Environmental and Microbial Biotechnology

Ram Prasad
Shi-Hong Zhang *Editors*

# Beneficial Microorganisms in Agriculture

Springer

# Environmental and Microbial Biotechnology

**Series Editor**

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ISSN 2662-1681 ISSN 2662-169X (electronic)
Environmental and Microbial Biotechnology
ISBN 978-981-19-0732-6 ISBN 978-981-19-0733-3 (eBook)
https://doi.org/10.1007/978-981-19-0733-3

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# Preface

"We also know there are known unknowns. That is to say, we know there are some things we do not know. But there are also unknown unknowns, the ones we don't know we don't know" said by Donald Henry Rumsfeld, U.S. Secretary of Defense, at the Iraq Weapons of Mass Destruction Press Conference. To microorganisms, their roles in agriculture seem to be uncertain and over neglected.

In the long history, people have been enjoying the material cycle and ecological balance promoted by microbial metabolism. The emergence of chemical fertilizers and pesticides, like the Hiroshima Atomic Bomb, has broken the silence of ecological balance. Chemical fertilizers and pesticides are double-edged swords for agriculture. And indeed, according to statistical data from UN Food and Agriculture Organization (FAO) and Ministry of Agriculture and Rural Affairs of the People's Republic of China (MOA), fertilizer contributes nearly 50% to the increase of world crop production, and pesticide use saves about 40% of the world's total crop production (MOA 2015, FAO 2015). However, fertilizers and pesticides lead to unwanted consequences, such as degraded soil fertility, excessive pesticide residues, and agricultural non-point source pollution. Particularly, excessive use of pesticides and fertilizers influences the safety of the ecological environment and agricultural production, and further threatens human health and sustainable agricultural development. People are crazy to pursue the pleasure brought by ultra-high output of crops but have to stand the cost of unhealthy food. With finite resources, the pressure of the growing global population, and human physical and mental health, we need a plan to stimulate action in areas of critical importance for agriculture.

In September 2015, the United Nations launched the Sustainable Development Goals. For developing countries with large populations such as China and India, this initiative has far-reaching significance. In order to deeply understand the connotation of the high-quality development of green agriculture and implement the ecological concept of "Nature is the true treasure" and in order to promote the pace of ecological civilization in the developing countries and accelerate the rapid development of global modern agriculture, the International Symposium on Soil Fertility Improvement and Ecological Restoration in the Great Bend of Yellow River—

Onsite Meeting for the Green Circulating Agriculture Based on Organic Fertilizer from Decomposed Straw was held in Tuoketuo County, Inner Mongolia, on July 31, 2019. The main theme of the conference is “Green, Cyclic, Health and Sustainability.” Fortunately, we both were invited to attend and make keynote speeches at the conference. The Yellow River has bred the Chinese national culture, and the Great Bend of Yellow River (Hetao in Chinese) has laid the material foundation of China. “Harmful sometime the yellow river flooding, but makes wealth in the great bend.” However, in today’s Hetao, fertile fields disappeared, instead of which salt thorns are clustered. This is caused by man-made and unscientific farming system, especially the over-utility of chemical fertilizers, pesticides, and secondary disaster by flood irrigation. As fungal biotechnologists, our research has direct applications that contribute towards solving these problems. During the conference, we discussed that a book should be published to emphasize the role of beneficial microorganisms in agriculture.

Modern agriculture should be sufficient, organic, and healthy agriculture. World agriculture is rapidly stepping into scale, intensiveness, and modernization. Certainly not limited to China, there are many technical problems facing in the development of modern agriculture, among which the problems of soil conservation tillage and fertility upgrading, and harmless treatment of crop straw and efficient utilization are the most urgent ones. Human beings have always benefited from beneficial microorganisms, but we don’t turn a blind eye to them until today. Therefore, a safe alternative to fertilizers and pesticides is becoming increasingly urgent.

Bacteria and fungi are beneficial for plants, the environment, and even across all aspects of human life. Soil microbes are vital for decomposing organic matter and recycling plant waste material. Some soil bacteria and fungi form relationships with plant roots that provide important nutrients like nitrogen, phosphorus, or micronutrients. Fungi/plant growth-promoting rhizobacteria can colonize rhizosphere region of plants and provide numerous benefits, including drought tolerance, heat tolerance, resistance to insects, and resistance to plant diseases. Besides, some endophytic microbes colonize in plant roots to bring forth the benefits.

Plant growth and productivity (PGP) is profoundly influenced by the interactions between plant roots and the surrounding soil, including the microbial populations within the soil. The plant rhizosphere harbors microorganisms that may have positive, negative, or no visible effect on plant growth. Although most rhizospheric microbes appear to be benign, deleterious microorganisms include pathogens and microbes producing toxins that inhibit root growth or those that remove essential substances from the soil. By contrast, the main mechanisms for plant growth promotion include suppression of disease (biocontrol); enhancement of nutrient availability; and production of plant hormones. Studies of PGP microbes indicate that multifunctionality is a hallmark of the most beneficial.

The indigenous rhizospheric microbial population of agricultural soils is importantly influenced by agricultural practices, crop plant species, cultivar and genotype, as well as soil type. Plant exudates may cause changes to soil characteristics such as pH and nutrient availability, impacting the diversity and activity of microbial populations. Bioaugmentation, the addition of microbes to agricultural soils, thus becomes a valuable influence on soil microbial processes. In light of this, the potential for successful application of biofertilization, biocontrol, and phytostimulation in plant production systems has attracted more and more attention.

Without a doubt, microorganisms offer numerous applications in sustainable environmental biotechnology; however, many of the processes still have not found industrial applications or received the attention they deserve. It is clear that despite the advances more researches are required to realize the potential of sustainable fungal environmental biotechnology. We sincerely hope this book contributes to the body of knowledge of sustainable agricultural applications of microorganism and serves as a useful reference for any agronomists and micrologists who work together with this fascinating group of organisms to improve the welfare of our planet and mankind.

Motihari, Bihar, India Ram Prasad
Shenyang, Liaoning, China Shi-Hong Zhang

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# Editors and Contributors

## About the Editors

**Ram Prasad, Ph.D** is associated with the Department of Botany, Mahatma Gandhi Central University, Motihari, Bihar, India. His research interest includes applied and environmental microbiology, plant–microbe interactions, sustainable agriculture, and nanobiotechnology. Dr. Prasad has more than 215 publications to his credit, including research papers, review articles and book chapters and six patents issued or pending, and edited or authored several books. Dr. Prasad has 12 years of teaching experience and has been awarded the Young Scientist Award and Prof. J.S. Datta Munshi Gold Medal by the International Society for Ecological Communications; FSAB fellowship by the Society for Applied Biotechnology; the American Cancer Society UICC International Fellowship for Beginning Investigators, USA; the Outstanding Scientist Award in the field of Microbiology by Venus International Foundation; BRICPL Science Investigator Award and Research Excellence Award, etc. Previously, Dr. Prasad served as Assistant Professor at Amity University, Uttar Pradesh, India; Visiting Assistant Professor, Whiting School of Engineering, Department of Mechanical Engineering at Johns Hopkins University, Baltimore, USA; and Research Associate Professor at the School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou, China.

**Shi-Hong Zhang, Ph.D** is a professor in the College of Plant Protection and the Director of the Fungal Genetics and Molecular Plant Pathology Program at Shenyang Agricultural University (SYAU), and a professor in the College of Plant Sciences at Jilin University, China. Prof. Zhang concurrently serves as the Director of the Key Laboratory for Extreme-Environmental Microbiology of Liaoning Province (SYAU), and Center for Extreme-Microorganisms (SYAU). Prof. Zhang is a pioneer in Extreme-Environmental Fungal identification and application in soil bioremediation by using halophilic fungi; also, he focuses on genetic research in *Magnaporthe oryzae* (pathogen) and *Aspergillus glaucus* CCHA (beneficial fungus) and is committed to promoting sustainable agricultural development through his research. His team is among the pioneers who used halophilic fungi for soil remediation and environmental management. By efficiently utilizing the effect of saline-alkaline fungi on soil ecological restoration and exerting the integrated effect of saline-alkaline microorganisms and agricultural organic wastes, the problems of serious lack of beneficial fungi in saline-alkali soil have been solved. After decades of efforts, the experimental area in Jilin, Liaoning, Heilongjiang, Shandong, Jiangsu, Hebei, and Inner Mongolia reached more than 40,000 mu in 13 counties and cities, which has achieved tremendous economic, environmental, and social benefits. From 2006 to 2016, Prof. Zhang has been the chairman of Science Committee of the College of Plant Sciences at Jilin University. Since 2020, he joined in the Shenyang Agricultural University. Prof. Zhang has more than 30 years of experience working in the field of plant protection, microbiology, and environment protection. Till now, he has published more than 100 peer-reviewed papers in his research fields and was the co-inventor of 40 granted Invention Patents of China, most of which are useful for crop-resistant improvement against bio- or abio-stress.

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# Chapter 1
# Molecular Approaches of Microbial Diversity in Agricultural Soil

**Belma Nural Yaman, Pınar Aytar Çelik, Blaise Manga Enuh, and Ahmet Çabuk**

**Abstract** Soil presents a highly heterogeneous medium, and the different components of the soil (sand, silt, clay, and organic matter) enable various habitats for microbial communities which are great potential tools for elucidating community interactions in microbial ecology. These communities are made up of a diversity of organisms from bacteria, archaea, and eukarya domains. Microbial diversity in soil has vital importance in understanding the function of natural and agriculture soils.

Soil bacteria and fungi play pivotal roles in sustainable agriculture for removal of toxins and in various biogeochemical cycles consisting of carbon, nitrogen, phosphorus, sulfur important for agricultural soils. Soil microorganisms also promote plant growth, increase resistance against stress, etc.

However, when researching the soil for identification and discovery, problems with pure cultures and enrichment are often encountered. These limitations could be overcome by methodological strategies including molecular techniques such as

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_1

indirect DNA techniques (cultivation of microorganisms and molecular identification), direct DNA techniques (polymerase chain reaction (PCR)-dependent methodologies such as Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), Terminal Restriction Fragment Length Polymorphism (T-RFLP), 16S–18S Clone Library, Amplified Ribosomal DNA Restriction Analysis (ARDRA), real-time PCR), fluorescence in situ hybridization, microarray, metagenomics and transcriptomics which can be used in the determination of diversity of soil bacteria.

Recently, current developments in next-generation DNA sequencing methods, such as pyrosequencing and shotgun metagenome using bioinformatics tools, have contributed to increasing scientific attention and understanding of the complexity of microbial communities, functional traits and the relationship between communities and external drivers including environmental factors in soil. This chapter will discuss molecular approaches used for determination of microbiota, challenges encountered, and also future trends in the application of molecular tools to study soil microbial diversity.

**Keywords** Microbial diversity · Molecular methods · Agriculture · Microbial communities · Soil microbes · Metagenome

## 1.1 Introduction

Environmental microbiology research is often needed to evaluate the composition and diversity of microbial populations. Cultivation-dependent techniques are important in many ways but are limited for use in this task because of the bias forced by laboratory medium conditions. A diversity of culture-independent techniques targeting ribosomal RNA (rRNA) that solve culture bias by analyzing the structures of microbial communities and diversity based on their phylogenies have been developed. PCR-dependent or -independent microbial population analysis methods offer worthful, cost-effective, and high-throughput measurement of community composition.

The soil has a heterogeneous structure which consists of different solid fraction components that include sand, silt, clay, and organic matter (van Elsas and Trevors 1997; Garbeva et al. 2004; McCauley et al. 2005; Al-Kaisi et al. 2017). Soil is often defined with respect to the area of interest, and the best definition is considered to be a medium which is composed of minerals, organic matter, countless organisms, liquid, and gases. It supports life by acting as food source and habitat, etc. (Al-Kaisi et al. 2017). Therefore, the soil system must be dynamic, stable, and composite to serve these purposes (Garbeva et al. 2004; Al-Kaisi et al. 2017). The progenitor materials and originating factors affect the soil environment and functions and they promote the physical, chemical, and biological characteristics of soils whose characteristic influenced primarily the parent materials, and secondarily on vegetation, topography, and time (Jenny 1941; McCauley et al. 2005; Al-Kaisi et al. 2017).

Physically, soil can be considered to have three phases which consist of the solid, liquid, and gaseous phase. The solid phase shapes the soil matrix, the liquid phase is described as soil solution consisting of water in the soil system, and gaseous phase is defined as the soil atmosphere (Al-Kaisi et al. 2017). The soil matrix comprises particles varying in size, shape, chemical orientation, and number (McCauley et al. 2005; Al-Kaisi et al. 2017). Amorphous substances, particularly organic matter, generate the chemical and mineralogical composition of the soil matrix. They attack the mineral gains and can bind each other. The originated structure is called soil aggregates (Hillel 2003). The three phases of soil are continuously dynamic with constantly changing proportions influenced by the weather, human management, and vegetation. The stability of the soil and aggregates formed within can be deeply affected by tilling and cropping (Al-Kaisi et al. 2017).

Agriculture that has vital importance to ensure food safety, decrease poverty, and protect natural resources is the foundation of human existence. As the world population continues to grow, the need to provide food for agriculture will become one of the biggest challenges facing the agricultural society. To meet this challenge, it is necessary to focus on studying the soil biological system and the entire agricultural ecosystem. Soil is an important natural resource that contributes to the success of sustainable agriculture and interacts with the flora, microbiota, and fauna in the ground. Soil quality can be defined as the soil's ability to fulfill the necessary functions, such as producing healthy crops, resisting erosion, and minimizing its impact on the environment (Sharma 2015).

Faced with climate change, agriculture faces enormous challenges in using limited natural resources to supply food to the growing population. This great challenge cannot be met without sustainable development that meets today's needs and without compromising the ability of future generations to meet their own needs. Sustainable agriculture is a set of strategies, especially management, that can improve or maintain the quality and quantity of food supply without harming the environment or crop productivity in the long run. Sustainable agriculture is very important as it tries to meet our long-term agricultural needs by using special breeding techniques that try to make full use of natural resources that traditional agriculture cannot achieve. The principle is environmentally friendly and provides safe and healthy agricultural products. Microorganisms can promote plant growth and stress resistance, improve soil contaminated with heavy metal, restore nutrients, long-term soil fertility management, and reduce rock and fertilizer mineralization, so they have potential roles in sustainable agriculture (Rashid et al. 2019).

Productive and potential soil microbiota is only suitable for sustainable farming methods and may not be suitable for other alternative methods. Crop rotation is an additional dimension to optimize our soil and crop management practices such as organic change, conservation tillage, crop residue recycling, soil fertility improvement, soil quality preservation, and biological control of plant diseases. If used correctly, microbial communities can greatly benefit from agricultural practices (Singh et al. 2011).

Sustainable agriculture is not a specific set of methods but a broad concept. It includes advances in agricultural management practices and technology and is increasingly recognized, indicating that traditional agriculture developed after the Second World War could not meet the needs of the growing population in the twenty-first century. Traditional agriculture is faced with reduced production or increased costs, or both. In agriculture, monoculture can cause topsoil depletion, affect soil viability, groundwater purity, beneficial microorganisms, insect life, and make crops vulnerable to parasites and pathogens (Singh et al. 2011).

Fundamental changes have occurred in global agricultural practices and food production. In the past, the main driving force was to increase the yield potential and productivity of food crops. Nowadays, the drive for productivity is increasingly coupled with the desire and even the need for sustainability. Sustainable agriculture involves the successful management of agricultural resources to meet human needs while preserving environmental quality and future natural resources. Improving agricultural sustainability requires the best use and management of soil fertility and its physical and chemical properties. Both depend on soil biological processes and soil microbial diversity. This increases the biological activity of the soil, increasing long-term soil fertility and crop health. This approach is of great concern to avoid degradation in marginal soils and restoration in degraded soils and areas where agriculture is not possible with high external inputs (Singh et al. 2011).

## 1.2 Microbial Diversity in Soil

Soil is a complex habitat for microorganisms in terms of typical qualities (Nannipieri and Badalucco 2003; Nannipieri et al. 2003; Pisa et al. 2011). The characteristics are grouped into four main headlines:

1. The microbial community of soil is highly variable owing to the rich environment (Nannipieri et al. 2003; Pisa et al. 2011). Soil microorganisms are made up of members of three domains: Eukarya, Bacteria, and Archaea (Fierer and Jackson 2006; Pisa et al. 2011). Microorganisms have easily colonized every area of the world because their genetics enable them to easily adapt. The genetic heterogeneity of microorganism communities causes widespread distribution in the world (Bouchez et al. 2016). So, there are fewer than one million bacteria species and 100,000 fungi species per gram of soil. However, there are a hundred thousand bacterial species in 1 ml of water and per 1 $m^3$ of air. These microbial communities also symbolize the large ratio of biomass in ecosystems (Bouchez et al. 2016). The bacterial diversity examinations are the most important methods to determine soil conditions according to nutrient cycle and productivity. The soil bacteria have a vital role in many processes consisting of decomposition, mineralization, biological nitrogen fixation, and denitrification (Boyle et al. 2008). Furthermore, some bacteria related to plants support their growth (Gray and Smith 2005; Pisa et al. 2011).
2. Soil is a poor system in the way of nutrient and energy source, compared to the appropriate nutrient medium in other habitats. However, the soil is a system that

consists of dissimilar elements and has no continuity (Stotzky 1997; Nannipieri et al. 2003).

3. The other unique property of soil as a microhabitat is the capability of adsorption of vital molecules such as proteins and nucleic acids by the solid phase (Nannipieri et al. 2003). Enzymes are absorbed by clay minerals or humic molecules, which protect nucleic acids against temperature, pH denaturation (Nannipieri et al. 1990, 2003).
4. The last but not least property is the avoidance of DNA denaturation. Clay, sand particles, and humic molecules are bound to DNA and protect it against the effect of nucleases degradation. The surface of soil mineral compounds has utmost important roles in reaction. However, electron transfer reactions are catalyzed by clay minerals, Mn (III and IV) and Fe (III) oxides. Also, abiotic reactions are catalyzed by clay minerals. These reactions are deamination, polymerization, polycondensation, and ring cleavage (Nannipieri et al. 2003).

The number of archaea and bacteria on the earth are $1.2 \times 10^{30}$ cells and are spread out in five big habitats including deep oceanic subsurface ($4 \times 10^{29}$), upper oceanic sediment ($5 \times 10^{28}$), deep continental subsurface ($3 \times 10^{29}$), soil ($3 \times 10^{29}$), and oceans ($1 \times 10^{29}$) (Flemming and Wuertz 2019). Microbial habitat is affected by the soil's physical and chemical environment, including water and gaseous behavior (Al-Kaisi et al. 2017; Flemming and Wuertz 2019). Therefore, the soil system impacts microbial diversity, efficiency, and performance (Nannipieri et al. 2003; Al-Kaisi et al. 2017). Soil bacteria have vital roles in the ecological and bioprocesses in contaminated and clean soils, including decomposition and transformation of soil substances, the cycles of carbon, nitrogen, and phosphorus (Su et al. 2012; Nema 2019). On the other hand, the contaminated soils have shown more microbial diversity compared to clean soils (Nema 2019). Microbial diversity is used often for expressing the distribution of bacteria, archaea, and fungi in different habitats. The term refers to genetic diversity which is related to the amount and distribution of genetic information among the microbial species. The microbial diversity is affected by various ecological and geographical factors (Nannipieri et al. 2003; Garbeva et al. 2004).

The aggregate arrangement of soil in different sizes allows shaping of diverse microbial communities in soil (Flemming and Wuertz 2019; Al-Kaisi et al. 2017). Macroaggregates behave as a defense for microaggregates which are opposite to activities of microorganisms in the soil. This situation is explained by the hierarchy theory of soil aggregate functions (Tisdall and Oades 1982; Al-Kaisi et al. 2017). Soil aggregates have pores of 50% total volume, which is an ideal condition for microorganisms to survive in soil systems. The pores are natural habitats for microbes occupying their walls. Water present in the soil allows microbes to move freely. Water movements in the soil perform an important function that promotes microbial life by also moving nutrients, gases, microbes, and their precursors (Al-Kaisi et al. 2017). To know the relationship between the soil, water, and microbial communities, the four critical elements need to be considered. These elements are (1) pH, (2) nutrient diffusion and flow rates, (3) mobility, and

(4) temperature (Standing and Killham 2007). Soil organic matter is found in different types, which affect the diffusion of food and energy for continuous microbial activities. Temperature is also an important element for the distribution of microorganisms in soil and is also related to the interaction of plant, animal, and microbes. The carbon sources of the rhizosphere depend heavily on temperature (Al-Kaisi et al. 2017). The pH of the soil is an indicative element for the generation and survival of various microbial types. The acidophiles grow best at low pH, and another group alkaliphiles prefer higher pH conditions (Staley et al. 2011).

## 1.3 Molecular Approach for Determination of Soil Diversity

Taxonomy is mostly used as an equivalent term of systematics or biosystematics. This has been divided into three parts: (a) classification, arrangement of microorganisms according to taxonomic groups, (b) naming of classified microorganisms, and (c) identification of undefined microorganisms (Agrawal et al. 2015). Two approaches of taxonomic classifications of microbes have been used. The culture-dependent technique is related to the phenotypic approach (Nural Yaman et al. 2019). The culture-independent techniques are informed on microbial diversity by using the phylogenetic markers (Agrawal et al. 2015; Panigrahi et al. 2019).

Culture-dependent techniques have been frequently used to do microbial diversity studies in natural and contaminated environments. However, these techniques are biased in evaluations of all microorganisms in the environment. The determinations of microorganisms by culture-dependent techniques have made known only about 1% of microorganisms. That is to say, there are no data on about 99% of the total number of microbes (Panigrahi et al. 2019). In microbial ecological studies, commercial media such as Nutrient Agar, Tryptic Soy Agar, Malt Extract Agar have been used to practice the traditional culture techniques. The media helped reveal a small part of microbial diversity. In any case, some culture medium conditions can be changed to optimize the growth conditions for the cultivation of different microorganisms. Despite the improvements of media, all microorganisms have not been successfully cultivated in the laboratory (Panigrahi et al. 2019).

Culture-independent techniques known as modern molecular approaches have been used to discover most of the unculturable microorganisms in laboratory conditions (Agrawal et al. 2015; Panigrahi et al. 2019). The primitive source of knowledge on culturable microorganisms consists of their biomolecules like nucleic acids, proteins, and lipids. The approaches related to nucleic acids have been performed using marker genes such as 16S and 18S rRNA (ribosomal RNA) for prokaryote and eukaryote microorganisms, respectively (Srivastava et al. 2019; Panigrahi et al. 2019). These biomolecules are phylogenetic markers used as a gold standard for the identification of taxonomic groups of microbial communities (Srivastava et al. 2019).

We will discuss different molecular determination techniques which are based on 16S/18S rRNA gene region amplifications as from the following headline.

### *1.3.1 DNA Extraction of Soil Microorganisms*

DNA extraction from soil samples is difficult because of high clay and humic material concentrations. Furthermore, DNA binds strongly to clay particles which block the isolation of DNA into the extraction supernatant (Frostegard et al. 1999; Cai et al. 2006). Humic material has also the same size as DNA; therefore, this material can bind to DNA and it may be brown-colored same as DNA extracts. The presence of humic material in DNA extracts blocks the activity of some enzymes including DNA polymerases (Dong et al. 2006). In addition, humic material affects the DNA quantification determined by spectrophotometric methods. Both DNA and humic material absorbance values are measured at 260 nm and 230 nm. Alternative fluorometric methods (Qubit, Thermo Fisher Scientific) are less effective to measure humic materials. Therefore, the concentration of DNA is guessed more accurately compared to other measurement methods (Lear et al. 2018).

Commercial kits, especially PowerSoil® DNA Isolation kit, can remove the PCR inhibitors from soil DNA such as humic acid, clay, etc. (Lear et al. 2018). Except for commercial kits, some precautions are applied to decrease the contaminants from extracted DNA. Firstly, the DNA may be diluted before the PCR amplification. This enables the PCR to be successful. Secondly, DNA is precipitated by PEG (polyethylene glycol) to reduce humic acids (Griffiths et al. 2000). Another precipitator may be glycogen which is effective for DNA precipitation. It can be combined with PEG or ethanol.

### *1.3.2 PCR-Dependent Methods*

Carl Woese reported the 16S rRNA gene region as a marker molecule for taxonomic studies towards the end of the 1970s. Then, life had been divided into three domains: Bacteria, Archaea, and Eukarya (Woese and Fox 1977; Woese et al. 1990). Just as 16S rRNA is a significant molecule for prokaryotic microorganisms, eukaryotic microorganisms also have the homolog molecules named as 18S rRNA gene region (Hughes et al. 2009).

Being responsible for the synthesis of proteins, ribosomes are found in every cell of organisms belonging to all three domains and they are considerably conserved. The ribosome comprises two main subunits which are small subunit (30S;16S rRNA) and large subunit (50S; 23S rRNA and 5S rRNA). These ribosomal RNA sequences have been used to identify microorganisms at the molecular level and are used to set up phylogenetic relationships (Aytar et al. 2015). Archaeal and bacterial systematics can use them because of their functional and structural stability. The genes are amplified from genomic DNAs of microorganisms by polymerase chain reaction (PCR). The universal primer pairs (Lane et al. 1985; Marchesi et al. 1998) are used in amplification. 16S rRNA has been often preferred instead of 23S rRNA due to full length and less favored region of 23S rRNA. *The 16S rRNA-based techniques are preferable and reliable to illuminate microbial diversity studies in culture-independent techniques (Tripathi et al. 2019).

The microbial phylogenetic identification of fungal species (eukaryote) is performed by 18S rRNA-based techniques or ITS region-based techniques. The 18S rRNA region is a variable sequence being in small subunits in fungal genomes. Primer designing is feasible because of the iterative sequences in this region. Therefore, the identification according to 18S rRNA has become ideal. On the other hand, because the ITS region is less conserved, it is a better biomarker than others for identification at the fungal species level. The gene regions are amplified by using a polymerase chain reaction. The universal primer pairs (Borneman and Hartin 2000; Martin and Rygiewicz 2005) are required for successful amplification.

The ITS region has been sequenced and used for microbial identification as it is being done for other biomarkers. A necessity for these methodologies is the use of universal primers for 18S rRNA region (Borneman and Hartin 2000) and ITS region (Martin and Rygiewicz 2005; Aytar et al. 2014a, b). This method identifies eukaryotes with high sensitivity and specificity in a short time. Whole genome sequencing studies are increasing due to the continuous decrease in sequencing costs over time.

16S rRNA- and/or 18S rRNA-based PCR techniques including DGGE, TGGE, SSCP, ARDRA, T-RFLP, etc. can reveal details on microbial population structure in ecological niches.

#### 1.3.2.1 Denatured Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE)

The differences in the 16S/18S rRNA gene regions of microbial communities in various environmental samples have been separated by DNA fingerprinting approach. This approach allows high-throughput sample and can be used for the marker sequences being phylogenetically or functionally important. DGGE or TGGE are DNA fingerprinting techniques that are most often used. The techniques are successfully performed to determine all microbial diversities (Zhao et al. 2011). Amongst often used techniques for microbial community study in environmental samples is PCR-DGGE that produces complex profiles of microbial communities in soil and rhizosphere (Fig. 1.1).

DGGE and TGGE are used to distinguish PCR-amplified ribosomal RNA fragments of microbial genomic DNA. The rRNA amplicons are the same length; however, variation in nucleotide compositions enables the distribution of microbial genetics fingerprinting on the gel (Rincon-Florez et al. 2013; Agrawal et al. 2015). Formamide and urea (in DGGE) or temperature (TGGE) has been used to melt the double-stranded DNAs, and melted DNAs have migrated partially on polyacrylamide gels by the electrophoretic mobility (Rincon-Florez et al. 2013). DNAs are extracted from samples and used as a template to amplify the amplicon with universal primer pairs targeted 16S or 18S rRNA regions. The forward primer has the GC clamps which are 30 bp lengths. The amplicons are loaded onto a polyacrylamide gel. The separation onto gel with different concentrations of denaturant agent

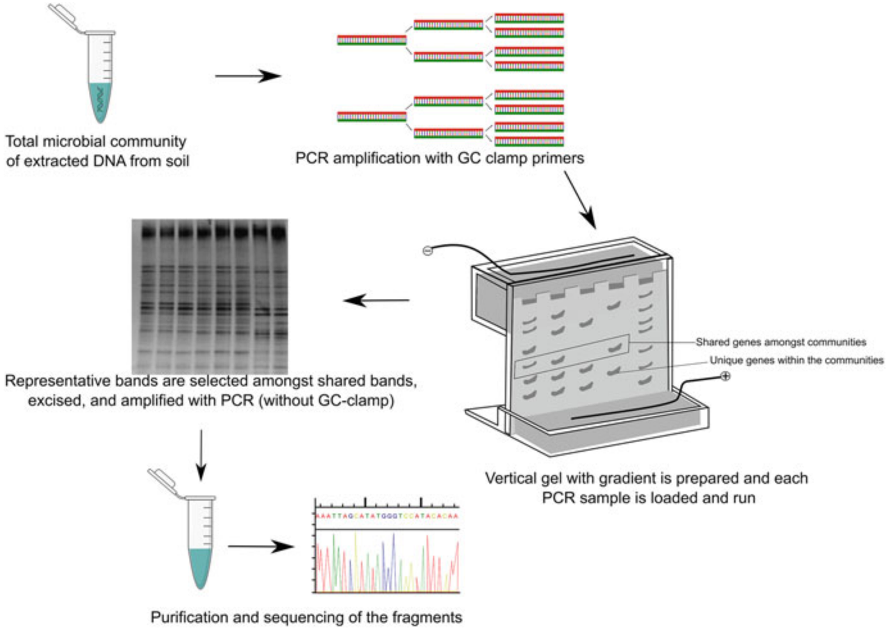

**Fig. 1.1** DGGE diagram for determining microbial diversity in soil

(formamide and urea) happens according to melting points of double-strand DNA. After denaturation, DNA fragments can migrate differentially from beginning to end of the polyacrylamide gel, and they stop at the different points on the gel (Muyzer et al. 1993). At the end of the running gel, DNA can be visualized by staining (Agrawal et al. 2015). DGGE and TGGE only reveal microbial abundance within a community. On the other hand, the results can be misleading because single bands could mean multiple species while multiple bands may represent a specie (Agrawal et al. 2015).

