indica* A. Juss.: isolation, diversity, and anti-microbial activity. Microb Ecol 57:749–756

Waghunde RR, Shelake RM, Sabalpara AN (2016) *Trichoderma*: a significant fungus for agriculture and environment. Afr J Agric Res 11:1952–1965

Waller F, Achatz B, Baltruschat H 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

Wang J, Wang G, Zhang Y et al (2014b) Isolation and identification of an endophytic fungus *Pezicula* sp. in *Forsythia viridissima* and its secondary metabolites. World J Microbiol Biotechnol 30:2639–2644

Wang Y, Yang X, Zhang X et al (2014a) Improved plant growth and Zn accumulation in grains of rice (*Oryza sativa* L.) by inoculation of endophytic microbes isolated from a Zn hyperaccumulator, Sedum alfredii H. J Agric Food Chem 62:1783–1791

Webber J (1981) A natural control of Dutch elm disease. Nature 292:449–451

Wei G, Kloepper JW, Tuzan S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508–1512

Yadav V, Kumar M, Deep DK et al (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. J Biol Chem 285:26532–26344

Zakhia F, Jeder H, Willems A et al (2006) Diverse bacteria associated with root nodules of spontaneous legumes in Tunesia and first report for *nifH*-like gene within the genera *Microbacterium* and *Starkeya*. Microbial Eco 51:375–393

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Zhang YF, He LY, Chen ZJ et al (2011) Characterization of ACC deaminase-producing endophytic bacteria isolated from copper-tolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus*. Chemosphere 83:57–62

Zhang WH, Chen W, He LY et al (2015) Characterization of Mn-resistant endophytic bacteria from Mn-hyperaccumulator *Phytolacca americana* and their impact on Mn accumulation of hybrid penisetum. Ecotoxicol Environ Saf 120:369–376

Efficacy of Entomopathogenic Fungi as Green Pesticides: Current and Future Prospects

17

Sardul Singh Sandhu, Harshita Shukla, Ravindra Prasad Aharwal, Suneel Kumar, and Shyamji Shukla

Abstract

The growing commercialization all over the world has led to a boost in the widespread use of chemical pesticides for crop protection in agricultural fields. It has not only contributed to an increase in food production, but its toxic and non-biodegradable character has also resulted in adverse effects on environment and nontarget organisms. Moreover, most of the pests have developed resistance against them. These drawbacks of conventional pesticides have led to an increase in the need for the search of some novel, non-harmful, eco-friendly pesticides. Natural pest control materials commonly known as biocontrol agents are the most promising of them. Biocontrol agents include macroorganisms as well as microorganisms. The microorganisms used are bacteria, fungi, viruses, nematodes and protozoan. The exploitation of these natural and renewable resources is essential for a successful biocontrol strategy. The present review focuses on the use of fungi as potential biocontrol agent for insect pest management. Different fungal formulations and metabolites that have been successfully implemented for pest control and some of the recent patents in this field are also discussed here.

Keywords

Entomopathogenic Fungi • Biocontrol • Pesticide • Insect pest • Patents • Pathogenicity • Green pesticides

S.S. Sandhu (✉) • H. Shukla • R.P. Aharwal • S. Kumar
Department of Biological Science, R.D. University, Jabalpur, Madhya Pradesh 482001, India
e-mail: ssandhu@rediffmail.com

S. Shukla
Department of Biotechnology, M.G.M.M College, Jabalpur, Madhya Pradesh 482002, India
e-mail: shyamshu@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_17

17.1 Introduction

Majority of agricultural products are destroyed by plant pests, thus making them the most important biotic agents (Heydari and Mohammad 2010). Insect pests are most harmful of all the pests, since they cause about 42% of the total crop damage (Oerke and Dehne 2004). A variety of diseases are caused by these pests which are generally controlled by chemical pesticides (Cook 1993). But the repeated use of these chemical pesticides for the control of insect pests has caused various hazardous effects on the environment, animals, humans and other nontarget organisms over many years (Mahr et al. 2001). It is estimated that the total usage of chemical pesticides for agriculture is about 2.5 million tons per annum resulting in a loss of about $100 billion annually (Koul et al. 2008). The main reasons for the undesirable effects of chemical pesticides include their toxic and non-biodegradable character and increasing resistance among insects towards them (French-Constant et al. 2004). As a consequence of these drawbacks associated with the use of chemical pesticides, there is a growing interest among the agriculturist to search for some novel and eco-friendly strategies for pest control. A considerable number of effective pest control methods have been developed and are presently in use (Cook 1993; Benhamon 2004; Islam et al. 2005; Heydari 2007). Use of biological control agents is the most attractive and nonhazardous alternative method for insect pest management (Nicholson 2007). Therefore, realizing the need and importance of biocontrol methods, the current chapter has been written on the use of entomopathogenic fungi as biocontrol agent for insect pest management and some recent patents on the same.

17.1.1 What Is Biological Control?

It is a method in which the pest population is regulated by the use of natural enemies against them, thus reducing the damage caused by them. It is defined as "The action of parasites, predators and pathogen in maintaining another organism's density at a lower average than would occur in their absence" (De Bach 1964). These natural enemies are called as biological control agents (BCAs). They can also be referred to as green pesticides since they reduce the pest population and increase food production in a safe and eco-friendly way (Koul et al. 2008). The natural enemies used for biocontrol of insect pests are divided into two main classes (Kibata 1996,) namely, the microbials and the macrobials. The former class includes microorganisms such as viruses, bacteria, fungi, nematodes, protozoa and rickettsia, while the latter includes macroorganisms such as parasitoids, predators, invertebrates and vertebrates (birds and mammals). Microbial biocontrol agents are more efficient than others since they have complex mode of action as a result of which insect pest does not easily develop resistance against them (Khan et al. 2012). Major microbial biocontrol agents being used include viruses, bacteria, nematodes and fungi.

17.2 Myco-Biocontrol: Fungi as Biocontrol Agent

Myco-biocontrol is the process of controlling insect population by using fungi with the aim of reducing infestation and consequently crop damage caused by them (Chet et al. 1993). It is an eco-friendly and efficient means of reducing insect pests. There is an increasing interest in exploiting the use of fungi as biopesticides from various fungal taxonomic groups to control agricultural pests, because of their diversity, easy engineering and delivery techniques, variety of intracellular as well as extracellular toxic metabolites, etc. (Butt et al. 2001; St Leger and Wang 2009). Besides these their broad spectrum nature in terms of disease control and yield makes them widely accepted biological control agents (Pandya and Saraf 2010). The biodiversity of fungi is enormous including 1.5 million species out of which 70,000 species are known (Zain et al. 2013). The use of fungus as microbial control agents is experimentally being tested by several researchers since late nineteenth century (Lacey et al. 2001). Their complex metabolic pathways, large amount of secreted enzymes and secondary metabolites have been exploited since many years for preparation of various natural products (Moore et al. 2011; Hawksworth 2001; Turner 2000). The bioactivity of the fungal secondary metabolites and genes responsible for their synthesis has drawn attention of microbiologists and pharmacologists towards them (Yu and Keller 2005; Zain et al. 2009; Awaad et al. 2012). Insecticidal activity of fungal secondary metabolites has been studied by a number of researchers. Recently insecticidal activity of five fungal strains *Acremonium cephalosporium*, *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viridae* and *Verticillum albo-atrum* was tested against house fly, *Musca domestica* (Al-Olayan 2013). The highest percentage of mortality to adults of house flies, 97.3 ± 3.52, was produced by 10^7 conidia ml^{-1} of *A. niger* with LT_{50} 3.49. While the lowest mortality percentage, 59.1 ± 2.38 with LT_{50} 6.91, was produced by 10^5 conidia ml^{-1} of *V. albo-atrum.* Fungi showing insecticidal activity mostly belong to *Hyphomycetes* group. *Beauveria bassiana* is most prominent member of the group and is used for preparation of various commercially available biopesticides such as Mycotrol O (Emerald BioAgriculture), Naturalis Home and Garden (H&G) and Naturalis-L (Troy BioSciences, Inc.) (Jim McNeil 2011).

17.2.1 Entomopathogenic Fungi as Biocontrol Agent

The term entomopathogenic fungi refer to fungi which induce disease symptoms in host insect. This domain does include the range of fungi from quick killers to absolute parasites that cause disease symptoms in the host and benefit at the host expense but does not diminish host's life span. Out of 700 species of fungi, about 90 genera are entomopathogenic (Khachatourians and Sohail 2008). Because of their wide range activity against a variety of sap sucking as well as chewing insect pests, entomopathogenic fungi are the first choice of fungal biocontrol agents (Butt 2002; Qazi and Khachatourians 2005; Fan et al. 2007; De Faria and Wraight 2007). Since these fungi are biological agents and do not produce any harmful effects on

the environment, i.e. they are eco-friendly in nature, they could be referred to as green pesticides. Entomopathogenic fungi such as *Verticillium lecanii*, *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces sp., Acremonium* sp. and *Fusarium* sp. are strongest natural enemies of insect pests and hence are most commonly used mycobiocotrol agents (Sandhu 1993; Roberts and St. Leger 2004; Wang et al. 2004; Thomas and Read 2007; Li and Sheng 2007). The bioactivities of these entomopathogenic fungi have been experimentally tested since many years. The activity of *Metarhizium anisopliae* against *Eutectona machaeralis* larva, a serious pest of teak, was analysed (Sandhu et al. 2000). Maximum mortality ca. 97.5 and 95% occurred in I and II instar larvae with LT_{50} of 72 h and 96 h, respectively. The larval mortality was rapid with the higher conidial concentrations. Similarly, *Nomuraea rileyi* caused 90% mortality in second instar larvae of *Spilosoma obliqua*, a cosmopolitan polyphagous pest damaging different cereals, fibres, pulses oilseeds, vegetables and ornamental plants in various parts of India. The mortality was caused by 8.97×10^7 conidia/ml with LT_{50} as 144 h (Mathew et al. 1998). In another study the honey bee-mediated delivery of *Metarhizium anisopliae* increased pollen beetle control (*Meligethes* spp.) in oilseed rape (Butt et al. 1998). Recently, activity of the entomopathogenic fungus, *Paecilomyces lilacinus* was evaluated against the adults of melon flies (*Bactrocera cucurbitae*). After spraying four different spore concentrations of the fungus, highest percentage mortality in adult flies was recorded at a spore concentration of 2.4×10^9 spores ml^{-1} (Amala et al. 2013). The high virulence and epizootic efficiency of entomopathogenic fungi towards insect pests (Agarwal et al. 1990), high sporulation rate and ability to adapt to changing environmental conditions make them more beneficial organisms for biopesticide production (Sharififard et al. 2011; Mwamburi et al. 2010; Lecouna et al. 2005; Kaufman et al. 2005). In another study the extraction conditions of bioactive metabolite from *Cordyceps militaris* 3936 were optimized (Tuli et al. 2014a, b). *Cordyceps militaris* is an entomopathogenic fungus that grows parasitically on lepidopteron larvae and insect pupae. The secondary metabolite of this fungus contains a novel bio-metabolite called cordycepin which has numerous pharmacological and therapeutic potentials. Some of the commonly developed mycoinsecticides used for control of various insect pests include *Beauveria bassiana* (Balsamo) Vuillemin (Babu et al. 2001; Sharma 2004), *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Alter and Vandenberg 2000; Avery et al. 2004) and *Verticillium lecanii* (Zimm.) Viegas (Butt et al. 2001). About 95% of migratory alate aphids are infected by *Beauveria bassiana* (Balsamo) Vuillemin (Chen et al. 2008). Some fungi developed for insect pest control are depicted in Table 17.1. These formulations of entomopathogenic fungi for the regulation of different types of insect pests are not only being commercialized but are also being patented by their inventors. For example, a formulation containing strains of *Beauveria bassiana* was prepared for controlling cockroaches, carpenter ants and pharaoh ants and patented (Stimac et al. 1997). Similarly, a composition containing strain of entomopathogenic fungus *Isaria fumosorosea* ccm 8367 (ccefo.011.pfr) for controlling insect and mite pests was developed and patented (Prenerova et al. 2011).

Table 17.1 Mycoinsecticides developed by using some entomopathogenic fungi

Fungus	Product	Target	Producer
Beauveria bassiana	Conidia	Coffee berry borer	Live Systems Technology, Colombia
Beauveria bassiana	Ostrinil	Corn borer	Natural Plant Protection (NPP), France
Beauveria bassiana	Corn Guard	European corn borer	Mycotech, USA
Beauveria bassiana	Mycotrol GH	Grasshoppers, locusts	Mycotech, USA
Beauveria bassiana	Mycotrol WP and BotaniGard	Whitefly, aphids, thrips	Mycotech, USA
Beauveria bassiana	Naturalis-L	Cotton pests including bollworms	Troy Biosciences, USA
Beauveria bassiana	Naturalis	White flies, thrips, white grubs	Troy Biosciences, US
Beauveria bassiana	Proecol	Army worm	Probioagro, Venezuela
Beauveria bassiana	Boverin	Colorado beetle	Former USSR
Beauveria bassiana	Bio-power	Mite, coffee green bug	Stanes
Beauveria bassiana	Racer BB	Aphids spittle bug, sugarcane	SOM Phytopharma
Beauveria bassiana	Trichobass-L, Trichobass-P	Aphids spittle bug, sugarcane	AMC Chemical/Trichodex
Beauveria brongniartii	Engerlingspilz	Cockchafer(s)	Andermatt, Switzerland
Beauveria brongniartii	Schweizer Beauveria	Cockchafer(s)	Eric Schweizer, Switzerland
Hirsutella thompsonii	Mycar	Eriophyid mites	Abbott Laboratories, USA
Lagenidium giganteum	Laginex	Mosquito larvae	AgraQuest, USA
Metarhizium anisopliae	BIO 1020	Vine weevil	Licenced to Taensa, USA
Metarhizium anisopliae	Biogreen	Scarab larvae on pasture	Bio-care Technology, Australia
Metarhizium anisopliae	Metaquino	Spittle bugs	Brazil
Metarhizium anisopliae	Bio-Blast	Termites	EcoScience, USA
Metarhizium anisopliae	Cobican	Sugarcane spittle bug	Probioagro, Venezuela
Metarhizium anisopliae	Biologic	Black vine weevil	Bayer AG, Germany

(continued)

Table 17.1 (continued)

Fungus	Product	Target	Producer
Metarhizium flavoviride	Green Muscle	Locusts, grasshoppers	CABI BioScience, UK
Paecilomyces fumosoroseus	PFR-97	Whitefly	ECO-tek, USA
Paecilomyces fumosoroseus	PFR-21	Whitefly	W.R. Grace, USA
Paecilomyces fumosoroseus	Pae-Sin	Whitefly	Agrobionsa, Mexico
Verticillium lecanii	Mycotal	Whitefly and thrips	Koppert, the Netherlands
Verticillium lecanii	Vertalec	Aphids	Koppert, the Netherlands

Khachatourians (1986), Burges (1998), Butt and Copping (2000), Butt et al. (1999, 2001), Whright et al. (2001), Bhattacharyya et al. (2004), Copping (2004), Zimmermann (2007) and Khan et al. (2012)

17.2.2 Some Potential Candidates of Entomopathogenic Fungi

17.2.2.1 *Beauveria bassiana*

It is a ubiquitous, filamentous, soil-borne fungus possessing high host specificity. This is the most promising candidate of entomopathogenic fungi having a broad range of host such as termites, whitefly, malaria-transmitting mosquitoes, scarabs, weevil, etc. (Sandhu et al. 2012). It causes white muscardine disease of insects and has been developed as microbial insecticide against many major insects like lepidopterans, orthopterans, coleopterans, etc. (Mustafa and Kaur 2009). It produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of *B. bassiana* are short and ovoid and terminate in a narrow apical extension called a rachis. The rachis elongates after each conidium is produced, resulting in a long zigzag extension. The conidia are single-celled, haploid and hydrophobic (Fig. 17.1a). Spores produced by this fungus are resistant to extreme environmental conditions. It causes infection by attaching to the cuticle of the host insect and then germinating upon arrival of favourable conditions. The hypha arising from spore then penetrates the cuticle by secreting cuticle degrading enzymes and grows inside the insect body (Baskar and Ignacimuthu 2011). Thereafter, it suppresses the host immune system by producing a toxin called beauvericin. In a recent study, strain GHA of *B. bassiana* was found to be more potent against a wood boring insect *Xyleborus glabratus* (Carrillo et al. 2015) than two strains of *Isaria fumosorosea* (Ifr 3581 and PFR). Similarly, higher mortality rate was observed in *Polyphylla fullo* larvae infected with *B. bassiana* formulation (Erler and Ates 2015) as compared to those infected with formulations containing *Metarhizium anisopliae.* These studies indicate about the better insecticidal efficiency of *B. bassiana* over other entomopathogenic fungi.

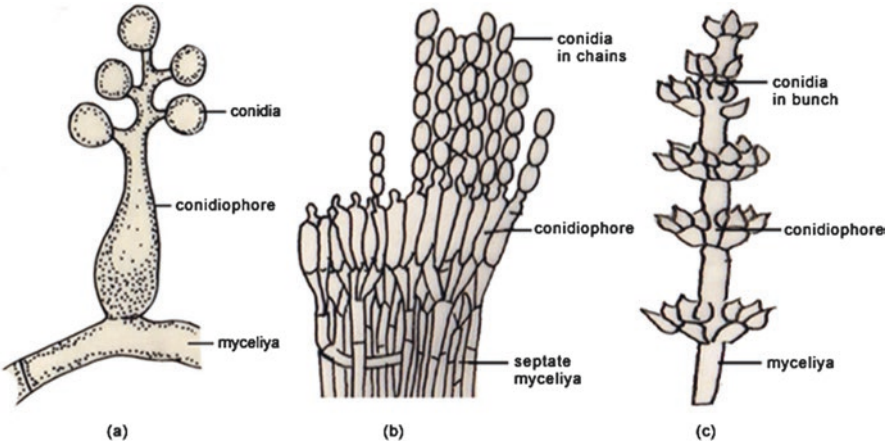


Fig. 17.1 Conidiophore with conidia: (**a**) *Beauveria bassiana* (**b**) *Metarhizium anisopliae* (**c**) *Nomuraea rileyi*

17.2.2.2 *Metarhizium Anisopliae*

It occurs naturally in soil and infects about 200 species of insects. It produces green cylindrical spores in chains from infected insects hence is the causative agent of "green muscardine" disease of insects (Cheraghi et al. 2013). The conidiophores are of variable length, penicillicate, in candle- or palisade-like arrangement, apically forming a sporulation layer, often aggregating into sporodochia (Fig. 17.1b). Its conidia are in long chains, often aggregated into prismatic columns, broadly ellipsoidal to cylindrical (Tzean et al. 1997). Like *B. bassiana* it also has a wide host range and infects some beneficial insects, for example, lady beetles and the teak pest *Eutectona machaeralis* (Sandhu et al. 2012).

17.2.2.3 *Nomuraea Rileyi*

It is yet another important entomopathogenic insect. It attacks mostly the larvae of rice insect. Other host insects of this fungus are leaffolder, stem border larva, green hairy caterpillar, army worm and caseworm (Rombach et al. 1994). It is composed of pale green to grey green conidiophores on a white basal felt of mycelium. The conidia are broadly ellipsoid and in dry chains (Padanad and Krishnaraj 2009). They are 3.5–4.5 × 2–3 μm long (Fig. 17.1c). The conidiophores have branches. Each branch contains 2–5 phialides or conidial chains (Humber 1997).

17.2.3 Beneficial Properties of Entomopathogenic Fungi

Some properties of entomopathogenic fungi which make them beneficial as compared to others (Sandhu et al. 2012) are:

(a) They are specific for particular insect species and do not infect other animals or plants.

(b) They have considerable epizootic potential and can spread quickly through an insect population and cause their collapse.
(c) They penetrate the insect body and infect sucking insects such as aphids and whiteflies that are not susceptible to bacteria and viruses.

17.3 Large-Scale Production of Fungi for Commercialization

The commercial use of entomopathogenic fungi for microbial control of insect pests require understanding of physiological aspect of growth, metabolic activity, genetic basis of virulence and host specificity. Several techniques for the mass production of entomopathogenic fungi are available, mostly designed to yield infective conidia; the conidia are harvested and formulated for storage and field use. Solid-state and liquid-state fermentation has gained significant importance in recent years for the same. Production of fungal conidiospores on large-scale trough is done by solid-state fermentation (Desgranges et al. 1993). Spore of *Metarhizium anisopliae* (ENT-12) has been produced on large scale by solid-state fermentation (Hasan et al. 2002). One of the major advantages of solid-state fermentation is the use of cheaper, easily available, agricultural-based and biodegradable substrate. Recently conidia of *Beauveria bassiana* Bb-202 were produced on rice by solid-state fermentation for the control of coleopteran pests (Xie et al. 2013). On the other hand, liquid-state fermentation is also beneficial in which mass production is carried out under controlled conditions. It has been successfully used for mass production of *Paecilomyces fumosoroseus* (Lozano-Contreras et al. 2007).

17.4 Mode of Action of Entomopathogenic Fungi

The different steps of fungal infection process are influenced by various intrinsic (fungal) and extrinsic (host, environmental) factors. These steps can be summarized as follows (Fig. 17.2):

17.4.1 Adhesion of Spore to the Host Cuticle

Adhesion is the most important prerequisite to infection. It involves the chemical and physical interactions of the insect epicuticle and the spore. For example, the airborne spores of some entomopathogenic fungi make contact where they land on insect surface, whereas zygospores of *Coelomycetes* locate their host by chemotaxis. Adhesion is normally achieved through secretion of cuticle degrading enzymes with the mucilage which interacts with and modifies epicuticular waxes. It also helps in host recognition and acts as cementing substance for pathogen and its substratum.

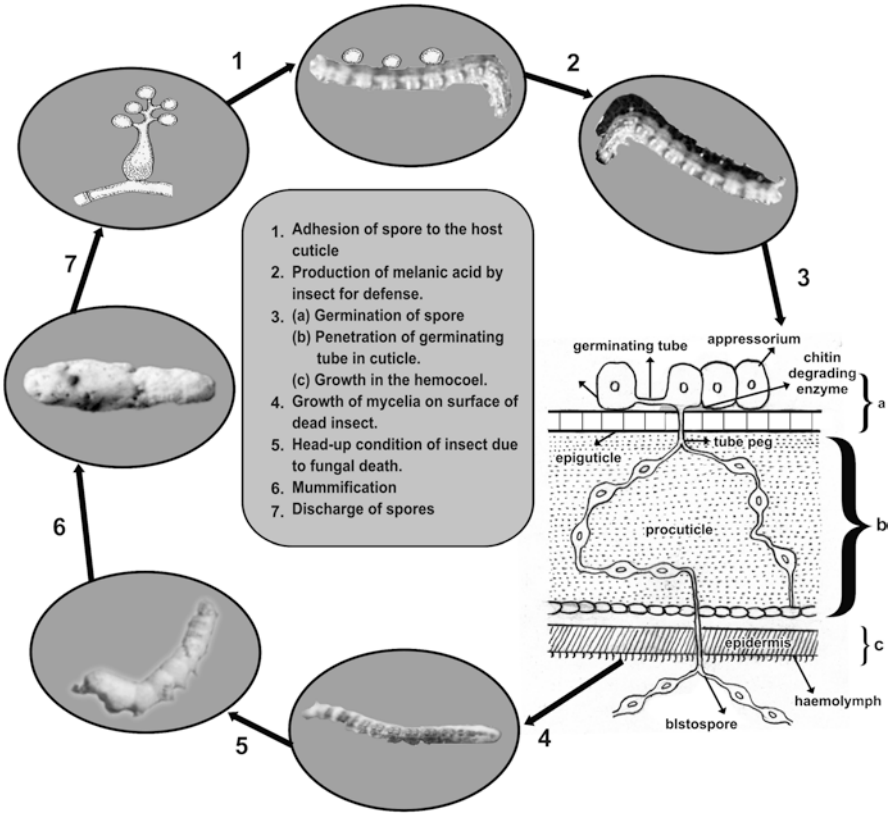


Fig. 17.2 Mechanism of action of entomopathogenic fungi

17.4.2 Defence Mechanism in the Host

Insect has several defence mechanisms which prevent the penetration and growth of the entomopathogenic fungus. One of the most common mechanisms of them is melanisation of the cuticle at the infection site. But it is initiated very late and thus is not able to stop the penetrating hyphae quickly (St. Leger et al. 1991).

17.4.3 Germination of Spore

A number of compounds have been found on the cuticle, which stimulate or inhibit germination (Latge et al. 1987). Nutrients accelerate germination, growth and development (Hassan et al. 1989). Fatty acids have a profound effect on the spore germination and differentiation either being toxic, fungistatic or stimulatory (Sandhu 1995).

17.4.4 Penetration of Cuticle

After adhesion, pathogenic fungi penetrate into the insect, the exact mechanism of which varies from species to species. A range of cuticle degrading enzymes are produced during penetration. Three most important classes of such enzymes are lipases, proteases and chitinases, which degrade the epicuticular waxes, followed by protein-chitin matrix (Smith et al. 1981). Besides this trypsin, chymotrypsin, elastases, collagenases and chymoelastases also play a role in penetration process (St. Leger et al. 1988; Bidochka and Khachatourians 1988).

17.4.5 Growth in the Haemocoel

Following penetration the fungus retaliates by rapid reproduction and tries to overcome the immune response in the haemocoel of insect body by various mechanisms:

(a) Formation of separate hyphal bodies by hyphal fission which are not as antigenic as hyphae (Pendland and Boucias 1986)
(b) Production of toxins by some members of *Deuteromycetes* such as *Beauveria* and *Metarhizium* (Roberts 1996)
(c) Development of wall less protoplast by *Coelomycetes* and members of the *Entomophthorales* in the haemocoel which are unrecognizable by the host (Sandhu 1993)

17.4.6 Death and Saprophytic Feeding

The toxin producers are quick killers and consequently secrete antibiotics so that they can continue their feeding saprobicly. Eventually all organs of the insect are consumed and replaced with hyphae. On the other hand, *Entomophthorales* are almost parasitic. The symptoms at the later stages of mycosis include physiological symptoms such as convulsions, lack of coordination, assuming lofty positions and outstretching of infected wings. These behavioural alterations are followed by death of the insect.

17.4.7 Hyphal Re-emergence and Sporulation

Upon favourable conditions hyphae repenetrate the cuticle and produce conidiophores on the outside of the insect producing spores both inside and outside of the cadaver (Sandhu 1995).

17.4.8 Mummification

After the death of insects infected with entomopathogenic fungi, fungal outgrowth from the insect body and coincidently the production and dispersal of spores to new host and environment occur (Hajek and Soper 1992).

17.5 Secondary Metabolites of Entomopathogenic Fungi as Potent Insecticidal Agent

Secondary metabolites are organic compounds which do not play a direct role in the growth and metabolism of organisms (Andersson 2012). Entomopathogenic fungi have been investigated as a source of a wide range of secondary metabolites possessing immense bioactivities against a broad range of insect pests. Diverse toxic metabolites have been described which display insecticidal properties against insect pests (Khan et al. 2012). Destruxins (A&B) produced by *Metarhizium anisopliae,* beauvercins, beauverolides, destruxins (dtx), bassianolides, bassianin and oosporein produced by *Beauveria bassiana* and *Nomuraea rileyi* are some examples of insecticidal metabolites of entomopathogenic fungi (Kodaira 1961). Table 17.2 below illustrates some commonly used insecticidal secondary metabolites of entomopathogenic fungi. Besides these compounds several extracellular enzymes produced by entomopathogenic fungi also play a significant role in their pathogenicity. Some important enzymes are chitinase, protease and lipase. Production of these enzymes has been reported in entomopathogenic fungi like *B. bassiana, Nomuraea rileyi* and *M. anisopliae* (Ali et al. 2011). Thus, special attention has been focussed on the isolation and purification of such enzymes from their respective entomopathogenic fungal strains and their use in biopesticide formulations. A few of these formulations have also been patented by their inventors. For example, an enzyme preparation comprising at least one protease derived from *Metarhizium*, *Beauveria*, *Verticillium* and *Aschersonia* was formulated and patented (US4987077) (Charnley et al. 1991). Similarly, a technology of controlling insect pest prepared with chitinolytic enzymes were patented (US6069299) (Broadway et al. 2000). Another invention which got patented involved an innovative combination of dormant spore of naturally occurring *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* fungus with enzymes, fats and growth-promoting molecules for controlling various foliage pest and soil-borne insect (WO2011099022 A1) (Patel 2011). Similarly, a combination of biopesticide and at least one exogenous cuticle degrading enzymes (e.g., a protease, chitinase, lipase and/or cutinase) was patented (US 20130156740A) (Leland 2013).

Table 17.2 List of metabolites having insecticidal activity produced by entomopathogenic fungi

S. No.	Fungi	Metabolite	Target Insect	Reference
1.	*Metarhizium anisopliae*	Destruxins (A & B)	*Spodoptera litura* (leafworm moth)	Kodaira (1961) and Hu et al. (2007)
2.	*Beauveria Bassiana* and *Nomuraea rileyi*	Beauvercins, beauverolides, destruxins (dtx), bassianolides, bassianin and oosporein	Various insects	Strasser et al. (2000)
3.	*Beauveria bassiana* and other species	Beauvericin (type A and B)	Various insects	Gupta et al. (1995)
		Bassianolide	Silkworm larva	Suzuki et al. (1977)
		Beauverolides	Unknown	Namatame et al. (1999)
		Bassianin, tenellin	Unknown	Mochizuki et al. (1993) and Jeffs and Khachatourians (1997)
4.	*Beauveria* spp. and other soil fungi	Oosporein (dibenzuoquinone)	Various insects	Eyal et al. (1994) and Wilson (1971)
5.	*Tolypocladium cylindrosporum*	Linear peptidic efrapeptins (types C to G)	Mites, beetle, budworm, moth	Weiser and Matha (1988) and Bandani et al. (2000)
6.	*Verticillium lecanii*	Vertilecanin A, B and C and their methyl ester	*Helicoverpa zea* (Corn earworm)	Soman et al. (2001)
7.	*Hirsutella thompsonii*	Hirsutellin A	*Galleria mellonella* (Wax-moth larvae)	Liu et al. (1995)
8.	Unidentified fungus (HF1)		*Oligonychus coffeae* (Tea Red Spider Mites)	Amarasena et al. (2011)
9.	*Hypocrella raciborskii* Zimm.	Ergosterol, dustanin (15α, 22 dihydroxyhopane) and 3β-acetoxy-15α,22-dihydroxyhopane	Spider Mite (*Tetranychus urticae* Koch)	Buttachon et al. (2013)

Hu et al. (2007), Strasser et al. (2000), Gupta et al. (1995), Suzuki et al. (1977), Namatame et al. (1999), Mochizuki et al. (1993), Jeffs and Khachatourians (1997), Eyal et al. (1994), Wilson (1971), Weiser and Matha (1988), Bandani et al. (2000), Soman et al. (2001), Liu et al. (1995), Amarasena et al. (2011), Buttachon et al. (2013)

17.6 Role of Biotechnology in Pest Management by Entomopathogenic Fungi

Recent developments in the field of genetic engineering provides new opportunities for the isolation of genes encoding pathogenicity and virulence and identification of markers for characterizing genome, thus allowing the genetic variation for strain improvement of entomopathogenic fungal population. The successful application of gene cloning technology to fungi of industrial and agricultural importance could be done by:

17.6.1 Development of an Efficient Transformation System

Nitrate reductase gene *(niaD)* of *Aspergillus niger* has been used for development of heterologous transformation system for entomopathogenic fungi *Beauveria bassiana* (Sandhu et al. 2001). Likewise, a heterologous transformation system for entomopathogenic fungus *Metarhizium anisopliae* was developed using the *cnx*-gene (cofactor for nitrate and xanthine dehydrogenase) of *Aspergillus nidulans* (Thakur and Sandhu 2003).

17.6.2 Protoplast Fusion Technique

It is yet another technique of biotechnology for production of potent entomopathogenic hybrid fungal strain. An intergeneric protoplast fusion of *Tolypocladium inflatum* and *Beauveria bassiana* was successfully accomplished to develop industrially as well as agriculturally important strain (Silawat et al. 2002).

17.6.3 Genetic Manipulation to Increase the Efficacy

Molecular biological studies on entomopathogenic fungi infection process have revealed that several genes are involved in the pathogenicity (Cho et al. 2007). Overexpression of such genes has resulted in the enhanced virulence of entomopathogenic fungal strains. Examples of some of these genes are subtilisin protease *PR1A* (St. Leger et al. 1996), subtilisin protease *PII* gene (Ahman et al. 2002) and chitinase gene *Bbchit1* (Fang et al. 2005). Besides these overexpression of genes for guanine nucleotide-binding proteins and its regulator (Fang et al. 2007, 2008), adhesin which helps in attachment of spore (Wang and St. Leger 2007), a perilipin-like protein that regulates appressorium turgor pressure and differentiation (Wang and St. Leger 2007) and a cell-protective coat protein helping in escaping the pathogen from the host immunity recognition have also increased the potency and ecological fitness of the engineered entomopathogenic fungal strains.

17.6.4 Development of Molecular Markers

Molecular markers are important tools for the identification and monitoring of specific fungal strains. Recently five microsatellite markers (Simple sequence repeats SSRs) were developed to monitor a commercialized isolate of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. in complex environmental samples such as bulk soil or plant DNA. Discriminatory power of these SSR markers was assessed in two commercialized *B. bassiana* isolates as well as in 16 *B. bassiana* isolates from a worldwide collection, and three of the five SSR markers were estimated to allow a confident discrimination among the given isolates (Reineke et al. 2014).

17.6.5 Development of Biochemical Markers

Such markers could be used for screening virulent strains of entomopathogenic fungi and then selecting the most promising candidates for biocontrol. In a recent study, the subtilisin-like protease *Pr1* activity of five *Metarhizium anisopliae* s.l. isolates was used as a biochemical tool to evaluate their virulence against *Rhipicephalus microplus* females. The isolates CG 629, CG 148 and CG 32 having higher protease activity showed higher virulence against *R. microplus* as compared to the isolates CG 112 or CG 347 with lower protease activity (Perinotto et al. 2014).

17.7 Recent Patents on Mycobiocontrol

With the increasing demand of new and eco-friendly biocontrol methods, several researchers have developed and patented their novel biocontrol strategies. Some of these employing the use of entomopathogenic fungi as biocontrol agents are listed in the Table 17.3.

17.8 Conclusion

Crop protection has relied mostly on synthetic chemical pesticides over many years. But their use is now declining owing to a number of factors like serious health problems caused due to their application, development of heritable resistance in pests and withdrawal of pesticide products by new health and safety legislation. Over 500 arthropod species now show resistance to one or more types of chemicals (Mota-Sanchez et al. 2002). This has forced the researchers to seek for some new pest control agents. Biological control agents have emerged as eco-friendly option for the management of insect pests. Numerous microbial candidates have been developed into biocontrol agents, but very few of these have been successful and persisted in the market place. This chapter clearly reveals the increasingly important role of entomopathogenic fungal biological control agents in the management of insect pest. The use of these agents will contribute in significant reduction of chemical pesticides usage in

Table 17.3 List of patents on use of entomopathogenic fungi for biocontrol of insect pest

Patent No.	Country	Inventor	Issue Date	Title
US2927060	United States	Oringer K	01 Mar. 1960	Refining of proteolytic enzymes
US3657414	United States	Paul et al.	18 Apr. 1972	Formulation of a boll weevil feeding stimulant mixture
US4027420	United States	McKibben et al.	07 Jun. 1977	Air dropped bait dispensers for attracting and killing the cotton boll weevil
US4293552	United States	Miesel JL	06 Oct. 1981	Novel 1-(mono-o-substituted benzoyl)-3-(substituted pyrazinyl) ureas
US4337271	United States	Jacobson M	29 Jun. 1982	Erythro-9,10-Dihydroxyoctadecan-1-ol acetate a boll weevil antifeedant
US4348385	United States	Synek J	07 Sep. 1982	Flowable pesticides
US4751082	United States	Schaerffenberg et al.	14 Jun. 1988	Insecticide and method for its distribution
US4797276	United States	Herrnstadt et al.	10 Jun. 1989	Control of cotton boll weevil, alfalfa weevil and corn rootworm via contact with a strain of *Bacillus thuringiensis*
US4908977	United States	Foster JP	20 Mar. 1990	Device for killing arthropods
US4925663	United States	Stimac JL	15 May 1990	Biological control of imported fire ants with a fungal pathogen
US4942030	United States	Osborne LS	17 Jul. 1990	Biological control of whiteflies and other pests with a fungal pathogen
US4987077	United States	Charnley et al.	22 Jan. 1991	Preparations of protease enzymes derived from entomopathogenic fungi
US5057316	United States	Gunner et al.	15 Oct. 1991	Method and device for the biological control of insects
US005360607A	United States	Eyal et al.	01 Nov. 1994	Method for production and use of pathogenic fungal preparation for pest control
US005413784A	United States	Wright et al.	09 May 1995	Biopesticide composition and process for controlling insect pests
US5516513	United States	Wright JC	14 May 1996	Biological ovicide for control of lepidopterous insects

(continued)

Table 17.3 (continued)

Patent No.	Country	Inventor	Issue Date	Title
EP0738317 A1	European	Clifford et al.	23 Oct. 1996	Formulations of entomopathogenic fungi for use as biological insecticides
USOO5683689A	United States	Stimac et al.	04 Nov. 1997	Controlling cockroaches, carpenter ants and pharaoh ants using strains of *Beauveria bassiana*
US005730973A	United States	Morales et al.	24 Mar. 1998	Water-dispersible granules of spores or live *Beauveria bassiana*
US006183733B1	United States	McKibben GH	13 Apr. 1999	Mycoinsecticide activity against grasshoppers produced by *Beauveria bassiana*
US005968504A	United States	Tahvonen et al.	19 Oct. 1999	Fungus *Gliocladium catenulatum* for biological control of plant diseases
US006069299A	United States	Broadway et al.	30 May 2000	Fungus and insect control with chitinolytic enzymes
US6254864 B1	United States	Stimac et al.	03 Jul. 2001	Method and formulations for control of pests
US006274157B1	United States	Lai et al.	14 Aug. 2001	Strains of *Streptomyces* and relevant uses thereof
US6280723 B2	United States	Stimac et al.	28 Aug. 2001	Methods and materials for control of termites
US006306386B1	United States	Cole et al.	23 Oct. 2001	Biological control formulations containing spores of nontoxigenic strains of fungi for toxin control of food crops
US006403085B1	United States	Stimac et al.	11 Jun. 2002	Method and formulations for control of pests
US006660290B1	United States	Stamets PE	09 Dec. 2003	Mycopesticides
US007037494B2	United States	Mattingly et al.	02 May 2006	Formulations and methods for insect control
US007402302B2	United States	Plato et al.	22 Jul. 2008	Composition of grandlure and dichlorvos for attracting and killing boll weevils in boll weevil traps
WO2009093261A2	India	Satyasayee et al.	30 Jul. 2009	Formulation of entomopathogenic fungus for use a biopesticide
EP2096926 A2	European	Samantha B	09 Sep. 2009	Use of entomopathogenic fungi as a means for the biological control of *Paysandisia archon*

(continued)

Table 17.3 (continued)

Patent No.	Country	Inventor	Issue Date	Title
EP2313488 A1	European	Prenerova et al.	27 Apr. 2011	Strain of entomopathogenic fungus *Isaria fumosorosea* ccm 8367 (ccefo.011.pfr) and the method for controlling insect and mite pests
US7943160 B2	United States	Borchert et al.	17 May 2011	Pest control methods
WO2011099022A1		Patel CS	18 Aug. 2011	Composition and method of preparation of fungal based bio insecticide from combination of *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* fungus with enzymes, fats and growth-promoting molecules for controlling various foliage pest and soil-borne insect
US008227224 B2	United States	Kalisz et al.	24 Jul. 2012	Method of making moulded part comprising Mycelium coupled to mechanical device
US8226938 B1	United States	Miekle et al.	24 Jul. 2012	Biocontrol of Varroa mites with *Beauveria bassiana*
US20130156740A1	United States	Leland JE	20 Jun. 2013	Biopesticide methods and compositions
US8501207B2	United States	Stamets P	06 Aug. 2013	Mycoattractants and mycopesticides

agriculture, horticulture and forest systems (Lacey and Goettel 1995). The pest control efficacy of these fungi is increased by the secondary metabolites produced by them (Vurro et al. 2001). Different extracellular enzymes are one of the most important secondary metabolites which could be used for the development of mycopesticides. Various entomopathogenic fungal strains have been exploited for their proteolytic enzymes. Recently, in a study a strain of *Verticillium lecanii* was found to be a good source of proteolytic as well as amylolytic and lipolytic enzymes, and their use as mycopesticide was rationally advocated (Hasan et al. 2013). With the development of modern techniques in the field of biotechnology now, it is possible to increase the efficacy of the entomopathogenic fungal strains by manipulating their desired traits. But still the research, development and final commercialization of fungal biological control agents continue to confront a number of obstacles which are needed to be removed for advancements in the field of myco-biocontrol of insect pests.

Acknowledgment The authors of this review would like to thank the Vice-Chancellor, R.D. University Jabalpur for his kind support and help. We would also like to thank the Head, Department of Biological Sciences, R.D. University, Jabalpur, India, for technical and linguistic assistance.