Fungal profile from different environmental samples such as soil has been determined by using DGGE or TGGE. DGGE or TGGE fingerprints of environmental DNA from the rhizosphere have discovered the relationship between fungal profile and its habitat (Zhao et al. 2011). TGGE approach uses increasing temperature and uniform denaturant inside of denaturant gradient in DGGE gel. Therefore, bacteria and fungi are detected by TGGE compared to other molecular techniques (Bruns et al. 1999; Felske et al. 1998; Takaku et al. 2006; Ishii et al. 2000; Agrawal et al. 2015).

#### 1.3.2.2 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP is another fingerprinting technique in which the forward or both forward and reverse primers are fluorescent-labeled (Fig. 1.2). Primers enable tagging targets

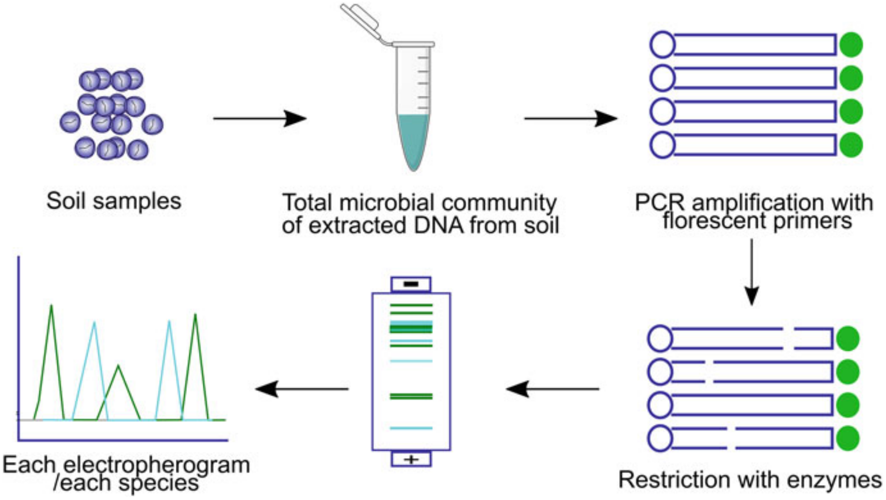

**Fig. 1.2** T-RFLP diagram for microbial diversity in soil

to be amplified, then digestion is followed by a restriction enzyme. The sample can be run on a sequencing gel electrophoresis to know the sizes of the labeled terminal restriction fragments. Diverse combinations of restriction analyses of different soil microbial communities will display due to the changes in the gene sequencing. The genes are specific regions for organisms (Rincon-Florez et al. 2013).

This terminal-restriction fragment length analysis has several benefits, hence its rapid popularity: it is compatible in the laboratory, and in the end, electrophoresis is easily practicable (Zhao et al. 2011). The easy practicality originates from physical capture, fluorescence scanning, and primer with 32 labels. Therefore, 16S rRNA gene for bacteria and archaea and ribosomal genes for fungi have been used to exhibit soil microbial community (Zhao et al. 2011).

T-RFLP has been derived from RFLP and ARDRA. Their principles are so similar to each other. The most important difference is using fluorescence-labeled primers in this technique.

The microbial diversity of different environments has been revealed by this technique (Srivastava et al. 2019). Castaneda and coworkers have performed to compare community diversity of microorganisms between forest and vineyards (Castañeda et al. 2015). Similarly, the fungal community has been reported by Kasel and coworkers using this technique (Kasel et al. 2008).

### 1.3.2.3 Clone Libraries

Clone libraries have benefits to identify and characterize the dominant bacterial or fungal types in soil and thereby provide a picture of diversity and this pioneers microbial diversity studies (Fig. 1.3). This method depends on cloning PCR amplified biomarker genes of prokaryotes and eukaryotes and then their gene fragments

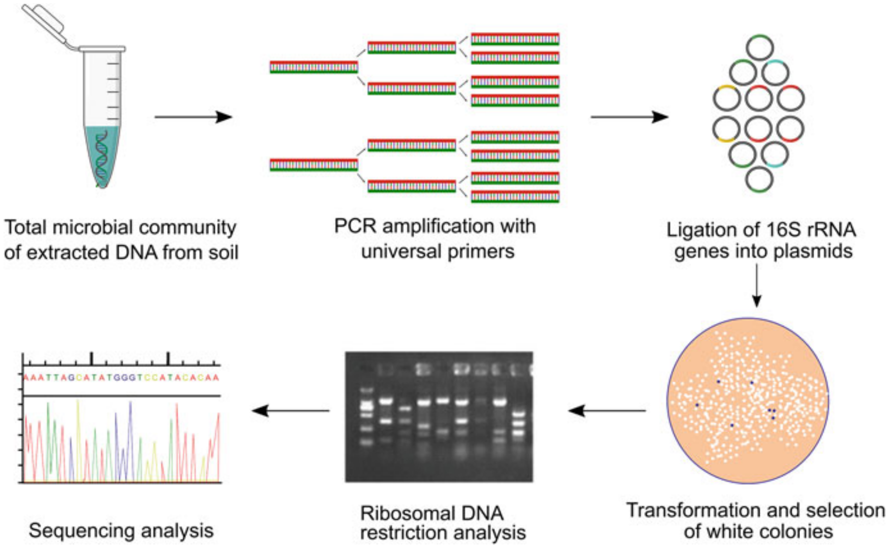

**Fig. 1.3** 16S–18S cloning diagram for microbial diversity in soil

sequences (Pal et al. 2019). The libraries should be large enough to describe the soil microbial community. There are a few studies about clone library constructions of soil environmental samples including hydrocarbon-contaminated soil (Dojka et al. 1998) because of some limitations and problems about a representative of soil microbiota (Garbeva et al. 2004; Sierra-Garcia et al. 2017).

#### 1.3.2.4 Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Amplified ribosomal DNA restriction analysis (ARDRA) has informed about the microbial diversity according to DNA polymorphism (Agrawal et al. 2015). ARDRA originates restriction fragments from the gene amplicons of 16S rRNA (Smit et al. 1997) and 18S rRNA (White et al. 1990), respectively, of bacterial-archaeal and fungal microbial populations in soil environments. Universal primers are not used to enable the knowledge about the specific organisms but are used to construct a pool of all microorganisms in soil environments (Rincon-Florez et al. 2013). The universal primers such as ITS-1 and ITS-4 are used in ARDRA-ITS (also termed ITS-RFLP). These primer pairs are specific for the evolutionary stable 18S and 28S rRNA genes region belonged to fungal ribosomes. 16S rRNA gene region is methodically used for bacterial and archaeal microorganisms, with appropriate primers (Choudhary et al. 2009).

Amplified marker genes were used in digestion reactions using restriction enzymes (Nocker et al. 2007; Rincon-Florez et al. 2013). The restriction enzymes (AluI, MspI, HaeIII, HinfI) recognize the region with four nucleotides and cut this region (Rincon-Florez et al. 2013; Agrawal et al. 2015; Srivastava et al. 2019). These fragments of digested amplicons are loaded on agarose gel and separated according to their sizes. The dendrograms are obtained after running the digestion fragments.

The ARDRA is a sensitive molecular technique to inform the pattern of phylogenetic groups (Srivastava et al. 2019), but it does not give enough information about the types of microorganisms present in the soil environmental samples (Liu et al. 1997; Heyndrickx et al. 1996; Sklarz et al. 2009). ARDRA is also used to screen rapidly both colonies of clone libraries and isolates obtained from culture-dependent techniques.

#### 1.3.2.5 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

One other approach which is used in the study of the diversity of microbial communities from environmental samples is Automated Ribosomal Intergenic Spacer Analysis. This method is based on the differentiation of the phylogenetic markers like the16S and 23S rRNA (Popa et al. 2009). An automated capillary laser detection system is used to determine the variation in the markers. The obtained peaks of the analysis are generated with universal primers (Nadarajah and Kumar 2019).

#### 1.3.2.6 Random Amplification of Polymorphic DNA (RAPD)

Random Amplification of Polymorphic DNA is used to evaluate the difference and diversity in microbial habitat (Nadarajah and Kumar 2019). The method is applied with random primer and generated varied lengths of products. These DNA fragments are distinguished on the gel by bands representing different polymorphisms of different organisms. Visualization and comparisons can be done at the level of bands. The bands indicate the polymorphisms of different organisms. They can be visualized and compared in the form of bands (Nadarajah and Kumar 2019; Gohil et al. 2019).

#### 1.3.2.7 Q- PCR

The Quantitative Polymerase Chain Reaction (Q-PCR) is generally used to determine the expression and abundance of marker gene regions. Marker gene used for this method might also be related to phylogenetic systematics in microbial communities. If the fluorescent stain (SYBR GREEN) or fluorescent probes (Taqman) are combined with conventional PCR conditions, this technique is called quantitative PCR (Rincon-Florez et al. 2013; Srivastava et al. 2019), and the amplicons can be measured in every cycle in real time (Smith and Osborn 2009). Many laboratory researchers start to use more frequently the Q-PCR because it is specific, sensitive, successful, reliable, and cost-effective. It can also be applied to detect the microbial composition even at RNA (Bustin et al. 2005). On the other hand, evaluation of soil communities such as acidobacterial population in rhizospheres can be performed by real-time PCR. At the same time, real-time PCR primers being specific to taxonomic groups are used to discover bacterial and fungal microorganisms from the soil in advance. However, it does not require post-PCR procedures to avoid contamination. Therefore, this is different from other PCR techniques.

#### 1.3.2.8 Single-Strand Cell Polymorphism (SSCP)

Single-strand conformation polymorphism (SSCP) analysis is a technique that is applied to detect differences in the sequence of single-stranded DNA as shown in Fig. 1.4 (Agrawal et al. 2015). The amplified fingerprint amplicons are loaded into a gel and separated by non-denaturating polyacrylamide gel electrophoresis (PAGE) (Srivastava et al. 2019). This approach has been performed to determine the

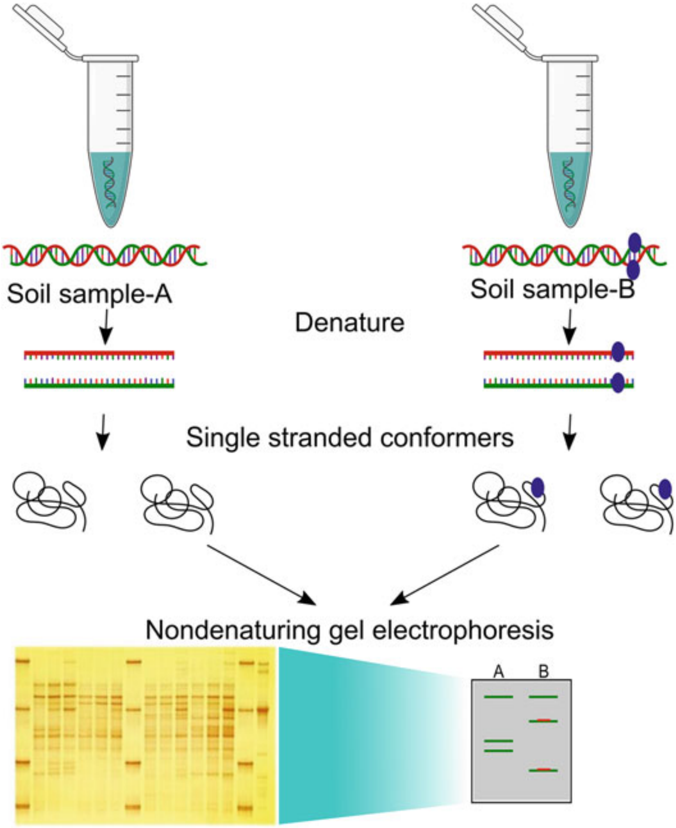

**Fig. 1.4** SSCP diagram for microbial diversity in soil

differentiation among the pure culture isolated from the rhizosphere, the investigation of microbial diversity and the functional gene in contaminated environmental samples (Schwieger and Tebbe 1998; Peters et al. 2000; Junca and Pieper 2004). The general procedure of SSCP consists of PCR amplification from the template DNA, amplified product denaturation with heat and denaturants, and sample separation by non-denaturing polyacrylamide gel electrophoresis (Orita et al. 1989). SSCP separates DNA molecules of the same size whose sequences have different nucleotide. These molecules are distinguished according to their mobility on the gel (Rawat et al. 2005).

Bacterial and fungal diversity in communities has been investigated via single-strand conformation polymorphisms (SSCPs). The PCR products have been amplified with universal primers for 16S rRNA (bacteria) and 18S rRNA (fungi), from the template environmental DNA (Peters et al. 2000). This approach may be a substitute for DGGE and TGGE. SSCP does not need gradient gels prepared with denaturants (Agrawal et al. 2015). For TGGE, there is usually a need for specific equipment like the temperature gradient incubation system for gels through trivial electrophoretic chambers with SSCP temperature controls that can be used for the same purpose.

While TGGE-specific equipment such as a temperature gradient incubation system for electrophoretic gels is also needed, regular electrophoretic chambers with temperature control for SSCP can be used. An additional positive side of SSCP over DGGE/TGGE is that useful SSCP primers do not require GC clamp when running the PCR (Droffner and Brinton 1995).

#### 1.3.2.9 Stable Isotope Probing (SIP)

Stable Isotope Probing (SIP) is a nucleic acid-based method used to identify bacterial communities in the environmental sample (McDonald et al. 2005; Schutte et al. 2008) (Fig.1.5). Either soil or plant is labeled with $^{13}$C, a $^{13}$C-labeled substrate is

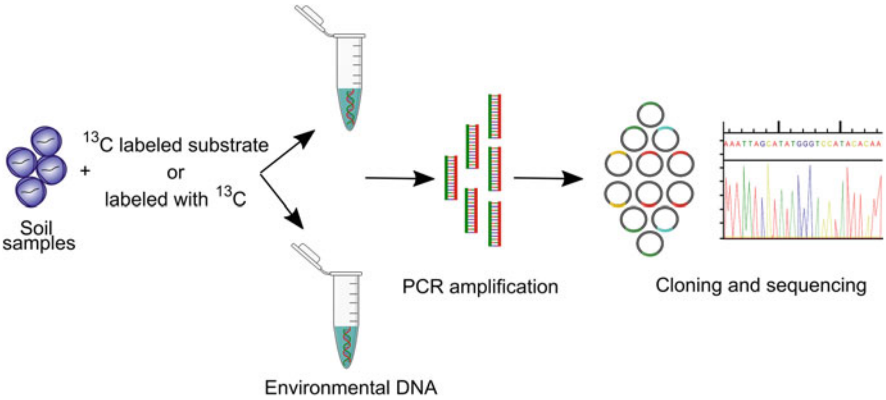

**Fig. 1.5** Isotope array

added to soil or plant is marked with $^{13}C\text{-}CO_2$. DNA in soil is extracted and a density gradient centrifuge is used to separate the $^{13}C$ marked DNA. Labeled DNA is the template to amplify PCR product which is cloned into a vector and this product is sequenced. Thus, the microbes that absorbed the marked substrates are identified. SIP approach has a big potential to identify microbes with functional activity. For this, the labeling degree should be very sensitive (Zhao et al. 2011).

This approach led to the understanding of how microorganisms vary in space in relation to carbon flow within the rhizosphere. The roles of fungal and bacteria interactions within communities have been investigated with SIP in the context of soil litter degradation. Different processes are followed by the method which also allows like matter fluxes and biochemical reactions in soil microbial samples. SIP may provide information related to carbon fluxes of soil microbial systems (Rincon-Florez et al. 2013).

#### 1.3.2.10 DNA Microarray

Microarrays are classified into three main headlines combined by the different probe types used to study microbial populations. These are community genome arrays, rRNA-based oligonucleotide microarrays, and functional gene arrays (Zhao et al. 2011).

The results of microbial communities of environmental samples obtained by DNA microarrays are high throughput and comprehensive when compared with other techniques. Total DNA extracted from the sample is used as a template for amplification. They are hybridized to molecular probes which are added to the microarray surface (Gentry et al. 2006). Positive signals are numbered by confocal laser scanning microscopy, after hybridization. This method is rapidly evaluated by the microbial population analyses. The cause of rapid completion is related to the analysis of thousands of DNA sequences in a single array (Agrawal et al. 2015).

It might be said that using microarrays to investigate microbial populations in the soil is limited in microorganisms due to available probes. Microarray data might be confirmed by other methods such as nucleic acid blot hybridization and/or Q-PCR (Rincon-Florez et al. 2013).

### *1.3.3 PCR-Independent Methods*

#### 1.3.3.1 DNA-DNA Hybridization

Hybridization of nucleic acids including DNA or RNA extracted from different biological sources is based upon sequence homology between DNA and/or RNA (Agrawal et al. 2015). Specific probes are used in hybridization, which provides useful qualitative and quantitative molecular data for bacterial ecological studies (Clegg et al. 2000; Theron and Cloete 2000). This hybridization approach can lead to the design of probes for extracted DNA or RNA but the mentioned design needs to use known model sequences consisted of a studied environmental sample. The oligo probes can be marked by fluorescent tags (Theron and Cloete 2000). On the other hand, the abundance of a specific group of microorganisms is determined by dot blot hybridization. This is a significant method to get information about the microbial community in environmental samples compared to similar ones (Agrawal et al. 2015). Large-scale study of DNA from the microbial community is performed by DNA reassociation kinetics to evaluate the diversity. In the case of DNA reassociation kinetics, the more complex the denatured DNA the slower the reassociation. This approach may be the only developed method that determines the total number of bacterial microorganisms in compost sample. This technique requires a good quantity of DNA which is always a challenge obtaining from soil constituting a major limitation (Torsvik et al. 2002).

#### 1.3.3.2 Fluorescent iIn Situ Hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a molecular cytogenetic technique for in situ detection of a specific gene, which has been used since the 1990s (Amann et al. 1995; Rincon-Florez et al. 2013; Srivastava et al. 2019). FISH is a generally implemented method for localizing, identifying, and isolating desired microbial taxa in environmental microbial ecology. Single-cell methods are committed to studying microbial population composition, and the efficiency of the method can be further improved through FISH technology (Amann and Fuchs 2008). Fluorescent stain or fluorochrome-labeled probes is preferred to detect the gene region of microorganisms in environmental soil samples. The complementary sequence and the fluorescent probe hybridize each other, and this group can be detected using fluorescence microscopy or confocal laser scanning microscopy. This technique helps to detect and visualize bacteria in the environment; at the same time, it is able to discover live cells by targeting the rRNA of microorganisms (Zhao et al. 2011). The results provide phylogenetical identification and counting in every cell. Diverse molecular probes (probes targeted Euc502, Eub338, and Arc915) have been directed towards the 16S rDNA genes of various taxa (Amann et al. 1995).

FISH technique is applied to study the cells of microorganisms with culture-independent techniques in laboratory conditions. FISH can reveal the taxonomic composition of a microbial population in contaminated soils (Ishii et al. 2004). This approach has been used to analyze the microbial diversity of agricultural soils with diverse pesticides and herbicides (Caracciolo et al. 2010). The FISH analysis is performed without cultivation of microorganisms, which has been reported firstly in 1989 (DeLong et al. 1989). These techniques are used often being reliable and rapid for soil samples (Sekar et al. 2003).

Studied soil microbial communities with fluorophore signal intensity is limited. To get over fluorescence problems in FISH technique, new methods use a single oligonucleotide combinatorial probe labeling, which is named multi-labeled FISH (MiL-FISH). In this approach, the technique will able to improve the signal intensity and visualize the quality of every microbe in environmental samples (Schimak et al. 2016).

## *1.3.4 Next-Generation Sequencing Approach*

Next-Generation Sequencing also called high-throughput sequencing is one of the culture-independent approaches and has been performed for determining of microbial diversity of complex environments such as soils. New technologies relating to DNA and/or RNA sequencing have been improved by advances in bioinformatics and other biotechnological methods. Metagenomics comprises DNA-based methods while metatranscriptomics comprises RNA-based methods. These methods play a major role in studying the microbial population in soil samples. Parallel sequencing platforms are performed most generally (Rincon-Florez et al. 2013). Metatranscriptome analysis reveals the enrichment and expression of genes in the soil environment (Pal et al. 2019) belonging to microorganisms. Metaproteome analysis has informed about protein complement of the microbial community in specific environmental conditions at a time point.

### 1.3.4.1 Metagenomics

Metagenomic is a culture-independent method that finds out the microbial community using only environmental DNAs (Srivastava et al. 2019; Demir et al. 2020; Nural Yaman et al. 2020). It can be called “environmental genomics” or “community genomics” according to Handelsman and coworkers (Handelsman et al. 2002). This technique does not require cultivation procedures. This term has been used firstly by Handelsman et al. (Handelsman et al. 1998) to explain the soil microbiota by using the concept of cloning of environmental DNA (Srivastava et al. 2019). It relies on shotgun sequencing and target gene sequencing, and their results generate two profiles of microbial community which are taxonomic profiling and functional profiling. This approach focused on the generation of taxonomic classification connecting to functional profiles of unculturable microorganisms (Rondon et al. 2000). Shotgun sequencing starts at the extraction of environmental DNA and continues to the cloning of environmental DNA to show the microbial habitat of environments. Then the constructed libraries are screened and can provide information about the microbial population at the taxonomic level (Srivastava et al. 2019; Nural Yaman et al. 2021; Aytar Çelik et al. 2021).

In targeted gene sequencing, the first step is the extraction of DNA from the soil environment. Then the 16S and/or 18S rRNA genes are amplified from soil DNA by using domain-specific primer, 341F/805R and 340F 915R (for prokaryotes, Herlemann et al. 2011), F1380/R1520 (for eukaryotes, Amaral-Zettler et al. 2009), and ITS3/ ITS4 (for fungi, White et al. 1990); then the products are purified and the adapters are added to amplicon. The fragments are both amplified and sequenced

(Sabale et al. 2019). The readings are blasted against the SILVA, Green Genes NCBI, and OTT (Balvočiūtė and Huson 2017). Identification of microbial communities living in environmental sites is completed.

Roche 454 Genome Sequencer (Roche Diagnostics Corp., Branford, CT, USA), HiSeq 2000 (Illumina Inc., San Diego, CA, USA), and AB SOLID™ System (Life Technologies Corp., Carlsbad, CA, USA) are used in metagenomic studies of soil samples. Other high throughput platforms are Ion Personal Genome Machine (Life Technologies, South San Francisco, CA, USA), Heliscope (Helicos Bioscience Corp., Cambridge, MA, USA), and PacBio RS SMRT system (Pacific Bioscience, Menlo Park, CA, USA) which are applied for metatranscriptomics (Rincon-Florez et al. 2013).

#### 1.3.4.1.1 Application of Soil Metagenomics

Soil habitats are the richest of all the other environments on earth with regard to microbial diversity. Soil is the most microbially diverse habitat and is considered the unlimited resource for finding out novel genes, enzymes, biomolecules, bioactive compounds, and bioprocesses (Sabale et al. 2019). Soil metagenomics approaches led to the understanding of microbial communities and their functional interactions. Therefore, this method can be preferred in the determination of microbial community and discovery of new functional genes that code for biocatalysts with industrial potential. Sustainable industry and bioeconomy have often needed candidate enzymes, biomolecules, and processes to modernize the industrial process. Soil metagenomics approach helps the researcher to identify candidate unculturable microorganisms having huge potential instead of culturable classic microorganisms. The next-generation sequencing methods are applied to figure out the problems of identifying diversity on the soil microbiota caused by the complex structure of the soil. The results of the two approaches provide the advance for soil health, industrial applications, antibiotic studies, agriculture and bioremediation topics, and so forth (Sabale et al. 2019).

### 1.3.4.2 Metatranscriptomics

Soil metagenomics provides both taxonomic and functional information about the microbial population in soils. However, it can inform the interaction community and functional activity in soil (Srivastava et al. 2019). The exact functional roles of microbial communities are given insight by studying the mRNA transcriptional profiles of microorganisms (Pal et al. 2019). Metatranscriptomic approach also reveal transcribed genes of active microbes by using the complement of RNA obtained from entire microbial communities (Zarraonaindia et al. 2013).

Total mRNA directly extracted from a single cell or the environment, such as soil sample, is called the transcriptome and metatranscriptome, respectively (Mason et al. 2012; Li et al. 2014; He et al. 2015; Bashiardes et al. 2016). Studies enable profiling the transcriptome of either the individual cell or the entire microbial community. The analysis in these approaches produces the information about the gene and microbes under specific environmental conditions such as soil and/or contaminated soil (Chistoserdova 2009; Bashiardes et al. 2016; Martinez et al. 2016). At the same time, active metabolic pathway(s) are found under studying environmental conditions (Srivastava et al. 2019).

In metatranscriptome analysis, total RNA is extracted firstly from the environmental sample. Complementary DNA (cDNA) is synthesized by using total RNA (Sahoo et al. 2019). The functional profile is constructed to use the map which generates RNA reads of functional gene sequences. Also, mRNA and rRNA are analyzed. mRNA is related to gene expression and rRNA is related to functional genes (Tveit et al. 2014). Environmental metatranscriptomics is studied on only mRNA that has been isolated from the environment and sequenced to show gene expression in the microbial community (Gosalbes et al. 2011).

#### 1.3.4.3 Metaproteomics

The direct determination of protein expression from mixed communities of microorganisms from environmental samples can be possible by developing the traditional proteomic techniques, and the mentioned technique is called metaproteomics (Chakraborty et al. 2014; Pal et al. 2019; Sahoo et al. 2019). Proteogenomics can also be known for this method (Armengaud et al. 2013). Metaproteomics also provides information about proteins related to the microbial community at a certain time point and particular environmental conditions (one example for contaminated soil; Guazzaroni et al. 2013) in microbial ecology studies (Pal et al. 2019).

The metaproteomic analyses have been applied in four significant steps: (1) extraction, purification, and concentration of proteins; (2) denaturation of protein and reduction; (3) separation of protein separation, digestion, and analysis; and (4) spectroscopic identification of proteins (Schneider and Riedel 2010).

The biochemical techniques are applied to determine the stability level of protein. The extracted protein is analyzed by SDS-PAGE gel electrophoresis (one or two dimensional). In this way, the proteofingerprint analysis of microbial population is generated. Then, mass spectroscopy (MaLDI-TOF MS) can be used combined with gel electrophoresis (Maron et al. 2007; Srivastava et al. 2019; Pal et al. 2019).

#### 1.3.4.4 Metabolomics

The profiles of whole metabolites in a single cell in a certain time and condition are studied in metabolomics. The next-generation technologies have widened to metabolomics technology, after the metagenomics, metatranscriptomics, and metaproteomics. The other -omics techniques, especially metagenomics, have demonstrated the power to determine the taxonomic and functional diversity of microbial communities of environmental samples in specific conditions (Malla et al. 2018).

The metabolome-based studies for environmental samples have shown microbial activities under the conditions where they live. In addition to this approach, other studies can be improved to profile the metabolic activity of communities according to changeable environmental factors. In general, secondary metabolites have been released under stress conditions. The metabolomics approach explains the functional roles of these metabolites (Malla et al. 2018).

#### 1.3.4.5 Functional Diversity

The role of a microorganism in the ecosystem can be described as its functional diversity. Some of the mentioned roles are competition, synergy in the microbial community, forming of species together, and communication in the ecosystem. The functional diversity is interested in the interaction between microbes indifferent conditions (Laureto et al. 2015; Petchey and Gaston 2006) and can be predicted rightly by selecting functional and important properties that affect and change the ecosystem's balance. To evaluate this, functional diversity uses some biochemical and traditional methods. Besides, molecular techniques can be also used. Extracted environmental and/or genomic DNA and amplified PCR products are evaluated in this approach (Srivastava et al. 2019).

Studies on functional diversity may also investigate the significance of the individual characteristics. It looks for the answers to two questions. (a) How do species influence the ecosystem? (b) How do species respond to environmental differences? (Laureto et al. 2015).

### *1.3.5 Microfluidic Chips*

The soil is a very diverse environment with so many different structural compositions harboring a large diversity of microorganisms. The study of these organisms has been very challenging because a large fraction of soil microbes are unculturable while others are found in very little amounts (Aleklett et al. 2018). Metagenomics has revealed a large functional diversity of soil microbial communities, but they do not replace culture techniques. Due to recent advances in microfluidics, high-throughput technologies, 3D bioprinting and single-cell analytics culture techniques have evolved from axenic to mixed cultures enabling the study of microbial communities and their underlying interactions. By creating microenvironments that mimic the natural environments, the behaviors of microorganisms can be studied in real time as in their natural environments (Nai and Meyer 2018). It is hoped that the exploration of the microbial dark matter will bring forth new antibiotics and beneficial metabolic pathways (Stanley et al. 2016).

Microfluidics has also improved cell sorting by producing far less damaged cells and higher precision. The possibility to customize the sorting process permits individual cells to be sorted and their roles as community members identified within large populations (Leung et al. 2012). Strategies to increase the precision of single-cell sorting from culture-independent techniques include PCR-activated cell sorting and digital PCRs based on genetic sequences rather than cell size and labeling. Cells can be sorted based on particular genetic traits for metabolism or antibiotic production which enables studying the transcriptome of individual cells within soil microbial communities (Lim et al. 2015; Ottesen et al. 2006). Furthermore, using microfluidic-based quantitative real-time PCR, it is also possible to quantify species within microbial communities making feasible the monitoring of species dynamics over time (Kleyer et al. 2017). With microfluidic droplet barcoding, sequencing of up to >50,000 cells per run has been demonstrated (Lan et al. 2017). With this, researchers are therefore able to identify unculturable microbes carrying particular traits that can lead to numerous research opportunities within the environment (Ottesen et al. 2006).

Besides, the applications of microfluidics in the understanding of plant microbiome interactions may also permit us to characterize various important microbial consortia contributing to plant nutrient uptake (Stanley and van der Heijden 2017).

### *1.3.6 Combined Methods for Microbial Diversity*

Some techniques can be combined to investigate microbial diversity in soil. For instance, FISH combined with microautoradiography is called FISH-MAR. This approach provides in situ identifications of microbial communities (Ouverney and Fuhrman 1999; Meyer et al. 2005). FISH-MAR detects the microbes, their activities, and specific substrate uptake profiles in the environmental microbial community (Lee et al. 1999). The sample obtained from the environment is incubated with compounds that have been tagged like 3H-acetate, 14C-pyruvate, 14C-butyrate, or 14C-bicarbonate after which it is fixed on a slide. Selected fluorescently labeled probes complementary to different 16S rRNA enable FISH analysis. Slide treatment with autoradiographic emulsion and silver particles allows for visualization with confocal scanning laser microscopy. Detecting radioactivity in combination with FISH allows for the detection of the metabolizers of the substrate of interest.

Furthermore, catalyzed reporter deposition (CARD) FISH is known as tyramide signal amplification, which also allows detection of microbes in the soil. CARD-FISH includes tyramide-labeled fluorochromes to amplify rRNA hybridization signals. Tyramide prevents the FISH staining, and many fluorescent probes come together at the target site (Pernthaler et al. 2002).

Another combined method is Chip-SIP, which contains stable isotope probing (SIP) and microarray approach. This technique utilizes the marker genes 16S and/or 18S rRNA genes and ion mass spectrometer which analyzes the relative isotope incorporation of the rRNA. This Chip-SIP approach helps in illuminating complex microbial diversity of environmental samples. Chip-SIP method is applied by comparing the different communities and/or different conditions. The researcher can make an analysis of these combinations: (a) the same community in different substrates/nutrition, (b) different communities in the same substrate(s)/nutrition, (c) response of microbial community against temperature or nutrient concentrations (Mayali et al. 2019).

DNA-SIP probing employing 15N and 14N isotopes can also be combined with density gradient centrifugation to detect different DNA. It is also possible to use other isotopes such as $^{2}H$ and $^{13}C$. For example, for the investigation of methylotrophs, substrates such as $^{13}CH_2OH$ and $^{13}CH_4$ have been included in soil samples to be investigated. Buoyant density gradient centrifugation showed good resolutions enabling effective separation of DNA that incorporated the labeled $^{13}C$ substrates. Using general PCR primers, the DNA can be amplified for further identification of the species by sRNA analyses. DNA-SIP can be further extended for use with multicarbon compounds which can allow for investigations of biodegradation rates (Dumont and Murrell 2005).

RNA-SIP has also been developed producing results even faster because lesser time is required in cells to synthesize RNA. Separation of RNA types can be achieved using cesium trifluoroacetate density gradient centrifugation. RT-PCR amplification can then be applied to obtain the corresponding DNA.

The investigation of microorganisms that are affected by root exudation for studies on rhizosphere-microorganism interactions can also be carried out using SIP techniques. Plants can be incubated with the stable isotope-containing substrates after which nucleic acids can be isolated from the rhizosphere. The DNA-containing isotopes can be obtained as mentioned above for further 16S rRNA analysis, development of metagenomic libraries to investigate functional genes, or other DGGE or microarray analysis (Dumont and Murrell 2005).

## 1.4 Challenges of Using Molecular Approaches for Analysis of Soil Environmental Samples

There are many types of techniques to determine the soil microbial diversity while they have numerous applications and importance in various analyses; their uses are also limited in many ways. Both culture-dependent and culture-independent approaches are very important separately. The two approaches do not replace each other and are more useful when applied together (Nai and Meyer 2018).

In amplification of marker genes, challenges or limitations affect the polymerase chain reaction in the soil system. DNA is used in a polymerase chain reaction as a template. Therefore, the inhibitor can bind to DNA during extraction. These inhibitors can also attack during the PCR leading to the generation of false-negative results. Nevertheless, wrong targets have also been amplified and false-positive PCR products have been produced. Another limitation is not being quantitative of the traditional form of PCR (Luby et al. 2016). However, RT-PCR method giving quantitative results has a limitation; this technique only allows a few gene(s) to be monitored per PCR reaction. As a solution to this problem, qPCR arrays can be used to allow the simultaneous quantification of hundreds of genes (Sen and Sarkar 2019).

DGGE, 16S rRNA-dependent technique has limitations related to artifacts of PCR (Dubey et al. 2020). PCR bias can be subjective and nondominant species can poorly resolute (Edet et al. 2017). Another limitation is that it is not possible to load all the samples on a single gel. Therefore, the gel variation can cause reproducibility (Dubey et al. 2020). PCR products from different organisms, despite differing nucleotide sequences, may also have the same melting point. This causes the generation of missing bands on the gel. To avoid the nonspecific binding, therefore, touchdown PCR is applied, and in this manner, specific binding might be increased (Gałązka and Grządziel 2016).

Another technique, SSCP works well for small fragments preferably 150–400 bp and is very simple and reliable. However, it is subject to PCR biases as well as DGGE. Besides, a major limitation of this technique appears to be the ability of some DNA strands to form multiple stable conformations. SIP methodology has also some problems such as the high cost of labeled substrates, labor-intensive, and low throughput.

Clone library, considered as a reliable technique, has also some limitations such as consuming time, labor-intensive, and cost (Sierra-Garcia et al. 2017). There are some restrictions in FISH technique, which is preferred for providing preliminary information. The signal intensity of the fluorophore used is the key limitation in FISH use. A multi-labeled FISH approach (MiL-FISH) employing combinatorial probe labeling is being proposed as a method to solve this fluorescence problem. The multi-labeled probe amplifies the signal from cells within the samples (Schimak et al. 2016).

Metagenomics approach can reveal the diversity of microbial communities from environmental samples. Recently developed tools which consist of microfluidics, bioprinting, high-throughput screening have been utilized to scrutinize microorganism identification and diversity. They should be applied with other culture-dependent methods to investigate and illuminate the diversity of microbial communities (Table 1.1) (Nai and Meyer 2018).

In metatranscriptome analysis, firstly, the RNA obtained directly from soil can restrict the process and its concentration might be often low. Therefore, additional amplification steps might be used to increase transcript concentrations (Frias-Lopez et al. 2008; Gilbert et al. 2011). Secondly, mRNA separation and the transcriptome of the sample can constrain. As a result, it may be possible to not obtain a statistically significant transcription pattern that represents most genes within a complex community. This therefore restricted earlier works to the more domineering species of the communities.

Metaproteomics has limitations: (1) the protein's source can be bad (e.g., soil sample); (2) molecules replicating proteins (unlike in DNA or RNA) may not have the ability; and (3) possibility for tedious protein identification and isolation (Sahoo et al. 2019).

Stable isotope probing techniques are faced with several limitations such as knowledge of the precise amounts of isotopes to be used to achieve effective resolution of DNAs on gels from complex communities, limited use to only 13C

**Table 1.1** Advantages and disadvantages of molecular approaches used for determination diversity of microorganisms

| Methods | Disadvantages | Advantages | References |
|---|---|---|---|
| DGGE/TGGE | PCR bias<br>Single band could represent multiple species<br>Same species could be represented by multiple bands<br>Time-consuming<br>Limited sequence information<br>Only detects dominant species<br>Dependent on DNA extraction efficiency | Rapid<br>Reliable<br>Reproducible<br>Large number of samples can be analyzed simultaneously<br>Bands can be excised, cloned, and sequenced for identification<br>High resolution for dominant taxa presents in the sample | Rincon-Florez et al. (2013); Agrawal et al. (2015); Pal et al. (2019); Srivastava et al. (2019) |
| ARDRA | PCR bias<br>More applicable to environments with low complexity<br>Unknown sequences often limit the optimization of restriction enzymes<br>Different bands can belong to the same group<br>Labor- and time-intensive<br>Several restrictions are needed for adequate resolution | Good comparison of microbial diversity in response to changing environmental conditions<br>No special equipment required<br>Highly reproducible microbial community<br>Profiles<br>Rapid monitoring of microbial communities over time | Rincon-Florez et al. (2013); Agrawal et al. (2015); Pal et al. (2019); Srivastava et al. (2019) |
| TRFLP | PCR biases<br>Type of DNA polymerase can increase variability<br>Underestimates community diversity because only a limited number of bands per gel can be resolved<br>Especially of spore formers during the extraction of community DNA leading to biasness in DNA amount<br>Choice of universal primers/restriction enzymes influences fingerprint data<br>Lower discriminatory power<br>Dependent on DNA extraction efficiency<br>Artefacts might appear as false peaks<br>Distinct sequences sharing a | Highly reproducible<br>Convenient way to store data and compare between different samples<br>Can be automated<br>Large number of samples can be analyzed simultaneously<br>Rapid, robust, inexpensive, less time-consuming | Rincon-Florez et al. (2013); Agrawal et al. (2015); Pal et al. (2019); Srivastava et al. (2019) |

(continued)

**Table 1.1** (continued)

| Methods | Disadvantages | Advantages | References |
|---|---|---|---|
| | restriction site will result in one peak | | |
| SSCP | PCR bias<br>Lack of reproducibility<br>Short fragments<br>More than one stable conformation possible for some ssDNA<br>Several factors like mutation and size of fragments can affect the sensitivity of the method<br>Several factors like mutation and size of fragments can affect the sensitivity of the method | Rapid, reliable, and Reproducible<br>Simultaneous analysis of a large number of samples<br>No gradient required<br>Possible to identify community members<br>Screening of potential variations in sequences<br>Helps to identify new mutations | Rincon-Florez et al. (2013); Agrawal et al. (2015); Srivastava et al. (2019) |
| Q-PCR | PCR bias<br>Can only be used for targeting of known genes<br>All inherent shortcomings of PCR | Speed, sensitivity, accuracy<br>Discrimination of gene numbers across a wider dynamic range than is found with end-point PCR | Pal et al. (2019); Srivastava et al. (2019) |
| Nucleic acid Reassociation | Dependent on DNA extraction efficiency<br>– Low sensitivity<br>– Requires high copy number sequences for detection | Total DNA extracted<br>– In situ study of DNA or RNA<br>– Not influenced by PCR biases. | Agrawal et al. 2015 |
| FISH | Autofluorescence of microorganisms<br>Sequence information is required for probe design<br>Limited number of probes could be used in a single hybridization experiment, low signal intensity, background fluorescence<br>Specific detection<br>FISH alone cannot provide any insight into the metabolic activities of microorganisms<br>Difficult to differentiate between live and dead cells<br>Difficult accessibility of target gene | DNA isolation and PCR bias independent<br>Highly sensitive and quantitative<br>Can use multiple fluorescent dyes to simultaneously detect different microorganisms<br>Taxonomic and phylogenetic identification<br>Visualization of uncultivable microorganisms<br>Highly sensitive and quantitative | Rincon-Florez et al. (2013); Pal et al. (2019); Srivastava et al. (2019) |

(continued)

**Table 1.1** (continued)

| Methods | Disadvantages | Advantages | References |
|---|---|---|---|
| Methods | Disadvantages | Advantages | References |
| SIP | Incubation and cycling of the stable isotope might cause biases within the microbial communities<br>Prerequisite DNA synthesis and cell division to incorporate sufficient label into DNA for gradient separation | High sensitivity<br>Provides evidence on the function of microorganisms in a controlled experimental setup<br>Less labor-intensive and minimal instruments requires<br>Phylogenetic resolution, provides ever-increasing resource for robust taxonomic and functional assignments | Rincon-Florez et al. (2013); Pal et al. (2019) |
| NGS | Massive data amount, a challenge for data analysis<br>Overestimation of taxonomic classification with short read lengths<br>High error rate | Provides more in-depth information about the composition and function of a whole microbial community | Pal et al. (2019) |
| DNA array | Culturing of organisms required<br>Only detect the most abundant species<br>– Culturing of the organisms required<br>Variation in major ecosystem type can cause variability in the detection of targeted bacterial cells<br>Difficulty in obtaining high-quality rRNA | Analyses a vast amount of genetic information simultaneously<br>Not influenced by PCR biases<br>Total DNA extracted<br>One protocol can be utilized to identify different targeted bacteria simultaneously on a single array<br>In situ study of DNA or RNA<br>Use of DNA fragments increases specificity<br>Large number of genes can be analyzed | Rincon-Florez et al. (2013); Agrawal et al. (2015); Pal et al. (2019); Srivastava et al. (2019) |
| Methods | Disadvantages | Advantages | References |
| Clone library | Labor intensive,<br>Time-consuming,<br>Expensive | More resolution | Sierra-Garcia et al. (2017); Pal et al. (2019) |
| RAPD | Low reproducibility | Rapid, inexpensive, and effortless, prior knowledge of sequence not needed | Gohil et al. (2019) |
| ARISA | PCR bias<br>Economic and relevant for microbial community structure | Better resolution | Kovacs et al. (2010); Likar et al. (2017) |

substrates, experiments are still carried out only in laboratory microcosms with limited applications in actual environments (Dumont and Murrell 2005).

## 1.5 Future Trends

Microbial diversity of environmental samples is investigated by culture-dependent and independent techniques. Culture-independent techniques can be divided into PCR-dependent and PCR-independent techniques. Culture-dependent techniques also use culture media and mimic the environmental conditions in the laboratory to isolate the microorganisms. However, this technique reveals only 1% of microorganisms from environments. On the other hand, molecular approaches investigate and determine more microorganisms than culture-dependent techniques. These approaches combined with bioinformatics tools analyze microbial communities. Nevertheless, they both have advantages and disadvantages.

In considering the future sustainability of agriculture, it is fundamental to evaluate and understand the roles that microbial communities play in the processes that govern ecological change in these ecosystems. Knowing soil microbiota and their applications in agriculture will promote sustainable agriculture, sustainable bioeconomy, enhancing product yield, providing healthy soil (Otwell et al. 2018). Techniques to be applied for figuring out interactions in microbial community and the ecological system should answer some questions:

- Do we investigate microorganisms that are used in the following processes (bioremediation, biosorption)?
- What is the role of microorganisms affecting product efficiency in the ecological system?
- When do we want to obtain the product, under which conditions, and which microorganisms will increase productivity?

New strategies can be followed to reveal the microbiota according to the changing environment selection. New methods to evaluate diversity may be in silico applications and systems biology approaches. Genome-scale metabolic models (GEMs) from in silico approaches are powerful tools to model an organism's/community's metabolic capabilities. The GEMs can be increased according to conditions and then can be used for comparisons. Before using the target-specific simulations to predict, these models should be repeatedly constructed and tried. Theories derived from lab-based studies can then be tested back in the field. The knowledge obtained from repetitive activities will allow for the computational understanding of field processes. This approach enables the understanding of interactions from microbial diversity to ecological and biogeochemical functions (Biggs et al. 2015; Oberhardt et al. 2011).

## 1.6 Conclusion

Soil structure is accepted as a very complex and composite environment. The determination of the interactions between microbial population and soil environment conditions is required because of improving new strategies about sustainable bioeconomy and industrial use, agriculture, bioremediation, and soil health.

Soil microbial population can be identified with culture-dependent techniques but it cannot mimic the environmental conditions in the laboratory conditions. Therefore, culture-independent techniques are applied to turn the disadvantages of cultivation. PCR-dependent or -independent techniques, next-generation sequencing technologies can exhibit the microbial community and relation between gene, protein, and this population. The target is microorganisms not arrived by cultivation in soil environmental samples.

The strategies discussed in this chapter have advantages and disadvantages that are related to one another. On the other hand, they have all been used to depict the microbial community in soil samples from the past to the present.

If we can get to know the oldest owners of the Earth's ecosystem better, we can make more use of them. For this, almost all of the soil microorganisms must be discovered. So, we will get to know the natives of the world and discover all the components in nature's toolbox. Thus, we will be able to make more use of nature to solve problems. According to industrial microbiologists, microorganisms are the first to come to mind when underground wealth is mentioned. Discovering and uncovering them is essential for a more livable world.

## References

Agrawal PK, Agrawal S, Shrivastava R (2015) Modern molecular approaches for analyzing microbial diversity from mushroom compost ecosystem. 3 Biotech 5(6):853–866

Aleklett K, Kiers ET, Ohlsson P, Shimizu TS, Caldas VE, Hammer EC (2018) Build your own soil: exploring microfluidics to create microbial habitat structures. ISME J 12:312–319

Al-Kaisi MM, Lal R, Olson KR, Lowery B (2017) Fundamentals and functions of soil environment. In: Soil health and intensification of agroecosytems. Academic, pp 1–23

Amann R, Fuchs B (2008) Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques. Nat Rev Microbiol 6:339–348. https://doi.org/10.1038/nrmicro1888

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Mol Biol Rev 59(1):143–169

Amaral-Zettler LA, McCliment EA, Ducklow HW, Huse SM (2009) A method for studying protistan diversity using massively parallel sequencing of V9 hyper- variable regions of small-subunit ribosomal RNA genes. PLoS One 4:e6372

Armengaud J, Marie Hartmann E, Bland CL (2013) Proteogenomics for environmental microbiology. Proteomics 13:2731–2742

Aytar Çelik P, Mutlu MB, Korkmaz F, Nural Yaman B, Gedikli S, Çabuk A (2021) Boron mine ponds: metagenomic insight to bacterial diversity. BioDicon 14(2):229–235

Aytar P, Aksoy DO, Toptas Y, Çabuk A, Koca S, Koca H (2014a) Isolation and characterization of native microorganism from Turkish lignite and usability at fungal desulphurization. Fuel 116: 634–664

Aytar P, Gedikli S, Buruk Y, Cabuk A, Burnak N (2014b) Lead and nickel biosorption with a fungal biomass isolated from metal mine drainage: Box–Behnken experimental design. Int J Environ Sci Technol 11(6):1631–1640

Aytar P, Kay CM, Mutlu MB, Çabuk A, Johnson DB (2015) Diversity of acidophilic prokaryotes at two acid mine drainage sites in Turkey. Environ Sci Pollut Res 22(8):5995–6003

Balvočiūtė M, Huson DH (2017) SILVA, RDP, greengenes, NCBI and OTT—how do these taxonomies compare? BMC Genomics 18:114

Bashiardes S, Zilberman-Schapira G, Elinav E (2016) Use of metatranscriptomics in microbiome research. Bioinform Biol Insights 10:19–25

Biggs MB, Gregory LM, Glynis LK, Jason AP (2015) Metabolic network modeling of microbial communities. Wiley Interdiscip Rev Syst Biol Med 7:317–334

Borneman J, Hartin RJ (2000) PCR primers that amplify fungal rRNA genes from environmental samples. Appl Environ Microbiol l66(10):4356–4360

Bouchez T, Blieux AL, Dequiedt S, Domaizon I, Dufresne A, Ferreira S, Martin-Laurent F (2016) Molecular microbiology methods for environmental diagnosis. Environ Chem Lett 14(4): 423–441

Boyle SA, Yarwood RR, Bottomley PJ, Myrold DD (2008) Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biol Biochem 40:443–451

Bruns MA, Stephen JR, Kowalchuk GA, Prosser JI, Paul EA (1999) Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled and successional soils. Appl Environ Microbiol 65:2994–3000

Bustin SA, Benes V, Nolan T, Pfaffl MW (2005) Quantitative real-time RT-PCR—a perspective. J Mol Endocrinol 34:597–601

Cai P, Huang Q, Zhang X, Chen H (2006) Adsorption of DNA on clay minerals and various colloidal particles from an Alfisol. Soil Biol Biochem 38:471–476

Caracciolo AB, Bottoni P, Grenni P (2010) Fluorescence in situ hybridization in soil and water ecosystems: a useful method for studying the effect of xenobiotics on bacterial community structure. Toxicol Environ Chem 92:567–579

Castañeda LE, Manzano M, Godoy K, Marquet PA, Barbosa O (2015) Comparative study between soil microbial structure communities from vineyards and sclerophyllous forest in central Chile. Ecol Evol 5(18):3857–3868. https://doi.org/10.1002/ece3.1652

Chakraborty A, Dasgupta CK, Bhadury P (2014) Chapter 4—application of molecular techniques for the assessment of microbial communities in contaminated sites. In: Das S (ed) Microbial Biodegradation and Bioremediation. Elsevier, Amsterdam

Chistoserdova L (2009) Functional metagenomics: recent advances and future challenges. Biotechnol Genet Eng Rev 26:335–352

Choudhary DK, Agrawal PK, Johri BN (2009) Characterization of functional activity in composted casing amendments used in cultivation of *Agaricus bisporus* (Lange) Imbach. Indian J Biotechnol 8:97–109

Clegg CD, Ritz K, Griffiths BS (2000) %G? C profiling and cross hybridization of microbial DNA reveals great variation in belowground community structure in UK upland grasslands. Appl Soil Ecol 14:125–134

DeLong EF, Wickham GS, Pace NR (1989) Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science 243(4896):1360–1363

Demir EK, Nural Yaman B, Aytar Çelik P, Şahinkaya E (2020) Iron oxidation in a ceramic membrane bioreactor using acidophilic bacteria isolated from an acid mine drainage. J Water Process Eng 38:101610

Dojka MA, Hugenholtz P, Haack SK, Pace NR (1998) Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. Appl Environ Microbiol 64:3869–3877

Dong D, Yan A, Liu H, Zhang X, Xu Y (2006) Removal of humic substances from soil DNA using aluminium sulfate. J Microbiol Methods 66:217–222

Droffner ML, Brinton WF (1995) Survival of *E. coli* and Salmonella populations in aerobic thermophilic composts as measured with DNA gene probes. Zentralbl Hyg Umweltmed 197: 387–397

Dubey RK, Tripathi V, Prabha R, Chaurasia R, Singh DP, Rao CS, Abhilash PC (2020) Methods for exploring soil microbial diversity. In: Unravelling the soil microbiome, SpringerBriefs in environmental science. Springer, Cham, pp 23–32

Dumont MG, Murrell JC (2005) Stable isotope probing—linking microbial identity to function. Nat Rev Microbiol 3:499–504

Edet UO, Antai SP, Brooks AA, Asitok AD, Enya O, Japhet FH (2017) An overview of cultural, molecular and metagenomic techniques in description of microbial diversity. J Adv Microbiol 7(2):1–19

Felske A, Wolterink A, Van Lis R, Akkermanns ADL (1998) Phylogeny of the main bacterial 16S rRNA sequences in Drentse A grassland soils. Appl Environ Microbiol 64(3):871–879

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103:626–631

Flemming HC, Wuertz S (2019) Bacteria and archaea on earth and their abundance in biofilms. Nat Rev Microbiol 17(4):247–260

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW, Delong EF (2008) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci USA 105: 3805–3810

Frostegard A, Courtois S, Ramisse V, Clerc S, Bernillon D, Le Gall F, Jeannin P, Nesme X, Simonet P (1999) Quantification of bias related to the extraction of DNA directly from soils. Appl Environ Microbiol 65(12):5409–5420

Gałązka A, Grządziel J (2016) The molecular-based methods used for studying bacterial diversity in soils contaminated with PAHs (the review). In: Larramendy ML, Soloneski S (eds) Soil contamination: current consequences and further solutions. IntechOpen, p 85

Garbeva PV, Van Veen JA, Van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270

Gentry RW, McCarthy J, Layton A, McKay L, Williams D, Koirala SR, Sayler GS (2006) *Escherichia coli* loading at or near base flow in a mixed-use watershed. J Environ Qual 35: 2244–2249

Gilbert JA, Field D, Huang Y, Edwards RA, Li W, Gilna P, Joint I (2011) Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. In: de Bruijn FJ (ed) Handbook of molecular microbial. Ecology II, metagenomics different habitats. Wiley-Blackwell, Hoboken, NJ, pp 277–286

Gohil N, Panchasara H, Patel S, Singh V (2019) Molecular biology techniques for the identification and genotyping of microorganisms. In: Microbial genomics in sustainable agroecosystems. Springer, Singapore, pp 203–226

Gosalbes MJ, Durban A, Pignatelli M, Abellan JJ, Jimenez-Hernandez N, Perez-Cobas AE et al (2011) Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS One 6(3):e17447

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66(12):5488–5491

Guazzaroni ME, Herbst FA, Lores I, Tamames J, Pelaez AI, Lopez-Cortes N et al (2013) Metaproteogenomic insights beyond bacterial response to naphthalene exposure and bio-stimulation. ISME J 7(1):122–136

Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5(10):R245–R249

Handelsman J, Liles M, Mann D, Riesenfeld C, Goodman RM (2002) Cloning the metagenome: culture-independent access to the diversity and functions of the uncultivated microbial world. Methods Microbiol 33:241–255

He Y, Feng X, Fang J, Zhang Y, Xiao X (2015) Metagenome and metatranscriptome revealed a highly active and intensive sulfur cycle in an oil-immersed hydrothermal chimney in Guaymas Basin. Front Microbiol 6:1236

Herlemann DPR, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5:1571–1579

Heyndrickx M, Vauterin L, Vandamme P, Kersters K, De Vos P (1996) Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) pattern in bacterial phylogeny and taxonomy. J Microbiol Methods 26:247–259

Hillel D (2003) Introduction to environmental soil physics. Elsevier 56(5), p 684

Hughes KW, Petersen RH, Lickey EB (2009) Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. New Phytol 182:792–794

Ishii K, Fukui M, Takii S (2000) Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J Appl Microbiol 89:768–777

Ishii K, Mußmann M, MacGregor BJ, Amann R (2004) An improved fluorescence in situ hybridization protocol for the identification of bacteria and archaea in marine sediments. FEMS Microbiol Ecol 50(3):203–213. https://doi.org/10.1016/j.femsec.2004.06.015

Jenny HF (1941) Factors of soil formation. McGraw-Hill, New York

Junca H, Pieper DH (2004) Functional gene diversity analysis in BTEX contaminated soils by means of PCR_SSCP DNA fingerprinting: comparative diversity assessment against bacterial isolates and PCR_DNA clone libraries. Environ Microbiol 6:95–110

Kasel S, Bennett LT, Tibbits J (2008) Land use influences soil fungal community composition across central Victoria, southeastern Australia. Soil Biol Biochem 40(7):1724–1732. https://doi.org/10.1016/j.soilbio.2008.02.011

Kleyer H, Tecon R, Or D (2017) Resolving species level changes in a representative soil bacterial community using microfluidic quantitative PCR. Front Microbiol 8:2017

Kovacs A, Yacoby K, Gophna U (2010) A systematic assessment of automated ribosomal intergenic spacer analysis (ARISA) as a tool for estimating bacterial richness. Res Microbiol 161(3):192–197

Lan F, Demaree B, Ahmed N, Abate AR (2017) Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding. Nat Biotechnol 35:640–646. https://doi.org/10.1038/nbt.3880

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci U S A 82(20):6955–6959. https://doi.org/10.1073/pnas.82.20.6955

Laureto LMO, Cianciaruso MV, Samia DSM (2015) Functional diversity: an overview of its history and applicability. Nat Conserv 13(2):112–116

Lear G, Dickie I, Banks J, Boyer S, Buckley HL, Buckley TR, Kamke J (2018) Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples. N Z J Ecol 42(1):10–50A

Lee N, Nielsen PH, Andreasen K, Juretschko S, Nielsen JL, Schleifer K-H, Wagner M (1999) Combination of fluorescent in situ hybridization and microautoradiography—a new tool for structure-function analysis in microbial ecology. Appl Environ Microbiol 65:1289–1297

Leung K, Zahn H, Leaver T, Konwar KM, Hanson NW, Pagé AP, Lo CC, Chain PS, Hallam SJ, Hansen CL (2012) A programmable droplet-based microfluidic device applied to multiparameter analysis of single microbes and microbial communities. PNAS 109:7665–7670

Li M, Jain S, Baker BJ, Taylor C, Dick GJ (2014) Novel hydrocarbon monooxygenase genes in the metatranscriptome of a natural deep-sea hydrocarbon plume. Environ Microbiol 16(1):60–71

Likar M, Stres B, Rusjan D, Potisek M, Regvar M (2017) Ecological and conventional viticulture gives rise to distinct fungal and bacterial microbial communities in vineyard soils. Appl Soil Ecol 113:86–95

Lim SW, Tran TM, Abate AR (2015) PCR-activated cell sorting for cultivation-free enrichment and sequencing of rare microbes. PLoS One 10(1):e0113549

Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522

Luby E, Ibekwe AM, Zilles J, Pruden A (2016) Molecular methods for assessment of antibiotic resistance in agricultural ecosystems: prospects and challenges. J Environ Qual 45(2):441–453

Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd Allah EF (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using—omics approaches. Front Microbiol 9:1132

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade WG (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 64:795–799

Maron PA, Ranjard L, Mougel C, Lemanceau P (2007) Metaproteomics: a new approach for studying functional microbial ecology. Microb Ecol 53:486–493

Martin KJ, Rygiewicz PT (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol 5(1):28

Martinez X, Pozuelo M, Pascal V, Campos D, Gut I, Gut M et al (2016) MetaTrans: an open-source pipeline for metatranscriptomics. Sci Rep 6:26447

Mason OU, Hazen TC, Borglin S, Chain PS, Dubinsky EA, Fortney JL, Han J, Holman HYN, Hultman J, Lamendella R, Mackelprang R (2012) Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J 6(9): 1715–1727