References

Agarwal GP, Rajak RC, Sandhu SS, Khan AK (1990) *Beauveria bassiana*: a potential pathogen of *Atteva fabriciella*, the insect of mahaneem. J Trop For 6:172–174

Ahman J, Johansson T, Olsson M, Punt PJ, van den Hondel CA, Tunlid A (2002) Improving the pathogenicity of a nematode trapping fungus by genetic engineering of a subtilisin with nematotoxic activity. Appl Environ Microbiol 73:295–302

Ali S, Zhen H, Wang Q, Shun XR (2011) Production and regulation of extracellular proteases from the Entomopathogenic fungus *Metarhizium anisopliae* (Cordycipitaceae; Hypocreales) in the presence of diamondback Moth cuticle. Pak J Zool 43:1203–1213

Al-Olayan EM (2013) Evaluation of pathogenicity of certain mitosporic ascomycete fungi to the house fly, *Musca domestica* L. (diptera: muscidae). J Saudi Chem Soc 17:97–100

Alter JA, Vandenberg JJD (2000) Factors that influencing the infectivity of isolates of *Paecilomyces* fumosoroseus against diamond back Moth. J Invertebr Pathol 78:31–36

Amala U, Jiji T, Naseema A (2013) Laboratory evaluation of local isolate of entomopathogenic fungus, *Paecilomyces lilacinus* Thom Samson (ITCC 6064) against adults of melon fruit fly, *Bactrocera cucurbitae* Coquillett (Diptera; Tephritidae). J Trop Agri 51:132–134

Amarasena S, Mohotti KM, Ahangama D (2011) A locally isolated entomopathogenic fungus to control tea red spider mites (*Oligonychus coffeae, Acarina tetranychidae*). Trop Agric Res 22:384–391

Andersson PF (2012) Secondary metabolites associated with plant disease, plant defense and biocontrol [Ph.D. thesis]. Uppsala: Swedish University of Agricultural Sciences, Uppsala

Avery PB, Faulla J, Simmands MSJ (2004) Effect of different photoperiods on the infectivity and colonization of *Paecilomyces fumosoroseus* on the greenhouse whitefly, *Trialeurodes vaporariorum*, using a glass slide bioassay. J Insect Sci 4:38

Awaad AS, Al-Jaber NA, Zain ME (2012) New antifungal compounds from *Aspergillus terreus* isolated from desert soil. Phytother Res 26:1872–1872

Babu V, Murugan S, Thangaraja P (2001) Laboratory studies on the efficacy of neem and the entomopathogenic fungus *Beauveria bassiana* on *Spodoptera litura*. Entomology 56:56–63

Bandani AR, Khambay BPS, Faull J, Newton R, Deadman M, Butt TM (2000) Production of efrapeptins by *Tolypocladium* species and evaluation of their insecticidal and antimicrobial properties. Mycol Res 104:537–544

Baskar K, Ignacimuthu S (2011) Bioefficacy of Violacein against Asian armyworm *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). J Saudi Soc Agric Sci 11:73–77

Benhamon N (2004) Potential of the mycoparasite, *Verticillium lecanii*, to protect citrus fruit against *Penicillium digitatum*, the causal agents of green, mold: a comparison with- the effect of chitosan. Phytopathology 94:693–670

Bhattacharyya A, Samal AC, Kar S (2004) Entomophagous fungus in pest management. News Letter 5:1–4

Bidochka MJ, Khachatourians GG (1988) Regulation of extracellular protease in the fungus *Beauveria bassiana*. Exp Mycol 12:161–168

Broadway RM, Gary EH, Cornell Research Foundation, Inc (2000, May 30) Fungus and insect control with chitinolytic enzymes. US Patent 6,069,299

Burges HD (1998) Formulation of microbial pesticides. Kluwer Academic Publishers, Dordrecht

Butt TM (2002) Use of entomogenous fungi for the control of insect pests. In: Esser K, Bennett JW (eds) Mycota. Springer, Berlin, pp 111–134

Butt TM, Copping L (2000) Fungal biological control agent. Pestic Outlook 11:186–191

Butt TM, Carreck NL, Ibrahim L, Williams IH (1998) Honey bee mediated infection of pollen beetle (*Meligethes* spp.) by the insect-pathogenic fungus, *Metarhizium anisopliae*. Biocontrol Sci Technol 8:533–538

Butt TM, Goettel MS, Papierok B (1999) Directory of specialists involved in the development of fungi as biocontrol agents. Warley, West Midlands

Butt TM, Jackson C, Magan N (2001) Introduction fungal biological control agents: progress, problems and potential. CAB International, Wallingford

Buttachon S, Angsumarn C, Winanda H, Anake K (2013) Acaricidal activity of *Hypocrella raciborskii* Zimm. (Hypocreales: Clavicipitaceae) crude extract and some pure compounds on *Tetranychus urticae* Koch (Acari: Tetranychidae). Afri J Microbiolo Res 7:577–585

Carrillo D, Dunlap CA, Avery PB, Navarrete J, Duncan RE, Jackson MA, Behle RW, Cave RD, Crane J, Rooney AP, Pena JE (2015) Entomopathogenic fungi as biological control agents for the vector the laurel wilt disease, the redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae). Biol Control 81:44–50

Charnley K, Richard MC, St. Leger RJ, Agriculture Genetics Company Ltd (1991January, 22) Preparations of protease enzymes derived from entomopathogenic fungi. US Patent 4,987,077

Chen C, Li ZY, Feng MG (2008) Occurrence of entomopathogenic fungi in migratory alate aphids in Yunnan Province of China. Biol Control 53:317–326

Cheraghi A, Behzad H, Mohammad SM (2013) Application of bait treated with the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin for the control of microcerotermes diversus Silv. Psyche 2013:5

Chet T, Schichler H, Haran S, Appenheim AB (1993) Cloned chitinase and their role in biological control of plant pathogenic fungi. In: Proceedings of the international symposium on chitin enzymology. Senigalia, Italy, pp 47–48

Cho EM, Kirkland BH, Holder DJ, Keyhani NO (2007) Phage display cDNA cloning and expression analysis of hydrophobins from the entomopathogenic fungus *Beauveria bassiana* (Cordyceps). Microbiology 153:3438–3447

Cook RJ (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. Annu Rev Phytopathol 31:53–80

Copping LG (2004) The manual of biocontrol agents, british crop protection council. Crop Prot 23:275–285

De Bach P (1964) Biological control of insect pest and weeds. Chapman and Hall, London

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

Desgranges C, Vergoignan C, Lereec A, Riba G, Durand A (1993) Use of solid state fermentation to produce *Beauveria bassiana* for the biological control of European corn borer. Biotechnol Adv 11:577–587

Erler F, Ates AO (2015) Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the june beetle. J Insect Sci 15:44

Eyal J, James FW, Grace WR, Co.-Conn (1994, November 1) Method for production and use of pathogenic fungal preparation for pest control. US Patent 5,360,607

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 over expressing 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* chitinase gene Bbchit1 and its application to improve fungal strain virulence. Appl Environ Microbiol 71:363–370

Fang W, Pei Y, Bidochka MJ (2007) A regulator of a G protein signalling (RGS) *gene*, cag8, from the insect-pathogenic fungus *Metarhizium anisopliae* is involved in conidiation, virulence and hydrophobin synthesis. Microbiology 153:1017–1025

Fang W, Scully LR, Zhang L, Pei Y, Bidochka MJ (2008) Implication of a regulator of G protein signalling (BbRGS1) in conidiation and conidial thermotolerance of the insect pathogenic fungus *Beauveria bassiana*. FEMS Microbiol Lett 279:146–156

French-Constant RH, Daborn PJ, Goff L (2004) The genetics and genomics of insecticide resistance. Trends Genet 20:163–700

Gupta S, Montillor C, Hwang YS (1995) Isolation of novel beauvericin analogues from the fungus *Beauveria bassiana*. J Nat Prod 58:733–738

Hajek AE, Soper RS (1992) Temporal dynamics of *Entomophaga maimaiga* after death of gypsy moth (Lepidoptera: Lymantriidae) larval hosts. Environ Entomol 21:129–135

Hasan S, Bhamra AK, Sil K, Rajak RC, Sandhu SS (2002) Spore production of *Metarhi-zium anisopliae* (ENT-12) by solid state fermentation. J Ind Bot Soc 8:85–88

Hasan S, Anis A, Abhinav P, Nausheen RK, Garima G (2013) Production of extracellular enzymes in the entomopathogenic fungus *Verticillium lecanii*. Bioinformation 9:238–242

Hassan AEM, Dillon RJ, Charnley AK (1989) Influence of accelerated germination of conidia of the pathogenicity of *Metarhizium anisopliae* for *Manduca sexta*. J Invert Patbol 54:277–279

Hawksworth D (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol Res 105:1422–1431

Heydari A (2007) Biological control of Turfgrass fungal disease. In: Pessarakli M (ed) Turfgrass management and physiology. CRC Press, Boca Raton

Heydari A, Mohammad P (2010) A review on biology control of fungal plant pathogen using microbial antagonists. J Biol Sci 10:273–290

Hu QB, Ren SX, An XC, Quain MH (2007) Insecticidal activity influence of destruxins on the pathogenicity of *Paecilomyces javanicus* against *Spodoptera litura*. J Appl Entomol 131:262–268

Humber RA (1997) Fungi: identification. In: Lacey L (ed) Manual of techniques in insect pathology. Academic, San Diego

Islam MT, Yasuyuki H, Abhinandan D, Toshinki I, Satoshi T (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 soil borne peronosporomycetes. Appl Environ Microbial 71:3786–3796

Jeffs LB, Khachatourians GG (1997) Toxic properties of *Beauveria* pigments on erythrocyte membranes. Toxicon 35:1351–1356

Jim McNeil (2011) Fungi for the biological control of insect pests fungi for the biological control of insect pests *eXtension* 4/4

Kaufman PE, Reasor C, Rutz DA, Ketzis JK, Arends J (2005) Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York state. J Bio Conf 33:360–367

Khachatourians GG (1986) Production and use of biological pest control agents. Tibtech 12:120–124

Khachatourians GG, Sohail SQ (2008) Entomopathogenic fungi. In: Brakhage AA, Zipfel PF (eds) Biochemistry and molecular biology, human and animal relationships. Springer, Berlin/Heidelberg

Khan S, Lihua G, Yushanjiang M, Mahmut M, Dewen Q (2012) Entomopathogenic fungi as microbial biocontrol agent. Mol Plant Breed 3:63–79

Kibata GN (1996) Diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), a problem pest of *Brassica* crop in Kenya. In: Farrel G, Kibata GN (eds) Proceedings of the 1st biennial crop protection conference, Nairobi, Kenya

Kodaira Y (1961) Biochemical studies on the muscardine fungi in the silkworms. J Fac Text Sci Technol Sinshu Univ Seric 5:1–68

Koul O, Suresh W, Dhaliwal GS (2008) Essential oils as green pesticides: potential and constraints. Biopestic Int 4:63–84

Lacey LA, Goettel MS (1995) Current developments in microbial control of insect pests and 871 prospects for the early 21st century. Entomophaga 40:1–25

Lacey LA, Frutos R, Kaya HK, Valis P (2001) Insect pathogens as biological control agents: do they have future? Biol Control 21:230–224

Latge JP, Sampedro L, Brey P, Diaquin M (1987) Aggressiveness of Conidiobolus obscures against the pea aphid – influence of cuticular extracts on ballistospore germination of aggressive and nonaggressive strains. J Gen Microbiol 133:1987–1997

Lecouna RE, Turica M, Tarocco F, Crespo DC (2005) Microbial control of *Musca domestica* (Diptera: Musciadae) with selected strains of *Beauveria bassiana*. J Med Entomol 42:332–336

Leland JE, Novozymes Biologicals Holidays A/S (2013, June 20) Bio-pesticide methods and compositions. US Patent US 20130156740A1

Li W, Sheng C (2007) Occurrence and distribution of entomo-phthoralean fungi infecting aphids in mainland China. Biocon Sci Technol 17:433–439

Liu WZ, Boucias DG, McCoy CW (1995) Extraction and characterization of the insecticidal toxin hirsutellin A produced by *Hirsutella thompsonii* var. *thompsonii*. Expt Mycol 19:254–262

Lozano-Contreras MG, Myriam E, Catalina R, Hugo AL, Luis JG, Maria G (2007) *Paecilomyces fumosoroseus* blastospore production using liquid culture in a bioreactor. Afri J biotechnol 6:2095–2099

Mahr SER, Raymond A, Cloyd D, Clifford SS (2001) Biological control of insects and other pests of greenhouse crops. North central regional publication 581

Mathew SO, Sandhu SS, Rajak RC (1998) Bioactivity of *Nomuraea rileyi* against *Spilosoma obliqua*: effect of dosage, temperature and relative humidity. J Indian Bot Soc 77:23–25

Mochizuki K, Ohmori K, Tamura H, Shizuri Y, Nishiyama S, Miyoshi E, Yamamura S (1993) The structures of bioactive cyclodepsipeptides, Beauveriollides I and II, metabolites of entomopathogenic fungi *Beauveria* sp. Bull Cheml Soc JPN 66:3041–3046

Moore D, Robson G, Trinci A (2011) Biochemistry and developmental biology of fungi. In: 21st Century guidebook to fungi. Cambridge University Press, Cambridge, pp 237–265

Mota-Sanchez D, Bills PS, Whalon ME, Wheeler WB (2002) Arthropod resistance to pesticides: status and overview. In: Wheeler WB (ed) Pesticides in agriculture and the environment. Marcel Dekker Inc, New York, pp 241–272

Mustafa U, Kaur G (2009) Remove from marked records effects of carbon and nitrogen sources and ratio on the germination, growth and sporulation characteristics of *Metarhizium anisopliae* and *Beauveria bassiana* isolates. Afri J Agric Res 4:922–930

Mwamburi LA, Laing MD, Miller RM (2010) Laboratory screening of insecticidal activities of *Beauveria bassiana* and *Paecilomyces lilacinus* against larval and adult house flies (*Musca domestica* L.) Afri Entomol 18:38–46

Namatame I, Tomoda H, Tabata N, Si SY, Omura S (1999) Structure elucidation of fungal Beauveriolide-III, a novel inhibitor of lipid droplet formation in mouse macrophages. J Antibiot 52:7–12

Nicholson GM (2007) Fighting the global pest problem: preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. Toxicon 49:413–422

Oerke EC, Dehne HW (2004) Safeguarding production – losses in major crops and the role of crop protection. Crop Prot 23:275–285

Padanad MS, Krishnaraj PU (2009) Pathogenicity of native entomopathogenic fungus *Nomuraea rileyi* against *Spodoptera litura*. Online Plant Health Prog. https://doi.org/10.1094/PHP-2009-0807-01-RS

Pandya U, Saraf M (2010) Application of fungi as a biocontrol agent and their biofertilizer potential in agriculture. J Adv Dev Res 1:90–99

Patel CS (2011, August 18) Composition and method of preparation of fungal based bio insecticide from combination of *Metarhizium anisopliae, Beauveria bassiana* and *Verticillium lecanii* fungus with enzymes, fats and growth promoting molecules for controlling various foliage pest and soil borne insect. WO Patent 2011099022A1

Pendland JC, Boucias DG (1986) Lactin binding characteristics of several entomogenous hyphomycetes. Possible relationship to insect hemagglutinisns. Mycologia 78:818–824

Perinotto WMS, Angelo IC, Golo PS, Camargo MG, Quinelato S, Santi L, Vainstein MH, da Silva WOB, Salles CMC, Bittencourt VREP (2014) *Metarhizium anisopliae* (Deuteromycetes: Moniliaceae) Pr1 activity: biochemical marker of fungal virulence in *Rhipicephalus microplus* (Acari: Ixodidae). Biocontrol Sci Technol:24, 123–132

Prenerova E, Rostislay Z, Lubomír V, Frantisek W, Biology Centre As Cr, V.v.i., Eva Prenerova (2011 April, 27) Strain of entomopathogenic fungus *Isaria fumosorosea* ccm 8367 (ccefo.011.pfr) and the method for controlling insect and mite pests. European Patent 2313488A1

Qazi SS, Khachatourians GG (2005) Insect pests of Pakistan and their management practices: prospects for the use of entomopathogenic fungi. Biopest Int 1:13–24

Reineke A, Bischoff-Schaefer M, Rondot Y, Galidevara S, Hirsch J, Uma Devi K (2014) Microsatellite markers to monitor a commercialized isolate of the entomopathogenic fungus *Beauveria bassiana* in different environments: technical validation and first applications. J Biol Control 70:1–8

Roberts DW (1996) Toxins from the entomogenous fungus *Metarhizium anisopliae* II. Symptoms and detection in moribund host. J Invert Patbol 8:222–227

Roberts DW, St. Leger RJ (2004) *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: mycological aspects. Adv Appl Microbiol 54:1–70

Rombach MC, Roberts DW, Aguda RM (1994) Pathogens of rice insects. In: Heinrichs EA (ed) Biology and management of rice insects. Wiley, New York, pp 613–655

Sandhu SS (1993) Mode of entry of *Beauveria bassiana* in *Helicoverpa armigera* larvae. Natl Acad Sci Lett 16:133–135

Sandhu SS (1995) Effect of physical factors on germination of entomopathogenic fungus *Beauveria bassiana* conidia. Natl Acad Sci Lett 18:1–5

Sandhu SS, Rajak RC, Hasija SK (2000) Bioactivity of *Metarhizium anisopliae* against Teak skeletonizer. Microbes Agric Indus Environ 2000:115–124

Sandhu SS, Kinghorn JR, Shiela EU, Rajak RC (2001) Transformation system of *Beauveria bassiana* and *Metarhizium anisopliae* using the nitrate reductase gene of *Aspergillus niger*. Indian J Exp Biol 39:650–653

Sandhu SS, Anil KS, Vikas B, Gunjan G, Priya B, Anil K, Sundeep J, Sharma AK, Sonal M (2012) Myco-biocontrol of insect pests: factors involved mechanism, and regulation. J Pathog 2012:1–10

Sharififard M, Mossadegh MS, Vazirian-zadhe B, Mahmoudabadi AZ (2011) Laboratory pathogenicity of entomopathogenic fungi, *Beauveria bassiana* (Bals) Vuill. and *Metarhizium anisopliae* (Metch.) Sorok. To larvae and adult of house fly, *Musca domestica* L. (Diptera: Muscidae). Asian J Bio Sci 4:128–137

Sharma K (2004) Bionatural management of pests in organic farming. Agrobios Newsl 2:296–325

Silawat N, Sandhu SS, Rajak RC (2002) Development of hybrid by intergeneric protoplast fusion of *Tolypocladium inflatum* and *Beauveria bassiana*. J Basic Appl Mycol 1:98–100

Smith RJ, Pekrul S, Grula EA (1981) Requirement for sequential enzymatic activities for penetration of the integument of the corn earworm (*Heliothis zea*). J Invert Pathol 38:335–344

Soman AG, Glor JB, Angawi RF, Wicklow DT, Dowd PF (2001) Vertilecanins: new phenopicolinic acid analogues from *Verticillium lecanii*. J Nat Prod 64:189–192

St. Leger RJ, Wang C (2009) Entomopathogenic fungi and the genomic era. In: Stock SP, Vandenberg J, Glazer I, Boemare N (eds) Insect pathogens: molecular approaches and techniques. CABI, Wallingford, pp 365–400

St. Leger RJ, Durrands PK, Cooper RM, Charnley AK (1988) Regulation of production of proteolytic enzymes by the entomopathogenic fungus *Metarhizium anisopliae*. Arch Microbiol 150:413–416

St. Leger RJ, Goettel M, Roberts DW, Staples RC (1991) Preparation events during infection of host cuticle by *Metarhizium anisopliae*. J Invert Pathol 58:168–179

St. Leger RJ, Joshi L, Bidochka MJ, Roberts DW (1996) Construction of an improved mycoinsecticide over expressing a toxic protease. Proc Natl Acad Sci USA 93:6349–6354

Stimac JL, Roberto P, University of Florida Research Foundation Inc (1997, November 4) Controlling cockroaches, carpenter ants, and pharaoh ants using strains of *Beauveria bassiana*. US Patent 5,683,689A

Strasser H, Vey A, Butt TM (2000) Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? Biocontrol Sci Technol 10:717–735

Suzuki A, Kanaoka M, Isogai A, Murakoshi S, Ichinoe M, Tamura S (1977) Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. Tetrahedron Lett 25:2167–2170

Thakur R, Sandhu SS (2003) Development of transformation system for entomopathogenic fungus *Metarhizium anisopliae* (ENT 12) based on cnx-gene. Ind J Microbiol 43:187–192

Thomas MB, Read AF (2007) Can fungal biopesticides control malaria? Nat Rev Microbiol 5:377–383

Tuli HS, Sandhu SS, Kashyap D, Sharma AK (2014a) Optimization of extraction compounds and antimicrobial potential of a bioactive metabolite, Cordycepin from *Cordyceps militaris*. World J Phar Pharmac Sci 3:1525–1535

Tuli HS, Sandhu SS, Sharma AK (2014b) Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin. 3 Biotech 4:1–12

Turner G (2000) Exploitation of fungal secondary metabolites old and new. Microbiol Today 27:118–120

Tzean SS, Hsieh LS, Wu WJ (1997) Atlas of entomopathogenic fungi from Taiwan Council of Agriculture, Taiwan, ROC. p 214

Vurro M, Zonno MC, Evidente A, Andolfi A, Montemurro P (2001) Enhancement of efficacy 836 of *Ascochyta caulina* to control *Chenopodium album* by use of phytotoxins and reduced rates 837 of herbicides. Biol Control 21:182–190

Wang C, St Leger RJ (2007) The *Metarhizium anisopliae* perilipin homolog MPL1 regulates lipid metabolism, appressorial turgor pressure, and virulence. J Biol Chem 282:21110–21115

Wang CS, Skrobek A, Butt TM (2004) Investigations on the destruxin production of the entomopathogenic fungus *Metarhizium anisopliae*. J Invertebr Pathol 85:168–174

Weiser J, Matha V (1988) Tolypin, a new insecticidal metabolite of fungi of the genus *Tolypocladium*. J Invertebr Pathol:51, 94–96

Whright SP, Jackson MA, De Kock SL (2001) Production stabilization and formulation of fungal biological agents. In: Butt TM, Jacktion C, Magan N (eds) Fungi as biocontrol agents. CABI, Wallinford, pp 253–287

Wilson BJ (1971) In: Ciegler A, Kadis S, Aje SJ (eds) Microbial toxins: a comprehensive treatise. Academic, New York, pp 288–289

Xie L, Hongmei C, Jibin Y (2013) Conidia production by *Beauveria bassiana* on rice in solid state fermentation using tray bioreactors. Adv Mater Res 610:3478–3482

Yu J, Keller N (2005) Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 43:437–458

Zain ME, Awaad AS, Razzak AA, Maitland DJ, Khamis NE, Sakhawy MA (2009) Secondary metabolites of *Aureobasidium pullulans* isolated from Egyptian soil and their biological activity. J Appl Sci Res 5:1582–1591

Zain ME, Amani SA, Monerah RA, Ahmed MA, Reham ME (2013) Biological activity of fungal secondary metabolites. Int J Chem Appl Biol Sci 1:14–22

Zimmermann G (2007) Review on safety of the entomopathogenic fungus *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci Teechnol 17:553–596

Premier Biocontrol Traits of Pseudomonads: Siderophores, Phenazines or What Else?

18

Bhushan L. Chaudhari, Sandeep N. Patil, Jayasinh S. Paradeshi, Mangal A. Chaudhari, and Charudatta S. Chaudhari

Abstract

Green revolution increased agricultural yields, but indiscriminate use of agrochemicals stagnated productivity and developed resistance among the pests. This provoked to search for effective biocontrol agents as a substitute to chemical pesticides. Among many biocontrol agents, ubiquitous pseudomonads can suppress plant diseases by inhibiting phytopathogens and promote plant growth. Pseudomonads possess a variety of traits that make them an appropriate biocontrol agent. The antimicrobial substances like hydrogen cyanide, 2,4-diacetylphloroglucinol, phenazines, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, etc. produced from pseudomonads are known to suppress fungal pathogens. Moreover, siderophores from pseudomonads also indirectly suppress fungal pathogens by making iron unavailable for their growth due to its chelation. The biosurfactants and hydrolytic enzymes from pseudomonads also support biocontrol mechanisms. Looking towards the overall importance of pseudomonads, the role of their metabolites in disease suppression is discussed here along with the effect of environmental factors and safety aspects.

Keywords

Pseudomonads • Secondary metabolites • Siderophores • Phenazines • Phloroglucinol

B.L. Chaudhari (✉) • S.N. Patil • J.S. Paradeshi • M.A. Chaudhari
Department of Microbiology, School of Life Sciences, North Maharashtra University, Jalgaon 425001, India
e-mail: blchaudhari@hotmail.com; blchaudhari@nmu.ac.in

C.S. Chaudhari
College of Horticulture, Shivaji Nagar, Pune 411005, Maharashtra, India

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_18

18.1 Introduction

Phytopathogenic microorganisms affecting health and productivity of plants are a major and serious obstruction to food production worldwide. As a result of the green revolution, agricultural production increased many folds over the past few decades; however, farmers become more dependent on the use of synthetic agrochemicals being more reliable and quick way of crop protection (Pingali 2012). Subsequently, increased application of chemical pesticides caused numerous negative impacts, like the development of resistance among phytopathogens against the chemical pesticides and non-target damage to environment due to these pesticides (de Weger et al. 1995; Gerhardson 2002; Compant et al. 2005). Moreover, the rising price of chemical pesticides, particularly in developing/low-income countries, as well as consumer demand for food which is either pesticide-free or with the minimum residue of pesticides has directed to explore for alternatives for chemical pesticides (Czaja et al. 2015). Biological control is thus being considered as a substitute or a complementary way of decreasing the use of chemicals pesticides in the agriculture (de Weger et al. 1995; Gerhardson 2002; Postma et al. 2003; Compant et al. 2005; Singh et al. 2011).

The word 'biocontrol' got renaissance since the world became conscious about the use of toxic chemical pesticides and agrochemicals. Several plant beneficial bacteria associated with plant roots play key roles in plant growth promotion by closely interacting with rhizospheric milieu and subsequently enhancing plant vigour through improved soil fertility. In addition to this, these bacteria help to control plant disease establishments by suppressing phytopathogenic microbes (Berendsen et al. 2012). Among these plant beneficial bacteria, pseudomonads are ubiquitous and have a variety of traits that make them an appropriate candidate for biocontrol of phytopathogens. Pseudomonads are known to suppress fungal pathogens by producing antimicrobial substances such as hydrogen cyanide, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, phenazines, tropolone, cyclic lipopeptides, etc.; moreover, production of siderophore by pseudomonads can indirectly suppress fungal pathogens by making iron unavailable for their growth and proliferation due to the advent of its chelation iron in the rhizospheric soil (O'sullivan and O'Gara 1992; Rai et al. 2017). Therefore, pseudomonads represent a potential alternative to toxic synthetic chemical pesticides. Looking towards the overall importance of antimicrobial metabolites of pseudomonads participating in disease suppression strategies is discussed here.

Pseudomonads are aerobic, Gram-negative bacteria known for their ubiquitous nature bearing an extraordinary ability to utilize various organic substances and sustain at various temperatures even though they are non-spore-bearing organisms (Weller 2007). They have extraordinary ability to circumvent the presence of other organisms by various mechanisms and also play a beneficial role for the plants. Pseudomonads have several traits that make them a good biocontrol and plant growth-promoting agent (Weller 1988; O'sullivan and O'Gara 1992; Panpatte et al. 2016) such as (1) ability to adhere to soil particles and proliferate in rhizosphere luxuriously; (2) ability to utilize root and seed exudates and prototrophy; (3) rapid

rhizosphere and spermosphere colonization; (4) ability to grow fast; (5) sensitive to chemotactic response through motility; (6) aggressive competitiveness for survival in environment; (7) adaptability to different environmental stresses, etc.; (8) short regeneration time; (9) easy multiplication and mass production; and (10) produce myriad of bioactive metabolites (i.e. antibiotics, siderophores, volatile compounds, hydrolytic enzymes, exopolysaccharides, plant growth-promoting substances, etc.). Among pseudomonads, fluorescent *Pseudomonas* spp. constitute a diverse group of bacteria that can usually be visually distinguished from other pseudomonads by their ability to produce a water-soluble yellow-green fluorescent pigment and belong to the rRNA group I of pseudomonads (Gomila et al. 2015; Khan et al. 2016). These fluorescent pseudomonads are most studied and have emerged as the largest and potentially most promising group of plant growth-promoting rhizobacteria involved in the biocontrol of plant diseases (O'sullivan and O'Gara 1992). The examples of biocontrol fluorescent pseudomonads are *P. aeruginosa*, *P. fluorescens*, *P. putida* and *P. syringae* (Bossis et al. 2000). Other pseudomonads with biocontrol potential are *P. chlororaphis*, *P. aurantiaca*, *P. aureofaciens*, etc. (Hu et al. 2014; Raio et al. 2017).

18.2 Biocontrol Potential of Pseudomonads Against Different Phytopathogens

Aggressive root colonization with the advent of prototrophy and competitive retention in the rhizosphere niches by pseudomonads is supported by the production of bacterial secondary metabolites, including antimicrobial compounds, biocidal organic volatiles, hydrolytic enzymes, detoxicating enzymes and iron-chelating agents; siderophores (Sturz and Christie 2003). These abilities of pseudomonads stipulated their role in biological control of phytopathogens as the antagonistic activity against the common fungal phytopathogens belonging to genera *Alternaria*, *Rhizoctonia*, *Fusarium*, *Phytopthora*, *Pythium*, *Sclerotinia*, *Colletotrichum*, *Botrytis*, *Aspergillus*, *Gaeumannomyces*, *Erwinia*, etc. (Khan et al. 2016, Panpatte et al. 2016). The potential of pseudomonads to suppress the diseases caused by phytopathogenic bacteria (belonging to genera *Xanthomonas*, *Ralstonia*, *Pseudomonas*, etc.) and nematodes is also widely studied and established. Table 18.1 summarizes the list of representative pseudomonads reported to offer biocontrol against different phytopathogens.

In addition to biocontrol, the production of indole acetic acid (IAA) and solubilization of inorganic phosphate by pseudomonads boost the growth of plants and ensure the significance in the management of the agro-environmental and phytopathological problems (Bano and Musarrat 2004).

18.2.1 Phytopathogenic Fungi

Pseudomonads suppressing or interfering the normal growth and physiology of phytopathogenic fungi are referred as a fungal antagonist. Usually, but not necessarily,

Table 18.1 Biocontrol potential of pseudomonads against different phytopathogens

Sr No	Pseudomonad strain	Phytopathogen	Disease	Plant/crop	References
1	*P. corrugata*	*Alternaria alternata*	Citrus black rot	Citrus plant	Trivedi et al. (2008).
2	*P. corrugata*	*Fusarium oxysporum*	Panama disease	Banana	
3	*Pseudomonas aeruginosa*	*Aspergillus niger*	Black mould of onions ornamental plants	Onion plant	Sayyed and Patel (2011).
				Human being	
			Aspergillosis	Peanut	
			Crown rot		
		Aspergillus flavus	Fusarium rot	Onion	
		Fusarium oxysporum	Target spot/early bligh	Tomato	
		Alternaria alternata	Citrus black rot	Citrus plant	
		Cercospora arachichola	Cercospora leaf spot	Peanut	
		Pseudomonas solanacearum	Bacterial wilt	Chilli	
4	*Pseudomonas fluorescens* strains	*Rhizoctonia solani*	Sheath blight	Rice	Ahmadzadeh and Tehrani (2009).
		Pythium ultimum	Pythium root rot	Ornamental species	
			Pythium damping-off		
5	*Pseudomonas putida* strains	*Rhizoctonia solani*	Sheath blight	Rice	
		Pythium ultimum	Pythium root rot	Ornamental species	
			Pythium damping-off		
6	*Pseudomonas fluorescens*	*Botrytis cinerea*	Legumes and fruit suffers	Different plant leaf and fruits	Trotel-Aziz et al. (2008).
7	*Pseudomonas chlororaphis*	*Verticillium microsclerotia*	Verticillium wilt	Eggplant, pepper, potato, peppermint, chrysanthemum, cotton, asters, fruit trees, strawberries, raspberries, roses, alfalfa	Debode et al. (2007).

(continued)

8	*Pseudomonas* sp.	*Rhizoctonia solani* and *Phytophthora capsici*	Sheath blight Black/brown lesion	Rice and chilli peppers (*Capsicum annuum*)	Arora et al. (2008).
9	*Pseudomonas fluorescens strain CV6*	*Phytophthora drechsleri*	Root rot	Raspberry	Maleki et al. (2010).
10	*Pseudomonas* spp.	*Clavibacter michiganensis* subsp. *michiganensis*	Bacterial canker	Tomato	Lanteigne et al. (2012).
11	*Pseudomonas* sp.	*Ralstonia solanacearum*	Wilting	Eggplant (*Solanum melongena* L.)	Ramesh et al. (2009).
12	*Pseudomonas syringae* *P. syringae*	*Pseudomonas syringae* *P. glycinea*	Bacterial speck	Tomato	Wensing et al. (2010).
13	*Pseudomonas fluorescens* strains	*Agrobacterium tumefaciens*	Crown gall tumours	Tomato plants	Dandurishvili et al. (2011).
14	*Pseudomonas brassicacearum*	*Ralstonia solanacearum*	Bacterial wilt	Potato, tomato, banana and pepper plants, as well as trees, such as eucalyptus	Zhou et al. (2012)
15	*P. fluorescens*	*Aspergillus niger*	Collar rot	Groundnut	Lukkani and Reddy (2014)
16	*Pseudomonas aeruginosa*	*Candida* sp. *A. flavus* *A. fumigatus*			Sudhakar et al. (2013)
17	*Pseudomonas fluorescens* strains	*Dickeya* phytopathogen	Soft rot diseases	Potato	Cigna et al. (2015)
18	*Pseudomonas putida*	*Dickeya* phytopathogen	Soft rot diseases	Potato	

(continued)

Table 18.1 (continued)

Sr No	Pseudomonad strain	Phytopathogen	Disease	Plant/crop	References
19	*Pseudomonas aeruginosa*	*Alternaria alternata*	Rot and wilt disease	Different cereal crops, Solanaceae plants. Grains, cereal crops, etc.	Patra (2012)
		A. solani	Sheath blight		
		Bipolaris australiensis			
		Colletotrichum acutatum			
		Curvularia andropogonis			
		Fusarium oxysporum			
		F. moniliforme			
		Pythium aphanidermatum			
		Rhizoctonia solani			
20	*Pseudomonas aeruginosa*	*Fusarium oxysporum* f. sp. *ciceri*, *Fusarium udum*, *Aspergillus niger*	Wilting of different plants	Chickpea	Sulochana et al. (2014)
				Pigeon pea	
				Groundnut	
21	*Pseudomonas protegens*	Myzus persicae (insect)	Decreased growth, shrivelling of the leaves and the death of various tissues	Solanaceae, celery, mustard, pepper, pumpkin, okra, corn and sunflower and other flower crops	Jang et al. (2013)

these pseudomonads isolated from suppressive soils offer a significant reduction in fungal disease severity even after adding to the rhizosphere of the plant. Such biocontrol pseudomonads with the capacity to suppress/antagonize fungal phytopathogens and thus preventing the development of plant diseases, represent a suitable alternative for chemical fungicides (Haas and Keel 2003). *Pseudomonas* isolates DGR22, MGR4 and MGR39 showed very high biocontrol potential (Cordero et al. 2012). *P. fluorescens* strains were reported to increase almost 10–13% alfalfa germination as well as the increased above-ground biomass of plant by 15 to 18% (Quagliotto et al. 2009). The isolate *P. brassicacearum* J12 produces 2,4-DAPG (2,4-diacetylphloroglucinol), HCN (hydrogen cyanide), siderophore(s) and protease (Zhou et al. 2012). *P. fluorescens* strain Psd produces phenazine-1-carboxylic acid (PCA) and pyrrolnitrin (Prn) whose genes were knockout where the resulting knockout strains did not produce PCA and Prn, respectively, resulting in the loss of antagonistic activity against phytopathogenic fungus *Fusarium oxysporum* which ensures and confirms that the PCA- and Prn-producing pseudomonads have a major role in antifungal activity of pseudomonads (Upadhyay and Srivastava 2011). The PCA synthesized from *Pseudomonas* sp. had shown in vitro fungicidal activity against phytopathogens including *Colletotrichum circinans*, *Colletotrichum dematium*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Patil et al. 2016). Cyclic lipodepsipeptides, pseudophomins A and B isolated from *Pseudomonas fluorescens* BRG100, have potential application in biocontrol of plant pathogens as well as weeds. *Phoma lingam*/*Leptosphaeria maculans* and *Sclerotinia sclerotiorum* were remarkably inhibited by pseudophomin B than pseudophomin A (Pedras et al. 2003). A member of *Peronosporomycete* family *Phytophthora capsici* is a phytopathogen that infects and results in damping-off and blight on vegetable crops, cucurbits and condiments like pepper, causing serious economic losses which has been significantly inhibited by *Pseudomonas* species moreover inducing excessive branching of plant, swelling and subsequent cellular disintegration of *P. capsici* (Zohara et al. 2015). Further, they have reported that the plant seeds treated with the same culture found to have enhanced resistance to the damping-off disease. The *Pseudomonas* sp. S4LiBe and S5LiBe isolates have shown remarkable mycelial growth inhibition against *Botrytis cinerea*, *Verticillium dahliae*, *Fusarium graminearum*, *Aspergillus niger* and *Aspergillus flavus* (growth inhibition between 88% and 48%). The antagonistic activity shown by two strains of *P. protegens* especially S4LiBe and S5LiBe was observed to produce chitinase and other polymer-degrading enzymes and PGPR through phytohormone indole acetic acid, siderophore production and phosphate solubilization together along with mycelial growth inhibition of *Botrytis cinerea*, *Verticillium dahliae*, *Fusarium graminearum*, *Aspergillus niger* and *Aspergillus flavus* (Bensidhoum et al. 2016). The *Pseudomonas chlororaphis* GBPI_507 was observed to solubilize phosphate and produce siderophores, HCN, ammonia, lytic enzymes (lipase and protease) and PCA which could inhibit *Alternaria alternate*, *Fusarium solani* and *F. oxysporum* (Jain and Pandey 2016). *Pseudomonas* spp. were characterized for their PGPR and biocontrol potential through the determination of in vitro activity against root-rotting fungi, viz. *Macrophomina phaseolina*, *Fusarium solani*, *Fusarium oxysporum* and *Rhizoctonia solani* (Noreen et al. 2015).

Pseudomonas aeruginosa FP6 showed in vitro antagonistic activity against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* over King's B media, with and without $FeCl_3$. When $FeCl_3$ was supplemented, it showed a significant reduction in *R. solani* than control (without $FeCl_3$), which enlightens the role of siderophore-mediated antagonistic activity against *R. solani*. But, in the case of *C. gloeosporioides*, antagonistic activity was not influenced by the presence of $FeCl_3$, suggesting the involvement of other antagonistic factors also (Sasirekha and Srividya 2016).

18.2.2 Phytopathogenic Bacteria

Even though fungi are dominant as phytopathogens, bacteria also act as pathogens. Many bacterial genera and species have been reported to be phytopathogenic in nature which can be arrested by the pseudomonads. The *Pseudomonas macerans* (strains BS-DFS and PF9) have potential use in potato bioprotection in an integrated bacterial wilt management as well as PGPR effect (Aliye et al. 2008). The *Pseudomonas fluorescens* mutant produced a higher amount of 2,4-diacetylphloroglucinol (DAPG) with higher colonization and in vitro inhibition effect on *Ralstonia solanacearum* than the wild type in tomato rhizosphere, while the consortium of both wild and mutant improved the colonization and biocontrol efficiency against tomato bacterial wilt (Zhou et al. 2014). The *Pseudomonas aeruginosa*-LN strain produced many bioactive components which upon evaluation against *Xanthomonas axonopodis* showed that the biofilm formation and cell morphology were severely affected. Some of the *P. fluorescens* strains resulted in induction systemic resistance (ISR) in *Arabidopsis thaliana* against bacterial speck caused by *Pseudomonas syringae* pv. tomato (Weller et al. 2012).

18.2.3 Phytopathogenic Nematodes

The root-knot nematode is one of the most economically important pests causing severe damages to a wide variety of crops worldwide (Siddiqui and Shaukat 2003). *Meloidogyne javanica* is obligatory parasitic nematode having many hosts and responsible for the severe loss of the crop productivity. Several pseudomonads have been reported bearing nematicidal activities. *Pseudomonas* sp. S4LiBe and S5LiBe isolates produce chitinase and other polymer-degrading enzymes. Moreover, insecticidal activities of gene fitD product tested against *Galleria* were tested positive (Bensidhoum et al. 2016). *Pseudomonas* isolates when used as a soil drench reduced root rot disease under greenhouse condition by maximum nematicidal activity against stage II juveniles of *Meloidogyne javanica* resulting in enhanced plant growth and yield in mung bean (Noreen et al. 2015). Similarly, *Pseudomonas aeruginosa* and other *Pseudomonas* strains when applied as either seed treatment or soil drench significantly reduced nematode population densities in soil and subsequent nematode-borne root-knot development under glasshouse conditions (Ali et al. 2002). Meyer et al. (2009) have stated that an antibiotic 2,4-diacetylphloroglucinol

(DAPG) of *Pseudomonas fluorescens* has shown good nematicidal activity against nematode like *Meloidogyne incognita*, but on the other hand, it could support the *Caenorhabditis elegans* for first few hours; however, DAPG bestows an additional advantage of imparting resistance to plants.

18.3 Biocontrol Traits of Pseudomonads

It is a well-established fact that not only plants but all the objects on earth except few are all time exposed to several microorganisms bearing a variety of characteristics. Even though the conditions are unfavourable, plants are compelled to interact with millions of microorganisms leading to several mutualistic benefits for both plants and microorganisms in view of survival under adverse conditions (Kamilova et al. 2006; Negi et al. 2011; Philippot et al. 2013). As the microorganisms in the soil are essentially important in recycling of nutrients, plant growth and the soil healthiness through a variety of interactions (Forni et al. 2017; Gepstein and Glick 2013; Ma et al. 2011; Mayak et al. 2004; Philippot et al. 2013; Rajkumar et al. 2013), the microbial diversity of the soil is considered as a key factor for soil fertility and plant health and productivity. These plant beneficial bacteria can perform mainly two roles, (1) plant growth promotion and (2) biological control of phytopathogen (Ma et al. 2016). Recently, the use of plant beneficial *Pseudomonas* spp. has received amplified attentions because of their perspective in detoxification of the inorganic pollutants, degradation of the xenobiotic compounds, bulk colonization in rhizospheric soil, synthesis of number of plant growth-promoting and antifungal substances and improvement in plant growth and subsequent yields (Glick 2014; Li et al. 2014; Ma et al. 2011; Nascimento et al. 2013; Negi et al. 2011; Rajkumar et al. 2010, 2012, 2013; Vessey 2003). These bacteria possess a variety of biocontrol traits to phytopathogens as shown in Fig. 18.1.

These traits fall under main three different mechanisms, (1) suppression of phytopathogens through competition for iron; (2) antagonistic action against phytopathogens through production of broad-spectrum antibiotics, volatile organic biocides and hydrolytic enzymes; and (3) induction of systemic resistance (Heil and Bostock 2002; Dwivedi and Johri 2003). However, suppression of phytopathogenic fungi and consequently plant disease suppression is a multifunctional feature; therefore, these three mechanisms are not exclusive and work concomitantly to achieve better results (Dwivedi and Johri 2003).

18.3.1 Siderophores

Although iron is the fourth most abundant element in the Earth's crust (Crichton and Charloteaux-Wauters 1987), it is largely in an insoluble form and thus is unavailable for direct assimilation by microbes (Saha et al. 2013). Hence, it is extremely limited in the heterogeneous environment like rhizosphere. Nearly all microorganisms need iron for their growth and existence in a diverse environment like rhizosphere. Microbes

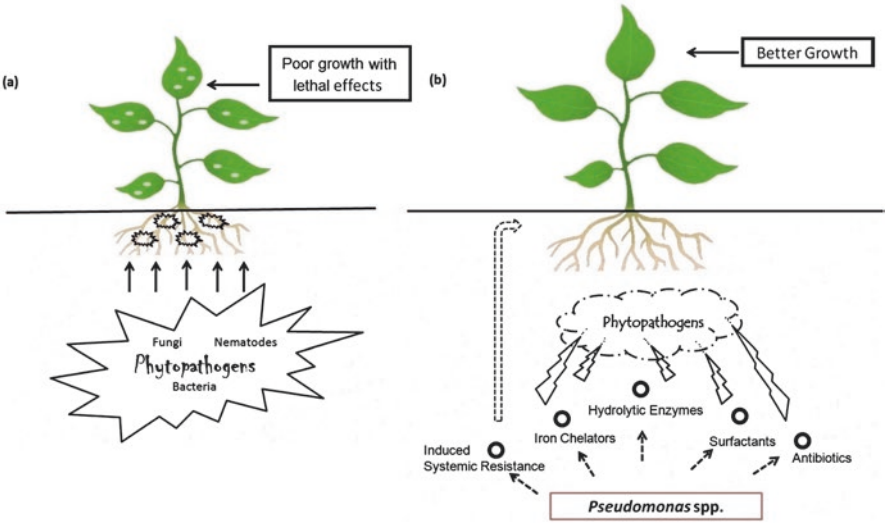


Fig. 18.1 Schematic representation of premier biocontrol traits of *Pseudomonas* leading to suppression of phytopathogens and enhanced plant growth

to fulfill demand mainly depend on their capacity to scavenge iron from a limited pool (O'sullivan and O'Gara 1992). Therefore, to trap traces of an insoluble form of iron (III) and form stable complexes, most microorganisms excrete molecules known as siderophores to overcome Fe-starvation conditions (Chincholkar et al. 2000). Siderophore is one of the premier secondary metabolites of pseudomonads that sequester iron in the vicinity and consequently inhibit the growth of pathogens by making it unavailable for metabolic activities. Pathogens such as *Fusarium oxysporum*, *Pythium ultimum* and many others causing wilt and root rot diseases in crops are well documented to be arrested by limiting the amount of iron owing to the presence of siderophores produced by plant beneficial bacteria like pseudomonads (Kloepper et al. 1980; Weller 2007; Sahu and Sindhu 2011). Some other examples of siderophore-producing pseudomonads such as *P. fluorescens* CHA0 (Couillerot et al. 2009), *P. putida* WCS strains (Weller 2007) and *P. syringae* pv. *syringae* strain 22d/93 (Wensing et al. 2010) have been proposed as biocontrol agents against soilborne plant diseases. The siderophore-metal binding reduces the formation of free radicals near the roots, leading to prevent the degradation of microbial auxins, thereby restoring the normal function of plant growth promotion (Dimkpa et al. 2008a, b).