Mayali X, Weber PK, Nuccio E, Lietard J, Somoza M, Blazewicz SJ, Pett-Ridge J (2019) Chip-SIP: stable isotope probing analyzed with rRNA-targeted microarrays and nanoSIMS. In: Stable isotope probing. Humana Press, New York, pp 71–87

McCauley A, Jones C, Jacobsen J (2005) Basic soil properties. Soil and water management module, 1(1), Bozeman: Montana State University Extension Service, 1–12

McDonald IR, Radajewski S, Murrell J (2005) Stable isotope probing of nucleic acids in methanotrophs and methylotrophs: a review. Org Geochem 36:779–787

Meyer RL, Zeng RJ, Giugliano V, Blackall LL (2005) Challenges for simultaneous nitrification, denitrification, and phosphorus removal in microbial aggregates: mass transfer limitation and nitrous oxide production. FEMS Microbiol Ecol 52:329–338

Muyzer G, deWaal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nadarajah K, Kumar IS (2019) Molecular microbial biodiversity assessment in the mycorrhizosphere. In: Mycorrhizosphere and pedogenesis. Springer, Singapore, pp 401–420

Nai C, Meyer V (2018) From axenic to mixed cultures: technological advances accelerating a paradigm shift in microbiology. Trends Microbiol 26(6):538–554

Nannipieri P, Badalucco L (2003) Biological processes. In: Bembi DK, Nieder R (eds) Handbook of processes in the soil–plant system: modelling concepts and applications. The Haworth Press, Binghamton, pp 57–76

Nannipieri P, Grego S, Ceccanti B (1990) Ecological significance of the biological activity in soil. In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 6. Marcel Dekker, New York, pp 293–355

Nannipieri P, Ascher J, Ceccherini M, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54(4):655–670

Nema V (2019) The role and future possibilities of next-generation sequencing in studying microbial diversity. In: Microbial diversity in the genomic era. Academic, pp 611–630

Nocker A, Burr M, Camper AK (2007) Genotypic microbial community profiling: a critical technical review. Microb Ecol 54:276–289

Nural Yaman B, Deniz Sonmez G, Aytar Celik P, Korkmaz F, Mutlu MB, Cabuk A (2019) Culture-dependent diversity of boron-tolerant bacteria from boron mine tailings pond and solid wastes. Water Environ J 33(4):574–581

Nural Yaman B, Aytar Çelik P, Mutlu MB, Çabuk A (2020) A combinational analysis of acidophilic bacterial diversity of an iron-rich environment. Geomicrobiol J 37(10):877–889

Nural Yaman B, Mutlu MB, Aytar Çelik P, Çabuk A (2021) Prokaryotic community determination of metal-rich acidic environment by comparative methods. Geomicrobiol J 38(6):504–514

Oberhardt MA, Puchałka J, Martins VAP, Santos D, Papin JA (2011) Reconciliation of genome-scale metabolic reconstructions for comparative systems analysis. PLoS Comput Biol 7: e1001116

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphism of human DNA by gel electrophoresis as single strand conformation polymorphisms. Proc Natl Acad Sci U S A 86:2766–2770

Ottesen EA, Hong JW, Quake SR, Leadbetter JR (2006) Microfluidic digital PCR enables multigene analysis of individual environmental bacteria. Science 314:1464–1467

Otwell AE, García L, de Lomana A, Gibbons SM, Orellana MV, Baliga NS (2018) Systems biology approaches towards predictive microbial ecology. Environ Microbiol 20(12):4197–4209

Ouverney CC, Fuhrman JA (1999) Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. Appl Environ Microbiol 65:1746–1752

Pal S, Roy A, Kazy SK (2019) Exploring microbial diversity and function in petroleum hydrocarbon associated environments through omics approaches. In Microbial diversity in the genomic era. Academic, pp 171–194

Panigrahi S, Velraj P, Rao TS (2019) Functional microbial diversity in contaminated environment and application in bioremediation. In Microbial diversity in the genomic era. Academic, pp 359–385

Pernthaler A, Pernthaler J, Amann R (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl Environ Microbiol 68(6): 3094–3101

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9:741–758

Peters S, Koschinsky S, Schwieger F, Tebbe CC (2000) Succession of microbial communities during hot composting as detected by PCR-single strand-conformation polymorphism based genetic profiles of small-subunit rRNA genes. Appl Environ Microbiol 66:930–936

Pisa G, Magnani GS, Weber H, Souza EM, Faoro H, Monteiro RA, Cruz LM (2011) Diversity of 16S rRNA genes from bacteria of sugarcane rhizosphere soil. Braz J Med Biol Res 44(12): 1215–1221

Popa R, Popa R, Mashall MJ, Nguyen H, Tebo BM, Brauer S (2009) Limitations and benefits of ARISA intra-genomic diversity fingerprinting. J Microbiol Methods 78:111–118. https://doi.org/10.1016/j.mimet.2009.06.005

Rashid MH, Kamruzzaman M, Haque ANA, Krehenbrink M (2019) Soil microbes for sustainable agriculture. In: Meena RS, Kumar S, Bohra JS, Jat ML (eds) Sustainable management of soil and environment. Springer, Singapore

Rawat S, Agrawal PK, Choudhary DK, Johri BN (2005) Microbial diversity and community dynamics of mushroom compost ecosystem. In: Satyanarayana T, Johri BN (eds) I.K. International Pvt. Ltd., New Delhi, p 1027

Rincon-Florez V, Carvalhais L, Schenk P (2013) Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5(3):581–612

Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR et al (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol 66:2541–2547

Sabale SN, Suryawanshi PP, Krishnaraj PU (2019) In: Hozzein WN (ed) Soil metagenomics: concepts and applications, metagenomics—basics, methods and applications. IntechOpen

Sahoo RK, Gaur M, Subudhi E (2019) Function profiling of microbial community. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 77–85

Schimak MP, Kleiner M, Wetzel S, Liebeke M, Dubilier N, Fuchs BM (2016) MiL-FISH: multilabeled oligonucleotides for fluorescence in situ hybridization improve visualization of bacterial cells. Appl Environ Microbiol 82(1):62–70

Schneider T, Riedel K (2010) Environmental proteomics: analysis of structure and function of microbial communities. Proteomics 10:785–798

Schutte UM, Abdo Z, Bent SJ, Shyu C, Williams CJ, Pierson JD, Forney LJ (2008) Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. Appl Microbiol Biotechnol 80(3):365–380

Schwieger F, Tebbe CC (1998) A new approach to utilize PCR-single-strand conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870–4876

Sekar R, Pernthaler A, Pernthaler J, Warnecke F, Posch T, Amann R (2003) An improved protocol for quantification of freshwater Actinobacteria by fluorescence in situ hybridization. Appl Environ Microbiol 69(5):2928–2935

Sen S, Sarkar K (2019) Molecular techniques for the study of microbial diversity with special emphasis on drug resistant microbes. In: Microbial diversity in the genomic era. Academic, pp 499–518

Sharma SS (2015) Molecular tools in the study of soil microbial diversity: with an emphasis on phosphate solubilizing microorganisms. In: Choudhary KK, Dhar DW (eds) Microbes in soil and their agricultural prospects. Nova Publishers, Hauppauge

Sierra-Garcia IN, Dellagnezze BM, Santos VP, Chaves B, Capilla MR, Santos Neto R et al (2017) Microbial diversity in degraded and nondegraded petroleum samples and comparison across oil reservoirs at local and global scales. Extremophiles 21:211–229

Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140(3–4): 339–353

Sklarz MY, Angel R, Gillor O, Soares MI (2009) Evaluating amplified rDNA restriction analysis assay for identification of bacterial communities. Antonie Van Leeuwenhoek 96(4):659. https://doi.org/10.1007/s10482-009-9380-1

Smit E, Leeflang P, Wernars K (1997) Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. FEMS Microbiol Ecol 23:249–261

Smith CJ, Osborn AM (2009) Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbiol Ecol 67:6–20

Srivastava N, Gupta B, Gupta S, Danquah MK, Sarethy IP (2019) Analyzing functional microbial diversity: an overview of techniques. In: Microbial diversity in the genomic era. Academic, pp 79–102

Staley BF, de los Reyes FL III, Barlaz MA (2011) Effect of spatial differences in microbial activity, pH, and substrate levels on methanogenesis initiation in refuse. Appl Environ Microbiol 77(7): 2381–2391

Standing D, Killham D (2007) The soil environment. In: van Elsas JD, Jansson JK, Trevors JT (eds) Modern soil microbiology, 2nd edn. CRC Press, New York

Stanley CE, van der Heijden MGA (2017) Microbiome-on-a-chip: new frontiers in plant–microbiota research. Trends Microbiol 25:610–613

Stanley CE, Grossmann G, Solvas XCI, deMello AJ (2016) Soil-on-a-chip: microfluidic platforms for environmental organismal studies. Lab Chip 16:228–241

Stotzky G (1997) Soil as an environment for microbial life. In: van Elsas JD, Trevors JT, Wellington EMH (eds) Modern soil microbiology. Marcel Dekker, New York, pp 1–20

Su C, Lei L, Duan Y, Zhang KQ, Yang J (2012) Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Appl Microbiol Biotechnol 93(3):993–1003

Takaku H, Kodaira S, Kimoto A, Nashimoto M, Takagi M (2006) Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and-independent approaches. J Biosci Bioeng 101(1):42–50

Theron J, Cloete TE (2000) Molecular techniques for determining microbial diversity and community structure in natural environments. Crit Rev Microbiol 26:37–57

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates. J Soil Sci 33:141–163

Torsvik V, Overas L, Thingstad T (2002) Prokaryotic diversity magnitude, dynamics, and controlling factors. Science 296:1064–1066

Tripathi V, Kumar P, Tripathi P, Kishore A, Kamle M (eds) (2019) Microbial genomics in sustainable agroecosystems, vol 2. Springer Nature

Tveit AT, Urich T, Svenning MM (2014) Metatranscriptomic analysis of Arctic peat soil microbiota. Appl Environ Microbiol 80:5761–5772

van Elsas JD, Trevors JT (1997) Modern soil microbiology. Marcel Dekker, New York

White TJ, Bruns T, Lee S, Taylor J (1990) 38—amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols. Academic, San Diego, pp 315–322

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci U S A 74:5088–5509

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains archaea, bacteria, and Eucarya. Proc Natl Acad Sci U S A 87(12):4576–4579

Zarraonaindia I, Smith DP, Gilbert JA (2013) Beyond the genome: community-level analysis of the microbial world. Biol Philos 28:261–282

Zhao L, Ma Z, Luan Y, Lu A, Wang J, Pan L (2011) Molecular methods of studying microbial diversity in soil environments. In: International conference on computer and computing technologies in agriculture. Springer, Berlin, pp 83–89

# Chapter 2
# Microorganisms Used as Growth Regulators in Modern Agriculture

**Sandra Pérez-Álvarez, Eduardo Fidel Héctor Ardisana, Marco Antonio Magallanes-Tapia, Cesar Marcial Escobedo Bonilla, Crescencio Urìas Garcia, Melisa Magaña González, María Antonia Flores-Cordova, and Sergio Guerrero-Morales**

**Abstract** Biofertilizers and biological products are increasingly being used to enlarge the productivity of crops. Of these, microbes known as Plant Growth-Promoting Microorganisms (PGPM) are the most valuable as biofertilizers, having the capacity to directly impact the growth and development of plants. Plant Growth-Promoting Fungi (PGPF) and Plant Growth-Promoting Bacteria (PGPB) help crops to face biotic and abiotic stresses by enhancing the defense system and several other parameters related to plant growth. This chapter is focused on explaining the function and positive influence of the PGPF and PGPB on several crops, and also to provide a general view of the application of microorganisms in modern agriculture.

**Keywords** Agriculture · Plant growth-promoting microorganism · Plant growth-promoting bacteria · Plant growth-promoting fungi · Growth regulator

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_2

## 2.1 Introduction

Nowadays, agriculture continues to use numerous chemicals (fertilizers and pesticides) to ensure the growth and development of plants. Many of these products have been related to the degradation and pollution of soils, water, and even crops themselves (Jiménez et al. 2011).

The excessive application of synthetic pesticides is the direct cause of the resistance of several organisms, and therefore, the loss of their effectiveness. Chemical control also produces decrease in populations of natural enemies, resurgence of pests with resistance against pesticides, and outbreaks of secondary pests (Pacheco et al. 2019).

One way to increase the efficiency of agronomic systems in the long term is through the application of microbial inoculants, which represent a new technology that can be considered as clean, aligned with the principles of sustainable agriculture, against the irrational increase in the use of pesticides and fertilizers (Naiman et al. 2009). An alternative to increase root growth in plants is by incorporating microorganisms that contribute to the implantation, production, and development of crops. Other factors include soil exploration, access to water and limiting nutrients for crops, reduction of processes for mobile nutrients loss, attenuating periods of moderate water stress and maintaining active growth rates of the crop, and improving its photosynthetic capacity (Díaz-Zorita and Fernández 2008).

Biofertilizers and biological products are increasingly being used to boost crop productivity, being the microbes that stimulate plant development, known as PGPM (Plant Growth-Promoting Microorganisms), most valuable as biofertilizers, having the capacity to directly increase the growth and development of crops (Bashan et al. 2014; Varma et al. 2012; Giri et al. 2019).

An example of PGPM are the Plant Growth-Promoting Bacteria (PGPB) which have been studied not only regarding their impact on the plant, but also relating to the mechanisms they use to promote growth and interact with the plant (Sgroy et al. 2009; Prasad et al. 2015). Some of the characteristics sought for the selection of bacteria with PGPB potential are: ability to solubilize inorganic phosphorus (genus *Paenibacillus*, genus *Enterobacter*), biological fixation of atmospheric nitrogen (species of *Rhizobium, Azotobacter, Bacillus, Clostridium*, and *Klebsiella*), production of plant growth inducers such as auxins, mineralization of organic phosphorus, ACC deaminase activity, production of siderophores (*Azotobacter vinelandii, Bacillus megaterium, Bacillus subtilis, Pantoea allii* and *Rhizobium radiobacter*), hydrocyanic acid and salicylic acid, among others (Glick et al. 2007; Sgroy et al. 2009).

Another group of PGPM are the Plant Growth-Promoting Fungi (PGPF). These microorganisms are gaining importance in organic agriculture because they are soilborne filamentous fungi which are innocuous to plants, and yet they are of great importance. These fungi act by colonizing the root of plants for development, improvement, protection, and growth (Hyakumachi 1994). Some examples of

PGPF are species of *Aspergillus*, *Trichoderma*, *Penicillium*, and several other endophytes (Mishra et al. 2015).

The PGPF remove or suppress pests in the rhizosphere area through the production of plant hormones and hydrolytic enzymes, and also through mineral solubilization (P, N, and Fe). Some other functions include stimulation of systemic resistance, competition for saprophytic colonization, and mycoparasitism (Lewis and Papavizas 1991).

All the above information demonstrates the importance of knowing the microbiota regardless of the function it performs in the plant (plant growth promoter, nitrogen fixer, or phosphate solubilizer), with the aim of maximizing the beneficial effects of biofertilization and biostimulation, to promote more sustainable agricultural production, and satisfy the rising demand for food with the required quality. In this way, using microorganisms able to promote plant growth is a great alternative for biofertilization. The aim of this chapter is to provide information on fungi and bacteria used as growth regulators in modern agriculture describing the species most commonly employed and their function in plant growth.

## 2.2 Microorganisms in Modern Agriculture

Microorganisms appeared on Earth 4 billion years ago. The conquest of the terrestrial surface emerged with the green algae, which evolution turned them into the first plants, an event that occurred about 3.5 billion years later. Afterwards, agriculture began in the Neolithic, about 10,000 years ago. These facts show that microbes have occupied the soil long before other organisms, and that the cultivation of agricultural species has always been associated with them. Nonetheless, only 300 years ago, we have known about the existence of microorganisms, and even less time has elapsed since they began to be considered important and even vital in various processes, including agriculture.

The discovery of the presence of microbes in soil, water, the plant rhizosphere, plant surface, and even inside plant tissues has motivated their in-depth study, in search of relationships and influences that enhance agricultural production. In recent decades, awareness of the contamination of agroecosystems by fertilizers and pesticides has opened up new avenues for taking advantage of the beneficial microorganisms that promote growth, to substitute or reduce the amount of those products used in agriculture.

Research on the relationships between plants and microorganisms, including many PGPBs and various genera of PGPF, have led to the discovery of two large groups of mechanisms (direct and indirect) in which this stimulation manifests itself, which will be reviewed below.

## 2.2.1 Direct Mechanisms

### 2.2.1.1 Biological Nitrogen Fixation and Supply of Other Nutrients

In soils low in nitrogen, the presence of bacteria of the genus *Rhizobium* in symbiosis with various legumes favors the growth of these plants. *Rhizobium* fixes atmospheric nitrogen in the form of ammonia, assimilated by the plant. The symbiosis begins with the emission of radical, exudates with a flavonoid structure that attracts microorganisms, and these respond by activating genes that synthesize nodulation factors (Oldroyd 2013). The plant-microbe association is genetically regulated by miRNA (Hoang et al. 2020) at various points of the symbiosis (Varma et al. 2020).

The miRNAs are small RNA fragments capable of controlling the expression of numerous and diverse genes through repression of translation or degradation of mRNA. The first miRNA was discovered in the nematode *Caenorhabditis elegans* (Lee et al. 1993). The regulation by miRNA in legumes occurs during the mutual recognition between the plant and the microbe, in the formation and growth of the nodules and the modulation of the synthesis and degradation of enzymes, reactive oxygen species (ROS), auxins, and cytokinins during the symbiotic process (Subramanian et al. 2008; Tóth and Stacey 2015; Wang et al. 2015; Yan et al. 2016; Tsikou et al. 2018).

Although the best-known nitrogen (N) fixation process is done by *Rhizobium*, another no less important N fixation is done by the actinobacteria *Frankia* in woody species (Van Nguyen and Pawlowski 2017). In this symbiosis, nodules develop on lateral roots, and the microorganism protects the nitrogenase enzyme complex from oxidation. Other bacterial genera also capable of fixing nitrogen include *Azotobacter* (Jnawali et al. 2015), *Azospirillum* (Fukami et al. 2018), *Bacillus* (Kuan et al. 2016; Yousuf et al. 2017), *Paenibacillus* (Shi et al. 2016), and others. Apparently, the expression of genes involved in N fixation is regulated by the presence of elements and substances such as oxygen and ammonia in the medium, as in *Paenibacillus* (Shi et al. 2016; Do Carmo et al. 2020).

Phosphorous and potassium, in addition to nitrogen, are macroelements consumed in large quantities by plants, and consequently are present in many synthetic chemical fertilizers. Phosphorus is an element commonly found in soils in an insoluble form, which cannot be assimilated by the plant. However, bacteria of the genera *Enterobacter, Pseudomonas, Rhizobium, and Bacillus* and fungi of the genera *Aspergillus, Penicillium*, and arbuscular mycorrhizae act as phosphorus mobilizers or solubilizers (Adhya et al. 2015; Alori et al. 2017; Satyaprakash et al. 2017). The solubilization pathway of phosphorus from phosphate compounds by bacteria such as *Pantoea* sp. is the acidification of the medium by the production of gluconic acid, which is transformed to 2-ketogluconic acid, solubilizer of phosphate (Castagno et al. 2011).

Potassium is the cation most absorbed by plants, but its availability in soils has decreased due to erosion and leaching (Sindhu et al. 2016). *A. tumefaciens* and *R. pusense* are rhizosphere bacteria that exhibit high levels of potassium solubilization (Meena et al. 2015) but a large variety of bacterial genera, as well as the fungi *P. frequentans* and *C. cladosporioides*, perform this metabolic activity (Sindhu et al. 2016). These microbes obtain potassium from the solubilization of minerals such as mica and feldspar, through methods like the production of organic acids, exchange reactions, and chelation (Etesami et al. 2017).

Even though iron is one of the most abundant elements in the earth's crust, its ferric form ($Fe^{3+}$) is not assimilable by plants. Instead, bacteria produce small organic molecules (siderophores) that can bind to this ion. Crowley (2006) showed that siderophores of microbial origin are the main source of iron for plants. In addition to the production of siderophores by *E. coli, Streptomyces* sp., and *Pseudomonas* sp. (Saha et al. 2015), its synthesis has also been demonstrated in the fungi *Trichoderma harzianum, Penicillium citrinum, Aspergillus niger* (Yadav et al. 2011), and *Trichoderma asperellum* (Qi and Zhao 2013). Zinc, mycorrhizal fungi (Gadd 2007), and some species of *Acinetobacter, Gluconacetobacter, Pseudomonas,* and mainly *Bacillus* solubilize this element from insoluble compounds (Sharma et al. 2012).

#### 2.2.1.2 Production of ACC Deaminase

In higher plants, excess ethylene can cause defoliation, wilting, rot, and death events. ACC (1-aminocyclopropane-1-carboxylate) is a precursor of ethylene, and one way to avoid the excessive increase of ethylene is its deamination by the enzyme ACC deaminase of rhizospheric microorganisms, synthesizing $NH_3$ that can be used by plants. In general, the activity of this enzyme is present in most microorganisms that live with plants, especially in environments considered stressful (Timmusk et al. 2011). In the contact among plants and numerous bacteria known to stimulate plant development, such as *Burkholderia, Pseudomonas,* and *Rhizobium*, and fungi like *Trichoderma asperellum*, the activity of microbial ACC deaminase plays an important role (Nascimento et al. 2014).

The high ACC-deaminase activity exhibited by PGPB participates in the reduction of ethylene levels that occur under conditions of flood stress, heavy metals, drought, and salinity. Maxton et al. (2017) found that *Burkholderiacepacia* showed the highest ACC-deaminase activity of three bacterial species tested, and also it induced the highest growth promotion in pepper (*Capsicum annuum* L.) under water and saline stress. The bacterium *Leclercia adecarboxylata* is ubiquitous, and previously considered to belong to the genus *Escherichia* until its reclassification (Tamura et al. 1986). Recently, its ability to reduce saline stress in tomato (*Solanum lycopersicum* L.), which seems to be associated with its high ACC-deaminase activity (Kang et al. 2019) has been demonstrated. Two bacterial lines isolated from the rhizosphere of garlic plants (*Allium sativum* L.), identified as *Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp., were the only ones among six that showed ACC-deaminase activity, and also the only ones capable of promoting *in vitro* growth of *Phaseolus vulgaris* plants under conditions of water and saline stress (Gupta and Pandey 2019).

The presence of ACC-deaminase activity in pathogenic microbes suggests that in these cases it could help the microorganism to overcome the plant defense responses to infection, which involve the ACC metabolic pathway (Singh et al. 2015). However, the use in agriculture of microorganisms with high ACC-deaminase activity is an option to increase tolerance to abiotic stress with more sustainable agricultural practices (Orozco et al. 2020).

#### 2.2.1.3 Synthesis and Modulation of Phytohormones

In one way or another, all the effects of PGPB and PGPF are linked to phytohormones, either through the synthesis of these compounds or through the modulation of pathways such as salicylic acid in systemic acquired resistance (Heil 2001) and ethylene and jasmonic acid in induced systemic resistance (Ongena et al. 2005; Bisen et al. 2016).

The synthesis of indoleacetic acid (IAA) by PPGB is a pathway by which bacteria stimulate the growth of lateral roots and root hairs (Jeyanthi and Kanimozhi 2018). The softening of the cell walls leads to cell growth and to an increase in the effective surface producing exudates useful for bacteria (Glick 2012a, b). Auxin synthesis by PGPB has been demonstrated in *Pseudomonas* (Egamberdiyeva 2007), *Azotobacter* (Jnawali et al. 2015), *Bacillus* (Kuan et al. 2016), *Azospirillum* (Fukami et al. 2018), and other genera. The stimulation of root growth by the fungi *Trichoderma harzianum* (Harman et al. 2004a) and *Trichoderma virens* (Contreras-Cornejo et al. 2009), as well as by other genera (*Penicillium, Aspergillus, Fusarium, Talaromyces* and *Mortierella*) (Murali et al. 2021) is mediated by auxin synthesis.

Cytokinins not only promote cell division and shoot growth, in which the microorganisms that synthesize them can cooperate with plants (Wang et al. 2018; Swarnalakshmi et al. 2020); their influence in plant-pathogen relationships have also been shown (Saleem Akhtar et al. 2020). Gibberellins, mainly synthesized by different genera of fungi, play a role in stem elongation and root colonization (Hossain et al. 2017a). However, the nature of the effects of both phytohormones remains to be clarified, since they have been proven to be produced by both beneficial and pathogenic microorganisms.

The modulation of hormone synthesis involved in acquired and induced systemic resistance (jasmonic acid, ethylene, and salicylic acid) can also be exerted by *Trichoderma* sp. (Houssien et al. 2010; Tucci et al. 2011; Nawrocka et al. 2018).

## 2.2.2 Indirect Mechanisms

### 2.2.2.1 Natural Enemy Suppressants

A great amount of fungi and bacteria synthesize substances that function as antagonists of plant pathogenic microorganisms. By reducing the chances of infection, they indirectly favor plant growth and development (Patil et al. 2021).

Abundant information exists on the pathogen-suppressive effects of the genera *Pseudomonas, Burkholderia,* and *Bacillus* (bacteria) and *Gliocladium* and *Trichoderma* (fungi). The genus *Burkholderia* contains a great amount of species that produce antibiotic substances of various types (Depoorter et al. 2016). The pyrrolnitrin synthesized by *Burkholderia* and *Pseudomonas* is known to be a broad-spectrum antibiotic, capable of acting against various genera of pathogenic microbes such as *Verticillium, Botrytis, Rhizoctonia*, and *Sclerotinia* (Raaijmakers and Mazzola 2012). The *Burkholderia tropica* line MTo431 synthesizes almost 20 volatile substances, several of them derived from toluene and terpenoids, capable of inhibiting to a greater or lesser extent the growth of the fungi *F. culmorum, F. oxysporum, S. rolffsi,* and *C. gloeosporioides*, pathogens of various plant species (Tenorio et al. 2013).

The 2,4-diacetylphloroglucinol produced by *Pseudomonas fluorescens* is recognized as an antibiotic against several species of pathogenic fungi, including *Gaeumannomyces graminis* var. *tritici* (Mazzola et al. 2004). However, it has also been found that other isolates of this same bacterium are capable of inhibiting the growth of this fungus through the production of hydrogen cyanide (HCN) and siderophores (Warren et al. 2016). The growth suppression of *Phytophthora infestans* in potato, mediated by *Pseudomonas protegens*, is also stronger in lines that synthesize HCN (Hunziker et al. 2014). *Pseudomonas chlororaphis* synthesizes other antibiotics such as pyrrolnitrin, diacetylphloroglucinol, rhizoxine, phenazines and their derivatives (Arrebola et al. 2019).

For its use as a biocontroller, *Bacillus* sp. has the advantages that it grows in numerous types of soil, it is easily cultivable under laboratory conditions, and several of its species produce antibiotics. Zwittermicin A is an antibiotic synthesized by *Baccillus cereus* (Savini 2016) and that acts on oomycetes such as *Phytophthora* (Singh et al. 2017). Iturin A produced by *Bacillus subtilis* is an effective antifungal against *Rhizoctonia solani* in tomato (Zohora et al. 2016). *Bacillus thuringiensis* synthesizes thuricin-17, a small peptide with biocidal or growth-retarding effects on many prokaryotes (Nazari and Smith 2020). In general, *Paenibacillus* and *Bacillus* species elaborate a whole variety of protein and peptide structures that have a deleterious action on other microbes (Olishevska et al. 2019; Miljaković et al. 2020).

Fungi of the genus *Trichoderma* are abundant in the rhizosphere and are capable of parasitizing other fungi and producing lethal effects on numerous microorganisms (Harman et al. 2004b). In recent years, documented reviews have been published on their ecology, mode of action, and use as biocontrollers of pathogens (Contreras et al. 2016; Ghazanfar et al. 2018; Singh et al. 2018; Al-Ani and Mohammed 2020).

The metabolites produced by *Trichoderma* sp. are very diverse, and include antibiotics, siderophores, and hydrolytic enzymes; they are effective against pathogens such as *Sclerotium rolfsii* (Evidente et al. 2003), *Gaeumannomyces graminis, Pythium ultimum, Rhizoctonia solani* (Vinale et al. 2006), *Botrytis allii, Colletotrichum lini, Fusarium caeruleum* (Reino et al. 2008), and *Phytophthora citrophthora* (Druzhinina et al. 2011), among others.

*Gliocladium* sp. is a genus of fungi that, due to its morphology and coexistence in the habitat, is often confused with *Hypocrea, Penicillium, Verticillium,* and *Trichod*erma, to the extent that some of its species have undergone reclassification (Castillo et al. 2016). The genus produces gliotoxin and gliovirin, antifungals against *R. solani* and *P. ultimum*, respectively (Keswani et al. 2017). *Gliocladium catenulatum* is effective against *Botrytis cinerea* (Van Delm et al. 2015); *Gliocladium virens* controls *Verticillium dhaliae, Curvularia lunata* (Rizk et al. 2017); *Gliocladium fimbriatum* reduces *Fusarium* infection by 48% (Fitrianingsih et al. 2019).

Although the antimicrobial effects of all these microorganisms are recognized, their massive application should be done with discretion, since some species of these genera are pathogens of plants, animals, and man, such as *Pseudomonas aeruginosa, Bacillus cereus, Bacillus anthracis* and *Burkholderia cepacia*, and they live in the same habitat of beneficial microorganisms.

#### 2.2.2.2 Induced Resistance

During their evolution, plants developed ways of recognizing the pathogens that attack them and defending themselves against those attacks. In addition, research on the interaction of crops with abiotic and biotic stresses has shown that there is another type of resistance, which depends on the contact of the plant with pathogenic or beneficial microorganisms. This has been called induced resistance (Carvalho et al. 2010).

Induced resistance phenomena could be classified into two large groups: systemic acquired resistance (SAR) and systemic induced resistance (SIR) (Peteira 2020). SAR is nonspecific and occurs as a result of attack by a pathogen; it is regulated through the salicylic acid pathway and pathogenesis-related proteins (PRP) participate in it (Heil 2001). PRPs are not involved in SIR, but it is controlled by ethylene and jasmonic acid. In addition, pathogens do not participate in this regulation, but growth-promoting bacteria (Ongena et al. 2005) and non-pathogenic growth-promoting fungi do participate (Bisen et al. 2016). Although the molecular signals, genes, and products involved are different, both mechanisms have a common result: the resistance of plants to stress (Peteira 2020).

Systemic induced resistance is particularly interesting, because despite involving harmless bacteria, it can increase resistance to pathogens and tolerance to abiotic stresses. Among the PGPB, the genera *Bacillus* and *Paenibacillus* have been the most studied. Inoculation with *Bacillus subtilis* decreased cucumber susceptibility to *Colletotrichum lagenarium* and of tomato to *Pythium aphanidermatum* (Ongena et al. 2005). In peanuts, the presence of *Paenibacillus polymyxa* in the rhizosphere contributed to the control of crown rot disease caused by *Apergillus niger* (Haggag 2007). *Bacillus cereus* induces resistance against *Pseudomonas syringae*, a pathogen of various crops (Nie et al. 2017). The synthesis of elicitors by the resistance-inducing microorganism plays a determining role in the process. Mutants of *Bacillus amyloliquefaciens* exhibiting deficiencies in the production of extracellular compounds are also deficient in the ability to induce resistance to *Pseudomonas syringae* and *Botrytis cinerea* in *Arabidopsis* (Wu et al. 2018a, b).

Not only bacteria are capable of causing SIR; fungi of the genera *Trichoderma, Penicillium, Phoma, Aspergillus, Fusarium,* and *Piriformospora* also induce this phenomenon (Hossain et al. 2017a). Undoubtedly, the best known and most used in agriculture is *Trichoderma* sp. (Yoshioka et al. 2012; Singh et al. 2014; Saxena et al. 2015; Bisen et al. 2016), but abundant examples exist of the effects of the remaining genera. Resistance to cucumber mosaic virus can be induced by *Penicillium simplicissimum* (Elsharkawy et al. 2012) and by *Phoma* sp. (Elsharkawy 2018). Two *Aspergillus* species increase the synthesis levels of defensive metabolites against various pathogens in corn (Mahapatra et al. 2014), while *Aspergillus terreus* induces resistance to *Pseudomonas syringae* in tomato (Yoo et al. 2018).

The defense spectrum provided by SIR can also include herbivorous insects (Rashid and Chung 2017) and abiotic stress. The increase in tolerance to salinity caused by NaCl due to the effects of *A. tumefaciens*, *Bacillus* sp., and *Pseudomonas* sp. has been reported (Gayathri et al. 2010; Rashid et al. 2012; Kumar et al. 2016).

#### 2.2.2.3 Bioremediation

Bioremediation is a biological process of decontamination of soils and water, which is carried out by taking advantage of the natural properties of bacteria, fungi, algae, and other organisms, so it is essentially a biotechnological procedure. These organisms convert waste and hazardous materials into less or nonhazardous substances (Singh et al. 2019) thereby reducing pollution of the ecosystem.

The pollutants most approached from science and practical application are pesticides, polycyclic aromatic hydrocarbons (PAH), and heavy metals (HM). Liu et al. (2017) reviewed the bacteria and fungi capable of reducing the presence of these contaminants and include genera well known to farmers, such as the bacteria *Bacillus, Pseudomonas,* and *Enterobacter*; and the fungi *Fusarium, Pleurotus, and Trichoderma*.

In bioremediation, bacteria, particularly PGPB, can act in two ways: a direct and an indirect (Kaur 2021). The first one includes mechanisms that allow bacteria to degrade pollutants (chelation, use of hydrolytic enzymes, biotransformation) and the second one relates to supporting plants that are capable of assimilating pollutants (particularly MH) in the process called phytoremediation (Sarkar et al. 2022; Prasad 2022; Sonowal et al. 2022). This collaboration mainly increases the bioavailability of these elements so they are absorbed by plants, and stimulating crops development through the production of phytohormones, with which crops increases its phytoremediation potential.

The enzymatic mechanisms used by fungi to degrade contaminants have been reviewed by several authors, and they include the activity of hydrolytic enzymes (such as lipases, cellulases, xylanases), the reduction of heavy metals to less toxic forms, their metabolic assimilation or immobilization in soil, among others (Deshmukh et al. 2016; Singh et al. 2019; Pérez and Héctor 2021).

### *2.2.3 The Microbiome Approach*

Although the mechanisms of plant growth stimulation by microorganisms are separately observed for their study, as well as the effects that one or another microbe can exert, this phenomenon is much more complex. Many studies show that the rhizosphere is an ecosystem itself, in which a great diversity of microorganisms interrelates with the roots of plants. These relationships are established through communication mechanisms through the segregation of chemical substances by the participating organisms (Mhlongo et al. 2018). Plants are capable of regulating which microorganisms live in the microbiome through radical exudates and, instead, certain microbial species have evolved to live in that environment (Jacoby et al. 2017).

Researchers have understood this, and that is why studies have been developed in which several microorganisms or substances synthesized by them are combined, seeking to enhance effects such as the acquisition of mineral elements from the soil. The combined inoculation of *Rhizobium leguminosarum* + arbuscular mycorrhizae (*Glomus mosseae*) increases $N_2$-fixation several times compared to the inoculation of these microorganisms separately (Meng et al. 2015). The co-inoculation of *Rhizobium* with the PGPB *Bacillus aryabhattai* and *Azotobacter vinelandii*, particularly with the latter, increases nitrogen fixation in *Trifolium repens* (Matse et al. 2020). The PGPB *Bacillus velezensis* increases the nodulation and N fixation produced by *Bradyrhizobium diazoefficiens* in soybeans (Sibponkrung et al. 2020).

Microbial and microbe interactions with plants are also important in defense against pathogens. One bacterium (*Streptomyces griseorubens*) and two fungi (*Gliocladium virens* and *Trichoderma harzianum*) cooperate with each other to control *Fusarium oxysporum* f. sp. *capsici*, *in vitro* and *in vivo* (Suryaminarsih et al. 2015). In alfalfa, the combination of the effects of the bacterium *Sinorhizobium medicae* and the mycorrhizal fungus *Funneliformis mosseae* reduces the severity of leaf spot caused by *Phomamedicaginis* (Gao et al. 2018). Co-inoculation of *Streptomyces atrovirens* and *Trichoderma lixii* is effective for the control of *Rhizoctonia solani* in infected soils (Solanki et al. 2019). The effects of the phytopathogenic fungus *Phytophthora capsici* on *Sechium edule* (Jacq.) Sw. are reduced by combined inoculation with *Rhizophagus intraradices* and *Azospirillum brasilense* (Aguirre et al. 2021).

Knowledge has also been achieved on the effect of the microbiome in reducing abiotic stress in plants. Inoculation with *Bradyrhizobium japonicum* combined with the addition of thuricin-17, produced by *B. thuringiensis*, protects soybeans from water stress (Prudent et al. 2015). Chickpea is more resistant to salinity after triple inoculation with *Rhizobium*, the endophytic bacterium *Stenotrophomonas maltophilia*, and arbuscular mycorrhizal fungi (Abd-Alla et al. 2019). The phytoremediation capacity of cadmium of *Sulla coronaria* is increased by co-inoculation with *Rhizobium sullae* and *Pseudomonas* sp. (Chiboub et al. 2020). By jointly inoculating *Rhizobium* and arbuscular mycorrhizae, the natural population of bacteria of the genera *Proteobacteria, Actinobacteria, Acidobacteria,* and *Chloroflexi* is increased, with which alfalfa plants take up more nutrients from the soil and better resist cadmium stress (Wang et al. 2021).

A very recent study by Sauer et al. (2021) in two medicinal plants illustrates the difficulty of relationships that could happen in the rhizosphere of plants: in the microbiome of *Hamamelis virginiana* L., 141 genera of fungi and 1,131 species of bacteria were identified, and in that of *Achillea millefolium* L., 161 genera of fungi and 1,168 bacterial species were observed. With such microbial populations, it is evident that the relationships between them and with the plant are very complex, and that very detailed studies are needed to effectively take advantage of the mechanisms governing these interactions.

#### 2.2.3.1 Fungi Used as Growth Regulators in Modern Agriculture

The use of PGPF in today's agriculture is becoming increasingly common because its use reduces the application of chemicals such as pesticides and fertilizers, and with this the accumulation of chemical residues in vegetable and fruits are diminished. Up to now, only a few studies have informed on PGPF because researchers pay more attention to their use for induction of resistance and plant development improvement by triggering induced systemic resistance (ISR) in crops (Fig. 2.1) (Zhang et al. 2018; Sindhu et al. 2018; Hossain et al. 2017b).

Some of the species that have been used as PGPF in agriculture as mentioned above are *Aspergillus*, *Trichoderma*, *Penicillium*, and also mycorrhizas.

#### 2.2.3.2 *Aspergillus* spp. as PGPF

The genus *Aspergillus* includes several endophytic fungi that are applied in many treatments and forms because they represent beneficial effects to crops growth promotion and protection. Some of the beneficial effects of *Aspergillus* spp. according to Hung and Lee (2016) are:

- Extracellular production of phytases [phytate is a phosphorylated derivative of myo-inositol important in the storage and retrieval of inositol, ions, and

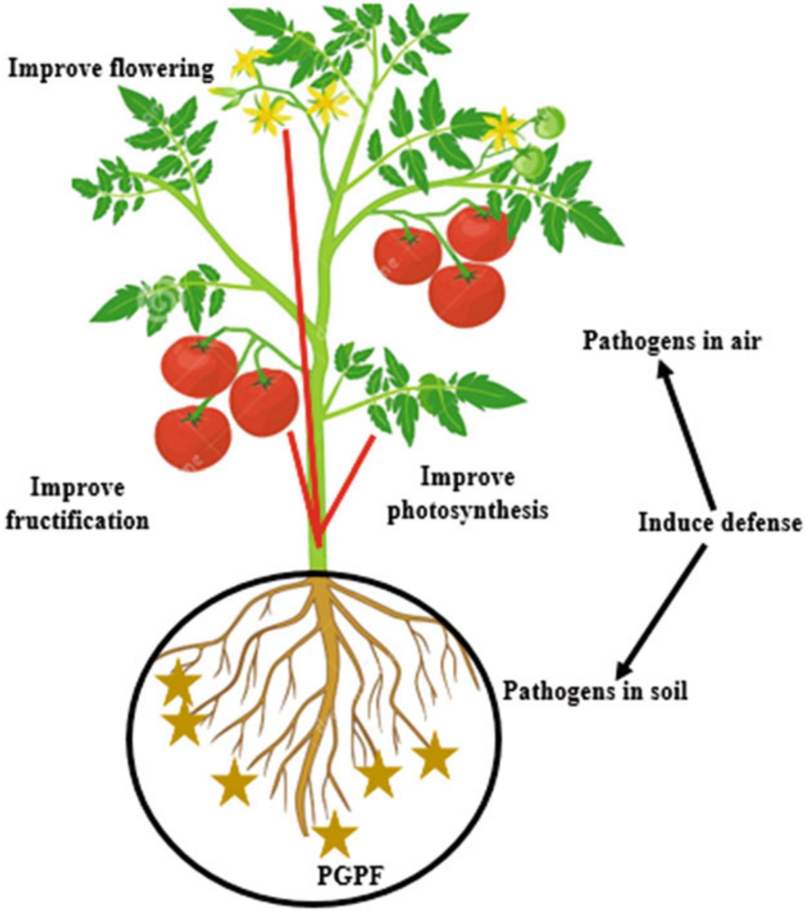

**Fig. 2.1** Stimulation of several physiological processes (flowering, fructification, growth) in tomato plant by Plant Growth-Promoting Fungi (PGPF)

phosphorus during plant germination and development (Raboy 2003)] during soil pretreatment

- Induction of growth promotion through the production of gibberellins, auxins, and other phytohormone-like compounds and secondary metabolites
- Reduce stress experienced by the plant

*Aspergillus ochraceus* Wilhelm (1877) was reported by Badawy et al. (2021) as providing salt stress tolerance (200 g $L^{-1}$), for which barley plants (*Hordeum vulgare* L.) were irrigated with seawater at 15% and 30% and inoculated with the fungus. Because of this, morphological parameters such as sugars, proteins, pigments, and yield characteristics increased, while the contents of hydrogen, proline, malondialdehyde, and peroxide besides the activities of antioxidant enzymes decreased. Another experiment addressing salt stress was done by Ali et al. (2021)

with plants of *Vigna radiate* (L.) R. Wilczek inoculated with *A. awamori* with the aim to evaluate ionic status of the plant, biochemical indices, seedling growth, antioxidant enzymes, and endogenous IAA.The main results showed that growing plants inoculated with 150 mM NaCl displayed growth promotion and increase of IAA contents.

Khushdil et al. (2019) inoculated plants of *Pennisetum glaucum* L. with *A. terreus* and they were grown under salt stress conditions, finding that under 100 mM salt stress, the plants significantly improved ($P = 0.05$) chlorophyll, relative water content, phenol, flavonoid, and soluble sugar because the fungus produced higher amounts of indole acetic acid (IAA).

Syamsia et al. (2021) studied the effect of six isolates of endophytic fungi on growth of the plant *Cucumis sativus* L. A combination of fungi isolates F6, F8, F9, and F12 induced an increase in cucumber plants height, whereas the isolate F8 improved the fresh weight of the plants and the isolate F4 improved root growth. The isolates were identified using molecular methods and it was found to be closely related to *Aspergillus foetidus, Daldinia eschscholtzii, Penicillium allahabadense, Sarocladium oryzae,* and *Rhizoctonia oryzae*.

Soybean (*Glycine max* L.) and sunflower (*Helianthus annuus* L.) seedlings were inoculated with *A. flavus* to analyze the plant response to thermic stress. Crops were grown in a thermal chamber with temperatures of 25 °C and 40 °C. Plants inoculated with the fungus exposed to high-temperature stress showed low levels of proline, abscisic acid (ABA), and high levels ascorbic acid oxidase, flavonoids, and phenols catalase. Also, an increase in dry weight, root-shoot length, and chlorophyll was registered in the inoculated plants. The results of this study suggested that *A. flavus* could be used in crops growth promotion under heat stress conditions (Hamayun et al. 2019).

Hamayun et al. (2020) identified the species *Aspergillus violaceofuscus* that also is useful under heat stress conditions. This species had higher quantities of secondary metabolites that increased biomass, plant height, and total chlorophyll content of *H. annuus* and *G. max* seedlings under heat stress. Conversely, the plants associated to *A. violaceofuscus* showed small levels of abscisic acid, proline, reactive oxygen species, ascorbic acid oxidase, catalase, and a general improvement of the nutritional value.

The inoculation of *Aspergillus ustus* on plants of *Arabidopsis thaliana* (L.), Heynh. and *Solanum tuberosum* L. induces changes in developmental stage and promotes growth mainly in roots (roots-induced root hair and lateral root numbers and also increased root and shoot growth). Authors also confirmed that *A. ustus* synthesizes gibberellins and auxins in liquid cultures (Salas-Marina et al. 2011).

#### 2.2.3.3 *Trichoderma* spp. *as PGPF*

*Trichoderma* is a genus of opportunistic symbiont fungi that proliferates in the rhizosphere of plants. Species of this genus can produce elicitors that activate plant defense against various pathogens, as well as induce the synthesis of plant

**Fig. 2.2** Experiment with tomato plants (*Solanum licopersicum* L.) at Facultad de Ciencias Agrícolas y Forestales of Universidad Autónoma de Chihuahua where (**a**) Tomato without *Trichoderma* spp. in a greenhouse, small fruits; (**b**) Tomato with *Trichoderma* spp. in a greenhouse, larger fruits (Source: Crescencio Urias Gracia and Melisa Magaña González)

growth-promoting substances and help phosphorus solubilization (Hohmann et al. 2011).

Species of *Trichoderma* predominate in agricultural soils or in forests (terrestrial ecosystems) with a low nutritional requirement and a temperature range of 25–30 °C for their growth (Sandle 2014). Also, they have a great adaptability to ecological conditions, for which they develop several substrates, which facilitates their massive production for use in agriculture (Ramos et al. 2008; Zeilinger et al. 2016).

Various species of this genus can promote crops development and growth (Fig. 2.2), due to the fact that they can be endophytically related or associated with the rhizosphere of crops. Further, they produce auxins and gibberellins, as well as organic acids (fumaric, citric, and gluconic) that influence the reduction of the pH in soil and can promote the solubilization of magnesium, phosphates, manganese, and iron; nutrients that are essential for plant functioning (Sharma et al. 2017).

Secondary metabolites produced by *Trichoderma* species function as plant growth regulators. As an example, a strain of *T. harzianum* (SQR-T037) released harzianolide, a growth inducer in tomato seedling in soil or in hydroponic system at very low concentrations (0.1 ppm and 1 ppm). Also, this metabolite can improve root development (Cai et al. 2013). Others species such as *T. atroviride* and *T. virens* can produce some auxin-related substances like IAA, a plant hormone that have many functions including induction of plant growth, root development, among others (Contreras-Cornejo et al. 2014).

The inoculation of the fungus *Trichoderma* sp. on plants such as *Arabidopsis* (Contreras-Cornejo et al. 2014) can have various effects such as increase in root tip, and shoots and root may have high iron levels (Yedidia et al. 2001). These results showed that the transport of this nutrient improved in plants, and also harzianic acid may control plant growth because of its Fe(III)-binding activity (Vinale et al. 2013).

One of the best roles of *Trichoderma* spp. is the development of roots, probably due to the control or production of hormones that can regulate this activity, for example harzianic acid, auxin, and harzionalide (Contreras-Cornejo et al. 2009; Vinale et al. 2013; Cai et al. 2013). Further, plants of cucumber (*C. sativus*) inoculated with *T. harzianum* considerably improved the root area on the 28th day, and also increased the concentrations of several nutrients like copper (Cu), phosphorus (P), zinc (Zn), iron (Fe), sodium (Na), and manganese (Mn) (Yedidia et al. 2001).

Chagas et al. (2019) studied the efficiency of *Trichoderma asperellum* (UFT 201 strain) as a plant growth promoter in soybean (*G. max*) by analyzing the possibility to synthesize IAA and to solubilize phosphate under greenhouse conditions. This study found that the production of IAA was higher (26.7%) in plants inoculated with *T. asperellum* UFT compared to the positive control. Also, soybean plants inoculated with the fungus showed higher biomass than controls. The inoculation of soybean plants with *T. asperellum* UFT 201 showed the high efficiency of this *Trichoderma* strain as a growth promoter.

In *Capsicum chinense* (Jacq.) var. 'Chichen Itza' the efficiency of *Trichoderma* spp. for the promotion of vegetative growth was analyzed and plants treated with *Trichoderma sp*. by foliar application at 28 days after germination had a biomass (dry = 0.13 g and plant$^{-1}$ fresh = 0.8 g plant$^{-1}$), higher stem diameter (2.6 mm), aerial height (11 cm), and root volume (dry = 0.04 g plant$^{-1}$and fresh = 0.13 g plant$^{-1}$), compared to the treatments evaluated (co-application of both a chemical fungicide (Captan®) and a commercial product (Tri-HB®: *Bacillus subtilis* and *Trichoderma harzianum*) (Larios et al. 2019).

Halifu et al. (2019) compared two species of *Trichoderma* spp. (*T. harzianum* E15 and *T. virens* ZT05) according to their effect on fungal community and plant growth rhizosphere soil nutrients of *Pinus sylvestris* var. mongolica annual seedlings. The results showed differences between the control and the two species studied. Parameters such as root structure index, seedling biomass, soil enzyme activity, and soil nutrients were considerably higher compared to the control

($p < 0.05$). For *T. harzianum* E15 treatment, the seedling total biomass, ground diameter and height were higher than that of *T. virens* ZT05 treatment. Regarding the results of enzyme activity and rhizosphere soil nutrient content, treatment with *T. virens* ZT05 showed higher values than those of *T. harzianum* E15.

Nuangmek et al. (2021) described morphological and phylogenetically a new species named *Trichoderma phayaoense*. The fungus promoted growth in the plant *Cucumis melo* L. by increasing plant shoot, root dry weight, and height. Also, *T. phayaoense* had positive effect on fruit quality by increasing its diameter, circumference, weight, and total soluble solid of fruit. Further, it is important to point out that *T. phayaoense* tolerated a frequently applied fungicide (metalaxyl) in recommended dosages for field applications.

#### 2.2.3.4 *Penicillium* spp. as PGPF

The genus *Penicillium* was first described by Link in 1809. Thom, in 1910, considered *P. expansum* as the type species of the genus. The species included in the genus *Penicillium* are ubiquitous, widely distributed throughout the world, and considered saprophytic. Many of them live in the soil or in decaying organic matter (Pitt 1981).

The species of *Penicillium* could be identified with high frequency (Domsch et al. 1993), but, very little information is available about the influence of these fungi in plant growth or interactions between species of the genus *Penicillium* and other soil fungi.

Ismail et al. (2021) performed a field study on *Phaseolus vulgaris* L. inoculated with fungal (*Penicillium commune* PF3 and *Alternaria sorghi* PF2) and endophytic bacterial (*Brevibacillusagri* and PB5, *Bacillus thuringiensis* PB2) strains compared to two hormones that were exogenously applied (benzyl adenine [BA] and IAA). The biochemical characteristics of the plants and their growth were evaluated. The plants inoculated with endophytic bacterial and fungal strains showed higher photosynthetic pigments, antioxidant enzyme activity, plant biomass, endogenous hormones, carbohydrate and protein contents, and yield, compared to plants with exogenous application of hormones (BA and IAA).

Surya and Yuwati (2020) inoculated *Penicillium citrinum* on Gerunggang (*Cratoxylon glaucum*) seedlings, and they found that only the height of the plants changed significantly, whereas leaf number and growth of plant diameter were not affected. To understand these responses, authors inoculated other plants with different application methods and dosage but the responses after 5 months were the same in plant growth parameters (leaf number, dry weight of plants, height, diameter, soil P content, and chlorophyll content). On the other hand, *Penicillium* spp. (*Penicillium neoechinulatum* or *Penicillium viridicatum*) isolated from zoysiagrass rhizospheres stimulated disease resistance and growth in cucumber plants (*Cucumis sativus* L.) (Hossain et al. 2014).

Species of *Penicillum* have also been reported as phosphate-solubilizing microorganisms. For this function, *Penicillium oxalicum* I1 is able to induce growth in maize plants (*Zea mays* L.) (Gong et al. 2014).

Nasim et al. (2012) reported several isolates of *Penicillium* that had a positive influence on growth of tomato plants (*S. licopersicum*) as follows:

- The nine isolates tested (*P. simplicissimum, P. citrinum, P. oxalicum, P. verrucosum var. cyclopium, Penicillium sp., P. billii, P. granulatum, P. expansum* and *P. implicatum*) significantly improved seed germination.
- Growth promotion increased up to 90% by the application of cultural extracts of *P. billi* and *P. expensum.*
- *P. oxlalicam* and *P. implicatum* expressively increased root development in tomato seedling.
- *P. granulatum, P. implicatum,* and *P. verrucosum* enhanced shoot length.
- *P. implicatum* improved root length and shoot in tomato seedlings.
- On seedling growth *P. citrinum* and *P. simplicissimium* were less effective.

Mushtaq et al. (2012) investigated the effect of several species of *Penicillium* (*Penicillium citrinum, Penicillium expansum, Penicillium oxalicum, Penicillium implicatum, Penicillium verrucosum, Penicillium simplicissimum* and *Penicillium bilaii*) in tomato plants (S. *licopersicum*), and they improved seed germination, plants' shoot and root system. On the other hand, *Penicillium chrysogenum* interrupted the dormancy of opuntia (*Opuntia streptacantha* Lem.) seeds (Delgado-Sánchez et al. 2011).

Salinity is one of the main stressors affecting plant growth. In this sense, one strain of *Penicillium citrinum*, KACC43900, was reported by Khan et al. (2009) as growth promoter in *Ixeris repens*. The strain was isolated from the same plant and this was the first report on the reduction of salinity stress in plants using *P. citrinum* KACC43900 (Khan et al. 2009). Another *Penicillium* species that reduced the negative effect of salinity stress in plants was *P. resedanum* LK6 specifically in *Capsicum annuum* L. from where it was isolated. Also, when this strain (LK6) was inoculated in *C. annuum* plants together with gibberellic acid treatment, an increase of several physiological parameters of plant growth was observed, such as shoot length, biomass, photosynthesis rate, and chlorophyll content (Khan et al. 2015).

### 2.2.3.5 Mycorrhizas as PGPF

Mycorrhizas constitute the most common synergy between microorganisms and plants called mycorrhizal symbiosis. According to Bonfante and Genre (2010), mycorrhizas (Arbuscular Mycorrhizal Fungi (AMF) endomycorrhiza, arbuscular mycorrhiza (AM)) are endophytic fungi belonging to the *Glomeromycota* genera that can colonize over 90% of higher plant families for symbiotic relationships (Prasad et al. 2017).

AMF improve the nutrient in plants and water uptake by spreading the root and so the absorbing zone and plants provide them carbohydrates to finish their life cycle. In turn, the AMF reduce in plants the negative effect of abiotic stresses like salinity, drought, heavy metals, and high temperatures (Kumar et al. 2017). The mechanisms by which AMF are adapted to these stresses are commonly related to increased gene regulation, hydromineral nutrition, production of osmolytes, synthesis of antioxidants, phytohormones, and ion selectivity. Additionally, they improve ecosystem stability and enhance bacterial communities, the quality of soil aggregation, and plant structure (El-Sawah et al. 2021).

Mycorrhizas could mitigate the stress caused by drought in plants because AMF can increase the area of plants for water absorption due to the symbiosis with roots of crops through AMF hyphae which allow them access to distant soil regions where water is retained by soil pores (Augé 2001). Several studies show the mitigation of drought stress by AMF due to the increase of nutrient contents and the efficient use of water in important agricultural crops such as tomato (*S. lycopersicum*) (Subramanian et al. 2006), *Allium cepa* L. (Nelsen and Safir 1982), *Triticum aestivum* L. (Allen and Boosalis 1983), *Trifolium repens* L. (Ortiz et al. 2015), *Lactuca sativa* L. (Ruiz-Lozano et al. 2015), and other crops.

Mycorrhizas can also mitigate the stress produced by salinity in plants, since plants inoculated with AMF have better capacity to absorb water and take nutrients, improving the ionic homeostasis (Khanam 2008; Munns and Tester 2008), inducing the accumulation of osmoregulators like sugars and proline (Yamato et al. 2008), and reducing the uptake of $Cl^-$ and $Na^+$ (Li et al. 2020a, b). Also, crops inoculated with AMF and exposed to salinity show a reduction in oxidative damage and enhanced stomatal conductance (Estrada et al. 2013; Pedranzani et al. 2015). Some examples are:

- *F. mosseae* inoculated on tomato plants (*S. lycopersicum*) exposed to saline water increased fruit fresh production, shoot contents of potassium (K), iron (Fe), zinc (Zn), phosphorous (P), copper (Cu), and plant biomass (Al-Karaki 2006);
- *F. mosseae* colonizing tomato plants (*S. lycopersicum*) roots diminished the concentration of sodium (Na) and increased several enzymes activity related to oxidative stress in plants [ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)] (Latef and Chaoxing 2011).
- AMF inoculation on wheat plants (*Triticum aestivum* L.) significantly reduced the oxidative damage (Talaat and Shawky 2014). Also, authors reported an increase in carbon and nitrogen metabolism.
- Plants of *Z. mays* under saline conditions were inoculated separately with three native AMF [*Claroideoglomus etunicatum* (Becker and Gerdemann 1977), *Rhizophagus intraradices* (Schenck and Smith 1982), and *Septoglomus constrictum* (Trappe 1977)] resulting in increased content of K and proline in shoots as well as plant biomass (Estrada et al. 2013).

In several important crops, the influence of AMF on plant physiological parameters and growth have been studied. Example of such crops include *Withania somnifera* (L.) Dunal (Parihar and Bora 2018), *Phaseolus vulgaris* L. (Ibijbijen et al. 1996), *Solanum lycopersicum* L. (Bona et al. 2016; Gamalero et al. 2004), *Cucurbita maxima* Duchesne (Al-Hmoud and Al-Momany 2017), *Sorghum bicolor* (L.) Moench (Nakmee et al. 2016; Kim et al. 2017), and others. In these species, AMF enhanced the uptake of important nutrients such as phosphorus and nitrogen (Jansa et al. 2019; Song et al. 2020), and growth parameters such as yield, quality, and nutritional value (Bona et al. 2016), root architecture (Gamalero et al. 2004), and root system morphology (Berta et al. 1995).

AMF were used by El-Sawah et al. (2021) as biofertilizers in guar (*Cyamopsis tetragonoloba* L.) to improve nutrients, soil microbial activity, and also the crop seed quality, growth, and yield. AMF were applied individually or in combination with *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 and *Bradyrhizobium sp*. Results showed a great increase in plant growth (plant dry weight, shoot length, root length, nutrient uptake, number of branches, chlorophyll content, and leaf area index (LAI)), as well as an increase in seed yield and improvement of fat, total protein, starch, and carbohydrate contents in seeds. In addition, the use of the biofertilizer improved the microbial activity of the soil. This research demonstrated that the use of biofertilizers with the correct doses can diminish the use of chemical fertilizers in about 25%.