Duijff et al. (1993) demonstrated the role of siderophore in the suppression of wilt caused by *Fusarium oxysporum* f. sp. *dianthi* in carnation roots. In the said study, treatments of carnation roots with pseudomonads were capable of producing siderophores that significantly reduced fusarium wilt, whereas mutants defective in siderophore biosynthesis (sid$^-$) were less effective in disease suppression suggesting the involvement of siderophores in majority along with some other metabolites. Vandenbergh and Gonzalez (1984) tested siderophore-producing *P. putida* strain NRRL-B-12537 and its mutant which was unable to produce siderophore against *F. oxysporum* in tomato rhizosphere.

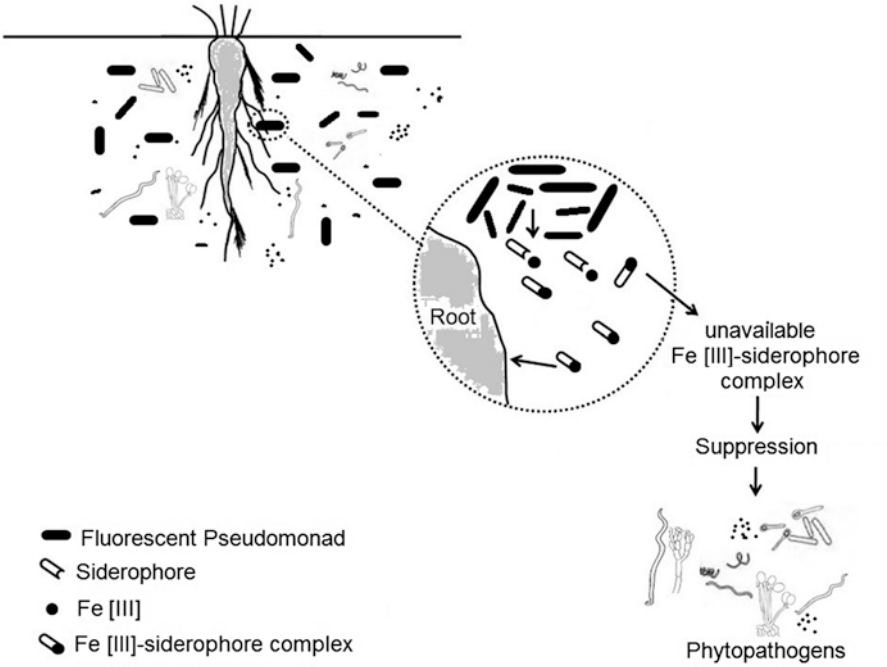


Fig. 18.2 Suppression of phytopathogens pseudomonads through siderophore-mediated iron deprivation

The results revealed that a wild-type strain was highly effective in suppressing *F. oxysporum* as compared to mutant-type (sid^-) *P. putida* strain. Another role of siderophores could be the prevention of the germination of fungal spores through iron deprivation, because a direct correlation has been observed between siderophore synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of *Fusarium oxysporum* under in vitro conditions (Elad and Baker 1985).

The siderophores produced by biocontrol pseudomonads arrest Fe in the surrounding area of roots and consequently inhibit the growth of phytopathogens by limiting the amount of iron required for the growth of these pathogens (Kloepper et al. 1980; Chincholkar et al. 2006; Weller 2007; Sahu and Sindhu 2011) as represented in Fig. 18.2.

The word siderophore is derived from a Greek word which means 'iron bearer'. These are low-molecular-weight compounds (400–1000 kDa) having greater affinity for iron (Neilands 1981a, b; Raymond and Dertz 2004; Skaar 2010). Broadly, based on the moiety that donates oxygen ligand for Fe (III) coordination, the siderophores can be classified into five categories as (1) catecholates, (2) phenolates, (3) hydroxymates, (4) carbooxylates and (5) mixed types (Miethke and Marahiel 2007). Soil pseudomonads usually produce fluorescent, yellow-green, water-soluble siderophores bearing both hydroxamate and phenolate groups which are either pyoverdines or pseudobactins. These types of siderophores from different fluorescent pseudomonads showed the main

difference in the composition, number and configuration of the amino acids in the peptide backbone (Neilands and Leong 1986). The fluorescent pseudomonads produce two unique siderophores as pyoverdine (Meyer and Abdallah 1978; Cox and Adams 1985; Poole and Mckay 2003; Jimenez et al. 2010) and pyochelins (Cox et al. 1981; Cobessi et al. 2005; Braud et al. 2009). Among the better-known siderophores, pyoverdine produced by fluorescent pseudomonads has a very high affinity towards Fe (III). The production of siderophores such as pyoverdine and pyochelin that mediate iron deprivation through its sequestration is one of the mechanisms behind the suppression of plant pathogens and their diseases by plant growth-promoting bacteria like pseudomonads (Kloepper et al. 1980; Chincholkar et al. 2006). Apart from disease suppression, siderophores have also been observed to increase the iron content in rice through siderophoregenic *P. putida* (Sharma et al. 2013).

18.3.2 Hydrolytic Enzymes

Hydrolytic enzymes specifically fungal cell wall-degrading enzymes such as chitinase, cellulases, glucanases, proteases, etc. have a significant role in the biocontrol potential of fluorescent pseudomonads. Many biocontrol pseudomonads show hyperparasitic action owing to its cell wall hydrolysis by enzymes compromising the integrity of cell wall and cell membrane leading to the death of the phytopathogens (Chernin and Chet 2002). Also, these enzymes are known to destroy oospores of those fungal phytopathogens which affect spore germination and germ-tube elongation (Sneh et al. 1984). A chitinase-producing *P. aeruginosa* strain GRC1 exhibited a strong reduction in stem rot of peanut caused by *S. sclerotiorum*, and the role of chitinase was clearly demonstrated through Tn5 mutagenesis (Gupta et al. 2006). The wild-type *P. fluorescens* strain BL915 capable of synthesizing chitinase inhibited growth of *R. solani*; however, its spontaneous pleiotropic mutant of which failed to synthesize chitinase did not inhibit the growth of *R. solani*, indicating a significant role of chitinase in biocontrol potential of BL915 (Gaffney et al. 1994). A significant relationship between the antagonistic potential of *P. fluorescens* against *R. solani* and its level of β-1,3-glucanase has been established by Nagarajkumar et al. (2004). A β-1,3 glucanase-producing bacterium *P. cepacia* decreased the incidence of diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium ultimum* out under greenhouse conditions (Fridlender et al. 1993).

18.3.3 Secondary Metabolites

Fluorescent pseudomonads act as an antagonist against a variety of phytopathogens mainly by producing antimicrobial secondary metabolites (Premchandra et al. 2016). Fluorescent pseudomonads capable of producing secondary metabolites that exhibit a wide range of antimicrobial compounds have been well documented for biocontrol of variety of phytopathogens (Dowling and O'Gara 1994; Ligon et al. 2000; Walsh et al. 2001; Haas and Défago 2005; Weller 2007; Santoyo et al. 2012; Subashri et al. 2013;

Saraf et al. 2014; Arseneault and Filion 2016). Role of different secondary metabolites in biocontrol of phytopathogens is summarized in Table 18.2**.**

There are roughly six classes of antibiotic agents of pseudomonads responsible for biocontrol having varied modes of action and characteristics, phenazines, phloroglucinols, pyrrolnitrin, pyoluteorin, hydrogen cyanide (HCN) and cyclic lipopeptides (Beneduzi et al. 2012), as discussed further.

18.3.3.1 Phenazines Antibiotics

One of the most studied biocontrol traits of pseudomonads is phenazine antibiotics apart from siderophores. Phenazines are nitrogen-containing heterocyclic low-molecular-weight compounds with bright colour and having a broad-spectrum antimicrobial activity (Chincholkar et al. 2009; Patil et al. 2016). They are known to be synthesized exclusively by bacteria especially those belonging to genus *Pseudomonas* (Turner and Messenger 1986; Thomashow et al. 1990). Phenazines have been known for their antifungal properties for a long time, e.g. pyocyanin (Budzikiewicz 1993). However, the role of phenazines in antagonism towards fungal phytopathogens came to notice in last quarter of the twentieth century because of increased concern about chemical pesticides and awareness about sustainable agriculture and increased the research interest in phenazines (Chincholkar et al. 2009). Phenazine antibiotic biosynthesis is another important biocontrol metabolite produced by many fluorescent pseudomonads to exert effective biocontrol against a variety of bacterial and fungal

Table 18.2 Pseudomonads and their metabolites involved in biocontrol of phytopathogens

Pseudomonas strain	Metabolite/mode involved	Effects on phytopathogen	References
P. fluorescens 2–79	Phenazine-1-carboxylic acid	Antifungal	Gurusiddaiah et al. (1986)
P. fluorescens 2–79			Thomashow et al. (1990)
P. aureofaciens 30–84			Thomashow and Pierson (1991)
P. fluorescens 2–79			Pathma et al. (2010)
P. aeruginosa PUPa3	Phenazine-1-carboxamide		Sunish Kumar et al. (2005)
P. chlororaphis PCL1391	2-hydroxyphenazine		Chin-A-Woeng et al. (1998)
P. aeruginosa PAO1	Pyocyanin		Baron et al. (1989)
P. fluorescens Pf-5,Q2-87CHAO, PFM2, Q8r1–96, F113	2,4-diacetylphloroglucinol	Antifungal	Howell and Stipanovic (1979)
		Antihelmenthic	
		Herbicidal	Shanahan et al. (1992)
			Keel et al. (1992)
			Levy et al. (1992)
			Flaishman et al. (1990)
			Raaijmakers and Weller (2001)

(continued)

Table 18.2 (continued)

Pseudomonas strain	Metabolite/mode involved	Effects on phytopathogen	References
P. fluorescens BL914, BL915	Pyrrolnitrin	Antifungal	Kirner et al. (1998)
			Ligon et al. (2000)
			Elander et al. (1968)
			Cartwright et al. (1995)
Pseudomonas sp.	Isopyrrolnitrin		Hashimoto and Hattori (1966a)
Pseudomonas sp.	Oxypyrrolnitrin		Hashimoto and Hattori (1966b)
P. pyrrolnitrica	Monodechloro-pyrrolnitrin		Hashimoto and Hattori (1968)
P. fluorescens Pf-5, CHA0	Pyoluteorin		Howell and Stipanovic (1979) and Keel et al. (1992)
P. borealis MA342	2,3-deepoxy-2,3-didehydro rhizoxin		Tombolini et al. (1999)
P. fluorescens Pf-5	Rhizoxin analogs		Loper et al. (2008)
P. fluorescens DR54	Viscosinamide		Nielsen et al. (1999)
P. fluorescens 96.578	Tensin		Nielsen et al. (2002)
Pseudomonas sp. DSS73	Amphisin		Sorensen et al. (2001)
P. fluorescens Pf-5, P5, P7, P8, P21	Hydrogen cyanide		Voisard et al. (1981)
P. pseudoalcaligenes P4	Hydrogen cyanide		Ayyadurai et al. (2007)
P. fluorescens	2,4 DAPG		Asadhi et al. (2013)
P. chlororaphis	Pyrrolnitrin		Park et al. (2011)
Pseudomonas PGC2	Lytic enzymes		Arora et al. (2008)
P. fluorescens 3551	Pyoverdine	Competitive inhibition of phytopathogens	Loper et al. (2008)
P. fluorescens CHAO	Pyoluteorin		Maurhofer et al. (1994)
P. fluorescens WCS374r	ISR		Van Wees et al. (1997)
P. aeruginosa PAO-1	Pyochelin		Cox et al. (1981)
P. fluorescens CHAO	Pyochelin		Buysens et al. (1996)
P. stutzeri KC	Pseudomonine		Lewis et al. (2000)
P. fluorescens ATCC 17400	Pseudomonine		Mossialos et al. (2000)
P. fluorescens WCS374	Pseudomonine		Mercado-Blanco et al. (2001)
P. fluorescens ATCC 17400	Quinolobactin		Matthijs et al. (2007)

phytopathogens (Pierson and Pierson 1996; Laursen and Nielsen 2004; Price-Whelan et al. 2006; Mavrodi et al. 2006; Arseneault and Filion 2016). The most common phenazine derivatives produced by pseudomonads are pyocyanin (PYC), phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN) and several hydroxy-phenazines (Turner and Messenger 1986; Patil et al. 2016). Very few number of phenazine derivatives like PCA, PYC, PCN and HP have been evaluated in biocontrol. PCA and PCN have been demonstrated to be effective against various fungal phytopathogens (Chin-A-Woeng et al. 1998). The most studied examples of beneficial phenazine producers are *P. fluorescens* and *P. chlororaphis* which are responsible for fungal disease suppression in plants (Pierson and Pierson 1996). Biocontrol strains of pseudomonads such as *P. fluorescens*, *P. aeruginosa* and *P. chlororaphis* often produce both PCA and PCN derivatives which play a crucial role in biological control of phytopathogens (Chin-A-Woeng et al. 1998). The proposed mechanisms states that PCA, PYC and PCN diffuse across cell membrane or get inserted into the membrane and then act as a reducing agent causing uncoupling of oxidative phosphorylation and the generation of toxic intracellular reactive oxygen species, specifically superoxide radicals, reactive nitrogen species, specifically peroxynitrite radicals and hydrogen peroxide which are detrimental to the organism (Turner and Messenger 1986; Chin-A-Woeng et al. 1998, Briard et al. 2015; Xu et al. 2015; Zhang et al. 2017). The hydroxy-phenazines (HP) have a completely different mode of action from that of PYC, PCA and PCN where it acts as iron chelator making it unavailable to fungi adversely affecting its growth in iron-limiting environment (Briard et al. 2015); however, the cumulative action of these phenazines producing oxidative stress is more important in pathogenic fungal cell and mitochondrial damage. The chelation of iron could not only be through HP, but also the siderophore must be interfering iron chelation under the dynamic environment with more possible events. This presumed antimicrobial mode of action of pseudomonads is schematically represented in the Fig. 18.3.

The biocontrol bacteria *P. fluorescens* 2-79 and *P. aureofaciens* 30-84 produce the antibiotic PCA and suppress take-all, an important root disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Thomashow et al. 1990). Marine *P.*

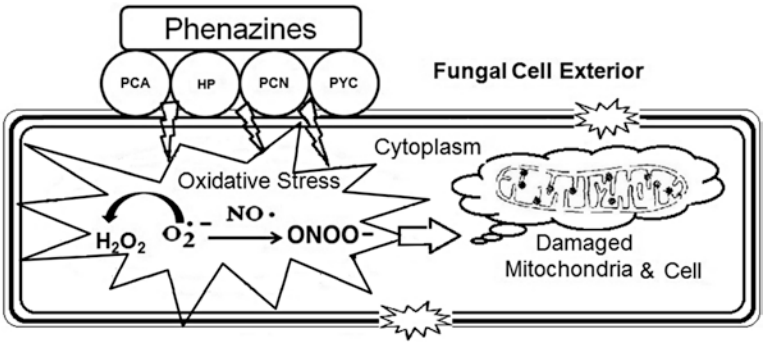


Fig. 18.3 Cumulative action of phenazines on phytopathogenic cells leading to biocontrol

aeruginosa strain GS-33 capable of producing PCA suppressed charcoal root caused by *Macrophomina phaseolina* in soybean under saline soil conditions (Patil et al. 2016). Tambong and Höfte (2001) demonstrated that both PCA and PCN produced by *P. aeruginosa* PNA1 were involved in biocontrol of *Pythium myriotylum*, the causative agent of root rot of cocoyam. Gurusiddaiah et al. (1986) reported that the fungi belonging to genera *Cochliobolus*, *Cortium*, *Gaeumannomyces*, *Rhizoctonia* and *Trametes* were most PCA-sensitive fungi (1–10 µg/mL). The antifungal activity of PCN under in vitro conditions was at least ten times higher than PCA (Chin-A-Woeng et al. 1998). PCN-producing bacterium *P. chlororaphis* strain PCL1391 found to be an efficient colonizer of tomato rhizosphere and an excellent biocontrol against tomato foot- and root rot-causing fungal pathogen *F. oxysporum* (Chin-A-Woeng et al. 1998). However, its phzB or phzH mutants were unable to produce PCN which failed to suppress tomato foot- and root rot-causing fungus *F. oxysporum*. Similarly, a mutant (PCA$^-$) of PCA-producing *P. fluorescens* 2-79 provided significantly less control of take-all than the wild type on wheat seedlings (Thomashow and Weller 1988). Mazzola et al. (1992) evaluated the role of phenazine biosynthesis in the ecological competence of *P. fluorescens* 2-79 and *P. aureofaciens* 30-84 in competitive soil and rhizosphere environments. They introduced 'Phz$^-$' mutants defective in phenazine production into the specific soil with and without amended with *G. graminis* var. *tritici*. It was observed that population sizes of 'Phz$^-$' mutants declined more rapidly than wild strain. This suggested that antibiotic contributes to the ecological competence of these strains (Mazzola et al. 1992). Thus, phenazines play an important role by acting as an antimicrobial agent against phytopathogens and contributing to ecological survival of biocontrol pseudomonads in the competitive rhizospheric milieu.

18.3.3.2 Phloroglucinol

Phloroglucinol is a naturally occurring benzenetriol compound found in certain plant species and is also produced by different microorganisms (Premchandra et al. 2016). In particular, 2, 4-diacetylphloroglucinol (DAPG) is a widely studied phloroglucinol produced by pseudomonads (Weller et al. 2007), while the biocontrol activity of many *P. fluorescens* isolates has been linked to the production of the DAPG (Brazelton et al. 2008). The DAPG exerts antimicrobial action via plasma membrane damage and inhibiting zoospore motility in oomycetes (de Souza et al. 2003). It has been demonstrated that root-associated fluorescent *Pseudomonas* spp. with the capacity to produce DAPG are the key components in biological control of wheat root disease 'take-all' (Raaijmakers and Weller 1998). The DAPG produced by the *Pseudomonas* sp. is a major contributing factor in the biocontrol potential, e.g. *Pseudomonas fluorescens* CHA0 acts against black root rot of tobacco caused by *Thielaviopsis basicola* while *P. fluorescens* F113 against 'damping-off' of sugar beet caused by *Pythium ultimum* (Dwivedi and Johri 2003).

Along with antifungal activity, DAPG produced by several strains of *P. fluorescens* also has antibacterial, anthelminthic and phytotoxic properties (Raaijmakers et al. 2002). Cronin et al. (1997) demonstrated that purified DAPG increased

hatching of cysts of the nematode *Globodera rostochiensis* and significantly reduced juvenile mobility. Better ecological fitness was observed among wild-type strains of *P. fluorescens* as compared to their DAPG-deficient mutants in the rhizosphere and in soil (Carroll et al. 1995; Cronin et al. 1997). DAPG not only exhibits antifungal activity but also acts as a plant growth stimulator. DAPG produced by *P. fluorescens* isolates can stimulate lateral root formation in tomato seedlings by inhibiting primary root development (Premachandra et al. 2016). A group of A.L. Iavicoli et al. (Hass and Keel 2003) found that in one plant-pathogen system, *Arabidopsis thaliana-Peronospora parasitica*, a DAPG-negative mutant of *P. fluorescens* strain CHA0 lost most of its capacity to trigger ISR as compared to wild-type strain, indicating role of DAPG in inducing systemic resistance in plant against phytopathogen.

18.3.3.3 Biosurfactants

Biosurfactants are organic chemical compounds produced by microorganisms that display surface activity and possess hydrophilic part usually made up of sugars, amino acids or polar functional groups like carboxylic acid groups, while the hydrophobic part is an aliphatic hydrocarbon chain of β-hydroxy fatty acids (Lang and Wullbrandt 1999). Production of biosurfactant is also an important biocontrol trait of fluorescent pseudomonads. From an agricultural viewpoint, biosurfactant-mediated biocontrol can also lead to beneficial effects. In the last decade, the biocontrol potential of *P. aeruginosa* strain PNA1 against the plant disease caused by *Pythium* sp. was reported to involve the production of rhamnolipids so as to exhibit the inhibition activities against the plant pathogen (Perneel et al. 2008). It is well known that pseudomonads can produce various types of biosurfactants which can act as a surfactant as well as antibiotics (Soberón-Chávez et al. 2005). An antimicrobial activity of biosurfactant pertains to its ability to penetrate cell wall or outer membrane via passive diffusion causing damage to the outer cell layer ultimately leading to the coagulation and leakage of intracellular constituents (Elshikh et al. 2016). Based on their physico-chemical properties, there are several types of biosurfactant such as glycolipids, lipopeptides, neutral lipids, and fatty acids that can be used in plant-pathogen elimination (Cameotra and Makkar 2010; Sachdev and Cameotra 2013). *P. aeruginosa* was the foremost reported producer of rhamnolipid (glycolipids) type of biosurfactants which have been widely investigated, and numerous reports on biosurfactants of pseudomonads are now available (Bergström et al. 1946; Maier and Soberón-Chávez 2000; Nitschke et al. 2005; Ochsner et al. 1996; Soberón-Chávez 2004; Soberón-Chávez et al. 2005, Abdel-Mawgoud et al. 2009). Debode et al. (2007) reported disruption of rhamnolipid and phenazine synthesis genes in the species *P. aeruginosa* and *P. chlororaphis* significantly reduced the ability of these species to suppress the fungal pathogen *Verticillium microsclerotia*. Recently, Dos et al. (2017) reported the synthesis of rhamnolipids from sugarcane bagasse using *P. aeruginosa*. The antimicrobial properties of rhamnolipids were reported since a long time and found active against a broad range of bacteria (Itoh et al. 1971; Lang et al. 1989).

Pseudomonads having biocontrol potential are known to produce lipopeptide biosurfactants (LPs) that are composed of lipid tails linked to a short linear or cyclic

oligopeptide. Among LPs, cyclic lipopeptides (CLP) are composed of a fatty acid tail connected to a short oligopeptide, which is cyclized to form a lactone ring between two amino acids in the peptide chain (Raaijmakers et al. 2006). CLPs are very diverse in both ways, structurally and functionally due to variations in the length and composition of the fatty acid tail and to variations in amino acids of the peptide moiety (de Bruijn and Raaijmakers 2009). Viscosinamide, one of the best studied CLPs with antifungal properties, was produced by *P. fluorescens* DR54, a sugar beet root isolate able to control *Pythium ultimum* and *Rhizoctonia solani* damping-off on sugar beet (Nielsen et al. 1999). CLPs are produced by numerous plant-associated *Pseudomonas* spp., such as *P. fluorescens* and *P. putida* (Nielsen et al. 2002; Nybroe and Sørensen 2004; Raaijmakers et al. 2006). Based on structural differences, the CLPs produced by pseudomonads are viscosin, amphisin, tolaasin, syringomycin, arthrofactin, putisolvins I and II, orfamide, pseudodesmins A and B, etc. (Roongsawang et al. 2003; Nybroe and Sørensen 2004; Kuiper et al. 2004; Paulsen et al. 2005; Kruijt et al. 2009). CLPs have received considerable attention for their antimicrobial, cytotoxic and surfactant properties. CLPs produced by pseudomonads play an important role in the antimicrobial activity, swarming motility and biofilm formation (Nielsen et al. 2002). CLPs, produced by pseudomonads can act as a broad-spectrum antibiotic agents, causing the damage of membranes leading to the death of phytopathogenic bacteria, fungi, oomycetes and viruses (Tapadar and Jha 2013).

18.3.3.4 Other Metabolites

Apart from siderophores, phenazines, and biosurfactants, biocontrol pseudomonads are known to produce a variety of small molecular weight antimicrobial compounds such as phloroglucinols (Phl), pyrrolnitrin (Prn), pyoluteorin (Plt), hydrogen cyanide (HCN), hydrolytic enzymes, etc. (Saraf et al. 2014). These metabolites have deleterious effects on pathogenic microorganisms and help beneficial pseudomonads to survive and grow under diverse environmental conditions.

Pyrrolnitrin (Prn) is a highly active broad-spectrum antifungal secondary metabolite produced from tryptophan by many fluorescent and nonfluorescent strains of the genus *Pseudomonas* (Kirner et al. 1998). The Prn production has been considered as one of the important mechanisms of biological control of phytopathogenic fungi by numerous *Pseudomonas* strains (Howell and Stipanovic 1979; Janisiewicz and Roitman 1988; Yoshihisa et al. 1989). Prn shows activity against a wide range of fungi including deuteromycetes, ascomycetes, and basidiomycetes. A phenyl pyrrol derivative of Prn has been reported as a potent agricultural fungicide, whereas other variants of Prn like isopyrrrolnitrin, oxypyrrolnitrin and monodechloropyrrolnitrin have lower fungicidal activities (Elander et al. 1968). The mechanism of action of pyrrolnitrin involves an initial attack on the cell membrane, by interacting with the phospholipids which alters the cell membrane permeability, and then it inhibits the synthesis of proteins and nucleic acids in the cell, thus leading to the death of microbes. Prn is also reported as a less potent inhibitor of electron transport chain in phytopathogens (Wong and Airall 1970) which does not readily diffuse and gets released only after lysis of host microbial cell (Nose and Arima 1969; Dwivedi and Johri 2003).

Pyoluteorin (Plt) is an aromatic polyketide secondary metabolite consisting of a resorcinol ring linked to a bichlorinated pyrrole moiety (Blender et al. 1999). Plt inhibits the growth of bacteria and fungi (Tekeda 1958) and is phytotoxic to certain plants (Maurhofer et al. 1992). Plt is produced by several *Pseudomonas* spp., including *P. fluorescens* strains, *P. putida*, *P. aeruginosa*, etc., those that suppress plant diseases caused by phytopathogenic fungi (Maurhofer et al. 1992; Maurhofer et al. 1994; Kraus and Loper 1995). The differential role of Plt in biological control is due to differential bacterial population and temporal patterns of Plt gene expression and production in the rhizosphere and spermosphere of different plant hosts (Maurhofer et al. 1994; Kraus and Loper 1995).

Hydrogen cyanide (HCN) is a volatile, secondary metabolite produced by many different bacterial genera including *Pseudomonas* (O'sullivan and O'Gara 1992, Siddiqui et al. 2006). Several reports suggest that production of HCN by certain fluorescent pseudomonads may also influence plant root pathogens and inhibit phytopathogenic nematodes (Schippers et al. 1991, Siddiqui et al. 2006). In case of tobacco plants, it has been proved that HCN production by fluorescent pseudomonads stimulated root hair formation (van Peer and Schippers 1989). HCN has been reported to suppress 'root-knot' and 'black rot' diseases of tomato and tobacco caused by the nematodes *Meloidogyne javanica* and *Thielaviopsis basicola* as reported by Voisard et al. (1981) and Siddiqui et al. (2006). HCN has also been observed to control termite *Odontotermes obesus* which is a pest in agriculture and forestry crops (Devi et al. 2007).

The mode of action of HCN can be attributed to its ability as a powerful inhibitor of many metalloenzymes, especially copper-containing cytochrome-C oxidases in the respiratory chain (Knowles 1976; Solomonson 1981). HCN mutant obtained by insertional inactivation of the wild-type *P. fluorescens* strain CHAO had lost its ability to suppress black root rot of tobacco (Voisard et al. 1981). Haas et al. (1991) demonstrated that the same mutation had no effect on the biocontrol performance of this strain against take-all disease in wheat. However, the role of HCN produced by fluorescent pseudomonads is contradictory. HCN produced by *Pseudomonas* in the rhizosphere inhibits the primary growth of roots in *Arabidopsis* due to the suppression of an auxin-responsive gene (Rudrappa et al. 2008). Few papers reported harmful effects of HCN in potato (Bakker and Schippers 1987) and lettuce roots (Alstrom and Burns 1989).

D'aes et al. (2011) demonstrated the involvement of both phenazines and CLPs during *Pseudomonas* CMR12a-mediated biocontrol of Rhizoctonia root rot of bean. They observed that *Pseudomonas* CMR12a wild-type strain could produce phenazines and CLPs which dramatically reduced severity of root rot of bean caused by two different anastomosis groups (AGs) of *R. solani*. However, a CLP-deficient and a phenazine-deficient mutant of CMR12a protected bean plants from root rot caused by *R. solani* (AGs) to a lesser degree as compared to the wild type, whereas a mutant deficient in both CLPs and phenazine completely lost their biocontrol activity. This indicated that both phenazines and CLPs together play an important role in biocontrol potential of pseudomonads.

Prn is also an important secondary metabolite of biocontrol pseudomonads which inhibits the growth of bacteria and fungi by membrane damage and inhibiting synthesis of nucleic acids and proteins in the cell (Wong and Airall 1970). A native isolate of *P. fluorescens* capable of producing Prn inhibited growth of *M. phaseolina* (Karunanithi et al. 2000). Prn-producing strains *P. fluorescens* BL915 and *P. cepacia* 5.5B have been reported as a biocontrol agent in cotton for the suppression of *R. solani* (Cartwright et al. 1995; Ligon et al. 2000). Like Prn, the Plt is also an important secondary metabolite produced by most of the fluorescent pseudomonads. Plt is the main inhibitor of oomycetous fungi, and it is strongly active against *Pythium ultimum*. Seeds priming with Plt-producing *P. fluorescens* Pf-5 decrease the severity of *Pythium* damping-off (Nowak-Thompson et al. 1999). As reported by Hassan et al. (2011), Plt-producing biocontrol bacteria *P. putida* strain NH-50 significantly reduced disease severity on sugarcane varieties under field conditions.

Although, there are several biocontrol traits of pseudomonads, a successful and superior antagonism against phytopathogens is achieved through a synergistic combination of different mechanisms responsible for a successful biocontrol (O'Sullivan and O'Gara 1992).

Apart from the above-stated metabolites rendering biocontrol activity to pseudomonads, there could be numerous metabolites reported contributing towards the phytopathogen suppression which could be the tip of the iceberg, while many would be unreported and unknown metabolites to the scientific community.

18.3.4 Induction of Systemic Resistance

Induction systemic resistance (ISR) is one of the important biocontrol traits of plant growth-promoting and biocontrol pseudomonads (Van Peer et al. 1991; Wei et al. 1991). Beneficial bacteria trigger ISR through specific signalling pathways leading to certain biochemical responses to activate the plant's defence system against a broad spectrum of phytopathogens (van Loon et al. 1998). Such signalling pathways involve salicylic acid, ethylene and jasmonic acid pathways (Bakker et al. 2007). Bacterial determinants like outer membrane lipopolysaccharides, flagella, iron-regulated metabolites, volatile compounds, antibiotics and cyclic lipopeptides are reported to activate ISR (Ongena et al. 2008; Subashri et al. 2013) which trigger the rapid accumulation of pathogenesis-related enzymes such as chitinase, glucanase, peroxidases, lyases, etc. and protect plants from pathogen attack.

Role of ISR in the suppression of root pathogen was demonstrated in split root system, by ensuring spatial separation between the *Pseudomonas* bacteria and the pathogen on the root system (Zhou and Paulitz 1994). However, a threshold population density of 10^5 colony forming units per gram of root was required for effectiveness of the resistance-inducing *Pseudomonas* strain (Raaijmakers et al. 1995). The *P. fluorescens* strain WCS374r capable of eliciting ISR significantly protected radish from *Fusarium* wilt and improved yield under commercial greenhouse conditions (Leeman et al. 1995). Role of ISR in disease suppression is demonstrated by

several workers by using mutants of *Pseudomonas* unable to express determinants of ISR (Bakker et al. 2007).

18.4 Impact of Environmental Factors on Biocontrol Potential

Several plant diseases arise due to phytopathogens which are harbouring in the soil. The management of plant diseases incited by soilborne phytopathogens increases the crop productivity. It can be done effectively through the application of pseudomonads. Use of such biocontrol agents is a foremost substratum of sustainable agriculture. However, the seemingly inherent variable performance of most *Pseudomonas* biocontrol strains in variable environments owing to field locations and cropping seasons has hampered its commercial development. Most of this variability has been attributed to differences in physical and chemical properties found in natural environments where biocontrol agents are applied (Howarth 1991; Duffy and Défago 1999). Different environmental factors many of the times adversely affect the biocontrol activity and antagonism of pseudomonads directly or indirectly. Understanding the appropriate environmental factors is important, and the way these influence disease suppression is a key to improve the levels and reliability of biocontrol agent.

Upadhyay et al. (1991) have elaborated influence of nutritional and environmental conditions on the antagonism of *Pseudomonas cepacia* against *Trichoderma viride*. While studying the nutritional impact, xylose and trehalose strongly enhanced the antifungal activity of *P. cepacia* as well as inhibited sporulation of fungi, but the effect of mannitol and glucose was minor. Antagonism of *P. cepacia* was better when ammoniacal nitrogen was present in the medium, while in case of nitrite or nitrate, there was only a little antagonism. At a wide range of temperature, the biocontrol activity of *P. cepacia* was good, but under acidic pH only, the activity was better against *T. viride*.

Presence of inorganic minerals in the surrounding influences the biocontrol potential of pseudomonads. Amendment of zinc and copper was found to improve biocontrol potential of *Pseudomonas fluorescens* strain against crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, whereas presence of ammonium molybdate did not have any effect (Duffy and Défago 1997).

Presence of mycotoxins in the soil also impacts the biocontrol potential of fluorescent pseudomonads as observed by Duffy and Défago (1997). Fusaric acid produced by the *Fusarium oxysporum*, a causative agent of the crown and root rot in tomato at a specific concentration, could repress the synthesis of 2,4-diacetylphloroglucinol by *Pseudomonas fluorescens* strain CHA0 which is a key factor in the biocontrol.

Ownley et al. in 2003 demonstrated that soil properties greatly influence biological control performance of phenazine-producing *Pseudomonas fluorescens* against take-all disease caused by *Gaeumannomyces graminis* var. *tritici* in wheat. The level of protection in the field varies per the location, and biocontrol activity of this bacterium was positively correlated with ammoniacal – nitrogen, availability of sand, soil pH, sodium (extractable and soluble), sulphate-sulphur, zinc, etc. In

contrast, biocontrol activity was negatively correlated with cation-exchange capacity (CEC), exchangeable acidity, iron, manganese, percent clay, percent organic matter, percent silt, total carbon and total nitrogen (Ownley et al. 2003).

De La Fuente et al. (2006) studied the effect of host plant genotype on the rhizosphere colonization performance of both indigenous and introduced DAPG-producing strains of *Pseudomonas fluorescens* having potent biocontrol activity against soilborne pathogens. Population densities differed among the rhizospheres of various crops (alfalfa, barley, bean, flax, lentil, lupine, oat, pea, and wheat) for different strains of *Pseudomonas fluorescens*.

Trivedi et al. (2008) reported the effect of pH and temperature on biocontrol performance of Himalayan soil isolate *Pseudomonas corrugate* from a temperate site having antagonistic activities against two phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*. The pH of the surrounding environment greatly influenced the antagonistic activities with no antagonistic activity at pH 8.5 or above, whereas better inhibition was found at acidic pH against both the fungi with maximum inhibition at pH 5.5. The temperature was also found to influence antagonistic activity of *P. corrugata* to a great extent with optimum activity at 21 °C. It was interesting to note that *P. corrugata* exerted good antagonistic effects at lower temperatures and this species has already been reported as a psychrotroph (Pandey et al. 2002).

Diverse environmental and nutritional conditions were found to modulate production of antibiotic PCN by *Pseudomonas chlororaphis* (van Rij et al. 2004). The production of antibiotics like DAPG, Plt, Pln and siderophores like salicylic acid and pyochelin by the model biocontrol bacterium *P. fluorescens* strain CHA0 was also greatly influenced by altering environmental and nutritional conditions under in vitro conditions (Duffy and Défago 1999).

The extent of disease suppression especially fusarium wilt of chickpea by rhizobacterial strains of *P. fluorescens* was observed to be modulated by soil temperature (Landa et al. 2004). They observed a positive linear trend between bacterial population density in the rhizosphere and temperature increase. However, the maximum inhibition of mycelial growth and conidial germination of *Fusarium oxysporum* under in vitro conditions occurs at a temperature range optimal for bacterial growth and production of antifungal secondary metabolites. In previous research, Landa et al. (2002) reported that *P. fluorescens* through soil treatment could suppress the fusarium wilt by delaying the development of disease symptoms and reducing the rate of disease increase at 20 and 30 °C, while the higher temperature was not supportive for disease suppression. Thus, numerous abiotic factors, such as pH, temperature, moisture, texture and inorganic and organic constituents, as well as biotic factors, like microbial population density, microbial diversity, and genotype of host plant, may influence the biocontrol potential of pseudomonads. The effect of environmental factors and medium ingredients surely affect the biocontrol potential of the pseudomonads; however, it is species specific and liable to alter its abilities depending on the environmental parameters and physico-chemical conditions.

18.5 Engineering Cells for Secondary Metabolites

Efficient biocontrol demands newer strategies where the foremost one is overproduction of secondary metabolites of biocontrol strains of pseudomonads through engineering the biocontrol agent. The first antibiotic genes cloned and manipulated were from *P. fluorescens* HV37a, which could produce oomycin-A. This antibiotic is primarily responsible for control of about 70% of *Pythium*-induced root infection of cotton seedlings by pseudomonads (Gutterson 1990). Hassani et al. (2012) reported the mutant strain named *P. aeruginosa* S300-8 showed the better productivity of pyocyanin than wild type.

Feklistova and Maksimova (2008) successfully obtained phenazine antibiotics overproducing strain by nitrosoguanidine-induced mutagenesis in *P. aurantiaca* B-162 and found that mutant strain produced phenazines three times more efficiently as compared to wild type; but the biocontrol potential of both mutants S300-8 and B-162 remains unexplored.

Improved levels of production of biosurfactant by mutants of *P. aeruginosa* have been reported as compared to their wild-type strains (Iqbal et al. 1995; Raza et al. 2007). Maurhofer et al. (1995) obtained Plt and DAPG overproducing strain *P. fluorescens* CHAO/pME3090 by insertion of recombinant cosmid pME3090 into *P. fluorescens* strain CHAO which was a good biocontrol agent acting against various phytopathogens. *P. fluorescens* CHAO/pME3090 increased production of Plt and DAPG three- to fivefold as compared to wild-type strain exhibiting increased protection of cucumber against *F. oxysporum* f. sp. *cucumerinum* and *Phomopsis sclerotioides*. As microbial cells are very specific for utilization of their own metabolites, this could be a hurdle in the development of a compatible consortium of biocontrol pseudomonads. This hurdle could be overcome by genetic manipulation as demonstrated by Marugg et al. (1989). They observed that rhizosphere-colonizing bacteria *P. fluorescens* WCS374 which initially was unable to take up a ferric pseudobactin produced by another rhizobacterium *P. putida* WCS358 under iron-limiting conditions could take it up when *P. fluorescens* WCS374 was inserted with a gene bank containing partial Sau3A DNA fragments from WCS358 constructed in cosmid pLAFR1. O'sullivan and O'gara (1991) isolated a mutant of fluorescent *Pseudomonas* sp. strain M114 that could produce siderophore even in the presence of iron and contributing to inhibition of bacteria and fungi under in vitro conditions which carries a high importance. Yang et al. (2017) constructed a recombinant strain of *P. fluorescens* strains HC1-07 and HC9-07 producing both PCA and CLP for the biocontrol of take-all disease of wheat. Initially, *P. fluorescens* strains HC1-07 and HC9-07 could produce only CLP and PCA, respectively, which was introduced with seven-gene operon for the synthesis of PCA from *P. synxantha* 2-79 and observed better biocontrol activity of the resultant recombinant strain HC1–07PHZ against 'take-all' disease-causing pathogen *G. graminis* var. *tritici* of wheat. Interestingly, recombinant strain HC1–07PHZ suppressed take-all better biocontrol than strains HC1-07rif and HC9-07rif applied either individually or in combination. This massive recombinant strain could provide better biocontrol strains of pseudomonads to provide effective disease suppression. Thus, the advancements in molecular biology

and genetic engineering have potential to offer avenues for improved production of biocontrol metabolites by pseudomonads under diverse filed conditions; however, it is up to the acceptance with its *pros* and *cons* by that society and the law in force by that specific country.

18.6 Safety Aspects of Application of Pseudomonads in Agriculture

The promising ability of pseudomonads to offer biocontrol against phytopathogenic microorganisms and simultaneous promotion of plant growth has made to exploit this potential for the benefit of agriculture. This lead to the idea of introducing beneficial pseudomonads into soil or the rhizosphere for biocontrol of soilborne crop diseases; additionally, in certain cases application of genetically modified (GM) strains with improved expression of biocontrol traits have been proposed to impart better biocontrol efficacy (Cook 1993; Dunne et al. 1996; Keel and De'fago 1997; Girlanda et al. 2001). However, there have always been the two sides of the coin where one would be favourable, while the other may not. Therefore, risk assessment is necessary before the application of huge populations of pseudomonads especially genetically modified strains into the field which may pose important safety concerns associated with the possible ecological consequences on nontarget native living populations (human, animals, insects, microbial flora, etc.) and ecosystem operation (De'fago et al. 1997; van Elsas and Migheli 2002). A human being has always been taking the risks in pursuit of the betterment of life. To fight with the situation of food scarcity, a lot of efforts have been invested improving agricultural productivity, and with the advent of the green revolution, toxic agrochemicals and pesticides have been overwhelmingly accepted across the world even though serious environmental consequences and medical risks are now learnt.

Presently, biological pesticides are becoming famous as a part of sustainable development bearing lower risks and environment friendly nature. Among the biological pesticides, agricultural sector bank upon pseudomonads a lot, even though; a few of the pseudomonads are either pathogens or opportunistic pathogens in nature. There has always been a risk of infections from these organisms especially lung infections like pneumonia to weaker animals and human beings when compromised with the immune system (Driscoll et al. 2007) or even the elderly people. *P. cepacia* has been implicated in nosocomial outbreaks involving septicemia and peritonitis and has been associated with respiratory tract infections (Tablan et al. 1985). *Pseudomonas aeruginosa* is an opportunistic pathogen that causes fatal nosocomial infections. People whose immune systems have been weakened by severe burns, cystic fibrosis, immunosuppressive or cancer chemotherapy act as an easy victim for *P. aeruginosa* infection (Gellatly and Hancock 2013). The *P. aeruginosa* is naturally resistant to most antibiotics and has an ability to develop resistance quickly to those commonly used. Faccone et al. (2014) reported the isolation of a strain of *P. chlororaphis* subs. *chlororaphis* from a blood culture of a patient with prolonged febrile syndrome and endocarditis. However, this identification was

based on just partial 16S rRNA gene sequencing (803 bp), raising some doubts about its reliability. The *P. fluorescens* is also emerging as nosocomial infectious bacteria, found not only in water or disinfectants but also in patient's normal flora (Picot et al. 2001). Blood or injectable pharmaceutic products contaminated with *P. fluorescens* have been reported to cause endotoxic shock (Sarubbi et al. 1978; Murray et al. 1987; Namnyak et al. 1999; Nishimura et al. 2017). One case of fatal infection by *P. fluorescens* in hepatic lesions has been reported in humans (Ramirez et al. 1989). This bacterium has been reported as a pathogen for causing lethal liver infections in birds (Jackson and Phillips 1996). Therefore, risk assessment of field releases is essential and becoming increasingly common.

Several schemes are currently in use in the USA (US Department of Agriculture, Food and Drug Administration and Environmental Protection Agency), the European Union (EU) and other countries or the Organization for Economic Cooperation and Development (OECD) (Mark et al. 2006) for the risk assessment and providing registrations to biocontrol agents. The OECD, for example, has prepared several consensus documents on monitoring regimes for the environmental release of organisms. Before registering *Pseudomonas* spp. as crop protection products, they must be assessed for their impact on human health as well as the environment. The European Union directive 91/414/EEC deals with the placing of crop protection products in the market and needs stringent testing of biocontrol strains which is equivalent to the registration process for chemical fungicides. Likewise, a separate scientific dossier as per Annex II of European Union directive 90/220/EEC needs to be submitted for registration of genetically modified strains, which deals with the intentional release of genetically modified organisms (GMOs) into the environment. An essential feature of the proposal to be submitted is for the assessment of the risk of the microorganism in the environment against a set of values that need to be protected, including human, animal and plant health. Additional values are added when appropriate, such as biodiversity as a source of natural variability and agronomic values.

Assessment of the impact of biocontrol pseudomonads on microbial species and total microbial populations is considered to evaluate its effect on rhizospheric microflora. Several studies have demonstrated that both wild-type and phloroglucinol overproducing *Pseudomonas* do not interfere with the symbiotic relationship between arbuscular mycorrhizal fungi and the majority of land plants (Barea et al. 1998; Edwards et al. 1998; Vázquez et al. 2000). Neimann et al. (1997) have reported that biocontrol *Pseudomonas* affect the growth and nodule occupancy of certain *Sinorhizobium meliloti* strains in gnotobiotic conditions; however, at commercial-scale field trials, there was no effect on nodulation or nutrient levels in the foliage of a red clover rotation crop (Moënne-Loccoz et al. 1998). The *P. fluorescens* wild-type strain and its phloroglucinol and pyoluteorin overproducing strain did not affect the frequency of dominant bacterial groups from total indigenous culturable bacteria (Natsch et al. 1998), whereas detectable (but very small) impact on the culturable fungal population in the cucumber rhizosphere has been observed (Girlanda et al. 2001) when compared with untreated plants. However, these culture-dependent methods for impact assessment are questionable because many of the

soil microorganisms cannot be isolated on laboratory media. A culture-independent method for the assessment of bacterial diversity demonstrated no effect on the rhizosphere bacterial population when lysozyme-tolerant *P. putida* strain inoculated to the genetically modified lysozyme-producing potatoes (Lottmann et al. 2000).

Based on the risk assessment, the Environmental Protection Agency in the USA and the Standing Committee on the Food Chain and Animal Health-Scientific Committee on Plant in Europe assessed *P. chlororaphis* strains for plant protection purposes and recommended it as '*Pseudomonas chlororaphis* strain 63-28 is a naturally occurring bacterium that can be used in controlling various fungi that attack crop roots. The bacterium has shown no toxicity or pathogenicity to humans, wildlife, or the environment. Its use is limited to vegetables and ornamental crops in containers in greenhouses' (European Food Safety Authority 2017). In the developing countries like India, the government reviewed the indiscriminate use of pesticides in the decade of sixties and through its Central Insecticide Board (CIB) formulated guidelines and rules for chemical as well as biopesticides (http://cibrc.nic.in). The CIB has also been instrumental in giving the guidelines for registration including comprehensive data requirements on the formulation, efficacy, toxicity and packaging for registration of antagonistic bacteria since with effect from 1 January 2011, and subsequently registered pseudomonads as biopesticides include *Pseudomonas fluorescens* in the majority from various manufacturers for sale. Likewise, *Pseudomonas fluorescens* CL 145 A and *Pseudomonas fluorescens* strain D7 are currently registered as microbial pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in the USA (https://ehs.umich.edu/wp-content/uploads/sites/37/2016/12/EPA-UnivMI-Workshop-Importation-of-Biologicals.pdf).

18.7 Conclusion and Future Perspectives

Pseudomonads possess potential antagonistic activity because of its ability to produce highly potent antimicrobial compounds like siderophores, phenazine and antibiotics, biosurfactants and hydrolytic enzymes and also induce systemic resistance to plants, etc. and exert cumulative action to act against various phytopathogens. These bacteria have highly specific action and are eco-friendly as well as cost-effective but dependent on their inherent properties and many environmental and physico-chemical factors. These bioinoculants represent a promising alternative to chemical pesticides for the agricultural system to enhance the productivity. In the current scenario of sustainable agriculture production, it is important to view the emerging bioinoculants *Pseudomonas* as a component of integrated pest management system instead of their stand-alone effect within the realm of differential biotic and abiotic stresses. Even though pseudomonads may prove to be the best organism in curbing the plant pathogens, it may prove to be a boomerang at any time since nature has its own reservations which invite the scientific communities to solve. Future technology development based on scientific temperaments shall help in formulating efficient biopesticides for curbing the pests with minimum risks, avoiding inconsistencies and disappointments with a goal of improving agro-productivity.

Acknowledgements Authors are thankful to University Grants Commission, New Delhi, for SAP-DRS-III infrastructural grant and DST-FIST grant of Dept. of Science and Tech., New Delhi.

References

Abdel-Mawgoud AM, Aboulwafa MM, Hassouna NA (2009) Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs20. Appl Biochem Biotechnol 157(2):329–345

Ahmadzadeh M, Tehrani AS (2009) Evaluation of fluorescent pseudomonads for plant growth promotion, antifungal activity against *Rhizoctonia solani* on common bean, and biocontrol potential. Biol Control 48(2):101–107

Ali NI, Siddiqui IA, Shaukat SS, Zaki MJ (2002) Nematicidal activity of some strains of *Pseudomonas* spp. Soil Biol Biochem 34(8):1051–1058

Aliye N, Fininsa C, Hiskias Y (2008) Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato (Solanum tuberosum) against bacterial wilt (Ralstonia solanacearum). Biol Control 47(3):282–288

Alström S, Burns RG (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol Fertil Soils 7(3):232–238

Arora NK, Khare E, JH O, Kang SC, Maheshwari DK (2008) Diverse mechanisms adopted by fluorescent Pseudomonas PGC2 during the inhibition of *Rhizoctonia solani* and *Phytophthora capsici*. World J Microbiol Biotechnol 24(4):581–585

Arseneault T, Filion M (2016) Phenazine-producing *Pseudomonas* spp. as biocontrol agents of plant pathogens. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, vol 2. Springer, New Delhi, pp 53–68

Asadhi S, Bhaskara Reddy BV, Sivaprasad Y, Prathyusha M, Murali Krishna T, Vijay Krishna Kumar K, Raja Reddy K (2013) Characterisation, genetic diversity and antagonistic potential of 2, 4-diacetylphloroglucinol producing *Pseudomonas fluorescens* isolates in groundnut-based cropping systems of Andhra Pradesh, India. Arch Phytopathol Plant Protect 46(16):1966–1977

Ayyadurai N, Naik PR, Sakthivel N (2007) Functional characterization of antagonistic fluorescent pseudomonads associated with rhizospheric soil of rice (*Oryza sativa* L.) J Microbiol Biotechnol 17(6):919–927

Bakker AW, Schippers B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp-mediated plant growth-stimulation. Soil Biol Biochem 19(4):451–457

Bakker PA, Pieterse CM, Van Loon LC (2007) Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology 97(2):239–243

Bano N, Musarrat J (2004) Characterization of a novel carbofuran degrading Pseudomonas sp. with collateral biocontrol and plant growth promoting potential. FEMS Microbiol Lett 231(1):13–17

Barea JM, Andrade G, Bianciotto V, Dowling D, Lohrke S, Bonfante P, O'gara F, Azcon-Aguilar C (1998) Impact on arbuscular mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. Appl Environ Microbiol 64(6):2304–2307

Baron SS, Terranova G, Rowe JJ (1989) Molecular mechanism of the antimicrobial action of pyocyanin. Curr Microbiol 18(4):223–230

Bender CL, Rangaswamy V, Loper J (1999) Polyketide production by plant-associated Pseudomonads 1. Annu Rev Phytopathol 37(1):175–196

Beneduzi A, Ambrosini A, Passaglia LM (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35(4):1044–1051

Bensidhoum L, Nabti E, Tabli N, Kupferschmied P, Weiss A, Rothballer M, Schmid M, Keel C, Hartmann A (2016) Heavy metal tolerant *Pseudomonas protegens* isolates from agricultural well water in northeastern Algeria with plant growth promoting, insecticidal and antifungal activities. Eur J Soil Biol 75:38–46

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486

Bergstrom S, Theorell H, Davide H (1946) Pyolipic acid, a metabolic product of pseudomonas-pyocyanea, active against mycobacterium-tuberculosis. Arch Biochem 10(1):165–166

Bossis E, Lemanceau P, Latour X, Gardan L (2000) The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: current status and need for revision. Agronomie 20(1):51–63

Braud A, Hannauer M, Mislin GL, Schalk IJ (2009) The *Pseudomonas aeruginosa* pyochelin-iron uptake pathway and its metal specificity. J Bacteriol 191(11):3517–3525

Brazelton JN, Pfeufer EE, Sweat TA, Gardener BB, Coenen C (2008) 2, 4-Diacetylphloroglucinol alters plant root development. Mol Plant Microbe Interact 21(10):1349–1358

Briard B, Bomme P, Lechner BE, Mislin GL, Lair V, Prévost MC, Latgé JP, Haas H, Beauvais A (2015) *Pseudomonas aeruginosa* manipulates redox and iron homeostasis of its microbiota partner *Aspergillus fumigatus* via phenazines. Sci Rep 5:8220

Budzikiewicz H (1993) Secondary metabolites from fluorescent pseudomonads. FEMS Microbiol Lett 104(3–4):209–228

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

Cameotra SS, Makkar RS (2010) Biosurfactant-enhanced bioremediation of hydrophobic pollutants. Pure Appl Chem 82(1):97–116

Carroll H, Moenne-Loccoz Y, Dowling DN, O'gara F (1995) Mutational disruption of the biosynthesis genes coding for the antifungal metabolite 2, 4-diacetylphloroglucinol does not influence the ecological fitness of *Pseudomonas fluorescens* F113 in the rhizosphere of sugarbeets. Appl Environ Microbiol 61(8):3002–3007

Cartwright DK, Chilton WS, Benson DM (1995) Pyrrolnitrin and phenazine production by *Pseudomonas cepacia*, strain 5.5 B, a biocontrol agent of *Rhizoctonia solani*. Appl Microbiol Biotechnol 43(2):211–216

Chernin L, Chet I (2002) Microbial enzymes in the biocontrol of plant pathogens and pests. In Enzymes in the environment: activity, ecology, and applications. CRC Press

Chin-A-Woeng TF, Bloemberg GV, van der Bij AJ, van der Drift KM, Schripsema J, Kroon B, Scheffer RJ, Keel C, Bakker PA, Tichy HV, de Bruijn FJ (1998) Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Mol Plant Microbe Interact 11(11):1069–1077

Chincholkar SB, Chaudhari BL, Talegaonkar SK, Kothari RM (2000) Microbial iron chelators: a sustainable tool for the biocontrol of plant diseases. In: Upadhyay RK, Mukerji KG, Chamol BP (eds) Biocontrol potential and its exploitation in sustainable agriculture crop disease, weeds, nematodes. Springer, Boston, pp 49–70

Chincholkar SB, Chaudhari BL, Rane MR, Sarode PO (2006) Fungal phytopathogen suppression using Siderophoregenic bioinoculants. In Biological control of plant diseases, p 401

Chincholkar SB, Chaudhari BL, Sarode PO, Rane MR (2009) Phenazines of Pseudomonas: tool for biological control of soilborne phytopathogens. In: Agriculturally important microorganisms, vol 1. Academic World International, Bhopal, pp 101–115

Cigna J, des Essarts YR, Mondy S, Hélias V, Beury-Cirou A, Faure D (2015) Draft genome sequences of *Pseudomonas fluorescens* strains PA4C2 and PA3G8 and *Pseudomonas putida* PA14H7, three biocontrol bacteria against Dickeya phytopathogens. Genome Announc 3(1):e01503–e01514

Cobessi D, Celia H, Pattus F (2005) Crystal structure at high resolution of ferric-pyochelin and its membrane receptor FptA from *Pseudomonas aeruginosa*. J Mol Biol 352(4):893–904

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

Cook RJ (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. Annu Rev Phytopathol 31(1):53–80

Cordero P, Cavigliasso A, Príncipe A, Godino A, Jofré E, Mori G, Fischer S (2012) Genetic diversity and antifungal activity of native Pseudomonas isolated from maize plants grown in a central region of Argentina. Syst Appl Microbiol 35(5):342–351

Couillerot O, Prigent-Combaret C, Caballero-Mellado J, Moënne-Loccoz Y (2009) *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. Lett Appl Microbiol 48(5):505–512

Cox CD, Adams PA (1985) Siderophore activity of pyoverdin for *Pseudomonas aeruginosa*. Infect Immun 48(1):130–138

Cox CD, Rinehart KL, Moore ML, Cook JC (1981) Pyochelin: novel structure of an iron-chelating growth promoter for *Pseudomonas aeruginosa*. Proc Natl Acad Sci 78(7):4256–4260

Crichton RR, Charloteaux-Wauters M (1987) Iron transport and storage. Eur J Biochem 164(3):485–506

Cronin D, Moenne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O'gara F (1997) Role of 2, 4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. Appl Environ Microbiol 63(4):1357–1361

Czaja K, Góralczyk K, Struciński P, Hernik A, Korcz W, Minorczyk M, Łyczewska M, Ludwicki JK (2015) Biopesticides–towards increased consumer safety in the European Union. Pest Manag Sci 71(1):3–6

D'aes J, Hua GK, De Maeyer K, Pannecoucque J, Forrez I, Ongena M, Dietrich LE, Thomashow LS, Mavrodi DV, Höfte M (2011) Biological control of Rhizoctonia root rot on bean by phenazine- and cyclic lipopeptide-producing Pseudomonas CMR12a. Phytopathology 101(8):996–1004

Dandurishvili N, Toklikishvili N, Ovadis M, Eliashvili P, Giorgobiani N, Keshelava R, Chernin L (2011) Broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* suppress Agrobacterium crown gall tumours on tomato plants. J Appl Microbiol 110(1):341–352

De Bruijn I, Raaijmakers JM (2009) Diversity and functional analysis of LuxR-type transcriptional regulators of cyclic lipopeptide biosynthesis in *Pseudomonas fluorescens*. Appl Environ Microbiol 75(14):4753–4761

De La Fuente L, Landa BB, Weller DM (2006) Host crop affects rhizosphere colonization and competitiveness of 2, 4-diacetylphloroglucinol-producing *Pseudomonas fluorescens*. Phytopathology 96(7):751–762

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 Weger LA, van der Bij AJ, Dekkers LC, Simons M, Wijffelman CA, Lugtenberg BJ (1995) Colonization of the rhizosphere of crop plants by plant-beneficial pseudomonads. FEMS Microbiol Ecol 17(4):221–227

Debode J, Maeyer KD, Perneel M, Pannecoucque J, Backer GD, Höfte M (2007) Biosurfactants are involved in the biological control of *Verticillium microsclerotia* by *Pseudomonas* spp. J Appl Microbiol 103(4):1184–1196

Défago G, Keel C, Moënne-Loccoz Y (1997) Fate of released Pseudomonas bacteria in the soil profile: implications for the use of genetically modified microbial inoculants. In: Heaven F (ed) Ecotoxicology: responses, biomarkers and risk assessment. SOS Publications, Fair Haven, pp 403–418

Devi KK, Seth N, Kothamasi S, Kothamasi D (2007) Hydrogen cyanide-producing rhizobacteria kill subterranean termite *Odontotermes obesus* (rambur) by cyanide poisoning under in vitro conditions. Curr Microbiol 54(1):74–78

Dimkpa CO, Svatoš A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008a) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces* spp. Chemosphere 74(1):19–25

Dimkpa C, Svatoš A, Merten D, Büchel G, Kothe E (2008b) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54(3):163–172

dos Santos Lopes V, Fischer J, Pinheiro TM, Cabral BV, Cardoso VL, Coutinho Filho U (2017) Biosurfactant and ethanol co-production using *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* co-cultures and exploded sugarcane bagasse. Renew Energy 109:305–310

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

Driscoll JA, Brody SL, Kollef MH (2007) The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. Drugs 67(3):351–368

Duffy BK, Défago G (1997) Zinc improves biocontrol of Fusarium crown and root rot of tomato by *Pseudomonas fluorescens*and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology 87(12):1250–1257

Duffy BK, Défago G (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. Appl Environ Microbiol 65(6):2429–2438

Duijff BJ, Meijer JW, Bakker PA, Schippers B (1993) Siderophore-mediated competition for iron and induced resistance in the suppression of *Fusarium* wilt of carnation by fluorescent *Pseudomonas* spp. Eur J Plant Pathol 99(5):277–289

Dunne C, Delany I, Fenton A, Lohrke S, Moe¨nne-Loccoz Y, O'Gara F (1996) The biotechnology and application of *Pseudomonas* inoculants for the biocontrol of phytopathogens, In: Stacey G, Mullin B, Gresshoff PM (eds) Biology of plant-microbe interactions. IS-MPMI/St. Paul, Minn pp 441–448

Dwivedi D, Johri BN (2003) Antifungals from fluorescent pseudomonads: biosynthesis and regulation. Curr Sci 85(12):1693–1703

Edwards SG, Young JPW, Fitter AH (1998) Interactions between *Pseudomonas fluorescens* biocontrol agents and *Glomus mosseae*, an arbuscular mycorrhizal fungus, within the rhizosphere. FEMS Microbiol Lett 166:297–303

EFSA (European Food Safety Authority) 2017 Conclusion on the peer review of the pesticide risk assessment of the active substance *Pseudomonas chlororaphis* strain MA 342. 15(1):4668–21 doi:https://doi.org/10.2903/j.efsa.2017.4668

Elad Y, Baker R (1985) The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp.by *Pseudomonas* spp. Phytopathology 75(9):1053–1059

Elander RP, MabeJA HRH, Gorman M (1968) Metabolism of tryptophans by *Pseudomonas aureofaciens*. VI. Production of pyrrolnitrin by selected *Pseudomonas* species. Appl Microbiol 16:753–758

Elshikh M, Marchant R, Banat IM (2016) Biosurfactants: promising bioactive molecules for oral-related health applications. FEMS Microbiol Lett 363(18):213

Faccone D, Pasteran F, Albornoz E, Gonzalez L, Veli O, Prieto M, Bucciarelli R, Callejo R, Corso A (2014) Human infections due to *Pseudomonas chlororaphis* and *Pseudomonas oleovorans* harboring new bla (VIM-2)-borne integrons. Infect Genet Evol 28:276

Feklistova IN, Maksimova NP (2008) Obtaining *Pseudomonas aurantiaca* strains capable of overproduction of phenazine antibiotics. Microbiology 77(2):176–180

Flaishman M, Eyal Z, Voisard C, Haas D (1990) Suppression of *Septoria tritici* by phenazine or siderophore deficient mutants of *Pseudomonas*. Curr Microbiol 20:121–124

Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410(1–2):335–356

Fridlender M, Inbar J, Chet I (1993) Biological control of soilborne plant pathogens by a β-1, 3 glucanase-producing *Pseudomonas cepacia*. Soil Biol Biochem 25(9):1211–1221

Gaffney TD, LamST LJ, Gates K, Frazelle A, MaioJD HS, Goodwin S, TorkewitzN AAM, Kempf HJ (1994) Global regulation of expression of antifungal factors by a *Pseudomonas fluorescens*-biological control strain. Mol Plant-Microbe Interact 7(4):455–463

Gellatly SL, Hancock RE (2013) *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis 67(3):159–173

Gepstein S, Glick BR (2013) Strategies to ameliorate abiotic stress-induced plant senescence. Plant Mol Biol 82(6):623–633

Gerhardson B (2002) Biological substitutes for pesticides. Trends Biotechnol 20(8):338–343

Girlanda M, Perotto S, Moenne-LoccozY BR, Lazzari A, Defago G, Bonfante P, Luppi AM (2001) Impact of biocontrol *Pseudomonas fluorescens*CHA0 and a genetically modified derivative on the diversity of culturable fungi in the cucumber rhizosphere. Appl Environ Microbiol 67(4):1851–1864

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39

Gomila M, Peña A, Mulet M, Lalucat J, García-Valdés E (2015) Phylogenomics and systematics in *Pseudomonas*. Front Microbiol 6:214

Gupta CP, Kumar B, Dubey RC, Maheshwari DK (2006) Chitinase-mediated destructive antagonistic potential of *Pseudomonas aeruginosa* GRC 1 against *Sclerotinia sclerotiorum* causing stem rot of peanut. BioControl 51(6):821–835

Gurusiddaiah S, Weller DM, Sarkar A, Cook RJ (1986) Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens*inhibitory to *Gaeumannomycesgraminis* var. tritici and *Pythium* spp. Antimicrob Agents Chemother 29:488–495

Gutterson N (1990) Microbial fungicides: recent approaches to elucidating mechanisms. Crit Rev Biotechnol 10(1):69–91

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3(4):307–319

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41(1):117–153

Haas D, Keel C, Laville J, Maurhofer M, Oberhänsli T, Schnider U, Voisard C, Wüthrich B, Defago G (1991) Secondary metabolites of *Pseudomonas fluorescens* strain CHA0 involved in the suppression of root diseases. In: Hennecke H, DPS V (eds) Advances in molecular genetics of plant-microbe interactions, vol 1. Springer, Dordrecht, pp 450–456

Hashimoto M, Hattori K (1966a) Isopyrrolnitrin: a metabolite from *Pseudomonas*. Bull Chem Soc Jpn 39:410

Hashimoto M, Hattori K (1966b) Oxypyrrolnitrin: a metabolite of *Pseudomonas*. Chem Pharm Bull 14:1314–1316

Hashimoto M, Hattori K (1968) A new metabolite from *Pseudomonas pyrrolnitrica*. Chem Pharma Bull 16:1144

Hassan MN, Afghan S, Hafeez FY (2011) Biological control of red rot in sugarcane by native pyoluteorin-producing *Pseudomonas putida* strain NH-50 under field conditions and its potential modes of action. Pest Manag Sci 67(9):1147–1154

Hassani HH, Hasan HM, Al-Saadi A, Ali AM, Muhammad MH (2012) A comparative study on cytotoxicity and apoptotic activity of pyocyanin produced by wild type and mutant strains of *Pseudomonas aeruginosa*. Eur J Exp Biol 2:1389–1394

Heil M, Bostock RM (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann Bot 89(5):503–512

Howarth FG (1991) Environmental impacts of classical biological control. Annu Rev Entomol 36(1):485–509

Howell CR, Stipanovic RD (1979) Control of *Rhizoctonia solani*on cotton seedlings with *Pseudomonas fluorescens*with an antibiotic produced by the bacterium. Phytopathology 69:480–482

Hu W, GaoQ HMS, Dawood DH, Zheng J, Chen Y, Ma Z (2014) Potential of *Pseudomonas chlororaphis* subsp. *aurantiaca* strain Pcho10 as a biocontrol agent against *Fusarium graminearum*. Phytopathology 104(12):1289–1297

Iqbal S, Khalid ZM, Malik KA (1995) Enhanced biodegradation and emulsification of crude oil and hyperproduction of biosurfactants by a gamma ray-induced mutant of *Pseudomonas aeruginosa*. Lett Appl Microbiol 21(3):176–179

Itoh S, Honda H, Tomita F, Suzuki T (1971) Rhamnolipids produced by *Pseudomonas aeruginosa* grown on n-paraffin (mixture of C12, C13 and C14 fractions). J Antibiot 24(12):855–859

Jackson MK, Phillips SN (1996) Necrotizing hepatitis in pet birds associated with *Pseudomonas fluorescens*. Avian Dis 40:473–476

Jain R, Pandey A (2016) A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. Microbiol Res 190:63–71

Jang JY, Yang SY, Kim YC, Lee CW, Park MS, Kim JC, Kim IS (2013) Identification of orfamide a as an insecticidal metabolite produced by *Pseudomonas protegens* F6. J Agric Food Chem 61(28):6786–6791

Janisiewicz WJ, Roitman J (1988) Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78(12):1697–1700

Jimenez PN, Koch G, PapaioannouE WM, Krzeslak J, Coenye T, Quax WJ (2010) Role of PvdQ in *Pseudomonas aeruginosa* virulence under iron-limiting conditions. Microbiology 156(1):49–59

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(3):250–256

Karunanithi K, Muthusamy M, Seetharaman K (2000) Pyrolnitrin production by *Pseudomonas fluorescens*effective against *Macrophomina phaseolina*. Crop Res Hisar 19(2):368–370

Keel C, Defago G (1997) Interactions between beneficial soil bacteria and root pathogens: mechanisms and ecological impact. In: Gange AC, Brown VK (eds) Multitrophic interactions in terrestrial systems. Blackwell Scientific Publishers, London, pp 27–46

Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger P, Wirthner P, Haas D, Defago G (1992) Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite, 2, 4-diacetylphloroglucinol. Mole Plant-Microbe Interact 5:4–13

Khan H, Parmar N, Kahlon RS (2016) *Pseudomonas*-plant interactions I: plant growth promotion and defense-mediated mechanisms. In: Kahlon RS (ed) *Pseudomonas*: molecular and applied biology. Springer International Publishing, New York, pp 419–468

Kirner S, Hammer PE, Hill DS, Altmann A, Fischer I, Weislo LJ, Lanahan M, van Pée KH, Ligon JM (1998) Functions encoded by pyrrolnitrin biosynthetic genes from *Pseudomonas fluorescens*. J Bacteriol 180(7):1939–1943

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Pseudomonas siderophores: a mechanism explaining disease-suppressive soils. Curr Microbiol 4(5):317–320

Knowles CJ (1976) Microorganisms and cyanide. Bacteriol Rev 40(3):652

Kraus J, Loper JE (1995) Characterization of a genomic region required for production of the antibiotic pyoluteorin by the biological control agent *Pseudomonas fluorescens* Pf-5. Appl Environ Microbiol 61(3):849–854

Kruijt M, Tran H, Raaijmakers JM (2009) Functional, genetic and chemical characterization of biosurfactants produced by plant growth-promoting *Pseudomonas putida* 267. J Appl Microbiol 107(2):546–556

Kuiper I, LagendijkEL PR, Derrick JP, Lamers GE, Thomas-Oates JE, Lugtenberg BJ, Bloemberg GV (2004) Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms. Mol Microbiol 51(1):97–113

Landa BB, Cachinero-Díaz JM, Lemanceau P, Jiménez-Díaz RM, Alabouvette C (2002) Effect of fusaric acid and phytoanticipins on growth of rhizobacteria and *Fusarium oxysporum*. Can J Microbiol 48(11):971–985

Landa BB, NavasCortés JA, JiménezDíaz RM (2004) Influence of temperature on plant–rhizobacteria interactions related to biocontrol potential for suppression of fusarium wilt of chickpea. Plant Pathol 53(3):341–352

Lang S, Wullbrandt D (1999) Rhamnose lipids–biosynthesis, microbial production and application potential. Appl Microbiol Biotechnol 51(1):22–32

Lang S, Katsiwela E, Wagner F (1989) Antimicrobial effects of biosurfactants. Eur J Lipid Sci Technol 91(9):363–366

Lanteigne C, Gadkar VJ, 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

Laursen JB, Nielsen J (2004) Phenazine natural products: biosynthesis, synthetic analogues, and biological activity. Chem Rev 104(3):1663–1686

Leeman M, Van PJA, Hendrickx MJ, Scheffer RJ, Bakker PAHM, Schippers B (1995) Biocontrol of Fusarium wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS374. Phytopathology 85(10):1301–1305

Levy E, Gough FJ, Berlin DK, Guiana PW, Smith JT (1992) Inhibition of *Septoria tritici* and other phytopathogenic fungi and bacteria by *Pseudomonas fluorescens* and its antibiotics. Plant Pathol 41:335–341

Lewis TA, Cortese MS, SebatJL GTL, Lee CH, Crawford RL (2000) A *Pseudomonas stutzeri* gene cluster encoding biosynthesis of the CCl4-dechlorination agent pyridine-2, 6-bis (thiocarboxylic acid). Environ Microbiol l2:407–416

Li K, Pidatala VR, Shaik R, Datta R, Ramakrishna W (2014) Integrated metabolomic and proteomic approaches dissect the effect of metal-resistant bacteria on maize biomass and copper uptake. Environ Sci Technol 48(2):1184–1193

Ligon JM, Hill DS, Hammer PE, Torkewitz NR, HofmannD KHJ, van Pee KH (2000) Natural products with antifungal activity from pseudomonas biocontrol bacteria. Pest Manag Sci 56:688–695

Loper JE, Henkels MD, Shaffer BT, Valeriote FA, Gross H (2008) Isolation and identification of rhizoxin analogs from *Pseudomonas fluorescens* Pf-5 by using a genomic mining strategy. Appl Environ Microbiol 74:3085–3093

Lottmann J, Heuer H, de Vries J, Mahn A, Düring K, Wackernagel W, Smalla K, Berg G (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 33:41–49

Lukkani NJ, Reddy ES (2014) Evaluation of plant growth promoting attributes and biocontrol potential of native fluorescent *Pseudomonas spp*. against *Aspergillus niger* causing collar rot of ground nut. IJPAES 4(4):256–262

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29(2):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

Maier RM, Soberon-Chavez G (2000) *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. Appl Microbiol Biotechnol 54(5):625–633

Maleki M, Mostafaee S, Mokhtarnejad L, Farzaneh M (2010) Characterization of '*Pseudomonas fluorescens*' strain CV6 isolated from cucumber Rhizosphere in Varamin as a potential biocontrol agent. AJCS 4(9):676

Mark GL, Morrissey JP, Higgins P, O'Gara F (2006) Molecular-based strategies to exploit *Pseudomonas* biocontrol strains for environmental biotechnology applications. FEMS Microbiol Ecol 56(2):167–177

Marugg JD, De Weger LA, Nielander HB, Oorthuizen M, Recourt K, Lugtenberg B, Weisbeek PJ (1989) Cloning and characterization of a gene encoding an outer membrane protein required for siderophore-mediated uptake of Fe^{3+} in *Pseudomonas putida* WCS358. J Bacteriol 171(5):2819–2826

Matthijs S, Abbaspour TK, Laus G, Jackson RW, Cooper RM, Cornelis P (2007) Thioquinolobactin, a *Pseudomonas* siderophore with antifungal and anti-Pythium activity. Environ Microbiol l9:425–434

Maurhofer M, Keel C, Schnider U, Voisard C, Haas D, Défago G (1992) Influence of enhanced antibiotic production in *Pseudomonas fluorescens*strain CHA0 on its disease suppressive capacity. Phytopathology 82(2):190–195

Maurhofer M, Keel C, Haas D, Défago G (1994) Pyoluteorin production by*Pseudomonas fluorescens*strain CHA0 is involved in the suppression ofPythium damping-off of cress but not of cucumber. Eur J Plant Pathol 100(3):221–232

Maurhofer M, Keel C, Haas D, Défago G (1995) Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHAO with enhanced antibiotic production. Plant Pathol 44(1):40–50

Mavrodi DV, Blankenfeldt W, Thomashow LS (2006) Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. Annu Rev Phytopathol 44:417–445

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

Mazzola M, Cook RJ, Thomashow LS, Weller DM, Pierson LS (1992) Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Appl Environ Microbiol 58:2616–2624

Mercado-Blanco J, Van der Drift KMGM, Olsson P, Thomas Oates JE, van Loon LC, Bakker PAHM (2001) Analysis of the pmsCEAB gene cluster involved in biosynthesis of salicylic acid and the siderophore pseudomonine in the biocontrol strain *Pseudomonas fluorescens* WCS374. J Bacteriol 183:1909–1920

Meyer JA, Abdallah MA (1978) The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. Microbiology 107(2):319–328

Meyer SL, Halbrendt JM, Carta LK, Skantar AM, Liu T, Abdelnabby HM, Vinyard BT (2009) Toxicity of 2, 4-diacetylphloroglucinol (DAPG) to plantparasitic and bacterial-feeding nematodes. J Nematol 41(4):274

Miethke M, Marahiel MA (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71(3):413–451

Moënne-Loccoz Y, Powell J, Higgins P, Britton J, O'Gara F (1998) Effect of the biocontrol agent *Pseudomonas fluorescens* F113 released as sugarbeet inoculant on the nutrient contents of soil and foliage of a red clover rotation crop. Biol Fertil Soils 27:380–385

Mossialos D, Meyer JM, Budzikiewicz H, Wolff U, Koedam N, Baysse C (2000) Quinolobactin, a new siderophore of *Pseudomonas fluorescens* ATCC 17400 whose production is repressed by the cognate pyoverdine. Appl Environ Microbiol 66:487–492

Murray AE, Bartzokas CA, AJN S, Roberts FM (1987) Blood transfusion-associated *Pseudomonas fluorescens* septicaemia: is this an increasing problem? J Hosp Infect 9(3):243–248

Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by Pseudomonas fluorescens in inhibition of Rhizoctonia solani, the rice sheath blight pathogen. Microbiol Res 159(1):73–81

Namnyak S, Hussain S, Davalle J, Roker K, Strickland M (1999) Contaminated lithium heparin bottles as a source of pseudobacteraemia due to *Pseudomonas fluorescens*. J Hosp Infect 41(1):23–28

Nascimento FX, Vicente CS, Barbosa P, Espada M, Glick BR, Mota M, Oliveira S (2013) Evidence for the involvement of ACC deaminase from *Pseudomonas putida* UW4 in the biocontrol of pine wilt disease caused by *Bursaphelenchusxylophilus*. BioControl 58(3):427–433

Natsch A, Keel C, Hebecker N, Laasik E, Défago G (1998) Impact of *Pseudomonas fluorescens* strain CHA0 and a derivative with improved biocontrol activity on culturable resident bacterial community on cucumber roots. FEMS Microbiol Ecol 27:365–380

Negi YK, Prabha D, Garg SK, Kumar J (2011) Genetic diversity among cold-tolerant fluorescent *Pseudomonas* isolates from Indian Himalayas and their characterization for biocontrol and plant growth-promoting activities. J Plant Growth Regul 30(2):128–143

Neilands JB (1981a) Iron absorption and transport in microorganisms. Annu Rev Nutr 1(1):27–46

Neilands JB (1981b) Microbial iron compounds. Annu Rev Biochem 50(1):715–731

Neilands JB, Leong SA (1986) Siderophores in relation to plant growth and disease. Annu Rev Plant Physiol 37(1):187–208

Neimann S, Keel C, Puhler A, Selbitschka W (1997) Biocontrol strain *Pseudomonas fluorescens* CHA0 and its genetically modified derivative with enhanced biocontrol capability exert comparable effects on the structure of a *Sinorhizobium meliloti* population in gnotobiotic systems. Biol Fertil Soils 25:240–244

Nielsen TH, Christophersen C, Anthoni U, Sorensen J (1999) Viscosinamide, a new cyclic depsipeptide with surfactant and antifungal properties produced by *Pseudomonas fluorescens* DR54. J Appl Microbiol 87(1):80–90

Nielsen TH, Sørensen D, Tobiasen C, Andersen JB, Christophersen C, Givskov M, Sørensen J (2002) Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent pseudomonas spp. from the sugar beet rhizosphere. Appl Environ Microbiol 68(7):3416–3423

Nishimura T, HattoriK IA, Ishii T, Yumoto T, Tsukahara K, Nakao A, Ishihara S, Nakayama S (2017) Bacteremia or pseudobacteremia? Review of *Pseudomonas fluorescens* infections. World J Emerg Med 8(2):151–154

Nitschke M, Costa SG, Contiero J (2005) Rhamnolipid surfactants: an update on the general aspects of these remarkable biomolecules. Biotechnol Prog 21(6):1593–1600

Noreen R, Ali SA, Hasan KA, Sultana V, Ara J, Ehteshamul-Haque S (2015) Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean. Crop Prot 75:18–24

Nose M, Arima K (1969) On the mode of action of a new antifungal antibiotic, pyrrolnitrin. J Antibiot 22(4):135–143

Nowak-Thompson B, Chaney N, Wing JS, GouldSJ LJE (1999) Characterization of the pyoluteorin biosynthetic gene cluster of *Pseudomonas fluorescens* Pf-5. J Bacteriol 181(7):2166–2174

Nybroe O, Sørensen J (2004) Production of cyclic lipopeptides by fluorescent pseudomonads. In: Ramos JL (ed) Pseudomonas, vol 3. Springer, Cham, pp 147–172

O'sullivan DJ, O'gara F (1991) Genetic improvement of siderophore production aimed at enhancing biocontrol in *Pseudomonas* strains. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth, vol 14. Springer, Dordrecht, pp 310–310

O'sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol Rev 56(4):662–676

Ochsner U, Hembach T, Fiechter A (1996) Production of rhamnolipid biosurfactants. In: Advances in biochemical engineering/biotechnology, vol 53. Springer, Berlin, pp 89–118

Ongena M, Jourdan E, Adam A, Schäfer M, Budzikiewicz H, Thonart P (2008) Amino acids, iron, and growth rate as key factors influencing production of the *Pseudomonas putida* BTP1 benzylamine derivative involved in systemic resistance induction in different plants. Microb Ecol 55(2):280–292

Ownley BH, Duffy BK, Weller DM (2003) Identification and manipulation of soil properties to improve the biological control performance of phenazine-producing *Pseudomonas fluorescens*. Appl Environ Microbiol 69(6):3333–3343

PandeyA PLMS, Mulkalwar P, Nadeem M (2002) Effect of temperature on solubilization of tricalcium phosphate by *Pseudomonas corrugata*. J Sci Ind Res 61:457–460

Panpatte DG, Jhala YK, Shelat HN, Vyas RV (2016) *Pseudomonas fluorescens*: a promising biocontrol agent and PGPR for sustainable agriculture. In: Singh PD, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, vol 1. Springer, New Delhi, pp 257–270

Park JY, SA O, Anderson AJ, Neiswender J, Kim JC, Kim YC (2011) Production of the antifungal compounds phenazine and pyrrolnitrin from pseudomonas chlororaphis O6 is differentially regulated by glucose. Lett Appl Microbiol 52(5):532–537

Pathma J, Ayyadurai N, Sakthivel N (2010) Assessment of genetic and functional relationship of antagonistic fluorescent pseudomonads of rice rhizosphere by repetitive sequence, protein coding sequence and functional gene analyses. J Microbiol 48:715–727

Patil S, Paradeshi J, Chaudhari B (2016) Suppression of charcoal rot in soybean by moderately halotolerant *Pseudomonas aeruginosa* GS-33 under saline conditions. J Basic Microbiol 56(8):889–899

Patra DD (2012) Production, purification, and characterization of antifungal metabolite from *Pseudomonas aeruginosa* SD12, a new strain obtained from tannery waste polluted soil. J Microbiol Biotechnol 22(5):674–683

Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GS, Mavrodi DV, DeBoy RT, Seshadri R, Ren Q, Madupu R, Dodson RJ (2005) Complete genome sequence of the plant commensal *Pseudomonas fluorescens*Pf-5. Nat Biotechnol 23(7):873–878

Pedras MS, Ismail N, Quail JW, Boyetchko SM (2003) Structure, chemistry, and biological activity of pseudophomins A and B, new cyclic lipodepsipeptides isolated from the biocontrol bacterium *Pseudomonas fluorescens*. Phytochemistry 62(7):1105–1114

Perneel M, D'hondt L, De Maeyer K, Adiobo A, Rabaey K, Höfte M (2008) Phenazines and biosurfactants interact in the biological control of soil-borne diseases caused by Pythium spp. Environ Microbiol 10(3):778–788

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nature Rev Microbiol 11(11):789–799

Picot L, Abdelmoula SM, Merieau A, Leroux P, Cazin L, Orange N, Feuilloley MG (2001) *Pseudomonas fluorescens*as a potential pathogen: adherence to nerve cells. Microb Infect 3(12):985–995

Pierson LS, Pierson EA (1996) Phenazine antibiotic production in *Pseudomonas aureofaciens*: role in rhizosphere ecology and pathogen suppression. FEMS Microbiol Lett 136(2):101–108

Pingali PL (2012) Green revolution: impacts, limits, and the path ahead. Proc Natl Acad Sci 109(31):12302–12308

Poole K, McKay GA (2003) Iron acquisition and its control in *Pseudomonas aeruginosa*: many roads lead to Rome. Front Biosci 8(1):661–686

Postma J, Montanari M, van den Boogert PH (2003) Microbial enrichment to enhance the disease suppressive activity of compost. Eur J Soil Biol 39(3):157–163

Premachandra D, Hudek L, Brau L (2016) Bacterial modes of action for enhancing of plant growth. J Biotechnol Biomater 6(3):1–8

Price-Whelan A, Dietrich LE, Newman DK (2006) Rethinking'secondary'metabolism: physiological roles for phenazine antibiotics. Nat Chem Biol 2(2):71–78

Quagliotto L, Azziz G, Bajsa N, Vaz P, Pérez C, Ducamp F, Cadenazzi M, Altier N, Arias A (2009) Three native Pseudomonas fluorescens strains tested under growth chamber and field conditions as biocontrol agents against damping-off in alfalfa. BioControl 51(1):42–50

Raaijmakers JM, Weller DM (1998) Natural plant protection by 2, 4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. Mol Plant Microbe Interact 11(2):144–152

Raaijmakers JM, Weller DM (2001) Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: characterization of superior rootcolonizing*P. fluorescens* strain Q8r1-96. Appl Environ Microbiol 67:2545–2554

Raaijmakers JM, Leeman M, Van Oorschot MM, Van der Sluis I, Schippers B, Bakker PAHM (1995) Dose-response relationships in biological control of fusarium wilt of radish by *Pseudomonas* spp. Phytopathology 85(10):1075–1080

Raaijmakers JM, Vlami M, De Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81(1):537–547

Raaijmakers JM, de Bruijn I, de Kock MJ (2006) Cyclic lipopeptide production by plant-associated *Pseudomonas* spp.: diversity, activity, biosynthesis, and regulation. Mol Plant Microbe Interact 19(7):699–710

Rai A, Rai PK, Singh S (2017) Exploiting beneficial traits of plant-associated fluorescent pseudomonads for plant health. In: Singh JS, Seneviratne G (eds) Agro-environmental sustainability, vol 1. Springer, Cham, pp 19–41

Raio A, Reveglia P, Puopolo G, Cimmino A, Danti R, Evidente A (2017) Involvement of phenazine-1-carboxylic acid in the interaction between *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71 and *Seiridium cardinal in vivo*. Microbiol Res 199:49–56

Rajkumar M, Ae N, MNV P, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574

Rajkumar M, Prasad MNV, Swaminathan S, Freitas H (2013) Climate change driven plant–metal–microbe interactions. Environ Int 53:74–86

Ramesh R, Joshi AA, Ghanekar MP (2009) Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.) World J Microbiol Biotechnol 25(1):47–55

Ramirez RJ, Quintana PP, Galvan JJC, Diaz JG (1989) Colestasis intrahepatica severa en el curso de sepsis por *Pseudomonas fluorescens*. Rev Clin Esp 185:106–107