Arbuscular Mycorrhiza (AM) as PGPF together with *Azotobacter* and *Pseudomonas sp*. as PGPB were used in *Capsicum frutescens* L, crop growing on infertile lateritic soil (deficient in nitrogen and phosphorous) and the results after the use of these three microorganisms together indicated an increased in growth (leaf area, leaf number, height, number of flowers, and root collar diameter), productivity (number of fruits, final dry and fresh yield), root colonization, and spore count of AM (Kulla et al. 2021).

Five species of AMF (*G. versiforme, Diversispora spurca*, *Acaulospora scrobiculata*, *G. mosseae*, and *Glomus etunicatum*) were used to determine their effect on leaf gas exchange, plant growth, root nutrient contents, and root morphology of walnut (*Juglans regia* L. Liaohe 1) seedlings. After 3 months, AMF colonized roots in 47.0% to 76.4%. Also, plants that were inoculated with *G. etunicatum*, *G. mosseae*, and *D. spurca* had greater projected area, volume, and root length. Four AMF (*D. spurca, G. etunicatum*, *G. mosseae,* and *A. scrobiculata*) improved transpiration rate, stomatal conductivity, and leaf photosynthesis rate, whereas leaf temperature and intercellular $CO_2$ concentrations were reduced (Huang et al. 2020).

## *2.2.4 Bacteria Used as Growth Regulators in Modern Agriculture*

### 2.2.4.1 The Rhizosphere and the Interaction of Beneficial Microorganisms

Among terrestrial ecosystems, the soil is one of the richest habitats in microbial taxa. More than 80% of the biological functions of this ecosystem are carried out by algae, bacteria, fungi, and nematodes. The classification of organisms, by their body size, are divided into microflora (<0.02 mm); microfauna (0.02–0.2 mm); mesofauna (0.2–10 mm); macrofauna (10–20 mm); and megafauna (>20 mm) (Wolters 2001; Wardle 2002). The rhizosphere is defined as the zone of soil that is located just in narrow zone of the plant roots, and is directly influenced by the root exudates with a high content of amino acids, sugars, carbohydrates, secondary metabolites, and organic acids that favor growth of microbial populations. The rhizosphere is divided into (1) endorizosphere, which corresponds to the endodermis, the root cortex and the apoplastic space between cells; (2) rhizoplane (root surface); (3) ectorizosphere, an area that extends from the rhizoplane to outside the area of the rhizosphere. Microbial groups found in the rhizosphere interfere with nutrient cycling, protect the plant from attack by pathogens, or act as plant parasites (Philippot et al. 2013; Ahkami et al. 2017; Vives-Peris et al. 2020).

The growth, health, and development of plants is influenced by the interactions that occur between microorganisms that inhabit the rhizosphere. Mycorrhizal fungi and plant growth-promoting bacteria (PGPB) play a key role in sustainable agriculture by reducing the use of chemical fertilizers and pesticides, solubilizing nutritional resources, and producing antagonistic compounds of potential phytopathogens (Genre et al. 2020; Phour et al. 2020; Trivedi et al. 2020; Molina-Romero et al. 2021). Mycorrhizae are fungi that, in combination with plant roots, externally or internally, form networks that capture nutrients and water from the soil (Varma et al. 2012). Ectomycorrhizae are mainly associated with trees and shrubs; while endomycorrhizae can be arbuscular (related to a great variety of taxa), ericoides (restricted to the order of Ericales plants), and orchids (associated with the Orchidaceae family). Some bacteria have the ability to modulate mycorrhizal symbiosis with the plant; as is the case of various species of the genus *Pseudomonas* that have the ability to help the mycorrhizal process, and are called mycorrhizal helper bacteria (Rigamonte et al. 2010; Xing et al. 2018; Genre et al. 2020). These synergistic interactions between both microorganisms can be useful to improve the growth and tolerance of plants in stressful environments (Moreira et al. 2016).

#### 2.2.4.2 Plant Growth-Promoting Bacteria (PGPB)

PGPBs are bacteria that inhabit the rhizosphere, from where they take glutamine, betaine, and trehalose sugars to improve their growth. PGPBs are divided into two groups: extracellular (ePGPB) and intracellular (iPGPB). The ePGPBs colonize the root surface or intercellular space of the cortex; while the iPGPBs produce special cells inside the roots called nodules and develop within these structures. Both groups of bacteria stimulate plant growth (Barber 1995; Yadav 2010). The genera *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Erwinia*, *Micrococcus*, *Pseudomonas,* and *Serratia* integrate the ePGPBs (Adesemoye and Egamberdieva 2013); iPGPBs include *Allorhizobium*, Azorhizobium, *Bradyrhizobium*, *Frankia*, *Mesorhizobium,* Ochrobactrum, *Rhizobium,* and *Sinorhizobium* (Quiza et al. 2015; Hakim et al. 2021; Stone et al. 2000). Plant growth, promoted by these bacterial groups, is carried out through indirect or direct mechanisms. The first is achieved by increasing nitrogen fixation, phosphate solubilization, the availability of iron and other essential nutrients, and improving regulation of the levels of phytohormones (abscisic acid, auxins, cytokinins, ethylene, and gibberellins) and siderophores (Gouda et al. 2018; Kalam et al. 2020; Rastegari et al. 2020; Singh et al. 2020). On the other hand, the indirect mechanism includes the increase of the enzymatic

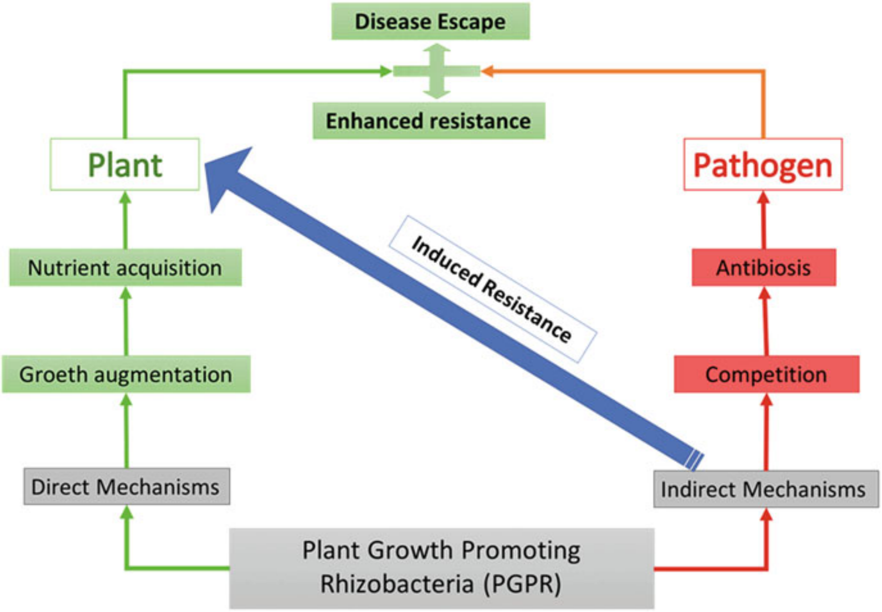

**Fig. 2.3** Mechanisms of action of plant growth-promoting bacteria (modified from Basu et al. 2021)

activity related to the defense of the plant such as proteases, β-1,3-glucanases, and chitinases, the reduction of ethylene (endogenous associated with stress), and the induced systemic resistance suppressing the development of root and foliar phytopathogens (Fig. 2.3) (Beneduzi et al. 2012; Glick 2014; Kour et al. 2020; Meena et al. 2020; Rana et al. 2020). PGPBs that exhibit both direct and indirect mechanisms have advantages in being more competent bioinoculants (Hernández et al. 2015).

#### 2.2.4.3 PGPB and Biotic and Abiotic Stress

PGPBs have the ability to convert infertile soils to fertile by mineralizing organic pollutants, and are used in soil bioremediation (Dessaux et al. 2016; Bibi et al. 2018). In addition, they confer a better adaptation of plants to various biotic factors such as diseases caused by plant pathogens (bacteria, fungi, insects, nematodes, viruses, among others) and abiotics that include drought, soil salinity, floods, extreme temperatures, and heavy metal contamination (Table 2.1) (Santoyo et al. 2017; Gimenez et al. 2018; Gamalero and Glick 2020). Various studies have mentioned that the inoculation of plants with PGPB consortia has a synergistic effect on their development by producing various defense compounds and reducing abiotic and biotic stress. The co-inoculation of *Bacillus megaterium* and *Paenibacillus*

**Table 2.1** Benefits of inoculating plant growth-promoting bacteria (PGPB)

| Bacterium | Benefits/type of stress | Hosts | References |
|---|---|---|---|
| *Alcaligenes feacalis* RZS2, *Bacillus* spp., *B. cereus*, *Enterobacter* sp. RZS5 *Ochrobactrum* sp., *Pseudomonas* spp., *P. fluorescens*, *P. aeruginosa* RZS3 | Bioremediation by pollutants and heavy metals/abiotic | *Withania somnifera*, *Arachis hypogaea*, *Zea mays, Oryza sativa* | Pandey et al. (2013), Sayyed et al. (2015), Das and Kumar (2016), Khan et al. (2016), Patel et al. (2016), Kalam et al. (2017) |
| *Paenibacillus polymyxa, Pantoea* sp. S32 | Increased nutrient absorption/ abiotic | *Capsicum chinense, Oryza sativa* | Pii et al. (2015), Castillo-Aguilar et al. (2017), Chen and Liu (2019) |
| *Bacillus subtilis*, *Rhizobium* spp., *B. cereus* | Improved soil fertility/abiotic | *Vigna radiata, Populus* sp. | Ahmad et al. (2011), Islam et al. (2016), Jang et al. (2017) |
| *Achromobacter piechaudii*, *Azospirillum* sp., *Bacillus megaterium, B. pumilus*, *Eneterobacter* sp. PR14, *Exiguobacterium oxidotolerans* | Tolerance to salinity/abiotic | *Bacopa monnieri*, *Eleusine coracana*, *Lactuca sativa*, *Oryza sativa* cv. *Sahbhagi*, *Solanum lycopersicum*, *Sorghum bicolor*, *Zeamays* | Mayak et al. (2004), Marulanda et al. (2010), Bharti et al. (2013), Fasciglione et al. (2015), Sagar et al. (2020) |
| *Achromobacter piechaudii ARV8, Azospirillum brasilense, Bacillus subtilis, Enterobacter hormaechei DR16, Paenibacillus polymyxa, Pseudomonas fluorescens DR11, P. migulae DR35, Phyllobacterium brassicacearum, Rhizobium tropici* | Drought tolerance/abiotic | *Setaria itálica*, *Zea mays*, *Phaseolus vulgaris*, *Arabidopsis thaliana*, *Solanum lycopersicum* cv. F144, *Capsicum annuum* cv. Maor, *Triticum aestivum* | Figueiredo et al. (2008), Yang et al. (2009), Bresson et al. (2013), Timmusk et al. (2014), Niu et al. (2018), De Lima et al. (2019) |
| *Bacillus amyloliquefaciens*, *Ochrobacttrum intermedium*, *Paenibacillus lentimorbus*, *P. xylanexedens*, *Pseudomonas* spp., *Streptomyces* sp. | Biocontrol/ biotic | *Oryzasativa*, *Pinus taeda*, *Solanum lycopersicum*, *Triticum aestivum* | De Vasconcellos and Cardoso (2009), Khan et al. (2012), Gowtham et al. (2016), Ilyas et al. (2020), Srivastava et al. (2016), Reshma et al. (2018) |
| *Rhizobium etli, Bacillus cereus, Serratia marcescens,* | | *Heterodera avenae*, *H. glycines*, *Meloidogyne* spp. | Reitz et al. (2000), Hamid et al. (2003), Siddiqui et al. (2005), |

(continued)

Table 2.1 (continued)

| Bacterium | Benefits/type of stress | Hosts | References |
|---|---|---|---|
| *B. coagulans, B. licheniformis, B. pumilus, B. megaterium, B. subtilis, B. pumilus* L1, *Pseudomonas aeruginosa, B. thuringiensis, P. stutzeri, P. fluorescens* CHA0 | | *M. incognita, M. arenaria, M. graminícola, M. javanica Bursaphelenchus xylophilus, Helicotylenchus multicinctus, Rotylenchulus reniformis, Radopholus similis* | Mohammed et al. (2008), Almaghrabi et al. (2013), Rahul et al. (2014), Khan et al. (2016), Fatima and Anjum (2017), Lastochkina et al. (2017), Basyony and Abo-Zaid (2018), Mostafa et al. (2018), Xiang et al. (2018), Ahmed (2019), El-Nagdi et al. (2019), Forghani and Hajihassani (2020), Jiang et al. (2020), Mazzuchelli et al. (2020) |
| *Arthrobacter protophormiae, Dietzia natronolimnaea, B. subtilis, Azospirillum lipoferum, Bacillus* sp. | Production of phytohormones | *Triticum aestivum, Solanum lycopersicum, Oryza sativa* | Barnawal et al. (2017), Tahir et al. (2017), Kalam et al. (2020), Cassán et al. (2001) |
| *Azotobacter chroococcum, Bacillus subtilis, B.pumilus, Exiguobacterium oxidotolerans, Pseudomonas putida* | Modulation of secondary metabolites | *Bacopa monnieri, Ocimum basilicum* | Banchio et al. (2009), Ordookhani et al. (2011) |
| *Azospirillum lipoferum, Bacillus subtilis, Brevundimonas diminuta, Pseudomonas fluorescens, P.putida, Providencia* sp., *Serratia marcences* | Improved seed germination | *Triticum aestivum, Zeamays* | Nezarat and Gholami (2009), Rana et al. (2011), Almaghrabi et al. (2014) |

*polymyxa*, in combination with *Rhizobium*, has shown an improved plant biomass of *Phaseolus vulgaris* compared to the individual inoculation of *Rhizobium* (Korir et al. 2017). Likewise, the application of *Pseudomonas* and *Rhizobium* increased the biomass and the yield of *Vigna radiata* (Ahmad et al. 2012).

#### 2.2.4.4 PGPB as Biological Control Agents for Pathogens

Biocontrol is a promising strategy to control plant pathogens and is an ecological alternative to chemical pesticides and fertilizers. In recent years, the application of PGPB as biocontrol agents for plant pathogens has been implemented in the world. This strategy provides a safe, economical, durable, and environmentally friendly alternative (Table 2.1) (Etesami 2019; Prasad et al. 2019). Bacteria of the genus *Bacillus* belong to the phyla Firmicutes, family Bacillaceae, and are gram positive. This group is characterized by forming rod-shaped endospores, which gives them the ability to adapt to adverse conditions in a wide variety of habitats (Ducrest et al. 2019; Kuebutornye et al. 2019; Liu et al. 2019; Li et al. 2020a, b). *Bacillus* spp., like PGPB, have been documented to confer numerous advantages in the agricultural sector (Radhakrishnan et al. 2017). In disease management, this bacterial genus controls the proliferation of phytopathogens by suppressing plant immunity or induced systemic resistance (Glick 2012a, b; Shafi et al. 2017). Likewise, it improves the immunity of plants by producing antimicrobial metabolites (directly) and antioxidant enzymes (indirectly) (Belbahri et al. 2017; Rais et al. 2017; Sarwar et al. 2018; Wu et al. 2018a, b).

The *Pseudomonas* group are found in the phyla Proteobacteria, family Pseudomonadaceae, and are Gram-negative, rod-shaped bacteria. *Pseudomonas* spp. is ubiquitous in agricultural soils and has many characteristics that promote plant growth. For this reason, they have been used on a large scale for biotechnological applications as biological control agents (*P. putida* and *P. fluorescens*) (Anayo et al. 2016; David et al. 2018; Kandaswamy et al. 2019). Some species of the genus *Pseudomonas* are pathogenic to plant (*P. syringae*) (Morris et al. 2008) and human (*P. aeruginosa*) (Diggle and Whiteley 2020). Bacteria of the genus *Enterobacter* belong to the phyla Proteobacteria, family Enterobacteriaceae. They are Gram-negative, rod-shaped, and do not form spores. Reports show that *Pseudomonas* sp. and *Bacillus* sp. are used in the management of nematode parasites of plants such as *Heterodera*, *Meloidogyne*, and *Rotylenchulus* (Siddiqui and Mahmood 1999; Kokalis-Burelle et al. 2002; Siddiqui et al. 2005).

The genus *Enterobacter* has potential as PGPB in agricultural systems (Jha et al. 2011), and even when the mechanisms for improving the yield and growth of plants due to *Enterobacter* spp. are not fully understood, it is inferred that they work by facilitating the absorption of certain nutrients from the soil, synthesizing particular compounds for plants, and reducing or preventing plant diseases through antagonism or growth-promoting activities (Kumar et al. 2020). The genus *Streptomyces*is is included in the row Actinobacteria, class Actinomycetes, family Streptomycetaceae. They are gram-positive, filamentous bacteria. Most *Streptomyces* species are efficient colonizers of the rhizosphere and rhizoplane. They can also act as endophytes that colonize the internal tissues of host plants (Sousa and Olivares 2016). This group has a high potential for biocontrol due to the production of antibiotics, volatile compounds, secondary metabolites (Som et al. 2017; Quinn et al. 2020), and production of extracellular enzymes (Gherbawy et al. 2012; Mukhtar et al. 2017).

The species of this bacterial genus grow as a mycelium of branched hyphae and reproduce in the form of mold sending aerial branches that become chains of spores (Chater 2006). *Streptomyces* spp. act as PGPB in plant development (Viaene et al. 2016; Vurukonda et al. 2018).

#### 2.2.4.5 Use of PGPB as Biofertilizers

During the last decades, the increasing use of fertilizers to improve crop yield has caused environmental pollution and deteriorated the biological and physicochemical characteristics of agricultural soils throughout the world. The use of PGPB as biofertilizers is of utmost importance to reduce the application of agrochemicals in crop production (Yang and Fang 2015; Bishnoi 2018; Dong et al. 2019; Anli et al. 2020). A biofertilizer is defined as a product that contains live or inactive microorganisms that, when applied to the soil, seeds, or plant surface, individually or in combination, colonizes the rhizosphere or the interior of it, and promotes their growth and performance, by increasing the availability of primary nutrients and secreting growth-promoting substances (Vessey 2003; Dineshkumar et al. 2018). Biofertilizers can fix $N_2$ from the atmosphere, solubilize the nutrients required by plants (phosphate, potassium, and zinc), and also secrete hormones and substances that promote plant growth (Table 2.1) (Borkar 2015; Kumar et al. 2018).

Due to the indirect and direct effects caused by PGPBs in plants, several microbial taxa have been commercialized as efficient biofertilizers (Table 2.2). However, these bacteria are affected by various factors such as the biological and physicochemical characteristics of the soil, crop rotation, natural selection, and the use of organic and chemical fertilizers. Mahajan and Gupta (2009) mentioned that some important measures must be carried out for the efficacy of the biofertilizer to be successful: (1) it is essential that its concentration contains at least $10^7$ viable cells per gram of inoculum when supplied in the field and that it comes from a reputable manufacturer; (2) it should only be applied to the crops specified in the product, since biofertilizers are highly specific; (3) all the remainder must be applied in the field so that the microorganisms of the inoculum begin to interact with other microbiota in the rhizosphere and begin their colonization; (4) the biofertilizer must be stored in shaded and cool places, at temperatures between 25 and 28 °C; (5) the contact of the biofertilizer with synthetic agrochemicals should be avoided; and (6) about 200 g of biofertilizer can be effectively used to treat 10 kg of seeds.

## 2.3 Conclusions

Plant growth-promoting microorganisms are increasingly being used in agriculture to reduce the application of chemicals and thus restore soils and reduce pollution of water and crops. The use of PGPF allows plants to produce bioactive substances, like plant hormones; decomposing organic matter through mineral solubilization;

**Table 2.2** Plant growth-promoting bacteria used as commercial biofertilizers

| Bacteria | Trade name | References |
|---|---|---|
| *Azospirillum* sp. | Nitrofix® | García-Fraile et al. (2015, 2017) |
| *Azospirillum* sp. | Rhizosum Aqua | García-Fraile et al. (2015, 2017) |
| *Azospirillum* spp. | Bio-N | Aloo et al. (2020), Uribe et al. (2010) |
| *Azospirillum* sp. | Ajay Azospirillum | Aloo et al. (2020) |
| *A. brasilense* | Azofer® | García-Fraile et al. (2017) |
| *A. brasilense* | Zadspirillum | Aloo et al. (2020) |
| *A. brasilense* B-4485 | Azotobacterin® | García-Fraile et al. (2015, 2017) |
| *A. brasilense, Azotobacter vinelandii, Bacllius megaterium* | BactoFil® A10 | Aloo et al. (2020) |
| *Azospirillum* sp., *Rhizobium* sp., *Acetobacter* sp., *Azotobacter* sp. | Symbion N | Macik et al. (2020) |
| *Azospirillumbrasilense*, *A. lipoferum* | Azo-N | Adeleke et al. (2019) |
| *Azospirillumbrasilense*, A. *lipoferum*, *Azotobacter chroococcum* | Azo-N Plus | Adeleke et al. (2019) |
| *Azorhizobium* sp., *Azoarcus* sp., *Azospirillum* sp. | TwinN® | Adeleke et al. (2019) |
| *Azorhizobium* spp., *Azoarcus* spp., *Azospirillum* spp. | TripleN® | Dal Cortivo et al. (2020) |
| *Azotobacter chroococcum* | Dimargon® | Uribe et al. (2010) |
| *Azotobacter vinelandii*, *Rhizophagus irregularis* | Rhizosum N® | García-Fraile et al. (2017), Dal Cortivo et al. (2020) |
| *Azotobacter chroococcum*, *Bacillus megaterium* | Phylazonit M | Macik et al. (2020) |
| *Azotobacter chroococcum*, *Azospirillum brasilense*, *Bacillus megaterium* | Azoter | Artyszak and Gozdowski (2020) |
| *Azotobacter chroococcum*, A. *vinelandii*, *Acetobacterdiazotrophicus*, *Azospirillum lipoferum*, *Rhizobium japonicum* | AgrilifeNitrofix | Mehnaz (2016) |
| *B. subtilis*, *Bradyrhizobium japonicum* | Nodulator® PRO | García-Fraile et al. (2017) |
| *Bradyrhizobium japonicum* | Nodulator® | García-Fraile et al. (2017) |
| *B. japonicum* | BactoFil®Soya | Mustafa et al. (2019) |
| *B. japonicum* | Nodulest 10 | Mehnaz (2016) |
| *B. japonicum* | Rizo-Liq Top | Adeleke et al. (2019) |
| *B. japonicum* | BiAgro 10® | Uribe et al. (2010) |
| *Bradyrhizobium* spp. | Nodumax | Adeleke et al. (2019), Aloo et al. (2020) |
| *Bradyrhizobium* sp., *Mesorhizobium ciceri*, *Rhizobium* spp. | Rizo-Liq | Adeleke et al. (2019), Aloo et al. (2020) |
| *Delftia acidovorans*, *Bradyrhizobium* sp. | Bioboost® | García-Fraile et al. (2015, 2017) |
| *Paenibacillus polymyxa* | Custom N2 | García-Fraile et al. (2017) |

(continued)

**Table 2.2** (continued)

| Bacteria | Trade name | References |
|---|---|---|
| *Pseudomonas fluorescens/putida*, *Klebsiella pneumoniae*, *Citrobacter freundii* | BioGro® | Uribe et al. (2010) |
| Rhizobia | Nitragin Gold® | García-Fraile et al. (2017) |
| Rhizobia | Cell-Tech® | García-Fraile et al. (2017) |
| Rhizobia | Mamezo® | García-Fraile et al. (2015, 2017) |
| Rhizobia | Biofix | Adeleke et al. (2019), Aloo et al. (2020) |
| Rhizobia, *Penicillium bilaii* | TagTeam® | García-Fraile et al. (2017) |
| *Rhizobium etli* | Rhizofer® | García-Fraile et al. (2017) |
| *Rhizobium* sp. | Nitrasec | Aloo et al. (2020) |
| *Rhizobium* sp., *Bradyrhizobium japonicum* | Legume Fix | Adeleke et al. (2019), Aloo et al. (2020) |
| *Bacillus megaterium* | Bio Phos® | Mehnaz (2016), Macik et al. (2020) |
| *B. megaterium* | Symbion vam Plus | Aloo et al. (2020) |
| *B. megaterium* var. *phosphaticum* | Phosphobacterin | Mahajan and Gupta (2009) |
| *Bacillus mucilaginosus*, *B. subtilis* | CBF | Uribe et al. (2010) |
| *Bacillus* spp., *Glomus intraradices* | CataPult | Mehnaz (2016) |
| *Bacillus megaterium*, *Frateuria aurantia*, *Rhizophagus irregularis* | Rhizosum PK® | García-Fraile et al. (2017), Dal Cortivo et al. (2020) |
| *Pseudomonas fluorescens* | Fosforina® | Uribe et al. (2010) |
| *Pseudomonas striata*, *Bacillus polymyxa*, *B. megaterium* | P Sol B | Mehnaz (2016), Macik et al. (2020) |
| *Azotobacter chroococcum*, *P. fluorescens* | Bio Gold | Mehnaz (2016), Macik et al. (2020) |
| PGPB consortia | EVL Coating® | García-Fraile et al. (2015) |
| PGPB consortia | Bioativo | Aloo et al. (2020) |
| *Pseudomonas azotoformans* | Amase® | Mehnaz (2016), Mustafa et al. (2019) |
| *Frateuria aurantia* | Rhizosum K | García-Fraile et al. (2015, 2017) |
| *F. aurantia* | K Sol B | Mehnaz (2016) |
| PGPR consortia | Biozink® | García-Fraile et al. (2017) |
| *Thiobacillus thiooxidans* | Zn Sol B | Mehnaz (2016) |

increasing plant defenses against biotic and abiotic stresses; improving photosynthetic processes through the increase of total chlorophyll content; increasing IAA contents, root-shoot length, plants' dry and fresh weight, nutrient uptake, and many other beneficial effects.

The PGPB are increasingly being used in sustainable agriculture with the aim of reducing the use of chemical products and also generating stability to the plant in presence of unfavorable conditions of abiotic and biotic origin. Using these bacteria allows a more natural way to cope with agricultural challenges. Moreover, these bacteria must be highly competent, environmentally friendly and compatible with other organisms in the rhizosphere. These features may allow the plant to produce bioactive substances and increase its defenses against extreme conditions and pest attacks. These biostimulant microorganisms are emerging as an innovative solution to the current crop-production crisis.