Raymond KN, Dertz EA (2004) Biochemical and physical properties ofsiderophores. In: Raymond KN, Dertz EA (eds) Iron transport in bacteria. American Society of Microbiology, Washington, pp 3–17

Raza ZA, Rehman A, Khan MS, Khalid ZM (2007) Improved production of biosurfactant by a *Pseudomonas aeruginosa* mutant using vegetable oil refinery wastes. Biodegradation 18(1):115–121

Roongsawang N, Hase KI, Haruki M, Imanaka T, Morikawa M, Kanaya S (2003) Cloning and characterization of the gene cluster encoding arthrofactin synthetase from *Pseudomonas* sp. MIS38. Chem Biol 10(9):869–880

Rudrappa T, Splaine RE, Biedrzycki ML, Bais HP (2008) Cyanogenic pseudomonads influence multitrophic interactions in the rhizosphere. PLoS One 3(4):2073

Sachdev DP, Cameotra SS (2013) Biosurfactants in agriculture. Appl Microbiol Biotechnol 97(3):1005–1016

Saha R, Saha N, Donofrio RS, Bestervelt LL (2013) Microbial siderophores: a mini review. J Basic Microbiol 53(4):303–317

Sahu G, Sindhu S (2011) Disease control and plant growth promotion of green gram by siderophore producing *Pseudomonas* sp. Res J Microbiol 6:735–749

Santoyo G, Orozco-Mosqueda MDC, Govindappa M (2012) Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. Biocontrol Sci Tech 22(8):855–872

Saraf M, Pandya U, Thakkar A (2014) Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. Microbiol Res 169(1):18–29

Sarubbi FA, Wilson MB, Lee M, Brokopp C (1978) Nosocomial meningitis and bacteremia due to contaminated amphotericin. B. JAMA 239(5):416–418

Sasirekha B, Srividya S (2016) Siderophore production by Pseudomonas aeruginosa FP6, a biocontrol strain for Rhizoctonia solani and Colletotrichum gloeosporioides causing diseases in chilli. Agric Nat Resour 50(4):250–256

Sayyed RZ, Patel PR (2011) Biocontrol potential of siderophore producing heavy metal resistant *Alcaligenes* sp. and *Pseudomonas aeruginosa* RZS3 vis-a-vis organophosphorus fungicide. Indian J Microbiol 51(3):266–272

Schippers B, Bakker AW, Bakker PAHM, Van Peer R (1991) Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth, vol 14. Springer, Dordrecht, pp 211–219

Shanahan PO, 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 A, Shankhdhar D, Shankhdhar SC (2013) Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. Plant Soil Environ 59(2):89–94

Siddiqui IA, Shaukat SS (2003) Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite, 2, 4-diacetylpholoroglucinol. Soil Biol Biochem 35(12):1615–1623

Siddiqui IA, Shaukat SS, Sheikh IH, Khan A (2006) Role of cyanide production by *Pseudomonas fluorescens*HA0 in the suppression of root-knot nematode, Meloidogyne javanica in tomato. World J Microbiol Biotechnol 22(6):641–650

Singh JS, Pandey VC, Singh DP (2011) Effecient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140(3):339–353

Skaar EP (2010) The battle for iron between bacterial pathogens and their vertebrate hosts. PLoS Pathog 6(8):1000949

Sneh B, Dupler M, Elad Y, Baker R (1984) Chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from a *Fusarium*-suppressive soil. Phytopathology 74(9):1115–1124

Soberón-Chávez G (2004) Biosynthesis of rhamnolipids. In: Ramos JL (ed) *Pseudomonas*. Springer, New York, pp 173–189

Soberón-Chávez G, Lépine F, Déziel E (2005) Production of rhamnolipids by *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 68(6):718–725
Solomonson LP (1981) Cyanide as a metabolic inhibitor. In: Vennesland B, Conn EE, Knowles CJ, Westley J, Wissing F (eds) Cyanide in biology. Academic, London, pp 11–28
Sorensen D, Nielsen TH, Christophersen C, Sorensen J, Gajhede M (2001) Cyclic lipoundecapeptide Amphisin from *Pseudomonas* sp. strain DSS73. Acta Crystallogr C 57:1123–1124
Sturz AV, Christie BR (2003) Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Res 72(2):107–123
Subashri R, Raman G, Sakthivel N (2013) Biological control of pathogens and plant growth promotion potential of fluorescent Pseudomonads. In: Maheshwari DK (ed) Bacteria in agrobiology: disease management. Springer, Berlin/Heidelberg, pp 77–110
Sudhakar T, Karpagam S, Shiyama S (2013) Analysis of pyocyanin compound and its antagonistic activity against phytopathogens. Int J ChemTech Res 5:1101–1106
Sulochana MB, Jayachandra SY, Kumar SK, Dayanand A (2014) Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. J Basic Microbiol 54(5):418–424
Sunish Kumar R, Ayyadurai N, Pandiaraja P, Reddy AV, Venkateswarlu Y, Prakash O, Sakthivel N (2005) Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. J Appl Microbiol 98:145–154
Tablan OC, Chorba TL, Schidlow DV, White JW, Hardy KA, Gilligan PH, Jarvis WR (1985) *Pseudomonas cepacia* colonization in patients with cystic fibrosis: risk factors and clinical outcome. J Pediatr 107(3):382–387
Tambong JT, Höfte M (2001) Phenazines are involved in biocontrol of Pythium myriotylum on cocoyam by *Pseudomonas aeruginosa* PNA1. Eur J Plant Pathol 107(5):511–521
Tapadar SA, Jha DK (2013) Disease management in staple crops: a bacteriological approach. In: Bacteria in agrobiology: disease management. Springer, Berlin/Heidelberg, pp 111–152
Tekeda R (1958) *Pseudomonas* pigments I. Pyoluteorin a new chlorine-containing pigment produced by *Pseudomonas aeruginosa*. Hakko Kogaku Zasshi 36:281–290
Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens*in biological control of *Gaeumannomyces graminis* var. tritici. J Bacteriol 170(8):3499–3508
Thomashow LS, Weller DM, Bonsall RF, Pierson LS (1990) Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. Appl Environ Microbiol 56(4):908–912
Thomashow LS, Pierson LS (1991) Genetic aspects of phenazine antibiotic production by fluorescent pseudomonads that suppress take-all disease of wheat. In: Advances in molecular genetics of plant-microbe interactions, vol 1. Springer Netherlands, Dordrecht, pp 443–449
Tombolini R, Vander Gaag DJ, Gerhardson B, Jansson JK (1999) Colonization pattern of the biocontrol strain *Pseudomonas chlororaphis*MA342 on barely seeds visualized by using green fluorescent protein. Appl Environ Microbiol 65:3674–3680
Trivedi P, Pandey A, Palni LMS (2008) In vitro evaluation of antagonistic properties of *Pseudomonas corrugata*. Microbiol Res 163(3):329–336
Trotel-Aziz P, Couderchet M, Biagianti S, Aziz A (2008) Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against *Botrytis cinerea*. Environ Exp Bot 64(1):21–32
Turner JM, Messenger AJ (1986) Occurrence, biochemistry and physiology of phenazine pigment production. Adv Microb Physiol 27:211–275
Upadhyay A, Srivastava S (2011) Phenazine-1-carboxylic acid is a more important contributor to biocontrol Fusarium oxysporum than pyrrolnitrin in *Pseudomonas fluorescens* strain Psd. Microbiol Res 166(4):323–335
Upadhyay RS, Visintin L, Jayaswal RK (1991) Environmental factors affecting the antagonism of *Pseudomonas cepacia* against *Trichoderma viride*. Can J Microbiol 37(11):880–884
van Elsas JD, Migheli Q (2002) Evaluation of risks related to the release of biocontrol agents active against plant pathogens. In: Albajes R, Guliino ML, van Lenteren JC, Elad Y (eds) Integrated

pest and disease management in greenhouse crops. Developments in plant pathology, vol 14. Springer, Dordrecht, pp 377–393

van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36(1):453–483

van Peer R, Schippers B (1989) Plant growth responses to bacterization with selected *Pseudomonas* spp. strains and rhizosphere microbial development in hydroponic cultures. Can J Microbiol 35(4):456–463

van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS 417 r. Phytopathology 81(7):728–734

van Rij ET, Wesselink M, Chin-A-Woeng TF, Bloemberg GV, Lugtenberg BJ (2004) Influence of environmental conditions on the production of phenazine-1-carboxamide by*Pseudomonas chlororaphis* PCL1391. Mol Plant Microbe Interact 7(5):557–566

van Wees SCM, Pieterse CMJ, Trijssenaar A, Van T, Westende YAM, Hartog F, van Loon LC (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol Plant-Microbe Interact 10:716–724

Vandenbergh PA, Gonzalez CF, Microlife Technics Inc (1984) Method for protecting the growth of plants employing mutant siderophore producing strains of *Pseudomonas putida*. US Patent 4,479,936, 30 Oct 1984

Vázquez MM, César S, Azcón R, Barea JM (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl Soil Ecol 15:261–272

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

Voisard C, Keel C, Haas D, Defago G (1981) Cyanide production in *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351–358

Walsh UF, Morrissey JP, O'Gara F (2001) *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Curr Opin Biotechnol 12(3):289–295

Wei G, Kloepper JW, TuZun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81(11):1508–1512

Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:379–407

Weller DM (2007) Pseudomonas biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97(2):250–256

Weller DM, Landa BB, Mavrodi OV, Schroeder KL, De La Fuente L, Bankhead SB, Molar RA, Bonsall RF, Mavrodi DV, Thomashow LS (2007) Role of 2, 4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in the defense of plant roots. Plant Biol 9(01):4–20

Weller DM, Mavrodi DV, van Pelt JA, Pieterse CM, van Loon LC, Bakker PA (2012) Induced systemic resistance in Arabidopsis thaliana against *Pseudomonas syringae* pv. tomato by 2,4-diacetylphloroglucinol-producing *Pseudomonasfluorescens*. Phytopathology 102(4):403–412

Wensing A, Braun SD, Büttner P, Expert D, Völksch B, Ullrich MS, Weingart H (2010) Impact of siderophore production by *Pseudomonas syringaepv. syringae* 22d/93 on epiphytic fitness and biocontrol activity against P*seudomonas syringae pv. glycinea* 1a/96. Appl Environ Microbiol 76(9):2704–2711

Wong DT, Airall JM (1970) The mode of action of antifungal agents: effect of pyrrolnitrin on mitochondrial electron transport. J Antibiot 23(2):55–62

Xu S, Pan X, Luo J, Wu J, Zhou Z, Liang X, He Y, Zhou M (2015) Effects of phenazine-1-carboxylic acid on the biology of the plant-pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae*. Pest Biochem Physiol 117:39–46

Yang M, Mavrodi DV, Mavrodi OV, Thomashow LS, Weller DM (2017) Construction of a recombinant strain of *Pseudomonas fluorescens*producing both phenazine-1-carboxylic acid and cyclic lipopeptide for the biocontrol of take-all disease of wheat. Eur J Plant Pathol 1–12

Yoshihisa H, Zenji S, Fukushi H, Katsuhiro K, Haruhisa S, Takahito S (1989) Production of antibiotics by *Pseudomonas cepacia* as an agent for biological control of soilborne plant pathogens. Soil Biol Biochem 21(5):723–728

Zhang L, Tian X, Kuang S, Liu G, Zhang C, Sun C (2017) Antagonistic activity and mode of action of phenazine-1-carboxylic acid, produced by marine bacterium *Pseudomonas aeruginosa* PA31x, against *Vibrio anguillarumin vitro* and in a Zebrafish *in vivo* model. Front Microbio l8:289

Zhou T, Paulitz TC (1994) Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. J Phytopathol 142(1):51–63

Zhou T, Chen D, Li C, Sun Q, Li L, Liu F, Shen Q, Shen B (2012) Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. Microbiol Res 167(7):388–394

Zhou T, Li CY, Chen D, Wu K, Shen QR, Shen B (2014) phlF− mutant of *Pseudomonas fluorescens* J2 improved 2, 4-DAPG biosynthesis and biocontrol efficacy against tomato bacterial wilt. BioControl 78:1–8

Zohara F, Akanda MAM, Paul NC, Rahman M, Islam MT (2015) Inhibitory effects of Pseudomonas spp. on plant pathogen Phytophthora capsici in vitro and in planta. Biocatal Agric Biotechnol 5:69–77

Rhizosphere Microorganisms: Application of Plant Beneficial Microbes in Biological Control of Weeds

19

Satyavir S. Sindhu and Anju Sehrawat

Abstract

Weeds usually result in average ~ 20–37% losses of the world's agricultural output, and therefore, weed control is indispensable in every crop production system. For weed management, usually chemical herbicides are applied, but their indiscriminate use causes environmental problems and human health hazards. Moreover, continuous use of herbicides may lead to evolution of resistant weed biotypes and shift in the weed flora. Thus, biological control of weeds is an alternate eco-friendly method of weed management, in which microorganisms or their products are used to suppress the growth of weed species. Many rhizosphere microorganisms including *Pseudomonas aeruginosa*, *P. fluorescens*, *Erwinia herbicola*, *Alcaligenes* sp., strains of *Xanthomonas campestris* pv. *poannua*, *Pseudomonas syringae* pv. *tagetis*, *Serratia plymuthica*, and *S. marcescens* as well as the fungi including *Colletotrichum gloeosporioides*, *Aeschynomene virginica*, *Phoma chenopodicola*, and *Exserohilum monoceras* have been characterized as bioherbicides. These rhizosphere microorganisms have been found to suppress the growth of weeds by reducing weed density, biomass, and its seed production. Various metabolites produced by microorganisms such as cyanide, organic acids, secondary metabolites (antibiotic 2, 4-diacetylphloroglucinol), and plant growth regulators, including auxins (indole acetic acid and δ-aminolevulinic acid), have been found to inhibit seed germination, seedling growth, and suppression of weed plant growth. Bacterial and fungal microbes also produce a wide array of phytotoxins that may cause mortality of weed plants. Many of the microorganisms have been released as commercial bioherbicides for different crops. Thus, there are immense possibilities for characterizing

S.S. Sindhu (✉) • A. Sehrawat
Department of Microbiology, CCS Haryana Agricultural University,
Hisar, Haryana 125004, India
e-mail: sindhuss58@gmail.com

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_19

and developing novel microbial bioherbicides that could reduce the application of chemical herbicides for weed control in sustainable agriculture.

Keywords
Bioherbicide • Weeds • Rhizosphere microorganisms • Antibiotics • Auxins • Biological control

19.1 Introduction

Weeds are unwanted useless plants that compete with crop plants for space, nutrients, water, sunlight, and other elements. Weeds are underestimated crop pests in agriculture, and they cause ~37% loss in the yields of crops (Ferreira and Reinhardt 2016). They decrease quantity and quality of produce/food, fiber, oil, forage/fodder, and animal products (meat and milk) and also cause health hazards to humans and animals. Weed management forces the use of large amounts of human labor and technology to prevent crop losses (Fickett et al. 2013). There are several ways of weed management, including weed prevention through crop rotation, crop competition, and cultivation. Direct management strategies involve mechanical weeding or herbicide treatment. Recently, labor has become nonavailable and costly due to intensification, diversification of agriculture, and urbanization. Therefore, chemical herbicides are applied under field conditions for successful weed management. The common herbicides used for chemical control of weeds include isoproturon, 2, 4-dichlorophenoxyacetic acid (2, 4-D), clodinafop, fenoxaprop, sulfosulfuron, tralkoxydim, tribenuron-methyl, etc. (Brar and Walia 1993). Recently, the herbicide component of all pesticides sold has increased from ~15% in the 1950s to ~20%. However, more health and environmental hazards have been created in nature with application of chemical herbicides (Soares and Porto 2009). Moreover, continuous herbicide use may lead to shift in weed flora and evolution of resistant weed biotypes (Singh 2007), threatening the efficacy of weed management in agriculture. These problems necessitated the search for an alternate eco-friendly and cost-effective method of weed management through the biological approach in which microorganisms or their products could be used to suppress the growth or population of the weed species (Templeton 1988; Kremer and Kennedy 1996; Gnanavel 2015).

In biological control of weeds, the use of rhizosphere microorganisms having herbicidal activity provides better alternative for reducing chemical inputs in agriculture. Rhizospheres host highly complex microbial communities (Schlaeppi and Bulgarelli 2015), which are affected by agricultural management practices (Kennedy 1999; Carbonetto et al. 2014; Lehman et al. 2015). Changing the crop management system toward reduced tillage, maintenance of high soil organic matter, and limited input of agrochemicals resulted in an increased prevalence of deleterious rhizosphere bacteria (DRB) associated with weed seedlings (Li and Kremer 2006). The microorganisms that specifically inhibit the development of weed seedlings thereby

prevent the establishment of weed population (Suslow and Schroth 1982). Specific effects of DRB include reduced seed germination, growth inhibition, reduced root elongation, and/or root deformation. Over the past two decades, there have been significant efforts aimed at the development and commercialization of microbial bioherbicides (bacteria, fungi, and viruses) to control both pre- and postemergent grass and broad-leaved weeds (Hynes and Boyetchko 2006; Bailey et al. 2010; Glare et al. 2012; Beckie et al. 2013). Thus, application of rhizospheric bacteria as weedicides/herbicides has reduced dependence on synthetic herbicides, lowered weed seed bank population through environment-friendly practices, and potentially reduced the costs of weed control in crop production, forestry, and aquatic systems (Kremer et al. 1990; Kennedy et al. 1991; Harding and Raizada 2015).

Several rhizobacteria such as *Pseudomonas*, *Xanthomonas* sp., *Enterobacter*, and *Serratia* have been developed as foliar bioherbicides and soil application bioherbicides (Kremer 2000). Similarly, some fungi including *Aeschynomene*, *Alternaria*, *Colletotrichum*, *Phoma*, and *Exserohilum* have been characterized to suppress the growth of weeds (Duke et al. 1991; Stewart-Wade and Boland 2005; Boyette and Hoagland 2015). The mode of action of these biocontrol agents is as varied as the microorganisms themselves (de Luna et al. 2011). They range from simple compounds like cyanide (Kremer and Souissi 2001; Owen and Zdor 2001) and organic acids to complex molecules with tertiary structure (Gurusiddaiah et al. 1994; Bouizgarne et al. 2006), secondary metabolites (Kroschel and Elzein 2004), and plant growth regulators such as auxins and ethylene (de Luna et al. 2005). Some deleterious bacteria and fungi also produce a wide array of phytotoxins with the potential to be used as herbicides (Duke et al. 1991). AAL toxin, a natural metabolite of the pathogen *Alternaria alternata* f. sp. *lycopersici*, has been tested on a range of crops and weed species and has been patented as herbicide (Abbas et al. 1995). Thus, inoculation of such rhizosphere microorganisms could minimize competition of weeds with crops, may reduce the use of chemical herbicides, and could benefit agriculture by contributing to increased crop yields.

19.2 Weeds Occurrence in Cereal and Legume Crops

Most of the weeds belong to the family Poaceae and Asteraceae. Majority of the weeds (~ 107 species) are terrestrial plants, a few (5 species) are aquatic weeds, and six of the species are parasitic weeds (Kostov and Pacanoski 2007). The broad-leaved weeds make growth during the cool season and compete with wheat crop for nutrition and other inputs. The competitiveness of broad-leaved weeds with small grain crops such as wheat depends on the type of weeds growing in the field and on whether the soil fertility, moisture, and temperature favor the crop or the weeds. Moreover, the continuous adoption of rice-wheat cropping system may further cause yield reduction of wheat to the level of ~30 to 80% depending upon the weed intensity (Brar and Walia 1993). The major weeds prevalent in winter season crop fields are dicot and monocots, viz., bathua (*Chenopodium album*), gazari (*Fumaria parviflora*), krishnneel (*Anagallis arvensis*), chetri (*Vicia sativa*), senji (*Melilotus*

indicus), matari (*Lathyrus aphaca*), satyanashi (*Argemone mexicana*), etc. Likewise, monocot weeds, viz., kanki/mandusi (*Phalaris minor*), wild oats (*Avena ludoviciana*), piazi (*Asphodelus tenuifolius*), etc., impose serious problem in wheat fields. In addition to these, doob (*Cynodon dactylon*) is a major perennial weed. Many biotypes have become resistant to isoproturon, with resistant biotypes from Haryana requiring up to 11 times the pre-susceptible dose of isoproturon to achieve ~50% weed control (Malik and Singh 1995), and farmers have to use costly herbicides, namely, clodinafop and sulfosulfuron (Singh 2006; Dhaliwal et al. 2007).

Annual late spring weeds are main invaders in the different crops such as soybean (*Glycine max* (L.) Merr.), spring forage pea (*Pisum sativum* L.), and spring vetch (*Vicia sativa* L.). These weeds account for 58–92% of the total weed infestation. The dominant weed species in various crop fields are redroot amaranth (*Amaranthus retroflexus*), bathua (*Chenopodium album*), horseweed (*Erigeron canadensis*), and black nightshade (*Solanum nigrum*) (Marinov-Serafimov 2005; Marinov-Serafimov and Dimitrova 2007). The most economically damaging weeds for temperate legumes are broomrape, in particular *Orobanche crenata*. Broomrape species such as *Orobanche foetida*, *Orobanche minor*, and *Phelipanche aegyptiaca* can also induce high local damage. Egyptian broomrape (*P. aegyptiaca*) is an important pest of legumes but also of many vegetable crops in the Middle East and Asia (Parker 2009). Other weeds such as cowpea witchweed (*Striga gesnerioides*) and yellow witchweed (*Alectra vogelii*) also decrease yield of legume crops (Rubiales and Fernández-Aparicio 2012). Dodders (*Cuscuta* spp.) are widely distributed, being a threat to alfalfa (*Medicago sativa* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.) in certain locations. The most important weed species is *Cuscuta campestris* Yunck.

19.3 Management of Weeds

The manual method of weed control is quite popular and effective in India. Usually, weed management takes away nearly one third of total cost of production of field crops. In elaborating strategies to control weeds, one must take into account the type of weed in presence and define the most convenient controlling agent to be used. Usually, four methods of weed control, i.e., physical, chemical, biological, and integrated weed management, are used (Liebman et al. 2001; Harding and Raizada 2015).

19.3.1 Physical Methods

In stale seedbed preparation technology, seeds of weeds are allowed to germinate through application of one to two pre-sowing irrigations. The emerged weed seedlings are then killed through plowing or by the use of nonselective herbicides such as paraquat, glyphosate, or glufosinate. This technique is effective not only in reducing weed emergence during the crop season but also in reducing the weed seed bank

(Kumar and Ladha 2011). Similarly, the crop rotations can cause a shift in weed species composition and are effective method of integrated weed management (Liebman et al. 2001). In addition, tillage also affects weed management, weed seed production, and pattern of soil disturbances. Weed management strategies, like tillage, generally alter soil structure along with changes in the microbial community. Once a weed population establishes in the field, the plants build up a close relationship with the available microorganisms. Weed and crop plants may interact differently with soil microorganisms. The development of new technologies for analyzing soil microbiomes under different management systems will help us to understand the functions of microorganisms involved in crop productivity, weed establishment, and weed prevention. Exploitation of the microbial ecology knowledge offers the possibility to search for new biocontrol methods against weeds based on soil and plant-associated microorganisms. For example, *P. minor,* which germinates from upper soil layers, can be buried by deep cultivation. Zero tillage technique integrated with timely planting of wheat (October sowing) has shown promising results in reducing *P. minor* infestation and is helpful in reducing the population of weeds (Chhokar et al. 2007; Franke et al. 2007).

A competitive crop species or cultivar maintains its yield well in the presence of weeds and is also able to reduce weed growth significantly (Olesen et al. 2004). Increasing the ability of crop cultivars to compete with weeds is an attractive control option for future weed control strategies (Lemerle et al. 2001). Recently, wheat variety PBW550 has been reported to be more competitive than DBW17 and PBW502 varieties due to its quick early growth. Moreover, the sowing time of crop should be recommended so that it is more favorable for crop growth and development, whereas it is least favorable for weed germination and growth. In addition, fertilizer timing and dose can be manipulated to reduce weed interference in crops. Nitrogen fertilizer is known to break weed seed dormancy and thus may directly affect weed densities. The growth response of many agricultural weeds to added nitrogen is similar to or greater than that of wheat (Blackshaw et al. 2004).

19.3.2 Chemical Control

In wheat, chemical method of weed control is preferred over manual and mechanical methods because of its better efficiency along with less cost and time involvement. Different chemical herbicides used are sulfosulfuron, clodinafop, fenoxaprop, tralkoxydim, pendimethalin, atlantis, and pinoxaden. Sulfosulfuron, atlantis, and pendimethalin are effective against both grass and non-grass weeds, whereas clodinafop, fenoxaprop, tralkoxydim, and pinoxaden are specific to grasses. However, sulfosulfuron and pendimethalin are not effective against *Rumex dentatus* and *Avena ludoviciana,* respectively. For control of broad-leaved weeds in wheat, three major herbicides used are metsulfuron, 2, 4-D, and carfentrazone (Chhokar et al. 2013). Many species of weeds were reported to acquire resistance against commercially available chemical herbicides. There are ~307 herbicide-resistant weed biotypes worldwide, 113 of these biotypes occur in the USA alone (Heap 2006). Some of the

common herbicides used for weed control include glyphosate and Roundup Renew (for annual and perennial grasses and broad-leaved weeds), Buster (for grasses, broad-leaved weeds, and clovers), versatile (for control of thistles, yarrow, clovers, and many difficult flat weeds), and interceptor (for control of annual weeds, grasses, and perennial weeds).

19.3.3 Biological Control

The reliance on synthetic agrochemicals to meet the growing food demand has led to the environment and health hazards. Residual toxicity of these xenobiotics has resulted in high incidences of cancer, hormonal and immunological disorders, and allergies apart from the effects on reproductive ability. Therefore, an alternative eco-friendly and cost-effective method of weed control using living organisms or bio-control agents is required (Chutia et al. 2006). Biological control refers to the introduction of natural predator or pathogen of a pest species into an ecosystem with the intention of controlling one or more undesirable species (Charudattan 2001; Bailey et al. 2010). The released organism should be able to persist in the environment and provide ongoing reduction of the pest species population throughout the entire ecosystem (Dane and Shaw 1996; Shaw et al. 2009). Biological weed control practices have been developed for the sustainable use of biodiversity for economic benefit toward mankind, and microorganisms have been used as a biological control agent of weeds (Li and Kremer 2006; Kennedy and Stubbs 2007; Patil 2013). Rhizosphere microorganisms and their metabolites have been evaluated as weed control agents in different crop systems (Norman et al. 1994; Mazzola et al. 1995; Gealy et al. 1996). For example, live cultures of *Pseudomonas syringae* strain 3366 were found to reduce weed root growth in controlled environment (Johnson and Booth 1983) and in field studies (Kennedy et al. 1991).

The classical biological or inoculative approach involves the introduction of a natural enemy from its native range to a new area where the weed or pest poses a problem. The biocontrol agent is released once into the new environment, and with time, the biocontrol organism builds up a population size that is able to reduce the pest or weed. In classical biological control of weeds, fungi have been favored over bacterial, viral, or other biocontrol agents (Morin et al. 2006). One of the most successful microbial biocontrol agents for weed control is the introduction of the rust fungus (*Puccinia chodrillina*) in Australia to control the rush skeleton weed (*Chondrilla juncea* L.). In the Mediterranean area, *P. chodrillina* was found to attack the narrow leaf form. Another example of microbial control of weeds is the introduction of the gall-forming fungus *Uromycladium tepperianum* to control the invasive tree *Acacia saligna* in the Cape Floristic Region in South Africa. Due to biological control, the weed density declined between 87% and 98% during the years 1991–2005 (Wood and Morris 2007). One drawback of the classical biocontrol approach of weeds is the development of resistant weed genotypes.

Another strategy called augmentation or inundative control refers to all forms of biological control in which natural enemies are applied periodically in high concentrations at the time when the pest or weed causes the problem, analogous to the use of a pesticide. *Colletotrichum gloeosporioides* f. sp. *aeschynomene* is another example of a bioherbicide based on the genus *Colletotrichum* to control northern joint vetch (*Aeschynomene virginica*) (TeBeest 1982). The bioherbicide was registered as Collego in the USA in 1997, reapproved in 2006, and then sold as LockDown (Bailey 2014). The fungus produces the phytotoxic metabolite ferricrocin, a kind of siderophore whose action mechanism has some relation with chelating activity (Ohra et al. 1995). Inoculation with *Bacillus* strain was found to suppress the growth of *Phalaris minor* weed species more effectively (Phour 2012), and inoculation of bacterial isolate WHA87 caused 21–81% decrease in root dry weight and 33–43% decrease in shoot dry weight of *Chenopodium album* at different stages of plant growth under pot house conditions (Khandelwal 2016). Similarly, inoculation of *P. fluorescens* strain G2-11 was found to suppress the growth of weeds but promoted the growth of wheat and soybean (Li and Kremmer 2006). Inoculation of the *Pseudomonas trivialis* strain X33d caused the growth suppression of great brome weed and promoted the growth of durum wheat (Mejri et al. 2010).

19.3.4 Integrated Weed Management

Integrated weed management (IWM) relies upon multiple chemical, physical, or biological weed management techniques to achieve an acceptable level of weed control. Agents that selectively suppress weeds but not the crops and that can be exploited in agriculture will be promising components for inclusion in IWM. Recently, lower doses of herbicides in combination with rhizosphere microorganisms (having herbicidal activity) are applied to effectively control the weeds under field conditions. Weissmann et al. (2003) reported that application of *Serratia plymuthica* strain A153 in a tank mix with another bacterial isolate or with reduced doses of herbicide showed good suppression of *Chenopodium album* in field tests. Li et al. (2016) reported that weak host plants had consistently lower mycorrhizal growth responses (MGRs) than strong host crops in both controlled and field conditions. Moreover, these differences in MGRs between weak weeds and strong host crops were more pronounced under mixed arbuscular mycorrhizal fungi (AMF) inoculum and low N and P nutrient availability. It was suggested that management practices affecting AMF diversity, crop, and weed mycorrhizal responses could be selected to improve the contribution of AMF to IWM (Li et al. 2016). Better understanding of crop-weed-AMF interactions and management practices is needed to enhance weed management.

The extracts prepared from *Zygophyllum coccineum* L. (family Zygophyllaceae) leaves inhibited seed germination and radical growth of *Chenopodium album* at 50 and 100 μg mL^{-1} concentration. Due to higher contents of bioactive compounds, the

inhibition of *C. album* was more significant with the extracts obtained from the desert plants as compared to that of coastal plants (El-Shora et al. 2016). Brassicas produce the allelopathic compound glucosinolate throughout their plant parts (Fahey et al. 2001). After the release, glucosinolate is decomposed into several biologically active compounds, such as isothiocyanate (Morra and Kirkegaard 2002), and suppressed the growth and development of weeds (Petersen et al. 2001). Allelopathic plants were found to suppress weeds and also showed positive effects on the soil environment by improved nutrient availability to crop plants through enhanced soil microbial activities (Wang et al. 2013; Zeng 2014). The allelopathic wheat cultivar 22 Xiaoyan was found to have higher concentrations of microorganisms and enzyme (catalase and urease) activity and also exuded carbon and nitrogen, which improved the allelopathic effects of soil microorganisms in the rhizosphere. Moreover, the allelochemicals excreted from the microorganisms further helped to suppress crop weeds and diseases (Zuo et al. 2014).

19.4 Microorganisms Associated with Weeds and Crop Plants

Agricultural practices like tillage and monoculture favor weed establishment. Seeds of weeds can stay in the soil for several years until conditions are favorable for germination. This is illustrated by the fact that significantly more plant growth-promoting bacteria were found in some weed species than in potato plants collected from a potato field (Sturz et al. 2001). The composition of plant microbiota depends on several factors such as the environment, climate, plant genotype, and developmental stage of the host plant (Bakker et al. 2012; Hardoim et al. 2015). Every plant species seems to select its own microbiome, and this influences plant competitiveness, health, and productivity (Berg et al. 2014; Agler et al. 2016) (Fig. 19.1).

During plant domestication and agricultural intensification, the cultivated plants may have lost traits linked to recruit host-specific root microorganisms (Perez-Jaramillo et al. 2015). On the other hand, weeds have more positive feedback interactions with soil microorganisms and seem to have a greater dependence on these associations than crops (Massenssini et al. 2014). About 20–30% of all grass species are colonized by *Neotyphodium* endophytes and their sexual relatives *Epichloe* (Leuchtmann 1997). These endophytes are mutualistic colonizers of leaves and stems and are vertically transmitted by seeds, contributing to their successful dissemination to the next generation (Sanchez Marquez et al. 2012). The loss of the fungal partner can be associated with the loss of important traits (Saikkonen et al. 2004). Likewise, some bacterial endophytes in plants such as *Chenopodium album* and *Stellaria media* seem to be vertically transmitted over generations via seeds (van Overbeek et al. 2011). Communities of endophytic bacteria have been extensively studied in staple crop plants such as rice, wheat, maize, and millet (Senthilkumar et al. 2011; Montanez et al. 2012; Sessitsch et al. 2012; Gupta et al. 2013), where they are being increasingly acknowledged for their functions in plant growth promotion, nutrient scavenging, nitrogen fixation, and pathogen antagonism (Gond et al. 2015). Weeds including wild crop relatives and other indigenous plants

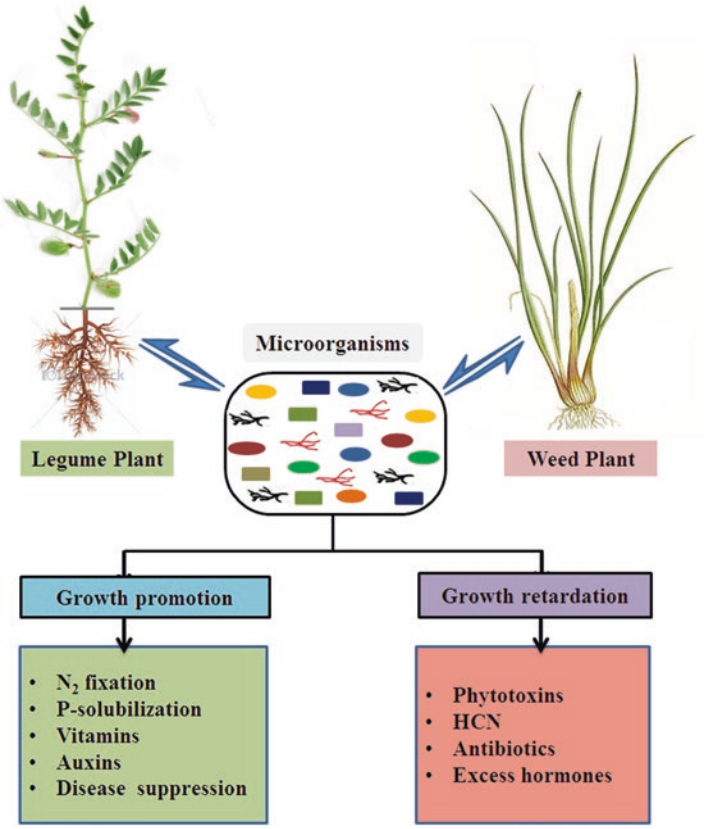


Fig. 19.1 Plant-microbe interactions in the rhizosphere of weed and legume plant

are targeted in inventories of plant-beneficial endophytes that may be applied on crops as inoculants and biofertilizers (Pérez-Jaramillo et al. 2015). For instance, diazotrophic endophytes belonging to the genera *Klebsiella*, *Enterobacter*, *Bradyrhizobium*, *Alcaligenes*, *Azospirillum*, *Herbaspirillum*, *Ideonella*, *Acetobacter*, and *Acinetobacter*, which are able to supply nitrogen to their host plants, have been isolated from wild rice (*Oryza alta*) plants (You and Zhou 1989; Baldani et al. 2000; Elbeltagy et al. 2001; Chaudhary et al. 2012).

19.4.1 Weed-Microbiota Interactions Affecting Weed Establishment

Belowground microbial communities play important roles in soil nutrient cycling. Plant-associated microbial communities in the rhizosphere are to a great extent shaped by the host plant because the plant provides nutrients in the form of exudates and mucilage-derived substances via the roots. There are a few studies showing that

soil microbial community structures change with plant invasions (Wolfe and Klironomos 2005), and such changes also implicate functional shifts. Rodrigues et al. (2015) identified major soil microbial community shifts brought about by three different invasive plant species, including a grass (*M. vimineum)*, a shrub (*Rhamnus davurica*), and a tree (*Ailanthus altissima*), which were investigated at three independent locations in the USA. For comparison, non-invaded reference areas were also investigated. Interestingly, all plant invasions shifted microbial communities in a similar way, resulting in increased abundance of several specific bacterial and fungal taxa (belonging to *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Ascomycota*). The study demonstrated an increased abundance of N-cycling taxa as well as N-cycling activity in the invaded areas.

Busby et al. (2016) investigated the symbiont composition of nodules obtained from an invasive legume in North America, *Lespedeza cuneata*, and from native *Lespedeza* species. Nodule bacterial composition differed greatly between native host and invasive *L. cuneata*, and the invasive plant contained a higher number of non-rhizobial taxa. In North America, it was shown that the dominance of the garlic mustard weed led to a decline of AMF (Roberts and Anderson 2001). Kourtev et al. (2002) reported a higher abundance of AMF associated with invasive plant species (Japanese barberry and Japanese stiltgrass) as compared to the co-occurring native blueberry plant. It seems that invasive plants are able to alter the soil microflora to their own benefit, e.g., by stimulating their own association with AMF (Callaway et al. 2004). *Phragmites australis* spp. *australis* is highly stress resistant, and it was suggested that fungal endophytes could confer stress resistance to their host (Fischer and Rodriguez 2013). The fungal endophytes were tested for their susceptibility to various fungicides to weaken competitiveness of the invasive plant. Response to fungicide treatment varied among fungal isolates, and fungicide-resistant phenotypes were encountered (Fischer and Rodriguez 2013). This approach has potential to be taken further, either by applying specific fungicides or preferably by the application of microorganisms outcompeting or antagonizing certain fungal endophytes or chemical molecules interfering with the growth of these fungi. Various mechanisms have been suggested for invasive plants to become more competitive in their invaded versus their native ranges (Broennimann et al. 2007), some of which are based on altered interspecific interactions. It appears that interactions with endophytes may significantly contribute to the plants' greater competitiveness in the invaded versus native ranges via effects on plant growth and resource allocation (Rout et al. 2013).

Weeds and invasive plants also modulate microbial populations in soil. New plant species may bring along novel microorganisms and interact with natural microbiota to favor the growth and competitiveness of the invader. On the other hand, they also contribute to a higher microbial diversity. During domestication, crop plants and weed-associated microorganisms may increase the richness and expanded the functional capacities of soil microbiota. Endophytes have recently been implicated to play a role also in herbicide tolerance of plants (Tetard-Jones and Edwards 2016). Several bacterial endophytes have been reported to degrade various herbicides. The endophyte and rhizosphere bacterium *Pseudomonas putida* strain

POPHV6, which was originally isolated from stems of poplar trees, showed degradation of 2,4-D and led to lower herbicide accumulation in aerial tissues (Shaw and Burns 2004; Germaine et al. 2006). Similarly, plant-associated bacteria have been identified that were able to degrade and thereby detoxify the atrazine or glyphosate herbicides (Kuklinsky-Sobral et al. 2005; Ngigi et al. 2012). Many fungal endophytes of grasses exhibit reactive oxygen species scavenging activity and enhanced antioxidant content (Cummins et al. 1999), which might be important for protecting plants from downstream toxicity induced by herbicides (Edwards et al. 2005).

19.4.2 Interactions of Rhizosphere Microorganisms with Plants

Plant species affect the root exudate composition, and it varies among different plant species and genotypes and further depends on age, nutritional status, and stress exposure (Compant et al. 2010; Pérez-Jaramillo et al. 2015). Some plants use root exudates to attract mutualistic microbes that can improve their nutrient supply (Parniske 2008; Oldroyd 2013). For example, to improve phosphate and nitrogen supply, plant roots release strigolactones to attract mycorrhiza (Akiyama et al. 2005), and legumes secrete specific combinations of flavonoids to establish symbioses with nitrogen-fixing rhizobia, respectively (Bertin et al. 2003; Hassan and Mathesius 2011). Soybeans secrete isoflavones in order to host the endosymbiotic nitrogen-fixing bacterium *Bradyrhizobium japonicum* (Morris et al. 1998). Other plants, such as maize (*Zea mays*), secrete a benzoxazinoid called 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one to attract the rhizobacterium *Pseudomonas putida* KT2440, which helps to repel other pathogenic microbes in the maize rhizosphere (Neal et al. 2012). Similarly, the infection of *Arabidopsis* by *Pseudomonas syringae* pv. *tomato* DC3000 is able to induce the expression of the L-malic acid (MA) transporter (aluminum-activated malate transporter 1) and increase the secretion of MA by roots (Lakshmanan et al. 2012). The abundance of malic acid in the rhizosphere recruits the beneficial rhizobacterium *B. subtilis* FB17 in a dose-dependent manner and promotes the biofilm formation of *B. subtilis* FB17 on *Arabidopsis* roots (Rudrappa et al. 2008; Lakshmanan et al. 2013) and produces a systemic resistance response against the pathogen. Besides MA, some bacteria secrete antimicrobial metabolites (e.g., cyclic lipopeptide surfactin and iturin A) that serve as a protective shield in roots against pathogenic fungi like *Rhizoctonia* spp. or pathogenic Gram-negative bacteria such as *P. syringae* (Asaka and Shoda 1996; Bais et al. 2004).

Plants can also alter the composition of root exudates, which may lead to a selective enrichment of respective microbes in the rhizosphere (Prikyrl et al. 1985; Bulgarelli et al. 2013). For example, some microbes such as *Pseudomonas* spp. are able to suppress the soilborne pathogen *Rhizoctonia solani* (Mendes et al. 2011), through secretion of phenazine-1-carboxylic acid and 2,4-DAPG (Raaijmakers et al. 1997). The production of lipoproteins by *Pseudomonas* and *Bacillus* spp. can also inhibit growth of a wide range of pathogens (Watrous et al. 2012; Zachow et al. 2015). *Pseudomonas* spp. that synthesize 2,4-DAPG have been implicated in take-all disease (TAD) suppression (Weller et al. 2002). Microbes produce secondary

metabolites to outcompete competitors that occupy similar niches and to establish at the rhizosphere or inside roots (van Loon and Bakker 2006; Kim et al. 2011). These metabolites include antibiotics, toxins, lytic enzymes, and siderophores (Bais et al. 2006).

Diverse species of the genus *Pseudomonas*, including *Pseudomonas cepacia*, *P. fluorescens*, *P. aeruginosa*, and *P. aureofaciens*, were demonstrated to produce hydrogen cyanide, 2,4-diacetylphloroglucinol, pyrrolnitrin, phenazine, oomycin A, and other compounds that help in protecting the plant against diseases (Raaijmakers and Weller 1998; Haas and Keel 2003). The production of these compounds depends on different factors; for instance, oomycin A and 2,4-diacetylphloroglucinol are stimulated by glucose (Duffy and Défago 1999), hydrogen cyanide is affected by light and temperature (Vickery et al. 1987), and an acidic pH seems to enhance the production of pyrrolnitrin (Hwang et al. 2002). Therefore, changes in the soil environment due to climate changes (Davidson and Janssens 2006; Frey et al. 2013) could affect antibiotic production from beneficial bacteria, making plants more resistant to pathogen attack.

Production of IAA has been found to affect plant growth in diverse ways, varying from pathogenesis and growth inhibition to plant growth stimulation (Spaepen et al. 2007; Park et al. 2015). Growth retardation effects were obtained when cuttings of sour cherry (*Prunus cerasus*) and black currant (*Ribes nigrum*) were inoculated with a recombinant strain of *Pseudomonas fluorescens* that produced increased amount of IAA (Dubeikovsky et al. 1993). A high density of bacterium inoculum on the roots of cherry cuttings inhibited root growth, whereas lower densities on black currant promoted growth. Sarwar and Kremer (1995) showed that an *Enterobacter taylorae* isolate with high auxin-producing potential (72 μg ml^{-1}) inhibited the growth of *Convolvulus arvensis*. On the other hand, the inhibitory effect of some deleterious rhizosphere bacteria through IAA secretion has been related to various bacterial species including *Enterobacter taylorae*, *Klebsiella planticola*, *Alcaligenes faecalis*, *Xanthomonas maltophilia*, *Pseudomonas* sp., and *Flavobacterium* sp. (Sarwar and Kremmer 1995; Suzuki et al. 2003). Mutants of *Pseudomonas putida* that produced high levels of IAA inhibited root growth of seedlings of canola (*Brassica campestris*) by ca. 33% (Xie et al. 1996).

19.5 Identification of Microorganisms having Bioherbicidal Properties

Biological control of weeds represents an effective and innovative means to manage troublesome weeds (Harding and Raizada 2015). It utilizes the naturally occurring rhizosphere microorganisms with deleterious/phytotoxic activity toward the seedling growth of weed due to production of secondary metabolites (Khattak et al. 2014; Sayed et al. 2014; Boyette and Hoagland 2015; Lakshmi et al. 2015). These compounds either kill or retard the growth of weeds so that beneficial plant species can gain a competitive advantage (Olesen et al. 2004). Various bacteria, fungi, and viruses have been characterized as potential weed control agents (Table 19.1).

Table 19.1 Application of various microorganisms having herbicidal activity against target weeds

Biological agent	Target weed	Intended system	References
Bacterial			
P. fluorescens strain D7	Downy brome (*Bromus tectorum*)	Field crops	Kennedy et al. (1991)
P. fluorescens strain BRG100	Green foxtail (*Setaria viridis*)	Not specified	Quail et al. (2002)
P. fluorescens strain WH6	Inhibits most of the species tested	Not specified	Banowetz et al. (2008)
Xanthomonas campestris pv. *poae* (JT-P482)	Annual bluegrass *Poa annua* and *Poa attenuata*	Turf	Imaizumi et al. (1997)
Fungal			
Colletotrichum gloeosporioides f. sp. *aeschynomene*	Northern joint vetch (*Aeschynomene virginica*)	Field crops: rice, soybean	Daniel et al. (1973) Boyette et al. (2011)
Colletotrichum orbiculare	Spiny cocklebur (*Xanthium spinosum*)	Pasture and field crops	Auld et al. (1988) Harata and Kubo (2014)
Colletotrichum truncatum	Hemp sesbania (*Sesbania exaltata*)	Field crops	Boyette (1991) Hynes et al. (2010)
Phoma chenopodicola	Lamb's quarters (*Chenopodium album*), creeping thistle (*Cirsium arvense*), green foxtail (*Setaria viridis*), annual mercury (*Mercurialis annua*)	Field crops such as sugar beet and corn	Cimmino et al. (2013)
Phoma herbarum	Dandelion (*Taraxacum officinale*)	Turf	Neumann and Boland (1999) Ray and Vijayachandran (2013)
Sclerotinia minor	Dandelion (*Taraxacum officinale*), white clover (*Trifolium repens)*	Turf	Riddle et al. (1991) Abu-Dieyeh and Watson (2007)
Viruses			
Tobacco mild green mosaic tobamovirus	Tropical soda apple (*Solanum viarum*)	Pastures	Ferrell et al. (2008), Font et al. (2009), EPA (2015)
Araujia mosaic virus	Moth plant (*Araujia hortorum*)	Ecosystem management	Elliott et al. (2009)
Obuda pepper virus	*Solanum nigrum*	Ecosystem management	Kazinczi et al. (2006)

19.5.1 Bacteria

Deleterious rhizosphere bacteria (DRB) that are associated with plant roots have the ability to inhibit the growth of weed plant (Kremer and Kennedy 1996). DRB usually cause reduced seed germination, growth inhibition, and reduced root elongation by producing phytotoxins, phytohormones, or cyanides. DRB can also reduce plant growth directly by competing with the weed plant for nutrients or indirectly by reducing the colonization of weed plants by beneficial rhizobia or mycorrhiza. Selection of those rhizospheric bacterial isolates that specifically inhibit growth of weeds, but not that of crop plants, could benefit agriculture by contributing to increased crop yields, by reducing weed competition, and by reducing the use of chemical herbicides (Li and Kremer 2006; Patil 2014).

Kennedy et al. (1991) screened 1000 isolates of pseudomonads for differential inhibition of downy brome (*Bromus tectorum*) and winter wheat. The filtrates obtained from 8% of the isolates inhibited root growth of downy brome on agar but did not affect root growth of winter wheat. However, when applied to soil (10^8 CFU mL^{-1}) under nonsterile conditions, only six isolates (~ 1%) inhibited growth of downy brome. In the field, two isolates (0.2%) suppressed downy brome by ~31–53%, and this treatment increased winter wheat yield by ~18–35%. *P. fluorescens* strain D7 was found to selectively inhibit growth and germination of a number of grassy weeds (Kennedy et al. 1991, 2001; Gealy et al. 1996). Conversely, *P. fluorescens* strain WH6 has been observed to significantly inhibit germination of all species tested (21 monocot species and 8 dicot species) with the exception of corn (*Zea mays*) hybrid. *Pseudomonas fluorescens* and *P. syringae* pv. *tabaci* and *tagetis* have also been reported to be potential biological agents for weeds (Daigle et al. 2002; Zidack and Quimby 2002; Zdor et al. 2005). The other bacterial species found to act as biological weed control agent is *Xanthomonas campestris*. The strain *X. campestris* pv. *poae* (JT-P482) was registered in Japan in 1997 for control of annual bluegrass (*Poa annua*) under the product name Camperico (Imaizumi et al. 1997; Tateno 2000).

Serratia plymuthica strain A153 showed strong growth-suppressing activities against a range of broad-leaved weeds after foliar spraying (Weissmann et al. 2003). In field tests of this *S. plymuthica* strain in spring wheat, spring barley, and potatoes, variable effects were achieved on a range of weeds including *Chenopodium album*, *Stellaria media*, *Polygonum convolvulus*, and *Galeopsis speciosa*. At one site, good suppression of *C. album* was observed when the strain was applied in a tank mix with another bacterial isolate or with reduced doses of herbicide. Li and Kremer (2006) demonstrated that *Pseudomonas fluorescens* strain G2-11 inoculated to wheat and soybean crops suppressed the growth of *Ipomoea* sp. and *Convolvulus arvensis* weeds, while promoting the growth of agricultural crops. Zermane et al. (2007) reported that *P. fluorescens* has potential for controlling *Orobanche crenata* and *O. foetida* (broomrape) in Northern Tunisia.

Fifteen potential deleterious rhizosphere bacteria were characterized from the rhizosphere of *Sida acuta* (Patil 2014). Five of these bacterial isolates significantly reduced the root and shoot lengths of weed seedlings compared to the crop plants on agar plate bioassay. *Xanthomonas* sp. was found to inhibit root and shoot length of crop plants in a range ~ 25–36% and 8–34%, respectively. Sayed et al. (2014) isolated actinobacterium *Streptomyces levis* strain LX-65 from cultivated soil, and it was found to produce extracellular metabolite that exhibited effective antibacterial, antifungal, and herbicidal activity against some weeds associated with the winter wheat (*Triticum aestivum* L.) and maize (*Zea mays*). The virulence and host range of a bacterial pathogen, *Xanthomonas campestris* (isolate LVA987), were evaluated as a bioherbicide against *Xanthium strumarium* L. (common cocklebur) (Boyette and Hoagland 2013a). The effects of environmental parameters on bioherbicidal activity of the bacterium *Xanthomonas campestris*, against glyphosate-resistant and glyphosate-susceptible *Conyza canadensis* (horseweed), were studied under greenhouse conditions (Boyette and Hoagland 2015). Rosette leaf-stage plants were found more susceptible than older plants, and increasing inoculum from 10^5 to 10^9 cells mL^{-1} caused significantly greater plant mortality and biomass reduction of plants in both the rosette and bolting growth stages. Recently, 4 strains named Ha1, Ha17, Ha38, and Ha384 showed herbicidal activity among 479 bacterial strains isolated from brine in Bohai, China (Juan et al. 2015). Strain Ha1 showed the highest herbicidal activity, and it was identified as *Serratia marcescens* based on 16S rDNA sequencing. Both the suppression of *Digitaria sanguinalis* and the cell viability of the Ha1 formulation in "pesta" were higher when stored at 4 °C than at 25 ± 2 °C.

19.5.2 Fungi

Most commercial biological weed control products have been based on formulations of fungal species. *Colletotrichum truncatum* showed the ability to control hemp sesbania (*Sesbania exaltata*) (Schisler et al. 1991) and *C. orbiculare* for its potential to control spiny cocklebur (*Xanthium spinosum*) (Auld et al. 1990). BioMal, a formulation of *Colletotrichum gloeosporioides* f. sp. *malvae*, was introduced for the control of round-leaved mallow (*Malva pusilla*) (Mortensen 1988; PMRA 2006), and *C. gloeosporioides* f. sp. *aeschynomene* was released for control of northern joint vetch (*Aeschynomene virginica*) in the USA in 1982 as Collego (Menaria 2007). Additionally, *Sarritor*, a formulation of *Sclerotinia minor*, was introduced for the control of dandelion (*Taraxacum officinale*), white clover (*Trifolium repens*), and broad-leaved plants *Plantago major* in turf (PMRA 2010). *Phoma herbarum*, a fungal pathogen originally isolated from dandelion leaf lesions in Southern Ontario, has been reported to control dandelions in turf (Stewart-Wade and Boland 2005), whereas *Phoma chenopodicola* was found to

act as a potential control agent for lamb's quarters (*Chenopodium album*). A phytotoxic diterpene, chenopodolin, has been isolated from this species, which was found to cause necrotic lesions on lamb's quarters, creeping thistle (*Cirsium arvense*), green foxtail (*Setaria viridis*), and annual mercury (*Mercurialis annua*) (Cimmino et al. 2013).

Echinochloa crus-galli is among the three most serious weeds of rice in Asian countries, and yield loss by *E. crus-galli* was reported ~41% in Malaysia. A total of 82 isolates from 12 fungus genera were isolated from diseased barnyard grass in paddy field. Fungal species were identified as *Exserohilum monoceras*, *E. longirostratum*, and *Curvularia lunata.* The fungus, *E. monoceras*, was found associated consistently with the disease (Tosiah et al. 2009, 2011). In Korea, *Colletotrichum graminicola* showed strong pathogenicity in a wide range of growth stages of *E. crus-galli* var. *praticola* and *E. crus-galli* var. *caudata* (Yang 2000). Kadir et al. (2003) reported that *E. longirostratum* has good control on *Rottboellia cochinchinensis* (itch grass) and *E. crus-galli* in Malaysia.

Khattak et al. (2014) isolated two fungi *Aspergillus* and *Penicillium* species from the rhizosphere of *Mentha piperita*. The extract of both fungi possessed potential agrochemical constituents which inhibited the growth of *Lemna minor* and *Silybum marianum* L. weed. Greenhouse and field experiments showed that conidia of the fungal pathogen, *Phoma commelinicola*, exhibited bioherbicidal activity against spreading dayflower (*Commelina diffusa*) seedlings when applied at concentrations of 10^6–10^9 conidia mL^{-1}. Maximal control (~ 80%) required longer dew periods (21 h), and ~90% plant dry weight reduction occurred at this dew period duration. More efficacious control occurred on younger plants (cotyledonary-first leaf growth stage) than older and larger plants. Mortality and dry weight reduction values in field experiments were ~70% and >80%, respectively, when cotyledonary-third leaf growth stage seedlings were sprayed with 10^8 or 10^9 conidia mL^{-1}. These results indicated that this fungus has potential as a biological control agent for controlling this problematic weed that is tolerant to the herbicide glyphosate (Boyette and Hoagland 2015).

19.5.3 Viruses

Viruses having the potential to control invasive or undesirable species include tobacco mild green mosaic tobamovirus for control of tropical soda apple (*Solanum viarum*) in Florida (Ferrell et al. 2008; Diaz et al. 2014) and *Araujia* mosaic virus for control of moth plant (*Araujia hortorum*) in New Zealand (Elliott et al. 2009). A virus resembling tobacco rattle virus has also been proposed as a control agent for *Impatiens glandulifera*, an invasive weed of Central and Western Europe (Kollmann et al. 2007). Similarly, Obuda pepper virus (ObPV) and Pepino mosaic virus (PepMV) have been proposed as viral agents to reduce overall populations of the weed *Solanum nigrum* (Kazinczi et al. 2006).

19.6 Mechanisms Involved in Conferring Herbicidal Activity

A wide range of rhizosphere microorganisms have been identified that possess herbicidal activity, and their inoculation reduced the need of herbicides for control of weeds under field conditions. A virulent strain of *X. campestris* (LVA987) was shown to control common cocklebur (*Xanthium strumarium* L.) (Boyette and Hoagland 2013a), which is an important weed in soybean, cotton, and peanut production. Many *Pseudomonas* strains are characterized as deleterious rhizosphere bacteria (DRB), which excrete exopolysaccharides and allelochemicals in the form of cyanide, phytohormones, siderophores, and phytotoxins that can negatively affect the metabolism of plants (Li and Kremer 2006) (Fig. 19.2). These rhizosphere microorganisms inhibit the growth of weeds by a variety of mechanisms.

19.6.1 Colonization of Roots and Leaves

Some biological control agents attach to the roots of weeds and release toxins that stunt root growth. Many fungi infect roots and disrupt water transport system, which reduces leaf growth. Beneficial insects and nematodes feed directly on the weed roots causing injury, which allows bacteria and fungi to penetrate. Insects that feed on leaves reduce the leaf surface available for energy capture. Similarly, fungi and bacteria that infect leaves reduce the ability of the leaf to make sugars. Severe infestations of biological control agents can cause damage on roots or leaves and may even kill the weeds. Fungi or insects that attack seeds can reduce the number of weed seeds stored in the soil, which in turn may reduce the size of future weed populations.

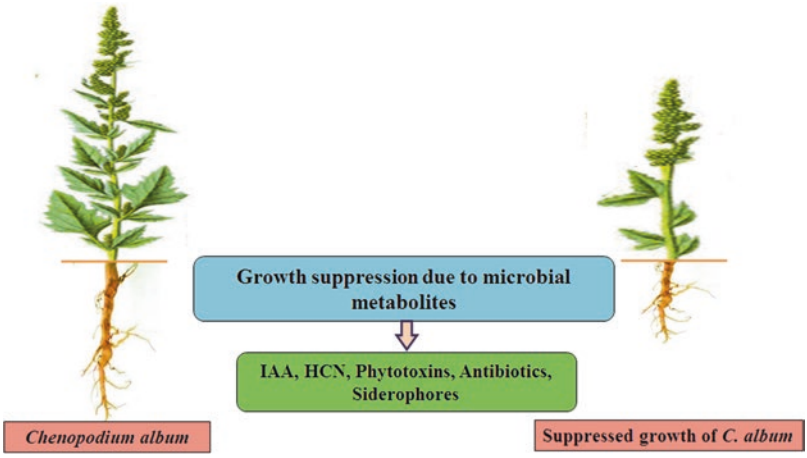


Fig. 19.2 Growth suppression effects of microorganisms on *Chenopodium album*

19.6.2 Antibiotic Production

Kataryan and Torgashova (1976) reported that the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) showed phytotoxic activity resembling to that of 2, 4-dichlorophenoxyacetate (2, 4-D). Geldanamycin and nigericin, phytotoxic metabolites, were obtained from a strain of *Streptomyces hygroscopicus*. Geldanamycin showed significant pre-emergence activity on proso millet, barnyard grass, garden cress, and giant foxtail. *Saccharothrix* sp. ST-888 produced phosphinothricin that inhibited the germination of graminaceous and broad-leaved weeds (Takahashi et al. 1995). Lee et al. (2003) reported that methoxyhygromycin antibiotic produced by *Streptomyces* sp. showed higher activity in the range of 90% at 0.25 kg ha^{-1} against monocotyledonous weeds such as large crabgrass (*D. sanguinalis*) and barnyard grass (*E. crus-galli*) than dicotyledonous weeds.

19.6.3 Indole Acetic Acid Production

Indole acetic acid (IAA) production is widespread among plants and bacteria (Malik and Sindhu 2011). Indole-3-acetic acid stimulates plant growth in lower concentrations, and in contrast, if the concentration becomes higher, the effect reverses, and elongation of root and shoot is inhibited (Grossmann 2010). Besides the concentration, also the plant tissue, physiological stage, and plant species determine the sensitivity to auxins. The plants react to elevated auxin with inhibition of root and shoot growth, decreased internode elongation and leaf growth, and intensified green leaf pigmentation, accompanied by stomatal closure and an increase of reactive oxygen species (Grossmann 2010). In addition, application of auxin promotes the susceptibility of the plant to bacterial pathogens and increases disease symptoms (Spaepen and Vanderleyden 2011).

Natural auxins have modes of action similar to many herbicides that interfere with plant growth such as 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) (Patten and Glick 1996). Sarwar and Kremer (1995) reported that auxins produced in high concentrations in the rhizosphere by deleterious rhizosphere bacteria (DRB) may contribute to reduced root growth of weeds. An *Enterobacter taylorae* isolate with high auxin-producing potential (72 mg L^{-1} IAA equivalents) was found to inhibit root growth of field bindweed (*Convolvulus arvensis* L.) by ~91% when combined with 10^{-5} M L-tryptophan compared with non-treated control. IAA production in *Bacillus japonicum* isolate GD3 resulted in suppression of morning glory growth (Kim and Kremer 2005). Mejri et al. (2010) studied the effect of rhizobacterial *Pseudomonas trivialis* strain X33d on growth suppression of weed great brome (*Bromus diandrus* Roth.). The specificity assay showed the suppressive activity of *P. trivialis* X33d against great brome, and it caused growth-promoting effect on most of the considered crops, especially durum wheat (*Triticum durum* Desf.). Great brome plants inoculated with X33d and co-seeded with durum wheat showed low root biomass, short root systems, and low surface area, volume, and number of tips. The

production of indole acetic acid by *P. trivialis* X33d was suggested to cause growth suppression of great brome and growth promotion of durum wheat.

Park et al. (2015) observed that two bacterial strains, I-4-5 and I-3, significantly reduced the seedling growth of radish in comparison to their controls. The highest rate of seedling growth inhibition was observed in bacterial isolate I-3 treatment in lettuce and radish. In vitro study revealed that culture exudate obtained from I-3 bacterial isolate and combined with tryptophan significantly decreased leaf length, leaf width, and root length and increased the number of lateral roots of lettuce. Similarly, ten rhizobacterial isolates, obtained from wheat rhizosphere soil, showed maximum retardation on fifth and tenth of seed germination of *Phalaris minor* on 0.8% water agar plates (Phour 2012). At 10th day of seed germination, ~ 15% bacterial isolates showed retardation of shoot growth and ~19% bacterial isolates retarded root growth. Screening of these rhizobacterial isolates for production of indole acetic acid showed that two isolates HWM49 and HWM35 produced 11.10 and 14.07 μg mL^{-1} IAA, respectively, and significant production of IAA (> than 25 μg mL^{-1}) was observed in isolates CPS67, CP43, and HWM13.

19.6.4 Aminolevulinic Acid Production

5-aminolevulinic acid (ALA) is a key intermediate in the biosynthesis of tetrapyrroles and is having a promoting effect on the growth and photosynthesis of crops and vegetables (Sasaki et al. 1993). ALA has recently drawn increasing attention as a photodynamic chemical, which can be used as a favorable biodegradable herbicide and insecticide, and it is harmless to crops, humans, and animals (Sasikala et al. 1994; Bhowmick and Girotti 2010; Kang et al. 2012). Herbicidal activity has been reported to increase accumulation of several chlorophyll intermediates, such as protochlorophyllide, protoporphyrin IX, and Mg-protoporphyrin IX, when plants were treated with exogenous ALA at relatively high concentrations (5–40 mM). However, low ALA concentrations, within the range of 0.06–0.6 mM, were found to promote the plant growth rather than damage by increasing nitrate reductase activity, by increasing fixation of CO_2 in the light, and by suppressing the release of CO_2 in darkness (Hotta et al. 1997). Zhang et al. (2006) reported that ALA at low concentrations of 0.3–3 mg L^{-1} promoted development and growth of potato microtubers in vitro and enhanced protective functions against oxidative stresses, but ALA at 30 mg L^{-1} and higher concentrations may induce oxidative damage. Khandelwal (2016) isolated 250 rhizosphere bacteria from the rhizosphere of wheat and mustard, and among these isolates, 96 rhizobacterial isolates showed significant stimulation or retardation effect on seed germination of weed *Chenopodium album* and *Asphodelus tenuifolius* on 0.8% water agar plates. Rhizobacterial isolates WSA38, MSA57, WSA68, WSA56, MSA42, MSA39, WHA98, and MSA11 showed >11.0 μg mL.$^{-1}$ production of δ-aminolevulinic acid, which contributed to growth retardation of *C. album* and *A. tenuifolius*. Forty-five isolates showed root growth inhibition on 5th day of seed germination in *C. album*. Nine rhizobacterial isolates caused shoot growth inhibition on 5th day, and seven bacterial isolates caused shoot

growth inhibition at 10th day of seed germination of *C. album*. In *Asphodelus tenuifolius*, 34 isolates showed root growth inhibition on 5th day, and 27 rhizobacterial isolates showed root growth inhibition at 10th day of seed germination.

19.6.5 Production of Secondary Metabolites

A polyketide secondary metabolite herboxidiene, produced by *Streptomyces chromofuscus*, showed potent and selective herbicidal activity against weeds but not against wheat (Miller-Wideman et al. 1992). A phytotoxic metabolite trans-4-aminoproline obtained from culture filtrates of *Ascochyta caulina* was found very effective in controlling *Chenopodium album* (L.) (Evidente et al. 2000). Javaid and Adrees (2009) reported that metabolites of *Alternaria alternata*, *Fusarium oxysporum*, *F. solani*, *Drechslera hawaiiensis*, *D. australiensis*, and *D. rostrata* were highly effective in controlling the growth of the noxious weed *Parthenium hysterophorus*. *P. fluorescens* strain BRG100 showed suppressive activity on the grassy weed green foxtail (*Setaria viridis*) (Quail et al. 2002; Caldwell et al. 2012). The herbicidal compounds produced by this species, referred to as pseudophomins A and B, have been characterized through chromatography, which are cyclic lipodepsipeptides. This strain can reduce the root growth in green foxtail by 73–79% and is able to colonize root hairs and the root except the root cap of green foxtail (Caldwell et al. 2011). The metabolite coronatine is a jasmonate analog produced by *Pseudomonas coronafaciencs* (Gerwick et al. 1997). It upregulated the jasmonate-controlled signaling pathways (Ichihara et al. 1977), and the typical symptom of this toxin is chlorosis of developing tissues. Cinnacidin, a product of the fungus *Nectria* sp. DA060097, has a similar mode of action to coronatine (Block et al. 2005). Gostatin, a product of *Streptomyces sumanensis* (Amagasa et al. 1994), is a potent aminotransferase inhibitor that is phytotoxic (Nishino et al. 1984). Pyridazocidin, a cationic compound from *Streptomyces* species, caused rapid plant necrosis and chlorosis, much like that of bipyridinium herbicides like paraquat (Oettmeier et al. 1990).

The germination-inhibiting activity of *P. fluorescens* strain WH6 has been attributed to the production of a compound originally referred to as germination arrest factor (GAF) (Banowetz et al. 2008). The active component of GAF was identified as 4-formylaminooxy-L-vinylglycine (McPhail et al. 2010). This class of compounds, the oxyvinylglycines, has been shown to interfere with enzymes that utilize pyridoxal phosphate as a cofactor, including enzymes involved in nitrogen metabolism and biosynthesis of the plant hormone ethylene (Berkowitz et al. 2006). The effects of cell-free supernatants (S) and anionic fractions (Q) obtained from three different strains of *Bacillus subtilis*, i.e., DN and Car13, as well as a non-promoting strain PY79, were evaluated on weed seed germination on pigweed (*Amaranthus hybridus* L.) and Johnson grass (*Sorghum halepense* L. Pers) (Mendoza et al. 2012). The application of anionic fractions QCar13, QDN, and QPY caused a drastic decrease in the germination rates of both pigweed and Johnson grass seeds in comparison to controls. Several *P. putida* strains were used to control velvetleaf and

Striga hermonthica (Del.) and *P. fluorescens* strains to control broomrape, wild radish, and *S. hermonthica* (Del.) (Stubbs and Kennedy 2012). *P. fluorescens* strain D7, which was isolated from roots of winter wheat, showed a reduction of downy brome (*Bromus tectorum* L.) biomass production of 18–54% in the field when the strain was applied to the soil (Ibekwe et al. 2010). This strain produces a complex of chromopeptides, peptides, fatty acids and a lipopolysaccharide matrix.

19.6.6 Hydrogen Cyanide Production

Hydrogen cyanide (HCN) production is found to be a common trait in strains of *Pseudomonas* (~ 89%) and *Bacillus* (~ 50%) obtained from the rhizospheric soil and plant root nodules (DeCoste et al. 2010; Ramyasmruthi et al. 2012; Ahemad and Kibret 2014). Due to the stimulation of ethylene biosynthesis caused by IAA, cyanide is formed as a coproduct (Grossmann 2010). Hydrogen cyanide effectively blocks the cytochrome oxidase pathway and forms metal complexes with functional groups of various enzymes. Cyanide is a potential inhibitor of enzymes involved in major plant metabolic processes including respiration, CO_2 and nitrate assimilation, and carbohydrate metabolism. Cyanide also interacts with the protein plastocyanin, which inhibits the photosynthetic electron transport (Kremer and Souissi 2001). The possible phytotoxic mechanism leading to significant growth reduction in plants has been reported in *Lactuca sativa* and *Echinochloa crus-galli* (Kremer and Souissi 2001; Zeller et al. 2007). *Pseudomonas aeruginosa* (HM195190) strain KC1 was isolated from the rhizosphere of castor plants (*Ricinus communis*) indigenous to agricultural fields of Bihar (Lakshmi et al. 2015). Strain KC1 was found to produce cyanide (4.78 nmol L^{-1}) over a period of 36 h. Seed bacterization with strain KC1 exhibited reduction in root and shoot length of *Amaranthus spinosus* and *Portulaca oleracea* weed seedlings, which was significant in both laboratory and glasshouse experiments. Biomass was also significantly reduced for the weed seedlings in glasshouse experiments. However, KC1 inoculated crop seedlings (*Triticum aestivum*) were found to be less inhibitory as compared to weed seedlings.

19.6.7 Phytotoxin Production

Bacterial and fungal microorganisms were found to produce various phytotoxins with the potential to be used as herbicides (Duke et al. 1991). The isolated phytotoxins may exhibit similar host and nonhost specificity to the pathogen. AAL toxin, a hydroxylated long-chain alkylamine containing a tricarboxylic acid moiety, is produced by *Alternaria alternata* f. sp. *lycopersici* and has been found to act as an effective herbicide on a range of crop and weed species (Abbas et al. 1995). Rhizobitoxine is produced by some *Bradyrhizobium* strains (Duke et al. 2011). It inhibits β-cystathionase, which is required for methionine synthesis. This toxin is phytotoxic enough to act as a commercial herbicide (Giovanelli et al. 1973). Since synthesis of the essential plant hormone ethylene is dependent on methionine,

therefore, it is expected that ethylene synthesis would be greatly inhibited in plants treated with rhizobitoxine.

The phytopathogenic fungus, *Bipolaris euphorbiae*, is the causal agent for the major disease of *E. heterophylla* in Brazil (Barreto and Evans 1998) and has been reported to be highly efficient and promising as a biological control agent for this weed as the best postemergence herbicides (Yorinori and Gazziero 1989). This fungus produced host-specific phytotoxin(s) that elicits its effect during germination and affects the leaves of susceptible *E. heterophylla* plants causing defoliation but does not affect soybeans (Barbosa et al. 2002). *P. syringae* pv. *tagetis* (Pst) produced the phytotoxin tagetitoxin, which caused symptom of apical chlorosis in infected plants. *P. syringae* strain CT99 isolated from *Cirsium arvense* (Canada thistle) was evaluated as a biological control agent for this invasive weed and other weeds in the family Asteraceae. Alternatively, tagetitoxin may be of value as a natural herbicide because of its impact on chloroplasts (Lydon et al. 2011). Several pathogens, including *Stagonospora cirsii* and *Ascochyta sonchi*, were found commonly on *Cirsium arvense* and *Sonchus arvensis*, and these fungi also produced phytotoxic metabolites. *Phyllosticta cirsii* and *Phomopsis cirsii*, belonging to two well-known toxin-producing genera, have also been proposed for biocontrol of *C. arvense* (Evidente et al. 2011).

LT toxin from *Lasiodiplodia theobromae* was reported to act as an effective herbicide to control *Parthenium hysterophorus*, duckweeds, jimson weed, prickly sida, and *Euphorbia hirsuta*. Phytotoxins which could control the weeds *Lantana camara* and *Parthenium hysterophorus* were isolated from *Alternaria alternata* f. sp. *lantanae* and patented for use as herbicide. Phyllostictine A is a powerful toxin produced by a mycoherbicide *Phyllosticta cirsii*, which is used for the biological control of *Cirsium arvense* (Zonno et al. 2008). Mevalocidin is another mobile phytotoxin, produced by *Fusarium* DA056446 and *Roselliana* strain DA092917. It is a broad-spectrum postemergence herbicide against grasses and broad-leaved plants (Gerwick et al. 2013).

More than 2000 species of the genus *Phoma* exist worldwide, and several of the species produce phytotoxic metabolites like phomalairdenone, nonenolides, epoxydonesters, and putaminoxin (Graupner et al. 2003). The bioherbicide based on *Phoma macrostoma* is used to control broad-leaved weeds in turfgrass, causing bleaching and chlorotic symptoms in infected plants (Zhou et al. 2004). The pathogen produces the phytotoxic metabolite macrocidins A and B, a new family of cyclic tetramic acids (Graupner et al. 2003). To control *Chenopodium album*, the species *P. chenopodicola* was proposed for biological control, and the fungus produced several phytotoxins in liquid culture (Cimmino et al. 2013; Evidente et al. 2015). When the toxins chenopodolan D and chenopodolin B are applied to leaf disks of nonhost weeds, a fast development of necrosis was observed, whereas chenisocoumarin and the 9-O-acetyl had no effects on leaf disks (Evidente et al. 2015).

19.7 Screening Approaches for Potential Bioherbicides

Several sampling strategies exist for obtaining appropriate microbial strains for further screening. The best way of finding microbes showing growth retardation effects on weeds is to look for sites with suppressed vegetation (Barazani and Friedman 2001). Screening for *Pseudomonas* rhizobacteria in weed-suppressive soil resulted in selection of 15 *Pseudomonas fluorescens* and *P. putida* strains that were able to significantly reduce the germination of *Striga hermonthica* (Del.) Benth (Ahonsi et al. 2002). Isolation of microorganisms from diseased weeds could lead directly to host-specific pathogens for potential use as bioherbicides (Boyette and Hoagland 2013a). Most of the plant pathogens are host specific and would be good candidates for selective herbicides, and all currently available fungal bioherbicides are plant pathogens with a narrow host range. In addition, bacterial pathogens like *Xanthomonas* sp. have been tested as bioherbicides (Imaizumi et al. 1997; Boyette and Hoagland 2013a). Kloepper et al. (2013) found endophytes in leatherleaf fern (*Rumohra adiantiformis*), which are responsible for the deformation of the leaves. The responsible fluorescent pseudomonads are present as latent endophytes also in healthy plants, but if they exceed a certain threshold, symptoms of leave distortion appear.

Stubbs and Kennedy (2012) proposed a screening procedure for bacterial biological control agents. In a first bioassay, the strains are tested for their activity against the weed. Selected strains that suppressed the growth or germination of the weed were tested against several crop plants in the next step. Only bacterial strains that do not suppress the crop plants are tested in soil, in the greenhouse, and in the field. The indicator technique for antimetabolite toxin production against *Escherichia coli* was also proposed (Gasson 1980) as an alternative method for the screening against target weeds. The mechanism of *E. coli* growth inhibition is similar to the phytotoxin-induced chlorosis of plant tissue (Gasson 1980). Similarly, IAA production is an important mechanism of bioherbicidal microorganisms, which may be tested by a colorimetric method, e.g., using the Salkowski reagent (Sarwar and Kremer 1995). Hydrogen cyanide (HCN), a volatile metabolite that negatively affects root metabolism and root growth, is produced by many *P. fluorescens* and *P. aeruginosa* strains (Blumer and Haas 2000). Bacteria produce different amounts of HCN, and the production is very tightly regulated. In cases where the plant is heavily colonized by *Pseudomonas* strains, the accumulated HCN concentration may have deleterious effects. Although colorimetric assays for the detection of HCN exist (Lorck 1948; Feigl and Anger 1966), the test proposed by Lakshmi et al. (2015) involves a paired plate assay and offers the possibility to screen bacteria for growth without knowing the volatile compound.

Like for other biocontrol agents, for bioherbicides also, risk assessments have to be carried out prior to registration. The risk associated with bioherbicides can be categorized in the risk to humans and mammalians, plant host range, and effects on nontarget organisms like competition or displacement of beneficial microbes. To address these issues, screenings for the toxin production and host range assays have been carried out. New approaches in molecular biology may facilitate the discovery of herbicidal compounds from metagenomic libraries targeting also microorganisms

difficult to cultivate (Kao-Kniffin et al. 2013). It is predicted that metagenomic tools together with new sequencing technologies will provide the basis for the discovery of new antibiotics and enzymes in biomedicine and industrial fields (Li and Vederas 2009). Using high-throughput sequencing techniques and advanced bioinformatic tools together with metabolomic analyses will allow the identification of genes and metabolites responsible for the production of herbicidal compounds.

19.8 Development of Commercial Bioherbicides

Bioherbicide DeVine, containing a Florida isolate of *Phytophthora palmivora*, is used for the control of *Morrenia odorata* (strangler vine or milkweed vine) for citrus plants in Florida. Collego, based on *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, is used to control *Aeschynomene virginica* (northern joint vetch), a leguminous weed in rice and soybean crops in Arkansas, Mississippi, and Louisiana. The fungal pathogen *Alternaria destruens* strain 059 was registered in the USA in 2005 for control of dodder (*Cuscuta* sp.) in field crops and ornamental plants. A stump-treatment product based on the wood-infecting basidiomycete, *Cylindrobasidium laeve*, under the commercial name Stumpout, is registered in South Africa to control resprouting of cut trees in tree plantations. Majority of bioherbicides are mycoherbicides with the exception of Camperico which is a bacterial bioherbicide. A wilt-inducing bacterium *Xanthomonas campestris* pv. poae isolate JT-P482, isolated from *Poa annua* (annual bluegrass or winter grass), has been registered in Japan as the bioherbicide Camperico to control annual bluegrass in golf courses (Imaizumi et al. 1999). Worldwide about 15 bioherbicide products have been developed and used commercially to manage weeds in various crops, including several horticultural crops (Table 19.2).

Currently, bioherbicides are being developed to manage weeds in citrus, vegetables, pastures, and natural areas, targeting pigweeds (*Amaranthus* sp.), purple nutsedge (*Cyperus rotundus* L.), several invasive grasses, dodder (*Cuscuta* sp.), and tropical soda apple (*Solanum viarum* Dunal) (Charudattan 2005). Loretta et al. (2006) described that seven species of *Amaranthus* had become resistant to a number of herbicides. But the combined application of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* as a mixture significantly decreased the weed species in the field and caused 100% mortality. Stumpout (*Cylindrobasidium laeve*), EcoClear™ (*Chondrostereum purpureum*), and Myco-Tech™ (*Chondrostereum purpureum*) paste are three commercially available bioherbicides (Barton 2005). *Colletotrichum gloeosporioides* f. sp. *aeschynomene* has been registered (previously Collego) under the commercial name LockDown for use in the rice in Arkansas, Louisiana, and Mississippi (Yandoc et al. 2006). All these herbicides have potential weed control capacity up to 100% in field condition though its efficacy is regulated by inoculum's concentration, formulation, spray parameters, target weed plant age, nontarget plant species, micro- and macroorganisms in the phyllosphere or rhizosphere, and pesticides applied in the area.

Table 19.2 Various bioherbicides developed on commercial scale

Bioherbicide trade name	Active microorganism	Target weed
CASST	*Alternaria cassia*	Sickle pod, coffee senna
Smolder	*Alternaria destruens*	Dodder
Chontrol	*Chondrostereum purpureum*	Alders and other hard woods
Myco-Tech	*Chondrostereum purpureum*	Deciduous tree species
BioChon	*Chondrostereum purpureum*	Woody weeds
Collego	*Colletotrichum gloeosporioides* f. sp. *aeschynomene*	Northern joint vetch
Hakatak	*Colletotridium acutatum*	*Hakea sericea*
Lubao	*Colletotridium gloeosporioides*	Dodder
BioMal	*Colletotrichum gloeosporioides* f. sp. *malvae*	Round-leaved mallow
Organo-Sol	*Lactobacillus* sp.	Leguminous weeds
Phoma	*Phoma macrostoma*	Broad-leaved weeds
DeVine	*Phytophthora palmivora*	Strangler vine
DrBioSedge	*Puccinia canaliculata*	Yellow nut sedge
Camperico	*Xanthomonas compestris* pv. *poae*	Annual bluegrass

19.9 Formulations to Improve Efficacy of Microbial Herbicides

Indiscriminate use of herbicides has resulted in development of herbicide-resistant weeds, which could be managed only with application of biocontrol agents. Adjuvants such as unrefined corn oil and Silwet L-77 may improve chances for success of mycoherbicides (Abbas et al. 2004; Boyette et al. 2006, 2007). Zhao and Shamoun (2005) tested combinations of gelatin and potato dextrose broth concentrations for optimum efficacy of *Phoma exigua* to control salal (*Gaultheria shallon*), a perennial evergreen shrub. *Fusarium oxysporum* f. sp. *orthoceras* (FOO) is known to suppress the root parasitic weed broomrape (*Orobanche cumana*) in sunflower. Hoagland et al. (2007) studied the formulation, application method, and growth media for control of kudzu (*Pueraria lobata*) using *Myrothecium verrucaria* fungi. Elzein et al. (2006) examined seed coatings containing *Fusarium oxysporum* isolates to control *Striga* and found that an ~ 40% gum arabic seed coating combined with dried chlamydospores is the most effective combination for causing disease in *Striga*. Zhang et al. (2010) analyzed the stability of pyoluteorin, a polyketide metabolite produced by fluorescent pseudomonads that showed potential to control weeds among other pests.

The success of applying bioherbicidal agents against weeds relies on the ability of the biological control agents to persist after its application and to remain viable after exposure to different environmental conditions. The persistence of bioherbicide formulated from multi-combination of the wild and mutant strain of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* under field condition was

determined (Oluwaseun et al. 2016). The viability of the formulated bioherbicides was in the following orders: BH4 > BH2 > BH6 > BH3 > BH1 > BH5 > control. BH4 showed the maximum number of viability with 4.0×10^5 CFU g^{-1} and 4.2×10^5 CFU g^{-1} at the two field trials after 12 weeks of application. The results revealed that multi-combination of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* into different "pesta" formulations greatly enhanced the viability of the bioherbicidal agent at two trial fields. Many mycoherbicides and bacteria have been processed to "pesta" formulations, such as *Fusarium oxysporum* (Kohlschmid et al. 2009), *Pseudomonas fluorescens* (Daigle et al. 2002), *Pseudomonas aeruginosa* (Yang et al. 2014), *Lasiodiplodia pseudotheobromae*, and *Pseudomonas aeruginosa* (Adetunji and Oloke 2013). A modified pesta granule was developed for *Pseudomonas fluorescens* BRG100, a bioherbicidal bacterium for grass weeds, green foxtail (*Setaria viridis*), and wild oat (*Avena fatua*) (Hynes and Boyetchko 2011). Both the suppression of *Digitaria sanguinalis* and the cell viability of the Ha1 formulation in "pesta" were higher when stored at 4 °C than at 25 ± 2 °C (Juan et al. 2015).

19.10 Inoculation Effect of Microorganisms with Bioherbicidal Activity on Plant Growth

Indigenous soil microorganisms in the soil habitat play key roles in ecosystem functioning through control of nutrient cycling reactions essential for maintaining soil fertility and also contributing to the maintenance of soil structure (Kirk et al. 2004; Wani et al. 2008; Khan et al. 2009). *Pseudomonas* strains isolated from the rhizosphere of different crops have emerged as effective plant growth-promoting rhizobacteria because they exhibit a wide range of beneficial properties, viz., production of phytohormones like indole acetic acid (IAA), gibberellic acid, and cytokinins, solubilization of phosphate and other nutrients (Vyas and Gulati 2009), siderophore production, and production of antibiotics such as 2,4-diacetylphloroglucinol, phenazines, pyrrolnitrin, and pyoluteorin, biocides such as hydrogen cyanide (Raaijmakers et al. 2002), and cell wall lytic enzymes (Haas and Défago 2005). Wani et al. (2007) tested the rhizosphere isolates for HCN producing ability in vitro and found that most of the isolates produced HCN and stimulated the plant growth. The bacterium *Pseudomonas entomophila* produced HCN with biocontrol properties (Ryall et al. 2009). The *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium, produced hydrogen cyanide, and the seed bacterization with the isolate significantly increased the percent germination, rate of germination, plant biomass, and nutrient uptake of wheat seedlings (Selvakumar et al. 2009).

Two *Pseudomonas* isolates suppressed downy brome by 31–53% and increased the yield of winter wheat by ~18–35% under field conditions (Kennedy et al. 1999). Li and Kremer (2006) showed that inoculation of *P. fluorescens* strain G2–11 on wheat and soybean roots promoted the growth of these crops and suppressed the growth of *Ipomoea* sp. and *Convolvulus arvensis* weeds. Mejri et al. (2010) reported the production of indole acetic acid by *Pseudomonas trivialis* strain X33d caused growth suppression of great brome weed and promoted the growth of durum wheat.

Twelve rhizobacterial isolates were tested for their effect on growth of wheat and weed under pot house conditions, and rhizobacterial isolates, i.e., SYB101, CPS67, and HWM11, were found to stimulate growth of wheat and inhibited the growth of *Phalaris minor* weed under pot house conditions (Phour 2012).

Inoculation of bacterial isolate WHA87 caused 21–81% decrease in root dry weight (RDW) and 33–43% decrease in shoot dry weight (SDW) of *Chenopodium album*, whereas its inoculation showed 94–182% increase in RDW and 30–340% increase in SDW of wheat at different stages of plant growth under pot house conditions (Khandelwal 2016). Rhizobacterial isolates, i.e., WHA87, MSA39, MHA75, and MSA56, were found to stimulate growth of wheat, whereas isolates MSA39 and WHA87 inhibited the growth of *Chenopodium album*, and isolates MHA75, MHA93, and MSA56 inhibited the growth of *Asphodelus tenuifolius* under pot house conditions. In another study, rhizobacterial isolates HMM76, HMM92, JMM24, JMM35, and SYB101 were found to stimulate growth of mustard and inhibited the growth of *Lathyrus aphaca* under pot house conditions (Phour 2016). At 75 days after sowing, inoculation of two bacterial isolates HMM92 and JMM24 showed 54–191% increase in RDW and SDW of mustard, whereas they caused 36–92% decrease in RDW and SDW of *Lathyrus aphaca.* These rhizobacterial isolates could be further tested for suppression of weed growth under field conditions for their subsequent application as bioherbicide.