## References

Abd-Alla MH, Nafady NA, Bashandy SR, Hassan AA (2019) Mitigation of effect of salt stress on the nodulation, nitrogen fixation and growth of chickpea (*Cicer arietinum* L.) by triple microbial inoculation. Rhizosphere 10:100148. https://doi.org/10.1016/j.rhisph.2019.100148

Adeleke RA, Raimi AR, Roopnarain A, Mokubedi SM (2019) Status and prospects of bacterial inoculants for sustainable management of agroecosystems. In: Giri B, Prasad R, Wu QS, Varma A (eds) Biofertilizers for sustainable agriculture and environment. Springer, Cham, pp 137–172

Adesemoye A, Egamberdieva D (2013) Beneficial effects of plant growth-promoting rhizobacteria on improved crop production: prospects for developing economies. In: Maheshwari D, Saraf M, Aeron A (eds) Bacteria in agrobiology: crop productivity. Springer, Berlin, pp 45–63. https://doi.org/10.1007/978-3-642-37241-4_2

Adhya TK, Kumar N, Reddy G, Podile AR, Bee H, Samantaray B (2015) Microbial mobilization of soil phosphorus and sustainable P management in agricultural soils. Curr Sci 108(7):1280–1287. http://www.jstor.org/stable/24905489

Aguirre JF, Cadena J, Olguín G, Aguirre JF, Andrade MI (2021) Co-inoculation of *Sechium edule* (Jacq.) Sw. plants with *Rhizophagusintraradices* and *Azospirillumbrasilense* to reduce *Phytophthora capsici* damage. Agriculture 11:391. https://doi.org/10.3390/agriculture11050391

Ahkami AH, White RA III, Handakumbura PP, Jansson C (2017) Rhizosphere engineering: enhancing sustainable plant ecosystem productivity. Rhizosphere 3:233–243. https://doi.org/10.1016/j.rhisph.2017.04.012

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 57:578–589. https://doi.org/10.1139/w11-044

Ahmad M, Zahir ZA, Asghar HN, Arshad M (2012) The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. Ann Microbiol 62:1321–1330. https://doi.org/10.1007/s13213-011-0380-9

Ahmed S (2019) *Bacillus cereus* a potential strain infested cereal cyst nematode (*Heteroderaavenae*). Pak J Nematol 37:53–61. https://doi.org/10.18681/pjn.v37.i01.p53-61

Al-Ani LKT, Mohammed AM (2020) Versatility of *Trichoderma* in plant disease management. In: Sharma V, Salwan R, Al-Ani LKT (eds) Molecular aspects of plant beneficial microbes in agriculture. Academic. https://doi.org/10.1016/B978-0-12-818469-1.00013-4

Al-Hmoud G, Al-Momany A (2017) Effect of four mycorrhizal products on squash plant growth and its effect on physiological plant elements. Adv Crop Sci Technol 5:1–6. https://doi.org/10.4172/2329-8863.1000260

Ali R, Gul H, Hamayun M, Rauf M, Iqbal A, Shah M, Hussain A, Bibi H, Lee H-J (2021) Aspergillus awamori ameliorates the physicochemical characteristics and mineral profile of mung bean under salt stress. Chem Biol Technol Agric 8:9. https://doi.org/10.1186/s40538-021-00208-9

Al-Karaki GN (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci Hortic 109:1–7. https://doi.org/10.1016/j.scienta.2006.02.019

Allen MF, Boosalis MG (1983) Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. New Phytol 93:67–76. https://doi.org/10.1111/j.1469-8137.1983.tb02693.x

Almaghrabi OA, Massoud SI, Abdelmoneim TS (2013) Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi J Biol Sci 20:57–61. https://doi.org/10.1016/j.sjbs.2012.10.004

Almaghrabi OA, Abdelmoneim TS, Albishri HM, Moussa TA (2014) Enhancement of maize growth using some plant growth promoting rhizobacteria (PGPR) under laboratory conditions. Life Sci J 11:764–772. https://doi.org/10.1016/j.sjbs.2012.10.004

Aloo BN, Makumba BA, Mbega ER (2020) Plant growth promoting rhizobacterial biofertilizers for sustainable crop production: the past, present, and future. Preprints, 2020090650, https://doi.org/10.20944/preprints202009.0650.v1

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971. https://doi.org/10.3389/fmicb.2017.00971

Anayo OF, Scholastica EC, Peter OC, Nneji UG, Obinna A, Mistura LO (2016) The beneficial roles of Pseudomonas in medicine, industries, and environment: a review. In: Pseudomonas aeruginosa-an armory within. IntechOpen, London. https://doi.org/10.5772/intechopen.85996

Anli M, Baslam M, Tahiri A, Raklami A, Symanczik S, Boutasknit A, Ait-El-Mokhtar M, Ben-Laouane R, Toubali S, AitRahou Y (2020) Biofertilizers as strategies to improve photosynthetic apparatus, growth, and drought stress tolerance in the date palm. Front Plant Sci 11: 516818. https://doi.org/10.3389/fpls.2020.516818

Arrebola E, Tienda S, Vida C, de Vicente A, Cazorla FM (2019) Fitness features involved in the biocontrol interaction of *Pseudomonas chlororaphis* with host plants: the case study of PcPCL1606. Front Microbiol 10:719. https://doi.org/10.3389/fmicb.2019.00719

Artyszak A, Gozdowski D (2020) The effect of growth activators and plant growth-promoting rhizobacteria (PGPR) on the soil properties, root yield, and technological quality of sugar beet. Agronomy 10:1262. https://doi.org/10.3390/agronomy10091262

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42. https://doi.org/10.1007/s005720100097

Badawy AA, Alotaibi MO, Abdelaziz AM, Osman MS, Khalil AMA, Saleh AM, Mohammed AE, Hashem AH (2021) Enhancement of seawater stress tolerance in barley by the endophytic fungus *Aspergillus ochraceus*. Metabolites 11:428–448. https://doi.org/10.3390/metabo11070428

Banchio E, Xie X, Zhang H, Paré PW (2009) Soil bacteria elevate essential oil accumulation and emissions in sweet basil. J Agric Food Chem 57:653–657. https://doi.org/10.1021/jf8020305

Barber SA (1995) Soil nutrient bioavailability: a mechanistic approach. Wiley, New York, p 384. isbn:978-0-471-58747-7

Barnawal D, Bharti N, Pandey SS, Pandey A, Chanotiya CS, Kalra A (2017) Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. Physiol Plant 161:502–514. https://doi.org/10.1111/ppl.12614

Bashan Y, de-Bashan LE, Prabhu SR, Hernández JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). Plant Soil 378:1–33. https://doi.org/10.1007/s11104-013-1956-x

Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, El Enshasy H (2021) Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustainability. 13:1140. https://doi.org/10.3390/su13031140

Basyony AG, Abo-Zaid GA (2018) Biocontrol of the root-knot nematode, *Meloidogyne incognita*, using an eco-friendly formulation from *Bacillus subtilis*, lab and greenhouse studies. Egypt J Biol Pest Control 28:87. https://doi.org/10.1186/s41938-018-0094-4

Belbahri L, ChenariBouket A, Rekik I, Alenezi FN, Vallat A, Luptakova L, Petrovova E, Oszako T, Cherrad S, Vacher S (2017) Comparative genomics of *Bacillus amyloliquefaciens* strains reveals a core genome with traits for habitat adaptation and a secondary metabolites rich accessory genome. Front Microbiol 8:1438. https://doi.org/10.3389/fmicb.2017.01438

Beneduzi A, Ambrosini A, Passaglia LM (2012) Plant growth-promoting rhizobacteria (PGPB): Their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051. https://doi.org/10.1590/s1415-47572012000600020

Berta G, Trotta A, Fusconi A, Hooker JE, Munro M, Atkinson D, Giovannetti M, Morini S, Fortuna P, Tisserant B, Gianinazzi-Pearson V, Gianinazzi S (1995) Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera*. Tree Physiol 15:281–293. https://doi.org/10.1093/treephys/15.5.281

Bharti N, Yadav D, Barnawal D, Maji D, Kalra A (2013) Exiguobacteriumoxidotolerans, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennell under primary and secondary salt stress. World J Microbiol Biotechnol. 29:379–387. https://doi.org/10.1007/s11274-012-1192-1

Bibi N, Hamayun M, Khan SA, Iqbal A, Islam B, Shah F (2018) Anthracene biodegradation capacity of newly isolated rhizospheric bacteria *Bacillus cereus* S13. PLoS One 13:e0201620. https://doi.org/10.1371/journal.pone.0201620

Bisen K, Keswani C, Patel JS, Sarma BK, Singh HB (2016) *Trichoderma* spp.: efficient inducers of systemic resistance in plants. In: Choudhary DK, Varma A (eds) Microbial-mediated induced systemic resistance in plants. Springer, Singapore. https://doi.org/10.1007/978-981-10-0388-2_12

Bishnoi U (2018) Agriculture and the dark side of chemical fertilizers. Environ Anal Ecol Stud 3: 198–201. https://doi.org/10.31031/EAES.2018.03.000552

Bona E, Cantamessa S, Massa N, Manassero P, Marsano F, Copetta A, Lingua G, D'Agostino G, Gamalero E, Berta G (2016) Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza 27: 1–11. https://doi.org/10.1007/s00572-016-0727-y

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48. https://doi.org/10.1038/ncomms1046

Borkar SG (2015) Microbes as bio-fertilizers and their production technology, 1st edn. WPI Publishing, New York. https://doi.org/10.1201/9780367805500

Bresson J, Varoquaux F, Bontpart T, Touraine B, Vile D (2013) The PGPR strain *Phyllobacteriumbrassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in Arabidopsis. New Phytol 200:558–569. https://doi.org/10.1111/nph.12383

Cai F, Yu G, Wang WZ, Fu L, Shen Q, Chen W (2013) Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. Plant Physiol Bioch 73:106–113. https://doi.org/10.1016/j.plaphy.2013.08.011

Carvalho F, Sagata E, de Castro LC, Juliatti FC (2010) Indução de resistência em plantas à fitopatógenos. Biosci J 26(2):231–239. http://www.seer.ufu.br/index.php/biosciencejournal/article/view/7071

Cassán FD, Lucangeli CD, Bottini R, Piccoli PN (2001) *Azospirillum* spp. metabolize [17,17-2H2] gibberellin A20 to [17,17-2H2] gibberellin A1 *in vivo* in dy rice mutant seedlings. Plant Cell Physiol. 42:763–767. https://doi.org/10.1093/pcp/pce099

Castagno LN, Estrella MJ, Sannazzaro AI, Grassano AE, Ruiz OA (2011) Phosphate-solubilization mechanism and *in vitro* plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). J Appl Microbiol 110(5):1151–1165. https://doi.org/10.1111/j.1365-2672.2011.04968.x

Castillo H, Rojas R, Villalta M (2016) *Gliocladium sp*., agente biocontrolador con aplicaciones prometedoras. Tecnología en Marcha. Ed. Esp. Biocontrol: 65-73, https://doi.org/10.18845/tm.v29i7.2707

Castillo-Aguilar C, Garruña R, Zúñiga-Aguilar JJ, Guzmán-Antonio AA (2017) PGPR inoculation improves growth, nutrient uptake and physiological parameters of *Capsicum chinense* plants. Phyton Int J Exp Bot 86:199–204. https://doi.org/10.32604/phyton.2017.86.199

Chagas AFJ, Borges LFC, de Oliveira LM, de Oliveira JC (2019) Efficiency of *Trichoderma asperellum* UFT 201 as plant growth promoter in soybean. Afr J Agric Res 14(5):263–271. https://doi.org/10.5897/AJAR2018.13556

Chater KF (2006) Streptomyces inside-out: a new perspective on the bacteria that provide us with antibiotics. Philos Trans R Soc B Biol Sci 361:761–768. https://doi.org/10.1098/rstb.2005.1758

Chen Q, Liu S (2019) Identification and characterization of the phosphate-solubilizing bacterium *Pantoea* sp. S32 in reclamation soil in Shanxi, China. Front Microbiol 10:2171. https://doi.org/10.3389/fmicb.2019.02171

Chiboub M, Jebara SH, Abid G, Jebara M (2020) Co-inoculation effects of *Rhizobium sullae* and *Pseudomonas* sp. on growth, antioxidant status, and expression pattern of genes associated with heavy metal tolerance and accumulation of cadmium in *Sulla coronaria*. J Plant Growth Regul 39:216–228. https://doi.org/10.1007/s00344-019-09976-z

Contreras HA, Macías L, del Val E, Larsen J (2016) Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiol Ecol 92(4):fiw036. https://doi.org/10.1093/femsec/fiw036

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: 1579–1592. https://doi.org/10.1104/pp.108.130369

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 27:503–514. https://doi.org/10.1094/MPMI-09-13-0265-R

Crowley DA (2006) Microbial siderophores in the plant rhizosphere. In: Barton LL, Abadia J (eds) Iron nutrition in plants and rhizospheric microorganisms. Springer, Netherlands. https://doi.org/10.1007/1-4020-4743-6_8

Dal Cortivo C, Ferrari M, Visioli G, Lauro M, Fornasier F, Barion G, Panozzo A, Vamerali T (2020) Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (*Triticum aestivum* L.) in the field. Front Plant Sci 11:72. https://doi.org/10.3389/fpls.2020.00072

Das AJ, Kumar R (2016) Bioremediation of petroleum contaminated soil to combat toxicity on *Withaniasomnifera* through seed priming with biosurfactant producing plant growth promoting rhizobacteria. J Environ Manag 174:79–86. https://doi.org/10.1016/j.jenvman.2016.01.031

David BV, Chandrasehar G, Selvam PN (2018) *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In: Crop improvement through microbial biotechnology. Elsevier, Amsterdam, pp 221–243. https://doi.org/10.1016/B978-0-444-63987-5.00010-4

De Lima BC, Moro AL, Santos ACP, Bonifacio A, Araujo ASF, de Araujo FF (2019) *Bacillus subtilis* ameliorates water stress tolerance in maize and common bean. J Plant Interact 14:432–439. https://doi.org/10.1080/17429145.2019.1645896

De Vasconcellos RLF, Cardoso EJBN (2009) Rhizosphericstreptomycetes as potential biocontrol agents of *Fusarium* and Armillaria pine rot and as PGPR for *Pinus taeda*. Biocontrol 54:807–816. https://doi.org/10.1007/s10526-009-9226-9

Delgado-Sánchez P, Ortega-Amaro MA, Jiménez-Bremont JF, Flores J (2011) Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae). Plant Biol 13:154–159. https://doi.org/10.1111/j.1438-8677.2010.00333.x

Depoorter E, Bull MJ, Peeters C, Coenye T, Vandamme P, Mahenthiralingam E (2016) Burkholderia: an update on taxonomy and biotechnological potential as antibiotic producers. Appl Microbiol Biotechnol 100(12):5215–5229. https://doi.org/10.1007/s00253-016-7520-x

Deshmukh R, Khardenavis AA, Purohit HJ (2016) Diverse metabolic capacities of fungi for bioremediation. Indian J Microbiol 56(3):247–264. https://doi.org/10.1007/s12088-016-0584-6

Dessaux Y, Grandclément C, Faure D (2016) Engineering the rhizosphere. Trends Plant Sci 21: 266–278. https://doi.org/10.1016/j.tplants.2016.01.002

Díaz-Zorita M, Fernández CMV (2008) Análisis de la producción de cereales inoculados con *Azospirillum brasilense* en la República Argentina. En: Cassán F, Garcia de Salamone IE (eds.). *Azospirillum sp.*: cell physiology, plant interactions and agronomic research in Argentina. Asociación Argentina de Microbiología. Buenos Aires. pp 155-166

Diggle SP, Whiteley M (2020) Microbe profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Microbiology 166:30–33. https://doi.org/10.1099/mic.0.000860

Dineshkumar R, Kumaravel R, Gopalsamy J, Sikder MNA, Sampathkumar P (2018) Microalgae as bio-fertilizers for rice growth and seed yield productivity. Waste Biomass Valor 9:793–800. https://doi.org/10.1007/s12649-017-9873-5

Do Carmo B, da Mota FF, Jurelevicius D, Seldin L (2020) Genetics and regulation of nitrogen fixation in *Paenibacillusbrasilensis* PB24. Microbiol Res 243:126647. https://doi.org/10.1016/j.micres.2020.126647

Domsch KH, Gams W, Anderson TH (1993) Compendium of soil fungi. IHW–Verlag, Regensburg, 859 p

Dong L, Li Y, Xu J, Yang J, Wei G, Shen L, Ding W, Chen S (2019) Biofertilizers regulate the soil microbial community and enhance Panax ginseng yields. Chin Med 14:20. https://doi.org/10.1186/s13020-019-0241-1

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(10):749–759. https://doi.org/10.1038/nrmicro2637

Ducrest PJ, Pfammatter S, Stephan D, Vogel G, Thibault P, Schnyder B (2019) Rapid detection of *Bacillus* ionophore cereulide in food products. Sci Rep 9:5814. https://doi.org/10.1038/s41598-019-42167-0

Egamberdiyeva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36(2–3):184–189. https://doi.org/10.1016/j.apsoil.2007.02.005

El-Nagdi WMA, Abd-El-Khair H, Soliman GM, Ameen HH, El-Sayed GM (2019) Application of protoplast fusants of *Bacillus licheniformis* and *Pseudomonas aeruginosa* on *Meloidogyne incognita* in tomato and eggplant. Middle East J Appl Sci 9:622–629

El-Sawah AM, El-Keblawy A, Ali DFI, Ibrahim HM, El-Sheikh MA, Sharma A, Alhai Hamoud Y, Shaghaleh H, Brestic M, Skalicky M, Xiong YC, Sheteiwy MS (2021) Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria enhance soil key enzymes, plant growth, seed yield, and qualitative attributes of guar. Agriculture 11:194. https://doi.org/10.3390/agriculture11030194

Elsharkawy MM (2018) Induced systemic resistance against Cucumber mosaic virus by *Phoma* sp. GS8-2 stimulates transcription of pathogenesis related genes in *Arabidopsis*. Pest Manag Sci 75:859–866. https://doi.org/10.1002/ps.5193

Elsharkawy MM, Shimizu M, Takahashi H, Hyakumachi M (2012) Induction of systemic resistance against Cucumber mosaic virus by *Penicillium simplicissimum* GP17-2 in *Arabidopsis* and tobacco. Plant Pathol 61:964–976. https://doi.org/10.1111/j.1365-3059.2011.02573.x

Estrada B, Aroca R, Maathuis FJM, Barea JM, Ruiz-Lozano JM (2013) Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant Cell Environ 36:1771–1782. https://doi.org/10.1111/pce.12082

Etesami H (2019) Plant growth promotion and suppression of fungal pathogens in rice (*Oryza sativa* L.) by plant growth-promoting bacteria. In: Field crops: sustainable management by PGPR. Springer, Cham, pp 351–383. https://doi.org/10.1007/978-3-030-30926-8_13

Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects—a review. J Soil Sci Plant Nutr 17(4):897–911. https://doi.org/10.4067/S0718-95162017000400005

Evidente A, Cabras A, Maddau L, Serra S, Andolfi A, Motta A (2003) Viridepyronone, a new antifungal 6-substituted 2H-pyran-2-one produced by *Trichoderma viride*. J Agric Food Chem 51(24):6957–6960. https://doi.org/10.1021/jf034708j

Fasciglione G, Casanovas EM, Quillehauquy V, Yommi AK, Gõ Ni MG, Roura SI, Barassi CA (2015) *Azospirillum* inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Sci Hortic 195:154–162. https://doi.org/10.1016/j.scienta.2015.09.015

Fatima S, Anjum T (2017) Identification of a potential ISR determinant from *Pseudomonas aeruginosa* PM12 against Fusarium wilt in tomato. Front Plant Sci 8:848. https://doi.org/10.3389/fpls.2017.00848

Figueiredo MVB, Burity HA, Martínez CR, Chanway CP (2008) Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacilluspolymyxa* and *Rhizobium tropici*. Appl Soil Ecol 40:182–188. https://doi.org/10.1016/j.apsoil.2008.04.005

Fitrianingsih A, Martanto EA, Abbas B (2019) The effectiveness of fungi Gliocladium fimbriatum and Trichoderma viride to control fusarium wilt disease of tomatoes (*Lycopersicum esculentum*). Indian J Agric Res 53(1):57–61. https://doi.org/10.18805/IJARe.A-363

Forghani F, Hajihassani A (2020) Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Front Plant Sci 11:1125. https://doi.org/10.3389/fpls.2020.01125

Fukami J, Cerezini P, Hungría M (2018) *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express 8:73. https://doi.org/10.1186/s13568-018-0608-1

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111(1):3–49. https://doi.org/10.1016/j.mycres.2006.12.001

Gamalero E, Glick BR (2020) The use of plant growth-promoting bacteria to prevent nematode damage to plants. Biology 9:1–13. https://doi.org/10.3390/biology9110381

Gamalero E, Trotta A, Massa N, Copetta A, Martinotti MG, Berta G (2004) Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. Mycorrhiza 14:185–192. https://doi.org/10.1007/s00572-003-0256-3

Gao P, Li Y, Guo Y, Duan T (2018) Co-inoculation of lucerne (*Medicago sativa*) with an AM fungus and a rhizobium reduces occurrence of spring black stem and leaf spot caused by *Phomamedicaginis*. Crop Past Sci 69(9):933–943. https://doi.org/10.1071/CP18135

García-Fraile P, Menéndez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. Aims Bioeng 2:183–205. https://doi.org/10.3934/bioeng.2015.3.183

García-Fraile P, Menéndez E, Celador-Lera L, Díez-Méndez A, Jiménez-Gómez A, Marcos-García M, Cruz-González XA, Martínez-Hidalgo P, Mateos PF, Rivas R (2017) Bacterial probiotics: a truly green revolution. In: Kumar V, Kumar M, Sharma S, Prasad R (eds) Probiotics and plant health. Springer, Singapore, pp 131–162. https://doi.org/10.1007/978-981-10-3473-2_6

Gayathri S, Saravanan D, Radhakrishnan M, Balagurunathan R, Kathiresan K (2010) Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt-marsh plant species. Indian J Biotechnol 9:397–402. http://nopr.niscair.res.in/bitstream/123456789/10438/1/IJBT%209(4)%20397-402.pdf

Genre A, Lanfranco L, Perotto S, Bonfante P (2020) Unique and common traits in mycorrhizal symbioses. Nat Rev Microbiol 18:649–660. https://doi.org/10.1038/s41579-020-0402-3

Ghazanfar MU, Raza M, Raza W, Qamar MI (2018) *Trichoderma* as potential biocontrol agent, its exploitation in agriculture: a review. Plant Prot 2(3):109–135. http://esciencepress.net/journals/PP

Gherbawy Y, Elhariry H, Altalhi A, El-Deeb B, Khiralla G (2012) Molecular screening of *Streptomyces* isolates for antifungal activity and family 19 chitinase enzymes. J Microbiol 50(3):459–468. https://doi.org/10.1007/s12275-012-2095-4

Gimenez E, Salinas M, Manzano-Agugliaro F (2018) Worldwide research on plant defense against biotic stresses as improvement for sustainable agriculture. Sustainability 10:1–19. https://doi.org/10.3390/su10020391

Giri B, Prasad R, Wu Q-S, Varma A (2019) Biofertilizers for sustainable agriculture and environment. Springer International Publishing (ISBN: 978-3-030-18932-7). https://www.springer.com/gp/book/9783030189327

Glick BR (2012a) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012: 963401. https://doi.org/10.6064/2012/963401

Glick BR (2012b) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 15 p. https://doi.org/10.6064/2012/963401

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res J 169:30–39. https://doi.org/10.1016/j.micres.2013.09.009

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119:329–339. https://doi.org/10.1007/s10658-007-9162-4

Gong M, Du P, Liu X, Zhu C (2014) Transformation of inorganic P fractions of soil and plant growth promotion by phosphate-solubilizing ability of *Penicillium oxalicum* II. J Microbiol 52(12):1012–1019. https://doi.org/10.1007/s12275-014-4406-4

Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res 206: 131–140. https://doi.org/10.1016/j.micres.2017.08.016

Gowtham HG, Hariprasad P, Nayak SC, Niranjana SR (2016) Application of rhizobacteria antagonistic to *Fusarium oxysporum* f. sp. *lycopersici* for the management of Fusarium wilt in tomato. Rhizosphere 2:72–74. https://doi.org/10.1016/j.rhisph.2016.07.008

Gupta S, Pandey S (2019) ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. Front Microbiol 10:1506. https://doi.org/10.3389/fmicb.2019.01506

Haggag WM (2007) Colonization of exopolysaccharide-producing *Paenibacilluspolymyxa* on peanut roots for enhancing resistance against crown rot disease. Afr J Biotechnol 6(13):1568–1577. https://academicjournals.org/journal/AJB/article-full-text-pdf/7700B356725

Hakim S, Imran A, Mirza MS (2021) Phylogenetic diversity analysis reveals Bradyrhizobiumyuanmingense and Ensiferaridi as major symbionts of mung bean (*Vigna radiata* L.) in Pakistan. Braz J Microbiol 52(1):311–324. https://doi.org/10.1007/s42770-020-00397-9

Halifu S, Deng X, Song X, Song R (2019) Effects of two Trichoderma strains on plant growth, rhizosphere soil nutrients, and fungal community of *Pinus sylvestris* var. mongolica annual seedlings. Forest 10(9):758. https://doi.org/10.3390/f10090758

Hamayun IM, Hussain A, Afzal SK, Iqbal A, Lee I-J (2019) *Aspergillus flavus* promoted the growth of soybean and sunflower seedlings at elevated temperature. BioMed Res

Hamayun IM, Hussain A, Khan SA, Iqbal A, Lee I-J (2020) An endophytic fungus *Aspergillus violaceofuscus* can be used as heat stress adaptive tool for *Glycine max* L. and *Helianthus annuus* L. J Appl Bot Food Qual 93:112–120. https://doi.org/10.5073/JABFQ.2020.093.014

Hamid M, SiddiquiI A, Shahid Shaukat S (2003) Improvement of *Pseudomonas fluorescens* CHA0 biocontrol activity against root-knot nematode by the addition of ammonium molybdate. Lett Appl Microbiol 36:239–244. https://doi.org/10.1046/j.1472-765X.2003.01299.x

Harman GE, Petzoldt R, Comis A, Chen J (2004a) Interactions between *Trichoderma harzianum strain* T22 and maize inbred line Mo17 and effects of this interaction on diseases caused by

*Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology 94(2):147–153. https://doi.org/10.1094/PHYTO.2004.94.2.147

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004b) *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56. https://doi.org/10.1038/nrmicro797

Heil M (2001) Induced systemic resistance (ISR) against pathogens—a promising field for ecological research. Persp Plant Ecol Evol Syst 4(2):65–79. https://doi.org/10.1078/1433-8319-00015

Hernández R, Rojas D, Contreras M, del Carmen Orozco-Mosqueda M, Macías LI, Reyes H (2015) Characterization of the antifungal and plant growthpromoting effects of diffusible and volatile organic compounds produced by *Pseudomonas fluorescens* strains. Biol Control 81:83–92. https://doi.org/10.1016/j.biocontrol.2014.11.011

Hoang NH, Tóth K, Stacey G (2020) The role of microRNAs in the legume–*Rhizobium* nitrogen-fixing symbiosis. J Exp Bot 71(5):1668–1680. https://doi.org/10.1093/jxb/eraa018

Hohmann P, Jones EE, Hill RA, Stewart A (2011) Understanding *Trichoderma* in the root system of *Pinus radiata*: associations between rhizosphere colonisation and growth promotion for commercially seedlings. Fungal Biol UK 115:759–767. https://doi.org/10.1016/j.funbio.2011.05.010

Hossain MM, Sultana F, Miyazawa M, Hyakumachi H (2014) The plant growth-promoting fungus *Penicillium spp*. GP15-1 enhances growth and confers protection against damping-off and anthracnose in the cucumber. J Oleo Sci 63(4):391–400. https://doi.org/10.5650/jos.ess13143

Hossain MM, Sultana F, Hyakumachi M (2017a) Role of ethylene signalling in growth and systemic resistance induction by the plant growth promoting fungus *Penicillium viridicatum* in Arabidopsis. J Phytopathol 165:432–441. https://doi.org/10.1111/jph.12577

Hossain MM, Sultana F, Islam S (2017b) Plant growth-promoting fungi (PGPF): phytostimulation and induced systemic resistance. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives, Microbial interactions and agro-ecological impacts, vol 2. Springer, Singapore. https://doi.org/10.1007/978-981-10-6593-4_6

Houssien AA, Ahmed SM, Ismail AA (2010) Activation of tomato plant defense response against *Fusarium* wilt disease using *Trichoderma harzianum* and salicylic acid under greenhouse conditions. Res J Agric Biol Sci 6(3):328–338. http://www.aensiweb.net/AENSIWEB/rjabs/rjabs/2010/328-338.pdf

Huang G-M, Zou Y-N, Wu Q-S, Xu Y-J, Kuča K (2020) Mycorrhizal roles in plant growth, gas exchange, root morphology, and nutrient uptake of walnuts. Plant Soil Environ 66:295–302. https://doi.org/10.17221/240/2020-PSE

Hung R, Lee SR (2016) Applications of *Aspergillus* in plant growth promotion. In: Gupta VK (ed) New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 223–227. https://doi.org/10.1016/B978-0-444-63505-1.00018-X

Hunziker L, Bönisch D, Groenhagen U, Bailly A, Schulz S, Weisskopf L (2014) *Pseudomonas* strains naturally associated with potato plants produce volatiles with high potential for inhibition of *Phytophthora infestans*. Appl Environ Microbiol 81(3):821–830. https://doi.org/10.1128/AEM.02999-14

Hyakumachi M (1994) Plant-growth-promoting fungi from turfgrass rhizosphere with potential for disease suppression. Soil Microorg 44:53–68. https://doi.org/10.18946/jssm.44.0_53

Ibijbijen J, Urquiaga S, Ismaili M, Alves BJR, Boddey RM (1996) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*). New Phytol 134:353–360. https://doi.org/10.1111/j.1469-8137.1996.tb04640.x

Ilyas N, Mumtaz K, Akhtar N, Yasmin H, Sayyed RZ, Khan W, El Enshasy HA, Dailin DJ, Elsayed EA, Ali Z (2020) Exopolysaccharides producing bacteria for the amelioration of drought stress in wheat. Sustainability 12:8876. https://doi.org/10.3390/su12218876

Islam S, Akanda AM, Prova A, Islam MT, Hossain MM (2016) Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol 6:1360. https://doi.org/10.3389/fmicb.2015.01360

Ismail MA, Amin MA, Eid AM, Hassan SED, Mahgoub HAM, Lashin I, Abdelwahab AT, Azab E, Gobouri AA, Elkelish A, Fouda A (2021) comparative study between exogenously applied plant growth hormones versus metabolites of microbial endophytes as plant growth-promoting for *Phaseolus vulgaris* L. Cells 10:1059. https://doi.org/10.3390/cells10051059

Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S (2017) The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. Front Plant Sci 8:1617. https://doi.org/10.3389/fpls.2017.01617

Jang JH, Woo SY, Kim SH, Khaine I, Kwak MJ, Lee HK, Lee TY, Lee WY (2017) Effects of increased soil fertility and plant growth-promoting rhizobacteria inoculation on biomass yield, energy value, and physiological response of poplar in short-rotation coppices in a reclaimed tideland: a case study in the Saemangeum area of Korea. Biomass Bioenergy 107:29–38. https://doi.org/10.1016/j.biombioe.2017.09.005

Jansa J, Forczek ST, Rozmoš M, Püschel D, Bukovská P, Hršelová H (2019) Arbuscular mycorrhiza and soil organic nitrogen: network of players and interactions. Chem Biol Technol Agric 6:10. https://doi.org/10.1186/s40538-019-0147-2

Jeyanthi V, Kanimozhi S (2018) Plant growth promoting rhizobacteria (PGPR)-prospective and mechanisms: a review. J Pure Appl Microbiol 12(2):733–749. https://doi.org/10.22207/JPAM.12.2.34

Jha CK, Aeron A, Patel BV, Maheshwari DK, Saraf M (2011) Enterobacter: role in plant growth promotion. In: Maheshwari DK (ed) Bacteria in agrobiology: plant growth responses. Springer, Berlin, 365 p. https://doi.org/10.1007/978-3-642-20332-9

Jiang C, Fan Z, Li Z, Niu D, Li Y, Zheng M, Wang Q, Jin H, Guo J (2020) *Bacillus cereus* AR156 triggers induced systemic resistance against *Pseudomonas syringae*pv. tomato DC3000 by suppressing MiR472 and activating CNLsmediated basal immunity in Arabidopsis. Mol Plant Pathol 21:854–870. https://doi.org/10.1111/mpp.12935

Jiménez C, de Albarracin NS, Altuna G, Alcano M (2011) Efecto de *Trichoderma harzianum* (Rifai) sobre el crecimiento de plantas de tomate (*Lycopersicon esculentum* L.). Rev Fac Agron 28:1–10

Jnawali AD, Ojha RB, Marahatta S (2015) Role of *Azotobacter* in soil fertility and sustainability—a review. Adv Plants Agric Res 2(6):250–253. https://medcraveonline.com/APAR/APAR-02-00069.pdf