19.11 Limitations and Future Prospective

Plant rhizosphere is a rich source of nutrients for different microorganisms present in the soil. These microorganisms provide the different nutrients and hormones for the plant growth, and some of the microbes produce the metabolites which suppress the pathogenic fungi and also suppress the growth of weeds. The interactions between the biocontrol agent, microbial population in the rhizosphere, the plant, and the environment are responsible for the variability observed in suppression/retardation of the growth of weeds and plant growth promotion. The persistence and survival of biocontrol agents/bioherbicides are major constraints to their widespread use in commercial agriculture. The application of microbial strains having better colonization capability to suppress the growth of weeds and the ability to promote the growth of crops will provide the pesticide-free food to ever-expanding human population. Therefore, more emphasis is required on the developments of bioherbicides and biofertilizers for their application in sustainable agriculture.

The multipartite interactions in the rhizosphere involving microbes, crop plants, and weeds lead to assembly and maintenance of highly complex and specific root microbiome. Many of these interactions are mediated by photo-assimilates that are excreted by plant roots. Besides providing nutrients for rhizosphere microorganisms, these root exudates serve numerous functions to control abiotic and biotic processes. These functions range from changing the chemical and physical properties of the soil, inhibiting the growth of competing plants, and regulating the microbial community (Lareen et al. 2016; Rasmann and Turlings 2016). In addition to

pathogens, plant roots interact with a plethora of nonpathogenic and symbiotic microorganisms. Therefore, a good understanding of how plant roots interact with the microbiome would be particularly important to engineer resistance to root pathogens without negatively altering root-beneficial microbe interactions. Therefore, understanding the potential for manipulation of soil microbial communities to increase crop yields is highly relevant.

A greater understanding of root microbiome community dynamics and communication between crop/weed plants has the potential to allow for more efficient exploitation of this largely untapped resource. Farming methods that support recruitment and maintenance of beneficial microbial communities in the rhizosphere could provide benefits to agriculture in the form of enhanced crop yields and suppression of diseases and growth of the weeds. Many more plant-microbe interactions remain to be uncovered, and a good understanding of the mechanisms and ecological implications could become the basis for exploitation and manipulation of these interactions for weed and disease control leading to improved crop productivity for sustainable agriculture.

References

Abbas HK, Tanaka T, Duke SO, Boyette CD (1995) Susceptibility of various crop and weed species to AAL-toxin, a natural herbicide. Weed Technol 9:125–130

Abbas H, Johnson B, Pantone D, Hines R (2004) Biological control and use of adjuvants against multiple seeded cocklebur (*Xanthium strumarium*) in comparison with several other cocklebur types. Biocontrol Sci Tech 14:855–860

Abu-Dieyeh M, Watson A (2007) Efficacy of *Sclerotinia minor* for dandelion control: effect of dandelion accession, age and grass competition. Weed Res 47:63–72

Adetunji C, Oloke J (2013) Efficacy of freshly prepared pesta granular formulations from the multicombination of wild and mutant strain of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa*. Agric Univ Tirana 12:555–563

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol 4(1):e1002352

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ahonsi MO, Berner DK, Emechebe AM, Lagoke ST (2002) Selection of rhizobacterial strains for suppression of germination of *Striga hermonthica* (Del.) Benth. Seeds. Biol Control 24:143–152

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Amagasa T, Paul RN, Heitholt JJ, Duke SO (1994) Physiological effects of cornexistin on *Lemna paucicostata*. Pesticide. Biochem Physiol 49:37–52

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

Auld BA, McRae CF, Say MM (1988) Possible control of *Xanthium spinosum* by a fungus. Agric Ecosyst Environ 21:219–223

Auld BA, Say MM, Ridings HI, Andrews J (1990) Field applications of *Colletotrichum orbiculare* to control *Xanthium spinosum*. Agric Ecosyst Environ 32:315–323

Bailey KL (2014) The bioherbicide approach to weed control using plant pathogens. In: Abrol DP (ed) Integrated pest management: current concepts and ecological perspective. Elsevier, San Diego, pp 245–266

Bailey KL, Boyetchko SM, Langle T (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 52:221–229

Bais HP, Fall R, Vivanco JM (2004) Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol 134:307–319

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. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Bakker M, Manter D, Sheflin A, Weir TL, Vivanco JM (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant Soil 360:1–13

Baldani VD, Baldani JI, Dobereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedica*e and *Burkholderia* spp. Biol Fertil Soils 30:485–491

Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI (2008) Germination-Arrest Factor (GAF): biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol Control 46:380–390

Barazani O, Friedman J (2001) Allelopathic bacteria and their impact on higher plants. Crit Rev Microbiol 27:41–55

Barbosa AM, Souza CGM, Dekker RFH, Fonseca RC, Ferreira DT (2002) Phytotoxin produced by *Bipolaris euphorbiae in-vitro* is effective against the weed *Euphorbia heterophylla*. Braz Arch Biol Technol 45:233–240

Barreto RW, Evans HC (1998) Fungal pathogens of *Euphorbia heterophylla* and *E. hirta* in Brazil and their potential as weed biocontrol agents. Mycopathologia 141:21–36

Barton J (2005) Bioherbicides: all in a day's work… for a superhero. Manaaki Whenua, Landcare Research Ltd., Lincoln, pp 4–6

Beckie HJ, Lozinski C, Shirriff S, Brenzil CA (2013) Herbicide-resistant weeds in the Canadian prairies: 2007 to 2011. Weed Technol 27:171–183

Berg G, Grube M, Schloter M, Smalla K (2014) Unraveling the plant microbiome: looking back and future perspectives. Front Microbiol 5:148

Berkowitz DB, Charette BD, Karukurichi KR, McFadden JM (2006) α-Vinylic amino acids: occurrence, asymmetric synthesis, and biochemical mechanisms. Tetrahedron Asymmetry 17:869–882

Bertin C, Yang X, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83. https://doi.org/10.1023/A:1026290508166

Bhowmick R, Girotti AW (2010) Cytoprotective induction of nitric oxide synthase in a cellular model of 5-aminolevulinic acid-based photodynamic therapy. Free Radic Biol Med 48:1296–1301

Blackshaw RE, Brandt RN, Janzen HH, Entz T (2004) Weed species response to phosphorus fertilization. Weed Sci 52:406–412

Block A, Schmelz E, Jones JB, Klee HJ (2005) Coronatine and salicylic acid: the battle between *Arabidopsis* and *Pseudomonas* for phytohormone control. Mol Plant Pathol 6:79–83

Blumer C, Haas D (2000) Mechanism, regulation and ecological role of bacterial cyanide biosynthesis. Arch Microbiol 173:170–177

Bouizgarne B, El-Maarouf-Bouteau H, Madiona K, Biligui B, Monestiez M, Pennarun A, Amiar Z, Rona J, Ouhdouch Y, El Hadrami I, Bouteau F (2006) A putative role for fusaric acid in biocontrol of the parasitic angiosperm *Orobanche ramosa*. Mol Plant-Microbe Inter 19:550–556

Boyette CD (1991) Host range and virulence of *Colletotrichum truncatum*, a potential mycoherbicide for hemp sesbania (*Sesbania exaltata*). Plant Dis 75:62–64

Boyette CD, Hoagland RE (2013a) Bioherbicidal potential of a strain of *Xanthomonas* spp. for control of common cocklebur (X*anthium strumarium*). Biocontrol Sci Tech 23:183–196

Boyette CD, Hoagland RE (2015) Bioherbicidal potential of *Xanthomonas campestris* for controlling *Conyza canadensis*. Biocontrol Sci Tech 25:229–237

Boyette CD, Reddy KN, Hoagland RE (2006) Glyphosate and bioherbicide interaction for controlling kudzu (*Pueraria lobata*), redvine (*Brunnichia ovata*) and trumpet creeper (*Campsis radicans*). Biocontrol Sci Tech 16:1067–1077

Boyette CD, Hoagland RE, Weaver MA (2007) Biocontrol efficacy of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) is enhanced with unrefined corn oil and surfactant. Weed Biol Manag 7:70–76

Boyette CD, Gealy D, Hoagland RE, Vaughn KC, Bowling AJ (2011) Hemp sesbania (*Sesbania exaltata*) control in rice (*Oryza sativa*) with the bioherbicidal fungus *Colletotrichum gloeosporioides* f.sp. *aeschynomene* formulated in an invert emulsion. Biocontrol Sci Tech 21:1399–1407

Brar LS, Walia US (1993) Bioefficacy of sulphonylureas against *Phalaris minor* Retz. in wheat. Indian. J. Weed Sci 25: 1–5.

Broennimann O, Treier UA, Muller-Scharer H, Thuiller W, Peterson AT, Guisan A (2007) Evidence of climatic niche shift during biological invasion. Ecol Lett 10:701–709

Bulgarelli D, Schlaeppi Spaepen S, Ver L, van Themaat E, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Busby RR, Rodriguez G, Gebhart DL, Yannarell AC (2016) Native *Lespedeza* species harbor greater non-rhizobial bacterial diversity in root nodules compared to the coexisting invader, *L. cuneata*. Plant Soil 401:427–436

Caldwell CJ, Hynes RK, Boyetchko SM, Korber DR (2011) Colonization and bioherbicidal activity on green foxtail by *Pseudomonas fluorescens* BRG100 in a pesta formulation. Can J Microbiol 58:1–9

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427:731–733

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(6):e99949

Charudattan R (2001) Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. BioControl 46:229–260

Charudattan R (2005) Ecological, practical and political inputs into selection of weed targets: what makes a good biological control target? Biol Control 35:183–196

Chaudhary H, Peng G, Hu M, He Y, Yang L, Luo Y, Tan Z (2012) Genetic diversity of endophytic diazotrophs of the wild rice, *Oryza alta* and identification of the new diazotroph, *Acinetobacter oryzae* sp. nov. Microb Ecol 63:813–821

Chhokar RS, Sharma RK, Jat GR, Pundir AK, Gathala MK (2007) Effect of tillage and herbicides on weeds and productivity of wheat under rice-wheat growing system. Crop Prot 26:1689–1696

Chhokar RS, Sharma RK, Gill SC (2013) Compatibility of herbicides against grassy weeds in wheat. Indian J Weed Sci 45:239–242

Chutia M, Mahanta JJ, Saikia R, Boruah AKS, Sarma TC (2006) Effect of leaf blight disease on yield of oil and its constituents of *Java citronella* and *in vitro* control of the pathogen using essential oils. World J Agri Sci 2:319–321

Cimmino A, Andolfi A, Zonno MC, Avolio F, Santini A, Tuzi A (2013) Chenopodolin: a phytotoxic unrearranged entpimaradiene diterpene produced by *Phoma chenopodicola*, a fungal pathogen for *Chenopodium album* biocontrol. J Nat Prod 76:1291–1297

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonisation, mechanisms involved and prospects for utilisation. Soil Biol Biochem 42:669–678. https://doi.org/10.1016/j.soilbio.2009.11.024

Cummins I, Cole DJ, Edwards R (1999) A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. Plant J 18:285–292

Daigle DJ, Connick JWJ, Boyetchko SM (2002) Formulating a weed suppressive bacterium in 'pesta'. Weed Technol 16:407–413

Dane F, Shaw JJ (1996) Survival and persistence of bioluminescent *Xanthomonas campestris* pv. *campestris* on host and non-host plants in the field environment. J Appl Bacteriol 80:73–80

Daniel JT, Templeton GE, Smith RJ, Fox WT (1973) Biological control of northern joint vetch in rice with an endemic fungal disease. Weed Sci 21:303–307
Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173
de Luna L, Stubbs T, Kennedy A, Kremer R (2005) Deleterious bacteria in the rhizosphere. In: Zobel R, Wright S (eds) Roots and soil management: interactions between roots and the soil. Monograph no. 48, Madison, pp 233–261
de Luna L, Kennedy A, Hansen J, Paulitz T, Gallagher R, Fuerst E (2011) Mycobiota on wild oat (*Avena fatua* L.) seed and their caryopsis decay potential. Plant Health Prog 10:1–8
DeCoste NJ, Gadkar VJ, Filion M (2010) *Verticillium dahliae* alters *Pseudomonas* spp. populations and HCN gene expression in the rhizosphere of strawberry. Can J Microbiol 56:906–915
Dhaliwal HS, Singh R, Brar LS (2007) Impact analysis of factors affecting *Phalaris minor* infestation in wheat in Punjab. Indian J Weed Sci 39:66–73
Diaz R, Manrique V, Hibbard K, Fox A, Roda A, Gandolfo D (2014) Successful biological control of tropical soda apple (*Solanales: Solanaceae*) in Florida: a review of key program components. Florida Entomol 97:179–190
Dubeikovsky AN, Mordukhova EA, Kochetkov VV, Polikarpova FY, Boronin AM (1993) Growth promotion of blackcurrant softwood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased amount of indole-3-acetic acid. Soil Biol Biochem 25:1277–1281
Duffy BK, Défago G (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. Appl Environ Microbiol 65:2429–2438
Duke SO, Abbas HK, Boyette CD, Gohbara M (1991) Microbial compounds with the potential for herbicide use. Proceeding Brighten Crop Protection Confenence Weeds, Brighton, pp 155–164
Duke SO, Evidente A, Fiore M, Rimando AM, Vurro M, Chistiansen N, Looser R, Grossmann K (2011) Effects of the aglycone of ascaulitoxin on amino acid metabolism in *Lemna paucicostata*. Pestic Biochem Physiol 100:41–50
Edwards R, Brazier-Hicks M, Dixon DP, Cummins I (2005) Chemical manipulation of antioxidant defences in plants. Adv Bot Res 42:1–32
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 67:5285–5293
Elliott MS, Massey B, Cui X, Hiebert E, Charudattan R, Waipara N (2009) Supplemental host range of Araujia mosaic virus, a potential biological control agent of moth plant in New Zealand. Australas Plant Pathol 38:603–607
El-Shora HM, El-Amier YA, Awad MH (2016) Antimicrobial activity and allelopathic potential of *Zygophyllum coccineum* L. on *Chenopodium album* L. British J Appl Sci Technol 15:1–10
Elzein A, Kroschel J, Leth V (2006) Seed treatment technology: an attractive delivery system for controlling root parasitic weed *Striga* with mycoherbicide. Biocontrol Sci Tech 16:3–26
EPA (2015) Biopesticides Registration Action Document: Tobacco mild green mosaic tobamo virus strain U2. PC Code: 056705. United States Environmental Protection Agency. Available at: http://www.regulations.gov/#! Document Detail; D-EPA-HQ-OPP-2013-0759-0017
Evidente A, Andolfi A, Vurro M, Zonno MC, Motta A (2000) Trans-4 aminoproline, a phytotoxic metabolite with herbicidal activity produced by *Ascochyta caulina*. Phytochemistry 53:231–237
Evidente A, Andolfi A, Cimmino A (2011) Relationships between the stereochemistry and biological activity of fungal phytotoxins. Chirality 23:674–693
Evidente M, Cimmino A, Zonno MC, Masi M, Berestetskyi A, Santoro E, Superchi S, Vurro M, Evidente A (2015) Phytotoxins produced by *Phoma chenopodiicola*, a fungal pathogen of *Chenopodium album*. Phytochemistry 117:482–488
Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:45–51
Feigl F, Anger V (1966) Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. Analyst 91:282–284

Ferreira MI, Reinhardt CF (2016) Allelopathic weed suppression in agroecosystems: a review of theories and practices. Afr J Agric Res 11(6):450–459

Ferrell J, Charudattan R, Elliott M, Hiebert E (2008) Effects of selected herbicides on the efficacy of Tobacco mild green mosaic virus to control tropical soda apple (*Solanum viarum*). Weed Sci 56:128–132

Fickett ND, Boerboom CM, Stoltenberg DE (2013) Predicted corn yield loss due to weed competition prior to postemergence herbicide application on Wisconsin farms. Weed Technol 27:54–62

Fischer MS, Rodriguez RJ (2013) Fungal endophytes of invasive *Phragmites australis* populations vary in species composition and fungicide susceptibility. Symbiosis 61:55–62

Font MI, Cordoba-Selles MC, Cebrian MC, Herrera-Vasquez JA, Alfaro-Fernandez A, Boubaker A (2009) First report of tobacco mild green mosaic virus infecting *Capsicum annuum* in Tunisia. Plant Dis 93:761–761

Franke AC, Singh S, McRoberts N, Nehra AS, Godara S, Malik RK, Marshall G (2007) *Phalaris minor* seed bank studies: longevity, seedling emergence and seed production as affected by tillage regime. Weed Res 47:73–83

Frey SD, Lee J, Melillo JM, Six J (2013) The temperature response of soil microbial efficiency and its feedback to climate. Nat Clim Chang 3:395–398

Gasson MJ (1980) Indicator technique for antimetabolic toxin production by phytopathogenic species of *Pseudomonas*. Appl Environ Microbiol 39:25–29

Gealy DR, Gurusiddah S, Ogg AGJ, Kennedy AC (1996) Metabolites from *Pseudomonas fluorescens* strain D7 inhibits downy brome (*Bromus tectorum*) seedling growth. Weed Technol 10:282–287

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

Gerwick BC, Fields SS, Graupner PR, Gray JA, Chapin EL, Cleveland JA, Heim DR (1997) Pyridazocidin, a new microbial phytotoxin with activity in the Mehler reaction. Weed Sci 45:654–657

Gerwick BC, Brewster WK, Deboer GJ, Fields SC, Graupner PR, Hahn DR, Pearce CJ, Schmitzer PR, Webster JD (2013) Mevalocidin, a novel phloem mobile phytotoxin from *Fusarium* DA 056446 and *Rosellina* DA092917. J Chem Ecol 39:253–261

Giovanelli J, Owens LD, Mudd SH (1973) β-cystathionase. *In vivo* inactivation by rhizobitoxins and role of the enzyme in methionine biosynthesis in corn seedlings. Plant Physiol 51:492–503

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L, Stewart A (2012) Have biopesticides come of age? Trends Biotechnol 30:250–258

Gnanavel I (2015) Eco-friendly weed control options for sustainable agriculture. Sci Int 3:37–47

Gond SK, Bergen MS, Torres MS, White JF Jr (2015) Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiol Res 172:79–87

Graupner PR, Carr A, Clancy E, Gilbert J, Bailey KL, Derby JA (2003) The macrocidins: novel cyclic tetramic acids with herbicidal activity produced by *Phoma macrostoma*. J Nat Prod 66:1558–1561

Grossmann K (2010) Auxin herbicides: current status of mechanism and mode of action. Pest Manag Sci 66:113–120

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

Gurusiddaiah S, Gealy D, Kennedy A, Ogg AJ (1994) Isolation and characterization of metabolites from *Pseudomonas fluorescens* strain D7 for control of downy brome (*Bromus tectorum* L.). Weed Sci 42:492–501

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41:117–153

Harata K, Kubo Y (2014) Ras GTPase activating protein CoIra1 is involved in infection-related morphogenesis by regulating cAMP and MAPK signaling pathways through CoRas2 in *Colletotrichum orbiculare*. PLoS One 9:e109045

Harding DP, Riazada MN (2015) Controlling weeds with fungi, bacteria and viruses: a review. Front Plant Sci 6:659–667

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 79:293–320

Hassan S, Mathesius U (2011) The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant-microbe interactions. J Exp Bot 63(9):3429–3444. https://doi.org/10.1093/jxb/err430

Heap I (2006) International survey of herbicide resistant weeds. https://www.weedscience.org/.in.asp

Hoagland R, Boyette C, Abbas H (2007) *Myrothecium verrucaria* isolates and formulations as bioherbicide agents for kudzu. Biocontrol Sci Tech 17:721–731

Hotta Y, Tanaka T, Takaoka H, Takeuchi Y, Konnai M (1997) New physiological effects of 5-aminolevulinic acid in plants: the increase of photosynthesis, chlorophyll content and plant growth. Biosci Biotechnol Biochem 61:2025–2028

Hwang J, Chilton WS, Benson DM (2002) Pyrrolnitrin production by *Burkholderia cepacia* and biocontrol of *Rhizoctonia* stem rot of poinsettia. Biol Control 25:56–63

Hynes RK, Boyetchko SM (2006) Research initiatives in the art and science of biopesticide formulations. Soil Biol Biochem 38:845–849

Hynes RK, Boyetchko SM (2011) Improvement to the "pesta" formulation to promote survival and dispersal of *Pseudomonas fluorescens* BRG100 green foxtail bioherbicide. Pest Technol 5:80–87

Hynes RK, Chumala PB, Hupka D, Peng G (2010) A complex coacervate formulation for delivery of *Colletotrichum truncatum* 00-003B1. Weed Technol 24:185–192

Ibekwe AM, Kennedy AC, Stubbs TL (2010) An assessment of environmental conditions for control of downy brome by *Pseudomonas fluorescens* D7. Int J Environ Technol Manag 12:27–46

Ichihara A, Shiraishi K, Sato H, Sakamura S, Nishiyama K, Sakai R, Furusaki A, Matsumotu T (1977) The structure of coronatine. J Chem Soc 99:636–637

Imaizumi S, Nishino T, Miyabe K, Fujimori T, Yamada M (1997) Biological control of annual bluegrass (*Poa annua* L.) with a Japanese isolate of *Xanthomonas campestris* pv. *poae* (JT-P482). Biol Control 8:7–14

Imaizumi S, Honda M, Fujimori T (1999) Effect of temperature on the control of annual bluegrass (*Poa annua* L.) with *Xanthomonas campestris* pv. *poae* (JT-P482). Biol Control 16:13–17

Javaid A, Adrees H (2009) *Parthenium* management by cultural filtrates of phytopathogenic fungi. Nat Prod Res 23:1541–1551

Johnson A, Booth C (1983) Plant pathologist's pocket book. 2nd ed., Surrey, Commonwealth Agricultural Bureaux, Slough

Juan Y, Wei W, Peng Y, Bu T, Zheng Y, Li-hui Z, Jin-gao D (2015) Isolation and identification of *Serratia marcescens* Ha1 and herbicidal activity of Ha1 'pesta' granular formulation. J Integr Agri 14:1348–1355

Kadir J, Ahmad A, Sariah M, Juraimi AS (2003) Potential of *Drechslera longirostrata* as bioherbicide for itch grass (*Rottboellia cochinchinensis*). Proceedings of the 19th Asian-Pacific Weed Science Society Conference, 17–21 Mar. 2003, Manila: Weed Science Society of the Philippines, pp 450–455

Kang Z, Zhang J, Zhou J, Qi Q, Du G, Chen J (2012) Recent advances in microbial production of δ-aminolevulinic acid and vitamin B12. Biotechnol Adv 30:1533–1542

Kao-Kniffin J, Carver SM, DiTommaso A (2013) Advancing weed management strategies using metagenomic techniques. Weed Sci 61:171–184

Kataryan BT, Torgashova GG (1976) Spectrum of herbicidal activity of 2, 4-diacetyl phloroglucinol. Dokl Akadmy Nauk Armyan SSR 63:109–112

Kazinczi G, Lukacs D, Takacs A, Horvath J, Gaborjanyi R, Nadasy M (2006) Biological decline of *Solanum nigrum* due to virus infections. J Plant Dis Protect 32:325–330
Kennedy A (1999) Bacterial diversity in agroecosystems. Agric Ecosyst Environ 74:65–76
Kennedy A, Stubbs T (2007) Management effects on the incidence of jointed goatgrass inhibitory rhizobacteria. Biol Control Theor Applic Pest Manag 40:213–221
Kennedy AC, Elliott LF, Young FL, Douglas CL (1991) Rhizobacteria suppressive to the weed downy brome. Am J Soil Sci Soc 55:722–727
Kennedy AC, Johnson BN, Stubbs TL (2001) Host range of a deleterious rhizobacterium for biological control of downy brome. Weed Sci 49:792–797
Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M (2009) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Res J Agric Biol Sci 1:48–58
Khandelwal A (2016) Evaluation of herbicidal potential of rhizosphere bacteria against bathu (*Chenopodium album)* and piazi (*Asphodelus tenuifolius)* weeds. Ph.D. thesis. Chaudhary Charan Singh Haryana Agricultural University, Hisar
Khattak SU, Iqbal Z, Lutfullah G, Bacha N, Khan AA, Saeed M, Ali M (2014) Phytotoxic and herbicidal activities of *Aspergillus* and *Penicillium* species isolated from rhizosphere and soil. Pakistan J Weed Sci Res 20:293–303
Kim SJ, Kremer RJ (2005) Scanning and transmission electron microscopy of root colonization of morning glory (*Ipomoea* spp.) seedlings by rhizobacteria. Symbiosis 39:117–124
Kim YC, Leveau J, McSpadden Gardener BB, Pierson EA, Pierson LS 3rd, Ryu C (2011) The multifactorial basis for plant health promotion by plant-associated bacteria. Appl Environ Microbiol 77:1548–1555. https://doi.org/10.1128/AEM.01867-10
Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomous JN, Lee H, Trevors JT (2004) Methods of studying soil microbial diversity. J Microbiol Methods 58:169–188
Kloepper JW, McInroy JA, Liu K (2013) Symptoms of fern distortion syndrome resulting from inoculation with opportunistic endophytic fluorescent *Pseudomonas* spp. PLoS One 8, e58531
Kohlschmid E, Sauerborn J, Muller-stover D (2009) Impact of *Fusarium oxysporum* on the holoparasitic weed *Phelipanche ramosa*: biocontrol efficacy under field-grown conditions. Weed Res 49:56–65
Kollmann J, Banuelos MJ, Nielsen SL (2007) Effects of virus infection on growth of the invasive alien *Impatiens glandulifera*. Preslia 79:33–44
Kostov T, Pacanoski Z (2007) Weeds with major economic impact on agriculture in Republic of Macedonia. Pakistan J Weed Sci Res 13:227–239
Kourtev PS, Ehrenfeld JG, Haggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166
Kremer RJ (2000) Growth suppression of annual weeds by deleterious rhizobacteria integrated with cover crops. In: Spencer NR (ed) Proceedings of the X international symposium on biological control of weeds. Montana State University, Bozeman, pp 931–940
Kremer RJ, Kennedy AC (1996) Rhizobacteria as biocontrol agents of weeds. Weed Technol 10:601–609
Kremer R, Souissi T (2001) Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. Curr Microbiol 43:182–186
Kremer RJ, Begonia MFT, Stanley L, Lanham ET (1990) Characterization of rhizobacteria associated with weed seedlings. Appl Environ Microbiol 56:1649–1655
Kroschel J, Elzein A (2004) Bioherbicidal effect of fumonisin B1, a phytotoxic metabolite naturally produced by *Fusarium nygamai*, on parasitic weeds of the genus *Striga*. Biocontrol Sci Tech 14:117–128
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
Kumar V, Ladha JK (2011) Direct seeding of rice: recent developments and future research needs. Adv Agron 111:299–413

Lakshmanan V, Kitto SL, Caplan JL, Hsueh YH, Kearns DB, Bais HP (2012) Microbe-associated molecular patterns-triggered root responses mediate beneficial rhizobacterial recruitment in *Arabidopsis*. Plant Physiol 160(3):1642–1661

Lakshmanan V, Castaneda R, Rudrappa T, Bais HP (2013) Root transcriptome analysis of *Arabidopsis thaliana* exposed to beneficial *Bacillus subtilis* FB17 rhizobacteria revealed genes for bacterial recruitment and plant defense independent of malate efflux. Planta 238(4):657–668

Lakshmi V, Kumari S, Singh A, Prabha C (2015) Isolation and characterization of deleterious *Pseudomonas aeruginosa* KC1 from rhizospheric soils and its interaction with weed seedlings. J King Saud Univ Sci 27:113–119

Lareen A, Burton F, Schafer P (2016) Plant root-microbe communication in shaping root microbiomes. Plant Mol Biol 90:575–587

Lee HB, Kim CJ, Kim JS, Hong KS, Cho KY (2003) A bleaching herbicidal activity of methoxyhygromycin (MHM) produced by an actinomycete strain *Streptomyces* sp. 8E-12. Lett Appl Microbiol 36:387–391

Lehman RM, Acosta-Martinez V, Buyer JS, Cambardella CA, Collins HP, Ducey TF, Halvorson JJ, Jin VL, Johnson JM, Kremer RJ, Lundgren JG (2015) Soil biology for resilient, healthy soil. J Soil Water Conserv 70(1):12A–18A

Lemerle D, Verbeek B, Orchard B (2001) Ranking the ability of wheat varieties to compete with *Lolium rigidum*. Weed Res 41:197–209

Leuchtmann A (1997) Ecological diversity in *Neotyphodium*-infected grasses as influenced by host and fungus characteristics. In: Bacon CW, Hill NS (eds) *Neotyphodium*/grass interactions. Springer, Boston, pp 93–108

Li J, Kremer RJ (2006) Growth response of weed and crop seedlings to deleterious rhizobacteria. Biol Control 39:58–65

Li JW-H, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325:161–165

Li M, Jordan NR, Koide RT, Yannarell AC, Davis AS (2016) Meta-analysis of crop and weed growth responses to arbuscular mycorrhizal fungi: implications for integrated weed management. Weed Sci 64:642–652

Liebman M, Mohler CL, Staver CP (2001) Ecological management of agricultural weeds. Cambridge University Press, Cambridge, p 532

Lorck H (1948) Production of hydrocyanic acid by bacteria. Physiol Plant 1:142–146

Loretta OR, Martin M, Williams II (2006) Conidial germination and germ tube elongation of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* on leaf surfaces of seven *Amaranthus* species: implications for biological control. Biol Control 38:356–362

Lydon J, Kong H, Murphy C, Zhang W (2011) The biology and biological activity of *Pseudomonas syringae* pv. *tagetis*. Pest Technol 5:48–55

Malik DK, Sindhu SS (2011) Production of indole acetic acid by *Pseudomonas* sp.: effect of coinoculation with *Mesorhizobium* sp. *Cicer* on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiol Mol Biol Plants 17:25–32

Malik RK, Singh S (1995) Little seed canary grass (*Phalaris minor* Retz.) resistance in India. Weed Technol 9:419–425

Marinov-Serafimoov P, Dimitrova T (2007) Dynamics and distribution of the main weeds in weed associations of some grain legume crops. Plant Sci 44:167–173

Marinov-Serafimov P (2005) Study on the competitive relationship between soybean and black nightshade (*Solanum nigrum* L.) under conditions of leached black earth in northern Bulgaria. Ph. D. thesis

Massenssini AM, Bonduki VH, Melo CA, Totola MR, Ferreira FA, Costa MD (2014) Soil microorganisms and their role in the interactions between weeds and crops. Planta Daninha 32(4):873–884

Mazzola M, Stahlman PW, Leach JE (1995) Application method affects the distribution and efficacy of rhizobacteria suppressive of downy brome (*Bromus tectorum*). Soil Biol Biochem 27:1271–1278

McPhail KL, Armstrong DJ, Azevedo MD, Banowetz GM, Mills DI (2010) 4-Formylaminooxyvinyl glycine, an herbicidal germination-arrest factor from *Pseudomonas* rhizosphere bacteria. J Nat Prod 73:1853–1857

Mejri D, Gamalero E, Tombolini R, Musso C, Massa N, Berta G, Souissi T (2010) Biological control of great brome (*Bromus diandrus*) in durum wheat (*Triticum durum*): specificity, physiological traits and impact on plant growth and root architecture of the fluorescent pseudomonad strain X33d. BioControl 55:561–572

Menaria BL (2007) Bioherbicides: an eco-friendly approach to weed management. Curr Sci 92:10–11

Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100. https://doi.org/10.1126/science.1203980

Mendoza EKM, Violante HGM, Inocencio CM, Salcedo GO, Madrigal HC, Portugal VO, Pérez MVA (2012) Effects of *Bacillus subtilis* extracts on weed seed germination of *Sorghum halepense* and *Amaranthus hybridus*. Afr J Microbiol Res 6:1887–1892

Miller-Wideman M, Makkar N, Tran M, Isaac B, Biest N, Stonard R (1992) Herboxidiene, a new herbicidal substance from *Streptomyces chromofuscus* A7847. Taxonomy, fermentation, isolation, physio-chemical and biological properties. J Antibiot 45:914–921

Montanez 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

Morin L, Evans KJ, Sheppard AW (2006) Selection of pathogen agents in weed biological control: critical issues and peculiarities in relation to arthropod agents. Aust J Entomol 45:349–365

Morra MJ, Kirkegaard JA (2002) Isothiocyanate release from soil-incorporated *Brassica* tissues. Soil Biol Biochem 34:1683–1690

Morris PF, Bone E, Tyler BM (1998) Chemotropic and contact responses of *Phytophthora sojae* hyphae to soybean isoflavonoids and artificial substrates. Plant Physiol 117(4):1171–1178

Mortensen K (1988) The potential of an endemic fungus, *Colletotrichum gloeosporioides*, for biological control of round-leaved mallow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*). Weed Sci 36:473–478

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(4):e35498

Neumann S, Boland GJ (1999) Influence of selected adjuvants on disease severity by *Phoma herbarum* on dandelion (*Taraxacum officinale*). Weed Technol 13:675–679

Ngigi AN, Getenga ZM, Boga HI, Ndalut PK (2012) Biodegradation of s-triazine herbicide atrazine by *Enterobacter cloacae* and *Burkholderia cepacia* sp. from long-term treated sugarcane-cultivated soils in Kenya. J Environ Sci Health B 47:769–778

Nishino T, Murao S, Wada H (1984) Mechanism of inactivation of pyridoxal phosphate-linked aspartate transaminase by gostatin. J Biochem 95:1283–1288

Norman MA, Patten KD, Gurusiddaiah S (1994) Evaluation of a phytotoxin(s) from *Pseudomonas syringae* for weed control in cranberries. Hortic Sci 29:1475–1477

Oettmeier W, Dostatni R, Majewski C, Hoefle G, Fecker T, Kunze B, Reichenbac H (1990) The aurachins, naturally occurring inhibitors of photosynthetic electron flow through photosystem II and cytochrome b6/f-complex. Z Natureforsch 45:322–328

Ohra J, Morita K, Tsujino Y, Tazaki H, Fujimori T, Goering M, Evans S, Zorner P (1995) Production of the phytotoxic metabolite, ferricrocin, by the fungus *Colletotrichum gloeosporioides*. Biosci Biotechnol Biochem 59:113–114

Oldroyd GED (2013) Speak, friend, and enter: signaling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

Olesen JE, Hansen PK, Berntsen J, Christensen S (2004) Simulation of above-ground suppression of competing species and competition tolerance in winter wheat varieties. Field Crop Res 89:263–280

Oluwaseun AC, Kola OJ, Isaac A (2016) Persistence of bioherbicidal agents formulated from the multi-combination of the wild and mutant strain of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa*. Am-Eurasian J Agric Environ Sci 16:1406–1416

Owen A, Zdor R (2001) Effect of cyanogenic rhizobacteria on the growth of velvetleaf (*Abutilon theophrasti*) and corn (*Zea mays*) in autoclaved soil and the influence of supplemental glycine. Soil Biol Biochem 33:801–809

Park J, Radhakrishnan R, Kang S, Lee I (2015) IAA producing *Enterobacter* sp. I-3 as a potent bio-herbicide candidate for weed control: a special reference with lettuce growth inhibition. Indian J Microbiol 55:207–212

Parker C (2009) Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Manag Sci 65:453–459

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6(10):763–775

Patil VS (2013) Rhizospheric bacteria with the potential for biological control of *Parthenium hysterophorus*. J Chem Biol Phys Sci 3:2679–2686

Patil VS (2014) Isolation, characterization and identification of rhizospheric bacteria with the potential for biological control of *Sida acuta*. J Environ Res Dev 8:411–417

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2015) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90:635–644. https://doi.org/10.1007/s11103-015-0337-7

Petersen J, Belz R, Walker F, Hurle K (2001) Weed suppression by release of isothiocyanates from turnip-rape mulch. Agron J 93:37–43

Phour M (2012) Biological control of *Phalaris minor* in wheat (*Triticum aestivum* L.) using rhizosphere bacteria. M. Sc. thesis. Chaudhary Charan Singh Haryana Agricultural University, Hisar

Phour M (2016) Aminolevulinic acid production by rhizobacteria: its role in salt tolerance and weed control in mustard [*Brassica juncea* (L.)]. Ph. D. thesis. Chaudhary Charan Singh Haryana Agricultural University, Hisar

PMRA (2006) "Re-evaluation of *Colletotrichum gloeosporioides* f.sp. *malvae* [CGM]" REV2006–10. Health Canada, Ottawa

PMRA (2010) "*Sclerotinia minor* strain IMI344141" RD2010-08. Health Canada, Ottawa

Prikyrl Z, Vančura V, Wurst M (1985) Auxin formation by rhizosphere bacteria as a factor of root growth. Biol Plant 27:159–163. https://doi.org/10.1007/BF02902155

Quail JW, Ismail N, Pedras MSC, Boyetchko SM (2002) Pseudophomins A and B, a class of cyclic lipodepsipeptides isolated from a *Pseudomonas* species. Acta Crystallographica, section C: crystal structure. Communications 58:268–271

Raaijmakers JM, Weller DM (1998) Natural plant protection by 2, 4- diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. Mol Plant-Microbe Interact 11:144–152

Raaijmakers JM, Weller AM, Thomashow LS (1997) Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. Appl Environ Microbiol 63(3):881–887

Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81:537–547

Ramyasmruthi S, Pallavi O, Pallavi S, Tilak K, Srividya S (2012) Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from *Solanaceae* rhizosphere effective against broad spectrum fungal phytopathogens. Asian J Plant Sci Res 2:16–24

Rasmann S, Turlings TCJ (2016) Root signals that mediate mutualistic interactions in the rhizosphere. Curr Opin Plant Biol 32:62–68

Ray P, Vijayachandran LS (2013) Evaluation of indigenous fungal pathogens from horse purslane (*Trianthema portulacastrum*) for their relative virulence and host range assessments to select a potential mycoherbicidal agent. Weed Sci 61:580–585

Riddle GE, Burpee LL, Boland GJ (1991) Virulence of *Sclerotinia sclerotiorum* and *S. minor* on dandelion (*Taraxacum officinale*). Weed Sci 39:109–118

Roberts KJ, Anderson RC (2001) Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. Am Midl Nat 146:146–152

Rodrigues RR, Pineda RP, Barney JN, Nilsen ET, Barrett JE, Williams MA (2015) Plant invasions associated with change in root-zone microbial community structure and diversity. PLoS One 10:e0141424

Rout ME, Chrzanowski TH, Westlie TK, DeLuca TH, Callaway RM, Holben WE (2013) Bacterial endophytes enhance competition by invasive plants. Am J Bot 100:1726–1737

Rubiales D, Fernández-Aparicio M (2012) Innovations in parasitic weeds management in legume crops. Agro Sustain Develop 32:433–449

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556. https://doi.org/10.1104/pp.108.127613

Ryall B, Mitchell H, Mossialos D, Williams HD (2009) Cyanogenesis by the entomopathogenic bacterium *Pseudomonas entomophila*. Lett Appl Microbiol 49:131–135

Saikkonen K, Wäli P, Helander M, Faeth SH (2004) Evolution of endophyte–plant symbioses. Trends Plant Sci 9(6):275–280

Sanchez Márquez S, Bills GF, Herrero N, Zabalgogeazcoa Í (2012) Non-systemic fungal endophytes of grasses. Fungal Ecol 5(3):289–297

Sarwar M, Kremmer RJ (1995) Enhanced suppression of plant growth through production of L-tryptophan compounds by deleterious rhizobacteria. Plant Soil 172:261–269

Sasaki K, Tanaka T, Nishio N, Nagai S (1993) Effect of culture pH on the extracellular production of 5-aminolevulinic acid by *Rhodobacter sphaeroides* from volatile fatty acid. Biotechnol Lett 15:859–864

Sasikala C, Ramana CV, Rao PR (1994) 5-aminolevulinic acid: a potential herbicide/insecticide from microorganisms. Biotechnol Prog 10:451–459

Sayed MHE, Aziz ZKA, Abouzaid AM (2014) Efficacy of extracellular metabolite produced by *Streptomyces levis* strain LX-65 as a potential herbicidal agent. J Am Sci 10:169–180

Schisler DA, Howard KM, Bothast RJ (1991) Enhancement of disease caused by *Colletotrichum truncatum* in *Sesbania exaltata* by coinoculating with epiphytic bacteria. Biol Control 1:261–268

Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. Mol Plant-Microbe Inter 28(3):212–217

Selvakumar G, Lenin M, Thamizhiniyan P, Ravimycin T (2009) Response of biofertilizers on the growth and yield of blackgram (*Vigna mungo*). Recom Res Sci Technol 1:169–175

Senthilkumar M, Anandham R, Madhaiyan M, Venkateswaran V, Sa T (2011) Endophytic bacteria: perspectives and applications in agricultural crop production. In: Bacteria in agrobiology: crop ecosystems. Springer, Berlin/Heidelberg, pp 61–96

Sessitsch A, Hardoim P, Doring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Inter 25:28–36

Shaw LJ, Burns RG (2004) Enhanced mineralization of [U-14C] 2, 4-dichlorophenoxyacetic acid in soil from the rhizosphere of *Trifolium pratense*. Appl Environ Microbiol 70:4766–4774

Shaw RH, Bryner S, Tanner R (2009) The life history and host range of the Japanese knot weed psyllid, *Aphalara itadori* Shinji: potentially the first classical biological weed control agent for the European Union. Biol Control 49:105–113

Singh S (2006) Herbicide resistance mechanism in *Phalaris minor* and its consequences on management strategies. Indian J Weed Sci 38:183–193

Singh S (2007) Role of management practices on control of isoproturon resistant little seed canary grass (*Phalaris minor*) in India. Weed Technol 21:339–346

Soares WL, Porto MFS (2009) Estimating the social cost of pesticide use: an assessment from acute poisoning in Brazil. Ecol Econ 68:2721–2728

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:a001438

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Stewart-Wade SM, Boland GJ (2005) Oil emulsions increase efficacy of *Phoma herbarum* to control dandelion but are phytotoxic. Biocontrol Sci Tech 15:671–681

Stubbs TL, Kennedy AC (2012) Microbial weed control and microbial herbicides. In: Alvarez-Fernandez R (ed.). INTECH DOI:https://doi.org/10.5772/32705

Sturz A, Matheson B, Arsenault W, Kimpinski J, Christie BR (2001) Weeds as a source of plant growth promoting rhizobacteria in agricultural soils. Can J Microbiol 47:1013–1024

Suslow TV, Schroth MN (1982) Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111–115

Suzuki S, Yuxi H, Oyaizu H, He Y (2003) Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. Curr Microbiol 47:138–143

Takahashi E, Kimura T, Nakamura K, Arahira M, Iida M (1995) Phosphonothrixin, a novel herbicidal antibiotic produced by *Saccharothrix* sp. ST 888, I. Taxonomy, fermentation isolation and biological properties. J Antibiot 48:1124–1129

Tateno A (2000) Herbicidal composition for the control of annual bluegrass. U.S. Patent No 6162763A. U.S. Patent and Trademark Office, Washington, DC

TeBeest D (1982) Survival of *Colletotrichum gloeosporioide*s f. sp. *aeschynomene* in rice irrigation water and soil [used as biocontrol for the weed]. Plant Dis 66:469–472

Templeton GE (1988) Biological control of weeds. Am J Altern Agri 3:69–72

Tetard-Jones C, Edwards R (2016) Potential roles for microbial endophytes in herbicide tolerance in plants. Pest Manag Sci 72:203–209

Tosiah S, Kadir J, Sariah M, Juraimi AS, Lo NP, Soetikno S (2009) Survey and evaluation of native fungal pathogens for biocontrol of barnyard grass (*Echinochloa crus-galli* complex). J Trop Agric Food Sci 37:119–128

Tosiah S, Kadir J, Sariah M, Juraimi AS, Soetikno S (2011) Efficacy of *Exserohilum monoceras*, a potential fungi for biocontrol of *Echinochloa* species. J Trop Agric Food Sci 39:117–124

van Loon LC, Bakker PAHM (2006) Root associated bacteria inducing systemic resistance. In: Gnanamanickam SS (ed) Plant associated bacteria. Springer, Dordrecht, pp 269–316

van Overbeek LS, Franke AC, Nijhuis EH, Groeneveld RM, da Rocha UN, Lotz LA (2011) Bacterial communities associated with *Chenopodium album* and *Stellaria media* seeds from arable soils. Microb Ecol 62:257–264

Vickery P, Wheeler J, Mulcahy C (1987) Factors affecting the hydrogen cyanide potential of white clover (*Trifolium repens* L.). Aust J Agric Res 38:1053–1059

Vyas P, Gulati A (2009) Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. BMC Microbiol 22:1–15

Wang P, Zhang X, Kong C (2013) The response of allelopathic rice growth and microbial feedback to barnyard grass infestation in a paddy field experiment. Eur J Soil Biol 56:26–32

Wani PA, Khan MS, Zaidi A (2007) Co-inoculation of nitrogen-fixing and phosphate solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. Acta Agron Hungarica 55:315–323

Wani PA, Khan MS, Zaidi A (2008) Impact of zinc-tolerant plant growth promoting rhizobacteria on lentil grown in zinc amended soil. Agron Sustain Dev 28:449–455

Watrous J, Roach P, Alexandrov T, Heath B, Yang JY, Kersten RD, van der Voort M, Pogliano K, Gross H, Raaijmakers JM, Moore BS, Laskin J, Bandeira N, Dorrestein PC (2012) Mass spectral molecular networking of living microbial colonies. Proc Natl Acad Sci U S A 109:1743–1752. https://doi.org/10.1073/pnas.1203689109

Weissmann R, Uggla C, Gerhardson B (2003) Field performance of a weed-suppressing *Serratia plymuthica* strain applied with conventional spraying equipment. Biol Control 48:725–742

Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348. https://doi.org/10.1146/annurev.phyto.40.030402.110010

Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. Bioscience 55:477–487

Wood AR, Morris MJ (2007) Impact of the gall-forming rust fungus *Uromycladium tepperianum* on the invasive tree *Acacia saligna* in South Africa: 15 years of monitoring. Biol Control 41:68–77

Xie H, Pasternack JJ, Glick BR (1996) Isolation and characterization of mutants of plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indole acetic acid. Curr Microbiol 32:67–71

Yandoc CB, Rosskopf EN, Pitelli A, Charudattan R (2006) Effect of selected pesticides on conidial germination and mycelia growth of *Dactylaria higginsii*, a potential bioherbicide for purple nutsedge (*Cyperus rotundus*). Weed Technol 20:255–260

Yang Z (2000) Maximum likelihood analysis of molecular adaptation in abalone sperm lysin reveals variable selective pressures among lineages and sites. Mol Biol Evol 17:1446–1455

Yang J, Cao HZ, Wang W, Zhang LH, Dong JG (2014) Isolation, identification and herbicidal activity of metabolites produced by *Pseudomonas aeruginosa* CB-4. J Integr Agric 13:1719–1726

Yorinori JT, Gazziero LP (1989) Control of milk weed (*Euphorbia heterophylla*) with *Helminthosporium* sp. In: Delfosse ES (ed) Proceedings 7th international symposium on biological control of weeds, 6–11 March, 1988. Istituto sperimentale per la patologia vegetale, Rome, pp 571–576

You C, Zhou F (1989) Non-nodular endorhizospheric nitrogen fixation in wetland rice. Can J Microbiol 35:403–408

Zachow C, Jahanshah G, de Bruijn I, Song C, Ianni F, Pataj Z, Gerhardt H, Pianet I, Lämmerhofer M, Berg G, Gross H (2015) The novel lipopeptide poaeamide of the endophyte *Pseudomonas poae* RE 1-1-14 is involved in pathogen suppression and root colonization. Mol Plant-Microbe Interact 28(7):800–810

Zdor R, Alexander C, Kremer R (2005) Weed suppression by deleterious rhizobacteria is affected by formulation and soil properties. Commun Soil Sci Plant Anal 36:1289–1299

Zeller S, Brandl H, Schmid B (2007) Host-plant selectivity of rhizobacteria in a crop weed model system. PLoS One 2:1–7

Zeng RS (2014) Allelopathy-the solution is indirect. J Chem Ecol 40:515–516

Zermane N, Souissi T, Kroschel J, Sikora R (2007) Biocontrol of broom rape (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.) by *Pseudomonas fluorescens* isolate Bf7-9 from the faba bean rhizosphere. Biocontrol Sci Tech 17:487–497

Zhang ZJ, Li HZ, Zhou WJ, Takeuchi Y, Yoneyama K (2006) Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers *in vitro*. Plant Growth Regul 49:27–34

Zhang J, Wang W, Lu X, Xu Y, Zhang X (2010) The stability and degradation of a new biological pesticide, pyoluteorin. Pest Manag Sci 66:248–252

Zhao S, Shamoun S (2005) Effects of potato dextrose broth and gelatin on germination and efficacy of *Phoma exigua*, a potential biocontrol agent for salal (*Gaultheria shallon*). Can J Plant Pathol 27:234–244

Zhou L, Bailey K, Derby J (2004) Plant colonization and environmental fate of the biocontrol fungus *Phoma macrostoma*. Biol Control 30:634–644

Zidack NK, Quimby PC (2002) Formulation of bacteria for biological control using the stabilize method. Biocontrol Sci Tech 12:67–74

Zonno MC, Vurro M, Luceretti S, Andolfi A, Perrone C, Evidente A (2008) Phyllostictine A, potential herbicide produced by *Phyllosticta cirsii*: *in vitro* production and toxicity. Plant Sci 175:818–828

Zuo S, Li X, Ma Y, Yang S (2014) Soil microbes are linked to the allelopathic potential of different wheat genotypes. Plant Soil 378:49–58

Biological Nitrogen Fixation: The Role of Underutilized Leguminous Plants

20

Olubukola Oluranti Babalola, Oluwaseyi Samuel Olanrewaju, Teresa Dias, Caroline Fadeke Ajilogba, Funso Raphael Kutu, and Cristina Cruz

Abstract

Soils in different parts of the world are generally being depleted of nitrogen (N), and this has now become a huge challenge to food production and security. Different sources of nutrients for enriching the soil have been evaluated in the past years especially the use of chemical fertilizers, but its usage is gradually dwindling as a result of numerous constraints, among which are environmental pollution, health challenges, and the negative impact of climate change. Better alternative strategies of replacing depleted soil N have been researched which include biological N fixation (BNF) using leguminous crops. Leguminous crops planted as cover crops, together with the symbiotic activities between root nodule bacteria and legumes, are the source of biologically fixed N. Because of the genetic diversity in legumes, there are so many underutilized leguminous crops whose potentials have not been fully tapped to understand their functionalities within the realm of BNF. This chapter brings to the limelight some of these legumes for biotechnological purpose in a bid to find a solution to soil infertility using the available cropping systems.

O.O. Babalola (✉) • O.S. Olanrewaju • C.F. Ajilogba • F.R. Kutu
Food Security and Safety Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa
e-mail: olubukola.babalola@nwu.ac.za

T. Dias • C. Cruz
Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências da Universidade de Lisboa,
Edifício C2, Piso 5, Sala 2.5.03, Campo Grande, 1749-016 Lisbon, Portugal

D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_20

20.1 Introduction

The well-being of plants revolves around their basic associations and the outcome of these associations with their immediate surroundings. The surroundings contain, among others, different organisms which might be beneficial, pathogenic, eukaryotes, and prokaryotes. Apart from the organisms, nutrients present in the soil are also a very important factor in relation to plant health and soil productivity. Many of these nutrients are either essential or nonessential and classified as macronutrients when required in large quantity or micronutrients when required in small quantity. One of the macronutrients (major nutrients) is nitrogen (N). Nitrogen is an essential macronutrient present in many life-sustaining biomolecules (Smil 2004; Hoffman et al. 2014). Although it is abundant in the atmosphere, most organisms still cannot metabolize and use it because of its inert nature as it exists in the dinitrogen (N_2) form. The only available form for its use by most organisms is in the fixed form either as ammonia or nitrate (Jia and Quadrelli 2014; Canfield et al. 2010; Thamdrup 2012; Santi et al. 2013). Sources of nitrogen fixation can be both biological and nonbiological. Such nonbiological nitrogen fixation sources include lightning, combustion, and industry, while sources of biological nitrogen fixation include agricultural lands, the sea and forests, and nonagricultural lands (Nna-Mvondo et al. 2005; Bhattacharyya and Jha 2012). Fixed N forms are always being separated into sediments making them unavailable, and they are also being converted to nitrogen gas through nitrification and denitrification. The conversion of N to ammonia is very essential to life, and it is termed N fixation (Jia and Quadrelli 2014; Thamdrup 2012). It occurs in three ways which are geochemical process, biological process, and industrial process (Canfield et al. 2010; Gruber and Galloway 2008; McGlynn et al. 2013; Haber 1922) (Fig. 20.1).

Biological process of N fixation is carried out by the actions of the nitrogenase enzyme, which is present in some microorganisms (Hoffman et al. 2014; Dos Santos et al. 2012), and it is commonly referred to as biological N fixation (BNF). Rhizobium

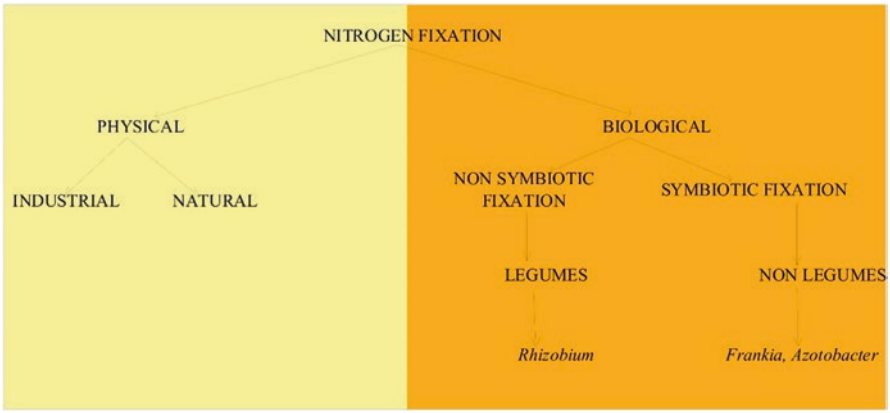


Fig. 20.1 Overview of nitrogen fixation

is a proteobacteria that makes use of the solar energy captured by plants to break the bond in dinitrogen forming reactive nitrogen species such as ammonium ion (Hoffman et al. 2014). Wagner (2012) indicated that microbes such as *Azotobacter*, *Frankia*, etc. also carry out nitrogen fixation in nonleguminous plants (Fig. 20.1).

20.2 Biological Nitrogen Fixation (BNF)

Biologically, different living organisms fix nitrogen in the soil and make it available to plants for proper functioning. Some of these organisms form symbiotic relationships with other plants, animals, or microorganisms (such as *Rhizobium* species in symbiosis with organisms like termites and protozoa, while others are free-living) (Remigi et al. 2016; Laranjo et al. 2014). Nitrogen is also fixed by the different activities of bacteria and fungi that break down organic matter in the soil and invariably release nitrogen that can be used by other organisms and in particular plants from the soil (Santi et al. 2013; Cooper and Scherer 2012). BNF changes inert N_2 into biologically useful NH_3 mediated in nature only by N-fixing bacteria such as *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (Lindström et al. 2015; Aserse 2013). Nitrogen fixation by legumes is a partnership between a bacterium and a plant. The process of BNF is bacteria-mediated and the product is accessible by plants (Doyle 2016). It is a process whereby an enzyme, nitrogenase, is used to reduce atmospheric N to ammonia (Liu et al. 2016). This bacteria-mediated process can be a result of microorganisms that are free-living in the soil and/or bacteria that are in symbiotic association with higher plants. Higher plants, in particular, the Leguminosae family, fix nitrogen in the soil by symbiotically relating and working with the rhizobia that inhabit the root nodules of legumes (Johnston-Monje and Raizada 2011). In the nodule of the root, the rhizobia get food and energy from the higher plant and, in return, utilize free N from the air and the soil and convert it to usable N which the plant can make use of to produce food (Laranjo et al. 2014; Karmakar et al. 2015).

20.3 Where and How Does BNF Take Place?

BNF takes place in the root nodules of the leguminous plants in the soil and within the rhizosphere of nonleguminous plants (Ahemad and Kibret 2014; Chanway et al. 2014). Within the nodules, N fixation is done by bacteria, and the NH_3 produced is absorbed by plants (Kennedy et al. 2005; Htwe and Yamakawa 2015). The process takes place in the presence of a bacteria called rhizobium with the help of a diazotroph which encodes the nitrogenase enzyme (Santi et al. 2013). In nonleguminous plants, such nitrogen fixation mostly results from the symbiotic association between the plants and rhizobia as seen in the association of *Azospirillum* spp., *Azoarcus* spp., and *Herbaspirillum* with cereal crops. Symbiotic relationships involving actinorhizals such as *Frankia* and cyanobacteria such as *Azolla* have been reported (Bergman et al. 2007; Kucho et al. 2009; Dawson 2007).

The process of BNF involves the reduction of atmospheric N and also requires large amount of energy because the N gas is joined together by three covalent bonds making it inert (Rahman and Yamin 2016). The total number of adenosine triphosphate (ATP) required by N-fixing bacteria is 16 moles which are either obtained from other organisms or from the product of photosynthesis (Wagner 2012). The sugar resulting from the photosynthesis is transferred to the root nodules which are then used by rhizobia for the N fixation (Jones et al. 2016; Courty et al. 2015). Nitrogen-fixing systems are sources of amino acids and proteins in the soil (Mueller et al. 2016). N_2 fixation requires more phosphorus than non-N_2-fixing systems (Chanway et al. 2014; Paerl and Otten 2016) because phosphorus is needed for plant growth, nodule formation, and ATP synthesis (Olofsson et al. 2016), which are very important for the N fixation (Dwivedi et al. 2015a). The sources of electron used in ATP synthesis are from small proteins such as ferredoxin, flavodoxin, nicotinamide, and adenine dinucleotide (ADP) (Roat-Malone 2014).

20.4 Why Is It Important?

The importance of nitrogen to plants, animals, and humans cannot be overemphasized. Plants need nitrogen for root nodulation, but it is not readily available. The constant application of nitrogen fertilizers to cover up for the unavailable nitrogen for plants by farmers shows the necessity of this nutrient. It is constantly being lost through erosion, leaching, and massive export during harvest. This subsequently affect yield if not replenished. BNF is important because it helps to make N available in a usable form to plants through the help of nitrogenase enzyme during which atmospheric N is converted to ammonia. The ammonia produced can lead to the formation of all the necessary biomolecules needed by the plants through amino acid production (Lugtenberg and Kamilova 2009). Among the essential major plant nutrients, N is uniquely different because its direct external input using mineral (inorganic) fertilizer following deficiency in soil may be reduced and/or completely avoided through replenishment as nodulated roots and soil incorporation of crop residues left after harvest. Hence, BNF could offer great advantage for farmers through the introduction of a legume-based cropping system where there is a serious threat of limited crop productivity existing due to N deficiency. Another problem faced by farmers is the cost of these fertilizers as most of them cannot afford them. The emergence of biological nitrogen fixers is a major boost for them. BNF is important in limiting environmental hazards. The leaching of chemical fertilizers into water bodies poses great risk to the environment in terms of good health. The water bodies are also contaminated, and water ecosystem is dramatically affected.

20.5 Biological Nitrogen Fixation in Legume-Based Cropping Systems

The main N sources in legume-based cropping systems are through BNF by the legume components, applied inorganic N fertilizers, and native soil N (Iannetta et al. 2016). Various methods have been used to quantify the amount of N that the legume-*Rhizobium* symbiosis contributes to legume-based cropping systems. The advantages and disadvantages of the different assessment methods have been discussed in various studies. Some of the methods used to assess BNF include N balance (Istfan et al. 1983), 15 N-isotopic techniques (Boddey et al. 2000), nodule evaluation (Hardy et al. 1968), ureide method (Herridge et al. 1996), acetylene reduction assay (ARA) (Navarro-Noya et al. 2012), and N fertilizer equivalence (Arthikala et al. 2014).

The amount of legume-fixed N in intercropping systems depends on the plant species, plant morphology, crop component density, type of management system adopted, and competitive abilities of the component crops (Stagnari et al. 2017). Variations in activities of some legumes toward N fixation have been reported in mono- and mixed cropping systems (Stagnari et al. 2017; Dwivedi et al. 2015b; Belel et al. 2014). Due to the high level of energy consumption in dinitrogen fixation, the photosynthate supply to the nodules cannot be lowered as this will be detrimental (Bottomley and Myrold 2014; Beatty et al. 2015). The height of the legume and nonlegume can affect the rate of photosynthesis and dinitrogen fixation depending on which one is taller in both (Nasielski 2015; Isaac et al. 2014). Total N fixed in a cowpea-maize system was more dependent on the type of cropping system than on the crop spacing (Dwivedi et al. 2015b).

20.6 Legumes and the Classification of Leguminous Plants

Legumes or pulses are a large group of angiosperm plants present in all continents and can grow in diverse aquatic and terrestrial environments under different conditions (Peix et al. 2015). According to Mulongoy (1995), out of about 1300 leguminous plants species worldwide, only about 87% have so far been examined and found to nodulate. This means that not all of them can be infected by rhizobia and invariably not all legumes can fix nitrogen biologically. For example *Gliricidia sepium* and *Vigna unguiculata* (cowpea) have been observed to nodulate freely, while others like *Cassia siamea* have no nodules in their roots (Dahlin and Rusinamhodzi 2014; Jonsson et al. 1988). This ability of legumes to or not to nodulate has formed part of the basis for their classification (Mulongoy 1995).

Legumes are flowering plants from the Fabaceae or Leguminosae family that have 690 genera and 18,000 species (Morris 2003). Fabaceae family is classified into subfamilies identified and differentiated by their types of flowers. They are Caesalpinioideae (bird-of-paradise subfamily), Mimosoideae (acacia subfamily), and Papilionoideae (bean or pea subfamily) that constitutes mainly edible legumes including the very popular ones such as soybean, chickpea, bean, and pea and the

less popular ones such as (Morris 365) clover, licorice, lentils, and peanut. Both Caesalpinioideae and Mimosoideae are represented by about 2800 species, while Papilionoideae is represented by over 12,000 species, which are mainly herbaceous (Legume 2017).

20.7 Examples of Leguminous Plants

Leguminous plants can be trees, shrubs, or herbs. Some are perennial or annual crops, while some are climbing, crawling, or growing like vine plants. Typical examples of leguminous plants from the Ceaesalpinioideae subfamily include the orchid tree (*Bauhinia* spp.), the shower tree (*Cassia* spp.), and the royal poinciana (*Delonix regia*) (Costa et al. 2013; McBride 2017; Kuppusamy et al. 2015). Similarly, examples from the Mimosoideae subfamily include wattles (*Acacia* spp.) or silk tree (*Albizia julibrissin*) (Mohamed 2016), while examples from the Papilionoideae subfamily include pea or bean as well as wisteria or coral pea vine (*Kennedia* spp.).

The bean family has four prominently cultivated genera, which are *Phaseolus*, *Vigna*, *Vicia*, and *Glycine* (Tobias 2004). Examples from the genus *Phaseolus* include species Tepary bean (Latin name *acutifolius*), runner bean (*coccineus*), lima bean (*lunatus*, so-called for its crescent shape), and common or pinto bean (*vulgaris*) (Gepts 2014). The *Vigna* species consist of plants like the moth bean (*aconitifolia*), azuki bean (*angularis*), urad bean (*mungo*), mung bean (*radiata*), rice bean (*umbellata*), and cowpea (*unguiculata*) under which label both black-eyed pea and yardlong bean fall (Chankaew et al. 2014). The *Vicia* genus only contains the broad or fava bean (*faba*). In the genus *Glycine*, only the plant soybean (*max)* is available. Others are important food species such as the grams (green gram (*V. radiata* (L.) R. Wilczek), also known as mung bean, and black gram (*V. mungo* Hepper); both of these species have many other local names) which are native to the Indian subcontinent (Sprent et al. 2010).

20.8 Role of Leguminous Plants in Promoting Improved Soil and Plant Health

The roles of Leguminosae are often overlooked as they concern the health of both soil and plant because a healthy soil makes a healthy plant. Legumes serve as cover crops and prevent excessive moisture loss from the soil and also protect the soil from excessive heat that could lead to soil dryness and hardening and further stunted growth of the plant. Litter produced from legumes including leaves and fodder not used as animal feed can decompose when returned back to the soil and add organic matter and nutrients to enrich the soil and consequently boost crop growth and yields (Gepts et al. 2005).

20.9 Specific Examples of Underutilized Leguminous Plant

Most plant species that are useful as food sources worldwide but currently not cultivated and fully utilized fall under the category of neglected and underutilized crop species (NUCS). They are very important to nutrition and food security (Dansi et al. 2012).

In the Republic of Benin, out of the 41 NUCS recorded, only 19 have not been researched, which are comprised of certain leguminous species including *Macrotyloma geocarpum*, *Vigna subterranea*, *Cajanus cajan*,and *Sphenostylis stenocarpa* (Dansi et al. 2012). In Nigeria, some of the underutilized leguminous crops include *Brachystegia eurycoma*, *Tamarindus indica*, and *Mucuna flagellipes* (Bhat and Karim 2009). *B. eurycoma* and *T. indica* are both from the Caesalpinioideae family, while *M. flagellipes* is from the family Papilionoideae, but all the rest are from the family Leguminosae. The *B. eurycoma* is a tree legume that can be found in both southwestern Nigeria and Cameroon (Adeyemi et al. 2015). The brownish buttery gum that exudes from *B. eurycoma* is used by the Igbo communities of Nigeria as an antihelminthic (Lawal et al. 2010). *T. indica*, popularly known as Tsamiya in northern Nigeria, is also a tree legume that is rich in sugars and vitamin B; its seeds are sometimes crushed and used as soup thickeners, and its pulp is widely used in food and beverages (Ajayi et al. 2006). *M. flagellipes*, a Papilionoideae, has leaves that are used to blacken cloth and pottery and has been shown to be important in pharmaceutical application for preparing suspensions of sulfadimidine and zinc oxide (Ajayi et al. 2006). Bambara groundnut (*Vigna subterranea*) is an underutilized leguminous crop found in many parts of sub-Saharan Africa and Asia that is receiving international research efforts (Karunaratne et al. 2011). It is considered a complete meal comprising of very important proteins.

20.10 Bambara Groundnut: A Case Study of Underutilized Legume in Soil Fertility

Bambara groundnut, a neglected and underutilized legume crop with African origin, is found to be important in various aspects ranging from nutrition to medicinal and agronomical. It is planted using different cropping systems. It is used in crop rotation; after planting other cereals, it is also interspersed with other cereals in intercropping, while it can also be grown in monoculture.

It thrives in very harsh weather and so its drought tolerant; it grows well and prefers to grow in infertile soil as it helps to add nitrogen to the soil by fixing atmospheric nitrogen. It also thrives well in the red laterite soil in Africa known to be acidic and unsuitable for growth of other tropical crops. It is an epitome of a sustainable crop, needing no fertilizer to enhance its productivity, and its soil is an array of genetic diversity. Its nitrogen needs are met during symbiotic nitrogen fixation, fixing up to100 kg/ha (Hillocks et al. 2012; Mohale et al. 2014). In Botswana, research carried out on Bambara groundnut revealed that nitrogen fertilization is not needed because the soil is fertile for crop yield increase, while phosphorus is sometimes applied when the soil is moist. The rhizosphere of Bambara groundnut was observed

to have nitrogen and phosphorus connections which have led to the increase in the growth of the crop and invariably yield (Nweke and Emeh 2013).

The issue of soil fertility is not just agronomic but also relates very importantly to socioeconomic issues. It has been reported that soil fertility problems in poor farmlands can be tackled using intercropping of cereals with legumes because when land productivity is enhanced, the issue of poor soil amelioration is already handled (Belel et al. 2014). Even though Legwaila et al. (2012) and Karikari (2004) did not record any appreciable yield increases when Bambara groundnut was intercropped with sorghum and maize, Ogah and Ogbodo (2012) had a very high and abundant yield when Bambara groundnut was intercropped with maize. Yield loss to stem borer was also significantly lower, while the number of stem borer larvae on cobs was very low. Bambara groundnut has been rotated with yam, pearl millet, sorghum, and maize in both South Africa and Botswana and has been shown to produce maximum yield when planted immediately after a fallow period. It was also observed that intercropping has helped to decrease nutrient loss as a result of monocropping with cereals such as maize and chemical contents such as potassium, nitrogen, and phosphorus were increased (Dahmardeh et al. 2010).

Among the outcomes of the problem of infertile soil are low crop yield which does sometimes occur as a result of continuous monocropping and lack of sufficient organic matter in the soil which is accompanied by drought or insufficient rainfall. Chemical fertilizers are not sufficient to maintain and improve soil fertility, but a continuous, consistent, and sustainable availability of nitrogen and other important minerals in the soil can ward off soil infertility (Ngwira et al. 2012). As legumes have been known to be cover crops and important in soil conservation which enhances fertility, Adeleke and Haruna (2012) observed that after cropping any of soybean, cowpea, lablab, and groundnut, total nitrogen in the soil increased when such lands were left to fallow. Bambara groundnut also in rotation cultivation with upland crops has been observed to have beneficial effect on soil fertility (Nyalemegbe and Osakpa 2012). In most cases, this increase in soil fertility as a result of increase in soil nitrogen content is due to the nitrogen-fixing ability of the microbes in the root nodules of the legumes through symbiotic activities. These symbiotic activities have been shown to contribute over 45 million tons of fixed nitrogen to agriculture each year which is valued at over 20% of the biological nitrogen fixed worldwide (Belel et al. 2014).

Cation exchange capacity (CEC) was higher in fields on which legumes were previously planted compared to fields where maize were previously cropped. Thus, soil fertility in addition to biological nitrogen fixation was enhanced also due to the dropping and decomposition of legume leaf litters and the addition of nutrients to the soil (Nana and Alemneh 2015).

20.11 Future Prospects and Recommendations

The different evidences from pockets of research and varying studies reveal that if Bambara groundnut is given much needed attention, it would also become a prominent crop in the nearest future taking cue from the rise of peanuts to prominence.

Bambara groundnut is highly resistant to pests and diseases and as such can be very important to food security not only in Africa but globally.

With more support of science and research, media and publicity, and government policies that can boost the production of both farmers and food processors and encourage investments, Bambara groundnut could be made a nutrition-enhancing and malnutrition-reducing food crop, which can help to increase the economy both at local and international levels.

It grows well in both Africa and Asia. Recent research by FAO has shown that it grows well too in parts of the Middle East. It has also been grown in parts of Europe and Florida in the USA, but now it should be encouraged in worldwide general production like peanuts.

Just like other crops that have gained worldwide attention (peanuts, soybean, cowpea), production of Bambara groundnut should be encouraged by improving the quality of cultivars and landraces available that can encourage abundant yield.

The field of engineering could be employed to develop harvesters peculiar to Bambara groundnut that can reduce the labor during harvesting because it has been found that harvesters like that of peanut will crush the pods and the seeds.

20.12 Conclusion

BNF has the potential of being the fertilizer of the future that can be used to enhance and strengthen crop yields leading to food security. Legumes and in particular NUCS like Bambara groundnut if well incorporated and integrated into the global market can be used as source of BNF and food security.

Acknowledgments South Africa's National Research Foundation granted OOB research funds (UID81192 and UID104015) that have supported her work in the laboratory. North-West University granted CFA a student bursary.

References

Adeleke MA, Haruna IM (2012) Residual nitrogen contributions from grain legumes to the growth and development of succeeding maize crop. ISRN Agron 2012:5

Adeyemi A, Ibe A, Okedimma F (2015) Tree structural and species diversities in Okwangwo Forest, Cross River State, Nigeria. J Res For 7(2):36–53

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ajayi IA, Oderinde RA, Kajogbola DO, Uponi JI (2006) Oil content and fatty acid composition of some underutilized legumes from Nigeria. Food Chem 99(1):115–120

Arthikala MK, Sánchez-López R, Nava N, Santana O, Cárdenas L, Quinto C (2014) RbohB, a Phaseolus vulgaris NADPH oxidase gene, enhances symbiosome number, bacteroid size, and nitrogen fixation in nodules and impairs mycorrhizal colonization. New Phytol 202(3):886–900

Aserse AA (2013) *Diversity and phylogeny of root nodule bacteria isolated from tree, shrub and food legumes of Ethiopia*. Academic dissertation, Faculty of Agriculture and Forestry, University of Helsinki, Finland. p 62

Beatty PH, Fischer JJ, Muench DG, Good AG (2015) Environmental and economic impacts of Biological Nitrogen-Fixing (BNF) cereal crops. In: Biological nitrogen fixation. Wiley, Hoboken, pp 1103–1116

Belel MD, Halim R, Rafii M, Saud H (2014) Intercropping of corn with some selected legumes for improved forage production: a review. J Agric Sci 6(3):48

Bergman B, Rai A, Rasmussen U (2007) Cyanobacterial associations. In: Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Springer, Dordrecht, pp 257–301

Bhat R, Karim A (2009) Exploring the nutritional potential of wild and underutilized legumes. Compr Rev Food Sci Food Saf 8(4):305–331

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(4):1327–1350

Boddey RM, Peoples MB, Palmer B, Dart PJ (2000) Use of the 15N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutr Cycl Agroecosyst 57(3):235–270

Bottomley PJ, Myrold DD (2014) Biological N inputs. Soil Microbiol Ecol Biochemist 3:365–388

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of Earth's nitrogen cycle. Science 330(6001):192–196

Chankaew S, Isemura T, Isobe S, Kaga A, Tomooka N, Somta P, Hirakawa H, Shirasawa K, Vaughan DA, Srinives P (2014) Detection of genome donor species of neglected tetraploid crop *Vigna reflexo-pilosa* (creole bean), and genetic structure of diploid species based on newly developed EST-SSR markers from azuki bean (*Vigna angularis*). PLoS One 9(8):e104990

Chanway C, Anand R, Yang H (2014) Nitrogen fixation outside and inside plant tissues. In: Ohyama T (ed) Advances in biology and ecology of nitrogen fixation, pp 3–23. Available from: http://cdn.intechopen.com/pdfs-wm/46070.pdf. Accessed 21 July 2015

Cooper JE, Scherer HW (2012) Chapter 16 – nitrogen fixation. In: Marschner P (ed) Marschner's Mineral nutrition of higher plants, 3rd edn. Academic, San Diego, pp 389–408

Costa CB, Costa JA, de Queiroz LP, Borba EL (2013) Self-compatible sympatric Chamaecrista (*Leguminosae-Caesalpinioideae*) species present different interspecific isolation mechanisms depending on their phylogenetic proximity. Plant Syst Evol 299(4):699–711

Courty PE, Smith P, Koegel S, Redecker D, Wipf D (2015) Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. Crit Rev Plant Sci 34(1–3):4–16

Dahlin S, Rusinamhodzi L (2014) Review of interventions and technologies for sustainable intensification of smallholder crop production in sub-humid sub-Saharan Africa, vol 5. Swedish University of Agricultural Sciences, Uppsala

Dahmardeh M, Ghanbari A, Syahsar B, Ramrodi M (2010) The role of intercropping maize (*Zea mays* L.) and Cowpea (*Vigna unguiculata* L.) on yield and soil chemical properties. Afr J Agric Res 5(8):631–636

Dansi A, Vodouhè R, Azokpota P, Yedomonhan H, Assogba P, Adjatin A, Loko YL, Dossou-Aminon I, Akpagana K (2012) Diversity of the neglected and underutilized crop species of importance in Benin. Sci World J 2012:19

Dawson J (2007) Ecology of actinorhizal plants. In: Nitrogen-fixing actinorhizal symbioses. Springer, Dordrecht, pp 199–234

Dos Santos PC, Fang Z, Mason SW, Setubal JC, Dixon R (2012) Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics 13(1):162

Doyle JJ (2016) Chasing unicorns: nodulation origins and the paradox of novelty. Am J Bot 103(11):1865–1868

Dwivedi SL, Sahrawat KL, Upadhyaya HD, Mengoni A, Galardini M, Bazzicalupo M, Biondi EG, Hungria M, Kaschuk G, Blair MW (2015a) Chapter one-advances in host plant and Rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. Adv Agron 129:1–116

Dwivedi A, Dev I, Kumar V, Yadav RS, Yadav M, Gupta D, Singh A, Tomar S (2015b) Potential role of maize-legume intercropping systems to improve soil fertility status under smallholder farming systems for sustainable agriculture in India. J Life Sci Biotechnol Pharma Res 4(3):145–157

Gepts P (2014) Beans: origins and development. In: Encyclopedia of global archaeology. Springer, New York, pp 822–827

Gepts P, Beavis WD, Brummer EC, Shoemaker RC, Stalker HT, Weeden NF, Young ND (2005) Legumes as a model plant family. Genomics for food and feed report of the cross-legume advances through genomics conference. Plant Physiol 137(4):1228–1235

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 451(7176):293–296

Haber F (1922) The production of ammonia from nitrogen and hydrogen. Naturwissenschaften 10:1041

Hardy RW, Holsten R, Jackson E, Burns R (1968) The acetylene-ethylene assay for N2 fixation: laboratory and field evaluation. Plant Physiol 43(8):1185–1207

Herridge D, Palmer B, Nurhayati D, Peoples M (1996) Evaluation of the xylem ureide method for measuring N 2 fixation in six tree legume species. Soil Biol Biochem 28(3):281–289

Hillocks R, Bennett C, Mponda O (2012) Bambara nut: a review of utilisation, market potential and crop improvement. Afr Crop Sci J 20(1)

Hoffman BM, Lukoyanov D, Yang Z-Y, Dean DR, Seefeldt LC (2014) Mechanism of nitrogen fixation by nitrogenase: the next stage. Chem Rev 114(8):4041–4062

Htwe AZ, Yamakawa T (2015) Enhanced plant growth and/or nitrogen fixation by leguminous and non-leguminous crops after single or dual inoculation of *Streptomyces griseoflavus* P4 with *Bradyrhizobium* strains. Afr J Microbiol Res 9(49):2337–2344

Iannetta PP, Young M, Bachinger J, Bergkvist G, Doltra J, Lopez-Bellido RJ, Monti M, Pappa VA, Reckling M, Topp CF (2016) A comparative nitrogen balance and productivity analysis of legume and non-legume supported cropping systems: the potential role of biological nitrogen fixation. Front Plant Sci 7

Isaac ME, Carlsson G, Ghoulam C, Makhani M, Thevathasan NV, Gordon AM (2014) Legume performance and nitrogen acquisition strategies in a tree-based agroecosystem. Agroecol Sust Food 38(6):686–703

Istfan N, Murray E, Janghorbani M, Young VR (1983) An evaluation of the nutritional value of a soy protein concentrate in young adult men using the short-term N-balance method. J Nutr 113(12):2516–2523

Jia H-P, Quadrelli EA (2014) Mechanistic aspects of dinitrogen cleavage and hydrogenation to produce ammonia in catalysis and organometallic chemistry: relevance of metal hydride bonds and dihydrogen. Chem Soc Rev 43(2):547–564

Johnston-Monje D, Raizada MN (2011) 4.58 – Plant and Endophyte relationships: nutrient management. In: Moo-Young M (ed) Comprehensive biotechnology, 2nd edn. Academic, Burlington, pp 713–727

Jones FP, Clark IM, King R, Shaw LJ, Woodward MJ, Hirsch PR (2016) Novel European free-living, non-diazotrophic *Bradyrhizobium* isolates from contrasting soils that lack nodulation and nitrogen fixation genes–a genome comparison. Sci Rep 6

Jonsson K, Fidjeland L, Maghembe J, Högberg P (1988) The vertical distribution of fine roots of five tree species and maize in Morogoro, Tanzania. Agrofor Syst 6(1–3):63–69

Karikari S (2004) A decade of bambara groundnut agronomic research at the Botswana College of Agriculture. UNISWA J Agric

Karmakar K, Rana A, Rajwar A, Sahgal M, Johri BN (2015) Legume-rhizobia symbiosis under stress. In: Plant microbes symbiosis: applied facets. Springer, New Delhi, pp 241–258

Karunaratne A, Azam-Ali S, Izzi G, Steduto P (2011) Calibration and validation of FAO-AquaCrop model for irrigated and water deficient Bambara groundnut. Exp Agric 47(03):509–527

Kennedy IR, Choudhury A, Kecskés ML, Roughley RJ, Hien NT (2005) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? In: Biological nitrogen fixation, sustainable agriculture and the environment. Springer, Dordrecht, pp 271–272

Kucho K-i, Kakoi K, Yamaura M, Higashi S, Uchiumi T, Abe M (2009) Transient transformation of Frankia by fusion marker genes in liquid culture. Microbes Environ 24(3):231–240

Kuppusamy S, Thavamani P, Megharaj M, Naidu R (2015) Environmental technology & innovation

Laranjo M, Alexandre A, Oliveira S (2014) Legume growth-promoting rhizobia: an overview on the *Mesorhizobium genus*. Microbiol Res 169(1):2–17

Lawal I, Uzokwe N, Igboanugo A, Adio A, Awosan E, Nwogwugwu J, Faloye B, Olatunji B, Adesoga A (2010) Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria. Afr J Pharm Pharmacol 4(1):001–007

Legume (2017) Scientific classification and characteristics Britannica online for kids. Encyclopædia Britannica, Inc, Chicago

Legwaila GM, Marokane TK, Mojeremane W (2012) Effects of intercropping on the performance of maize and cowpeas in Botswana. Int J Agric For 2(6):307–310

Lindström K, Aserse AA, Mousavi SA (2015) Evolution and taxonomy of nitrogen-fixing organisms with emphasis on rhizobia. Biol Nitrogen Fixation:21–37

Liu J, Kelley MS, Wu W, Banerjee A, Douvalis AP, Wu J, Zhang Y, Schatz GC, Kanatzidis MG (2016) Nitrogenase-mimic iron-containing chalcogels for photochemical reduction of dinitrogen to ammonia. Proc Natl Acad Sci 113(20):5530–5535

Lugtenberg B, Kamilova F (2009) Plant growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

McBride JR (2017) Composition. In: The world's urban forests. Springer, Cham, pp 71–139

McGlynn SE, Boyd ES, Peters JW, Orphan VJ (2013) Classifying the metal dependence of uncharacterized nitrogenases. Front Microbiol 3:419

Mohale KC, Belane AK, Dakora FD (2014) Symbiotic N nutrition, C assimilation, and plant water use efficiency in Bambara groundnut (*Vigna subterranea* L. *Verdc*) grown in farmers' fields in South Africa, measured using 15N and 13C natural abundance. Biol Fertil Soils 50(2):307–319

Mohamed MIE (2016) Characterization of Flavonoids from Albizia amara leaves and their biological activity on microbes. Sudan University of Science and Technology, Khartoum

Morris B (2003) Legumes. In: Encyclopedia of food and culture, vol 3. Charles Scribner & Sons, New York

Mueller TJ, Welsh EA, Pakrasi HB, Maranas CD (2016) Identifying regulatory changes to facilitate nitrogen fixation in the nondiazotroph *Synechocystis sp*. PCC 6803. ACS Synth Biol 5(3):250–258

Mulongoy K (1995) Technical paper 2: biological nitrogen fixation. Food and agriculture Organization of the United Nations (FAO) corporate document repository, ILRI training manual 2

Nana EM, Alemneh AA (2015) Rhizobia strain and legume genome interaction effects on nitrogen fixation and yield of grain legume: a review. Mol Soil Biol 6(4):1–12

Nasielski J (2015) Soybean N2-fixation rates and yield in tree-based intercropping systems: effects of water limitations and environmental modifications. University of Toronto

Navarro-Noya YE, Hernández-Mendoza E, Morales-Jiménez J, Jan-Roblero J, Martínez-Romero E, Hernández-Rodríguez C (2012) Isolation and characterization of nitrogen fixing heterotrophic bacteria from the rhizosphere of pioneer plants growing on mine tailings. Appl Soil Ecol 62(0):52–60

Ngwira AR, Aune JB, Mkwinda S (2012) On-farm evaluation of yield and economic benefit of short term maize legume intercropping systems under conservation agriculture in Malawi. Field Crops Res 132:149–157

Nna-Mvondo D, Navarro-González R, Raulin F, Coll P (2005) Nitrogen fixation by corona discharge on the early Precambrian Earth. Orig Life Evol Biosph 35(5):401–409

Nweke I, Emeh H (2013) The response of Bambara ground nut (*Vigna subterranean* L) to phosphate fertilizers levels in Igbariam South East Nigeria. IOSR J of Agric & Vet Sci 2(1):28–34

Nyalemegbe K, Osakpa T (2012) Rotation of maize with some leguminous food crops for sustainable production on the vertisols of the Accra plains of Ghana. West Afr J Appl Ecol 20(2):33–40
Ogah E, Ogbodo E (2012) Assessing the impact of biodiversity conservation in the management of maize stalk borer (Busseola fusca F.) in Nigeria. Curr Trends Technol Sci II II: 234–238
Olofsson M, Egardt J, Singh A, Ploug H (2016) Inorganic phosphorus enrichments in Baltic Sea water have large effects on growth, carbon fixation, and N2 fixation by Nodularia spumigena. Aquat Microb Ecol 77(2):111–123
Paerl HW, Otten TG (2016) Duelling 'CyanoHABs': unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria. Environ Microbiol 18(2):316–324
Peix A, Ramírez-Bahena MH, Velázquez E, Bedmar EJ (2015) Bacterial associations with legumes. Crit Rev Plant Sci 34(1–3):17–42
Rahman A, Yamin A (2016) A genomic analysis of *Paenibacillus macerans* ATCC 8244, a gram positive nitrogen fixing bacterium. Universiti Sains Malaysia, George Town
Remigi P, Zhu J, Young JPW, Masson-Boivin C (2016) Symbiosis within symbiosis: evolving nitrogen-fixing legume symbionts. Trends Microbiol 24(1):63–75
Roat-Malone R (2014) Biological inorganic chemistry; inorganic chemistry of biological compounds. Springer, New York
Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot 111(5):743–767
Smil V (2004) Enriching the earth: Fritz Haber, Carl Bosch, and the transformation of world food production. MIT Press, Cambridge, MA
Sprent JI, Odee DW, Dakora FD (2010) African legumes: a vital but under-utilized resource. J Exp Bot 61(5):1257–1265
Stagnari F, Maggio A, Galieni A, Pisante M (2017) Multiple benefits of legumes for agriculture sustainability: an overview. Chem Biol Technol Agric 4(1):2
Thamdrup B (2012) New pathways and processes in the global nitrogen cycle. Annu Rev Ecol Evol Syst 43:407–428
Tobias R (2004) Beans. In: The Oxford encyclopedia of food and drink in America, 2nd edn. Oxford University Press, Oxford
Wagner S (2012) Biological nitrogen fixation. Nat Educ Knowl 3(10):15