Kalam S, Basu A, Ankati S (2017) Plant root-associated biofilms in bioremediation. In: Ahmad I, Husain FM (eds) Biofilms in plant and soil health. Wiley, Chichester, pp 337–355. https://doi.org/10.1002/9781119246329.ch18

Kalam S, Basu A, Podile AR (2020) Functional and molecular characterization of plant growth promoting *Bacillus* isolates from tomato rhizosphere. Heliyon 6:e04734. https://doi.org/10.1016/j.heliyon.2020.e04734

Kandaswamy R, Ramasamy MK, Palanivel R, Balasundaram U (2019) Impact of Pseudomonas putida RRF3 on the root transcriptome of rice plants: insights into defense response, secondary metabolism and root exudation. J Biosci 44(4):98. https://doi.org/10.1007/s12038-019-9922-2

Kang SM, Shahzad R, Bilal S, Khan AL, Park YG, Lee KE, Asaf S, Khan MA, Lee IJ (2019) Indole-3-acetic-acid and ACC deaminase producing *Leclerciaadecarboxylata* MO1 improves *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. BMC Microbiol 19:80. https://doi.org/10.1186/s12866-019-1450-6

Kaur J (2021) PGPR in management of soil toxicity. In: Kumar V, Prasad R, Kumar M (eds) Rhizobiont in bioremediation of hazardous wastes. Springer. https://doi.org/10.1007/978-981-16-0602-1_14

Keswani C, Bisen K, Chitara MK, Sarma BK, Singh HB (2017) Exploring the role of secondary metabolites of *Trichoderma* in tripartite interaction with plant and pathogens. In: Singh JS, Seneviratne G (eds) Agro-environmental sustainability. Springer. https://doi.org/10.1007/978-3-319-49724-2_4

Khan SA, Hamayun M, Kim HY, Yoon HJ, Seo JC, Choo YS, Lee IJ, Kim SD, Rhee IK, Kim JG (2009) A new strain of *Arthrinium phaeospermum* isolated from *Carex kobomugi* Ohwi is capable of gibberellin production. Biotechnol Lett 31(2):283–287. https://doi.org/10.1007/s10529-008-9862-7

Khan N, Mishra A, Nautiyal CS (2012) *Paenibacilluslentimorbus* B-30488 r controls early blight disease in tomato by inducing host resistance associated gene expression and inhibiting *Alternaria solani*. Biol Control 62:65–74. https://doi.org/10.1016/j.biocontrol.2012.03.010

Khan AL, Hussain J, Al-Harrasi A, Al-Rawahi A, Lee IJ (2015) Endophytic fungi: resource for gibberellins and crop abiotic stress resistance. Crit Rev Biotechnol 35(1):62–74. https://doi.org/10.3109/07388551.2013.800018

Khan MR, Mohidin FA, Khan U, Ahamad F (2016) Native *Pseudomonas* spp. suppressed the root-knot nematode in in vitro and in vivo and promoted the nodulation and grain yield in the field grown mungbean. Biol Control 101:159–168. https://doi.org/10.1016/j.biocontrol.2016.06.012

Khanam D (2008) Influence of flooding on the survival of Arbuscular Mycorrhiza. Bangladesh J Microbiol 25:111–114. https://doi.org/10.3329/bjm.v25i2.4872

Khushdil F, Gul Jana F, Gul J, Hamayun M, Iqbal A, Hussain A, Bibi N (2019) Salt stress alleviation in *Pennisetum glaucum* through secondary metabolites modulation by *Aspergillus terreus*. Plant Physiol Biochem 144:127–134. https://doi.org/10.1016/j.plaphy.2019.09.038

Kim SJ, Eo J-K, Lee E-H, Park H, Eom A-H (2017) Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology 45:20–24. https://doi.org/10.5941/MYCO.2017.45.1.20

Kokalis-Burelle N, Mahaffee WF, Rodriguez-Kabana R, Kloepper JW, Bowen KL (2002) Effects of switchgrass (*Panicum virgatum*) rotations with peanut (*Arachis hypogaea* L.) on nematode populations and soil microflora. J Nematol 34(2):98

Korir H, Mungai NW, Thuita M, Hamba Y, Masso C (2017) Coinoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front Plant Sci 8:141. https://doi.org/10.3389/fpls.2017.00141

Kour D, Rana KL, Yadav AN, Yadav N, Kumar M, Kumar V (2020) Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal Agric Biotechnol 23:101487. https://doi.org/10.1016/j.bcab.2019.101487

Kuan KB, Othman R, Abdul Rahim K, Shamsuddin ZH (2016) Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PLoS One 11(3):e0152478. https://doi.org/10.1371/journal.pone.0152478

Kuebutornye FKA, Abarike ED, Lu Y (2019) A review on the application of *Bacillus* as probiotics in aquaculture. Fish Shellfish Immunol 87:820–828. https://doi.org/10.1016/j.fsi.2019.02.010

Kulla D, Mal B, Mondal S, Ghosh S, Biswas G (2021) Effect of an indigenous AM and PGPR combination on chilli growth and productivity in lateritic soil. Biotecnol Veg 20(3):177–188, issn:2074-8647

Kumar A, Singh V, Singh M, Singh P, 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. Biocat Agric Biotechnol 8:1–7. https://doi.org/10.1016/j.bcab.2016.07.002

Kumar M, Prasad R, Kumar V, Tuteja N, Varma A (2017) Mycorrhizal fungi under biotic and abiotic stress. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 57–70

Kumar SM, Reddy CG, Phogat M, Korav S (2018) Role of bio-fertilizers towards sustainable agricultural development: a review. J Pharm Phytochem 7(6):1915–1921

Kumar V, Jain L, Jain SK, Chaturvedi S, Kaushal P (2020) Bacterial endophytes of rice (*Oryza sativa* L.) and their potential for plant growth promotion and antagonistic activities. S Afr J Bot 134:50–63. https://doi.org/10.1016/j.sajb.2020.02.017

Larios EJL, Wilmer JJVN, Chan WC, García FAL, Manzo GS, Buenrostro MTN (2019) Biocontrol of Damping off and promotion of vegetative growth in plants of *Capsicum chinense* (Jacq) with *Trichoderma spp*. Rev Mex Cienc Agríc 10(3):471–483. https://doi.org/10.29312/remexca.v10i3.332

Lastochkina O, Pusenkova L, Yuldashev R, Babaev M, Garipova S, Blagova D, Khairullin R, Aliniaeifard S (2017) Effects of Bacillus subtilis on some physiological and biochemical parameters of *Triticum aestivum* L. (wheat) under salinity. Plant Physiol Biochem 121:80–88

Latef AAHA, Chaoxing H (2011) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Sci Hortic 127:228–233. https://doi.org/10.1016/j.scienta.2010.09.020

Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854. https://doi.org/10.1016/0092-8674(93)90529-y

Lewis JA, Papavizas GC (1991) Biocontrol of plant diseases: the approach of tomorrow. Crop Prot 10:95–105. https://doi.org/10.1016/0261-2194(91)90055-V

Li Y, Li X, Jia D, Liu J, Wang J, Liu A, Liu Z, Guan G, Liu G, Luo J (2020a) Complete genome sequence and antimicrobial activity of *Bacillus velezensis* JT3-1, a microbial germicide isolated from yak feces. 3 Biotech 10:231. https://doi.org/10.1007/s13205-020-02235-z

Li Z, Wu N, Meng S, Wu F, Liu T (2020b) Arbuscular mycorrhizal fungi (AMF) enhance the tolerance of *Euonymus maackii* Rupr. at a moderate level of salinity. PLoS One 15:e0231497. https://doi.org/10.1371/journal.pone.0231497

Liu SH, Zeng GM, Niu QY, Liu Y, Zhou L, Jiang LH, Tan XF, Xu P, Zhang C, Cheng M (2017) Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: a mini review. Bioresour Technol 224:25–33. https://doi.org/10.1016/j.biortech.2016.11.095

Liu J, Cui X, Liu Z, Guo Z, Yu Z, Yao Q, Sui Y, Jin J, Liu X, Wang G (2019) The diversity and geographic distribution of cultivable *Bacillus*-like bacteria across black soils of northeast China. Front Microbiol 10:1–11. https://doi.org/10.3389/fmicb.2019.01424

Macik M, Gryta A, Frac M (2020) Biofertilizers in agriculture: an overview on concepts, strategies and effects on soil microorganisms. In: Sparks DL (ed) Advances in agronomy, vol 162. Academic, Cambridge, pp 31–87. https://doi.org/10.1016/bs.agron.2020.02.001

Mahajan A, Gupta RD (2009) Bio-fertilizers: their kinds and requirement in India. In: Mahajan A, Gupta RD (eds) Integrated nutrient management (INM) in a sustainable rice-wheat cropping system. Springer, Dordrecht, pp 75–100. https://doi.org/10.1007/978-1-4020-9875-8

Mahapatra R, Jampala SSM, Patel DR (2014) Induction of systemic acquired resistance in Zea mays L. by aspergillus flavus and A. parasiticus derived elicitors. Arch Phytopathol Plant Protect 48 (2):120–134. https://doi.org/10.1080/03235408.2014.884523

Marulanda A, Azcón R, Chaumont F, Ruiz-Lozano JM, Aroca R (2010) Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays* L.) plants under unstressed and salt-stressed conditions. Planta 232:533–543. https://doi.org/10.1007/s00425-010-1196-8

Matse DT, Hunag CH, Huang YM, Yen MY (2020) Effects of coinoculation of *Rhizobium* with plant growth promoting rhizobacteria on the nitrogen fixation and nutrient uptake of *Trifolium repens* in low phosphorus soil. J Plant Nutr 43:739–752. https://doi.org/10.1080/01904167.2019.1702205

Maxton A, Singh P, Masih S (2017) ACC deaminase-producing bacteria mediated drought and salt tolerance in *Capsicum annuum*. J Plant Nutr 41(5):574–583. https://doi.org/10.1080/01904167.2017.1392574

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. https://doi.org/10.1016/j.plaphy.2004.05.009

Mazzola M, Funnell DL, Raaijmakers JM (2004) Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil populations. Microb Ecol 48(3):338–348. https://doi.org/10.1007/s00248-003-1067-y

Mazzuchelli RCL, Mazzuchelli EHL, de Araujo FF (2020) Efficiency of *Bacillus subtilis* for root-knot and lesion nematodes management in sugarcane. Biol Control 143:104185. https://doi.org/10.1016/j.biocontrol.2020.104185

Meena VS, Maurya BR, Verma JP, Aeron A, Kumar A, Kim K, Bajpai VK (2015) Potassium solubilizing rhizobacteria (KSR): isolation, identification, and K-release dynamics from waste mica. Ecol Eng 81:340–347. https://doi.org/10.1016/J.ECOLENG.2015.04.065

Meena M, Swapnil P, Divyanshu K, Kumar S, Tripathi YN, Zehra A, Marwal A, Upadhyay RS (2020) PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: current perspectives. J Basic Microbiol 60:828–861. https://doi.org/10.1002/jobm.202000370

Mehnaz S (2016) An overview of globally available bioformulations. In: Arora N, Mehnaz S, Balestrini R (eds) Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 268–281. https://doi.org/10.1007/978-81-322-2779-3_15

Meng L, Zhang A, Wang F, Han X, Wang D, Li S (2015) Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Front Plant Sci 6:339. https://doi.org/10.3389/fpls.2015.00339

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (2018) The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112. https://doi.org/10.3389/fpls.2018.00112

Miljaković D, Marinković J, Balešević-Tubić S (2020) The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms 8:1037. https://doi.org/10.3390/microorganisms8071037

Mishra M, Prasad R, Varma A (2015) Endophytic fungi: biodiversity and functions. Int J Pharm BioSci 6(1):18–46

Mohammed S, Saedy M, Enan M, Ibrahim NE, Ghareeb A, Moustafa S (2008) Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita*. J Cell Mol Biol 7:57–66

Molina-Romero D, Juárez-Sánchez S, Venegas B, Ortíz-González CS, Baez A, Morales-García YE (2021) A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. Front Sustain Food Syst 4:293. https://doi.org/10.3389/fsufs.2020.616757

Moreira H, Pereira SIA, Marques APGC, Rangel AOSS, Castro PML (2016) Mine land valorization through energy maize production enhanced by the application of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi. Environ Sci Pollut Res 23:6940–6950. https://doi.org/10.1007/s11356015-5914-4

Morris CE, Sands DC, Vinatzer BA, Glaux C, Guilbaud C, Buffière A, Yan S, Dominguez H, Thompson BM (2008) The life history of the plant pathogen Pseudomonas syringae is linked to the water cycle. ISME J 2:321–334. https://doi.org/10.1038/ismej.2007.113

Mostafa FAM, Khalil AE, Nour El-Deen AH, Ibrahim DS (2018) The role of *Bacillus megaterium* and other bioagents in controlling root-knot nematodes infecting sugar beet under field conditions. Egypt J Biol Pest Control 28:66

Mukhtar S, Zaheer A, Aiysha D, Abdulla Malik K, Mehnaz S (2017) Actinomycetes: a source of industrially important enzymes. J Proteomics Bioinform 10:316–319. https://doi.org/10.4172/jpb.1000456

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Murali M, Naziya B, Ansari MA, Alomary MN, AlYahya S, Almatroudi A, Thriveni MC, Gowtham HG, Singh SB, Aiyaz M, Kalegowda N, Lakshmidevi N, Amruthesh KN (2021) Bioprospecting of rhizosphere-resident fungi: their role and importance in sustainable agriculture. J Fungi 7:314. https://doi.org/10.3390/jof7040314

Mushtaq S, Nasim G, Khokhar I, Mukhtar I (2012) Effects of *Penicillium* extracts on germination vigour in subsequent seedling growth of tomato (*Solanum lycopersicum* L.). Arch Phytopathol Plant Protect 45:932–937. https://doi.org/10.1080/03235408.2011.603965

Mustafa S, Kabir S, Shabbir U, Batool R (2019) Plant growth promoting rhizobacteria in sustainable agriculture: from theoretical to pragmatic approach. Symbiosis 78:115–123. https://doi.org/10.1007/s13199-019-00602-w

Naiman AD, Latronico AE, García de Salamone IE (2009) Inoculation of wheat with *Azospirillumbrasilense* and *Pseudomonas fluorescens*: impact on the production and rhizospheric microflora. Eur J Soil Biol 45:44–51. https://doi.org/10.1016/j.ejsobi.2008.11.001

Nakmee PS, Techapinyawat S, Ngamprasit S (2016) Comparative potentials of native arbuscular mycorrhizal fungi to improve nutrient uptake and biomass of *Sorghum bicolor* Linn. Agric Nat Resour 50:173–178. https://doi.org/10.1016/j.anres.2016.06.004

Nascimento FX, Rossi MJ, Soares CRFS, McConkey BJ, Glick BR (2014) New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. PLoS One 9(6):e99168. https://doi.org/10.1371/journal.pone.0099168

Nasim G, Mushtaq S, Mukhtar I, Khokhar I (2012) Effect of *Penicillium extractsa* on germination vigor in subsequent seedling growth of tomato (*Solanum lycopersicum* L.). Plant Breed Seed Sci 65:71–77. https://doi.org/10.2478/v10129-011-0049-3

Nawrocka J, Małolepsza U, Szymczak K, Szczech M (2018) Involvement of metabolic components, volatile compounds, PR proteins, and mechanical strengthening in multilayer protection of cucumber plants against *Rhizoctonia solani* activated by *Trichoderma atroviride* TRS25. Protoplasma 255(1):359–373. https://doi.org/10.1007/s00709-017-1157-1

Nazari M, Smith DL (2020) A PGPR-produced bacteriocin for sustainable agriculture: a review of Thuricin 17 characteristics and applications. Front Plant Sci 11:916. https://doi.org/10.3389/fpls.2020.00916

Nelsen CE, Safir GR (1982) Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154:407–413. https://doi.org/10.1007/BF01267807

Nezarat S, Gholami A (2009) Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize. Pak J Biol Sci 12:26–32. https://doi.org/10.3923/pjbs.2009.26.32

Nie P, Li X, Wang S, Guo J, Zhao H, Niu D (2017) Induced systemic resistance against *Botrytis cinerea* by *Bacillus cereus* AR156 through a JA/ET- and NPR1-dependent signaling pathway and activates PAMP-triggered immunity in *Arabidopsis*. Front Plant Sci 8:238. https://doi.org/10.3389/fpls.2017.00238

Niu X, Song L, Xiao Y, Ge W (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol 8:2580. https://doi.org/10.3389/fmicb.2017.02580

Nuangmek W, Aiduang W, Kumla J, Lumyong S, Suwannarach N (2021) Evaluation of a newly identified endophytic fungus, *Trichoderma phayaoense* for plant growth promotion and biological control of gummy stem blight and wilt of muskmelon. Front Microbiol 12:1–14. https://doi.org/10.3389/fmicb.2021.634772

Oldroyd G (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263. https://doi.org/10.1038/nrmicro2990

Olishevska S, Nickzad A, Déziel E (2019) *Bacillus* and *Paenibacillus* secreted polyketides and peptides involved in controlling human and plant pathogens. Appl Microbiol Biotechnol 103(3):1189–1215. https://doi.org/10.1007/s00253-018-9541-0

Ongena M, Duby F, Jourdan E, Beaudry T, Jadin V, Dommes J, Thonart P (2005) *Bacillus subtilis* M4 decreases plant susceptibility towards fungal pathogens by increasing host resistance associated with differential gene expresión. Appl Microbiol Biotechnol 67(5):692–698. https://doi.org/10.1007/s00253-004-1741-0

Ordookhani K, Sharafzadeh S, Zare M (2011) Influence of PGPR on growth, essential oil and nutrients uptake of sweet basil. Adv Environ Biol 5:672–677

Orozco MC, Glick BR, Santoyo G (2020) ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. Microbiol Res 235:126439. https://doi.org/10.1016/j.micres.2020.126439

Ortiz N, Armada E, Duque E, Roldan A, Azcón R (2015) Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. J Plant Physiol 174:87–96. https://doi.org/10.1016/j.jplph.2014.08.019

Pacheco LH, Reséndiz JFM, Arriola VJ (2019) Organismos entomopatógenos como control biológico en los sectores agropecuario y forestal de México: una revisión. Revista Mexicana de Ciencias Forestales 10(56):1–27. https://doi.org/10.29298/rmcf.v10i56.496

Pandey S, Ghosh PK, Ghosh S, De TK, Maiti TK (2013) Role of heavy metal resistant *Ochrobactrum* sp. and *Bacillus* spp. strains in bioremediation of a rice cultivar and their PGPR like activities. J Microbiol 51:11–17. https://doi.org/10.1007/s12275-013-2330-7

Parihar P, Bora M (2018) Effect of mycorrhiza (*Glomus mosseae*) on morphological and biochemical properties of Ashwagandha (*Withaniasomnifera*) (L.) Dunal. J Appl Nat Sci 10:1115–1123. https://doi.org/10.31018/jans.v10i4.1797

Patel P, Shaikh S, Sayyed R (2016) Dynamism of PGPR in bioremediation and plant growth promotion in heavy metal contaminated soil. Indian J Exp Biol 54:286–290

Patil LP, Navasare MG, Kambale OS, Jagtap GP (2021) Role of bacterial biofertilizers in agriculture for prevention of plant disease. AgriCos e-Newsletter 2(3):102–104. https://3b3ad16b-16bf-4401-956b39e03266b7cf.filesusr.com/ugd/93e822_f5d69faeb68649a58994833c97d72486.pdf?index=true

Pedranzani H, Rodríguez-Rivera M, Gutierrez M, Porcel R, Hause B, Ruiz-Lozano JM (2015) Arbuscular mycorrhizal symbiosis regulates physiology and performance of *Digitariaeriantha* plants subjected to abiotic stresses by modulating antioxidant and jasmonate levels. Mycorrhiza 26:141–152. https://doi.org/10.1007/s00572-015-0653-4

Pérez S, Héctor E (2021) Biotechnology of beneficial bacteria and fungi in agriculture. In: Prasad R, Nayak SC, Karwar RN, Dubey NK (eds) Mycoremediation and environmental sustainability, vol. 3. Springer. https://doi.org/10.1007/978-3-030-54422-5_12

Peteira B (2020) La resistencia inducida como alternativa para el manejo de plagas en las plantas de cultivo. Rev Prot Veg 35(1):1–12. http://revistas.censa.edu.cu/index.php/RPV/article/view/1084

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799. https://doi.org/10.1038/nrmicro3109

Phour M, Sehrawat A, Sindhu SS, Glick BR (2020) Interkingdom signaling in plant-rhizomicrobiome interactions for sustainable agriculture. Microbiol Res 241:126589. https://doi.org/10.1016/j.micres.2020.126589

Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 51:403–415. https://doi.org/10.1007/s00374-015-0996-1

Pitt JI (1981) The genus *Penicillium* and its telemorphic states *Eupenicillium* and *Talaromyces*. Academic, New York. https://doi.org/10.1002/jobm.19810210822

Prasad R (2022) Phytoremediation for environmental sustainability. Springer, Singapore (ISBN: 978-9811656200). https://doi.org/10.1007/978-981-16-5621-7

Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant growth-promoting rhizobacteria (PGPR) and medicinal plants. Springer International Publishing, Cham, pp 247–260

Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 1–7

Prasad M, Srinivasan R, Chaudhary M, Choudhary M, Jat LK (2019) Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. In: PGPR amelioration in sustainable agriculture. Woodhead Publishing, Cambridge, pp 129–157. https://doi.org/10.1016/B978-0-12-815879-1.00007-0

Prudent M, Salon C, Souleimanov A, Emery RJN, Smith DL (2015) Soybean is less impacted by water stress using *Bradyrhizobium japonicum* and thuricin-17 from *Bacillus thuringiensis*. Agron Sust Dev 35(2):749–757. https://doi.org/10.1007/s13593-014-0256-z

Qi W, Zhao L (2013) Study of the siderophore producing Trichoderma asperellum Q1 on cucumber growth promotion under salt stress. J Basic Microbiol 53(4):355–364. https://doi.org/10.1002/jobm.201200031

Quinn GA, Banat AM, Abdelhameed AM, Banat IM (2020) *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. J Med Microbiol 69:1040–1048. https://doi.org/10.1099/jmm.0.001232

Quiza L, St-Arnaud M, Yergeau E (2015) Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering. Front Plant Sci 6:507. https://doi.org/10.3389/fpls.2015.00507

Raaijmakers JM, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Ann Rev Phytopathol 50(1):403–424. https://doi.org/10.1146/annurev-phyto-081211-172908

Raboy V (2003) Myo-inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry 64(6):1033–1043. https://doi.org/10.1016/s0031-9422(03)00446-1

Radhakrishnan R, Hashem A, Abd Allah EF (2017) Bacillus: a biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol 8:667. https://doi.org/10.3389/fphys.2017.00667

Rahul S, Chandrashekhar P, Hemant B, Chandrakant N, Laxmikant S, Satish P (2014) Nematicidal activity of microbial pigment from *Serratia marcescens*. Nat Prod Res 28(17):1399–1404. https://doi.org/10.1080/14786419.2014.904310

Rais A, Jabeen Z, Shair F, Hafeez FY, Hassan MN (2017) *Bacillus* spp. a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyriculariaoryzae*. PLoS One 12:e0187412. https://doi.org/10.1371/journal.pone.0187412

Ramos EYA, Navarro RIZ, Zumaqué LEO, Violeth JLB (2008) Evaluación de sustratos y procesos de fermentación sólida para la producción de esporas de *Trichoderma sp*. Rev Colomb Biotechnol X(2):23–34

Rana A, Saharan B, Joshi M, Prasanna R, Kumar K, Nain L (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol 61:893–900. https://doi.org/10.1007/s13213-011-0211-z

Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN (2020) Endophytic fungi from medicinal plants: biodiversity and biotechnological applications. In: Microbial endophytes. Woodhead Publishing, Cambridge, pp 273–305. https://doi.org/10.1016/B978-0-12-819654-0.00011-9

Rashid MH, Chung YR (2017) Induction of systemic resistance against insect herbivores in plants by beneficial soil microbes. Front Plant Sci 8:1816. https://doi.org/10.3389/fpls.2017.01816

Rashid S, Charles TC, Glick BR (2012) Isolation and characterization of new plant growth promoting bacterial endophytes. Appl Soil Ecol 61:217–224. https://doi.org/10.1016/j.apsoil.2011.09.011

Rastegari AA, Yadav AN, Yadav N (2020) New and future developments in microbial biotechnology and bioengineering: trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, p 345, isbn:9780128205280

Reino JL, Guerrero RF, Hernáandez R, Collado IG (2008) Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem Rev 7(1):89–123. https://doi.org/10.1007/s11101-006-9032-2

Reitz M, Rudolph K, Schröder I, Homann-Hergarten S, Hallmann J, Sikora RA (2000) Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. Appl Environ Microbiol 66: 3515–3518. https://doi.org/10.1128/AEM.66.8.3515-3518.2000

Reshma P, Naik MK, Aiyaz M, Niranjana SK, Chennappa G, Shaikh SS, Sayyed RZ (2018) Induced systemic resistance by 2,4-diacetylphloroglucinol positive fluorescent Pseudomonas strains against rice sheath blight. Indian J Exp Biol 56:207–212

Rigamonte TA, Pylro VS, Duarte GF (2010) The role of Mycorrhization helper bacteria in the establishment and action of ectomycorrhizae associations. Braz J Microbiol 41:832–840. https://doi.org/10.1590/S1517-83822010000400002

Rizk IMH, Mousa IE, Ammar MM, Abd-ElMaksoud I (2017) Biological control of *Fusarium oxysporum* and *Verticillium dahliae* by *Trichoderma harzianum* and *Gliocladium virens*of two mint species. Res J Appl Biotechnol 3(2):24–36. https://doi.org/10.21608/rjab.2017.104581

Ruiz-Lozano JM, Aroca R, Zamarreño ÁM, Molina S, Andreo-Jimenez B, Porcel R, García-Mina JM, Ruyter-Spira C, López-Ráez JA (2015) Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant Cell Environ 39:441–452. https://doi.org/10.1111/pce.12631

Sagar A, Sayyed RZ, Ramteke PW, Sharma S, Marraiki N, Elgorban AM, Syed A (2020) ACC deaminase and antioxidant enzymes producing halophilic *Enterobacter* sp. PR14 promotes the growth of rice and millets under salinity stress. Physiol Mol Biol Plants 26:1847–1854. https://doi.org/10.1007/s12298-020-00852-9

Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2015) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res Int 23(5):3984–3999. https://doi.org/10.1007/s11356-015-4294-0

Salas-Marina MA, Silva-Flores MA, Cervantes-Badillo MG, Rosales-Saavedra MT, Islas-Osuna MA, Casas-Flores S (2011) The plant growth-promoting fungus *Aspergillus ustus* promotes growth and induces resistance against different lifestyle pathogens in *Arabidopsis thaliana*. J Microbiol Biotechnol 21(7):686–696. https://doi.org/10.4014/jmb.1101.01012

Saleem Akhtar S, Mekureyaw MF, Pandey C, Roitsch TG (2020) Role of cytokinins for interactions of plants with microbial pathogens and pest insects. Front Plant Sci 10:1777. https://doi.org/10.3389/fpls.2019.01777

Sandle T (2014) Trichoderma. In: Batt CA, Tortorello ML (eds) Encyclopedia of food microbiology. London, pp 644–646

Santoyo G, Hernández-Pacheco C, Hernández-Salmerón J, Hernández-León R (2017) The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review. Spanish J Agric Res 15:13. https://doi.org/10.5424/sjar/2017151-9990

Sarkar S, Basu S, Prasad R, Kumar G (2022) Phytoremediation: mechanistic approach for eliminating heavy metal toxicity from environment. In: Prasad R (ed) Phytoremediation for environmental sustainability. Springer, pp 513–543

Sarwar A, Hassan MN, Imran M, Iqbal M, Majeed S, Brader G, Sessitsch A, Hafeez FY (2018) Biocontrol activity of surfactin a purified from *Bacillus* NH-100 and NH-217 against Rice Bakanae Disease. Microbiol Res 209:1–13. https://doi.org/10.1016/j.micres.2018.01.006

Satyaprakash M, Nikitha T, Reddi EUB, Sadhana B, Satya Vani S (2017) Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. Int J Curr Microbiol App Sci 6(4):2133–2144. https://doi.org/10.20546/ijcmas.2017.604.251

Sauer S, Dlugosch L, Kammerer DR, Stintzing FC, Simon M (2021) The microbiome of the medicinal plants *Achillea millefolium* L. and *Hamamelis virginiana* L. Front Microbiol 12: 696398. https://doi.org/10.3389/fmicb.2021.696398

Savini V (2016) *Bacillus cereus* biocontrol properties. In: Savini V (ed) The diverse faces of *Bacillus cereus*. Elsevier. https://doi.org/10.1016/B978-0-12-801474-5.00010-4

Saxena A, Raghuwanshi R, Singh HB (2015) *Trichoderma* species mediated differential tolerance against biotic stress of phytopathogens in *Cicer arietinum* L. J Basic Microbiol 55(2):195–206. https://doi.org/10.1002/jobm.201400317

Sayyed RZ, Patel PR, Shaikh SS (2015) Plant growth promotion and root colonization by EPS producing *Enterobacter* sp. RZS5 under heavy metal contaminated soil. Indian J Exp Biol 53: 116–123

Sgroy V, Cassán F, Masciarelli O, Del Papa MF, Lagares A, Luna V (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. Appl Microbiol Biotechnol 85:371–338. https://doi.org/10.1007/s00253-009-2116-3

Shafi J, Tian H, Ji M (2017) *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnol Equip 31:446–459. https://doi.org/10.1080/13102818.2017.1286950

Sharma SK, Sharma MP, Ramesh A, Joshi OP (2012) Characterization of zinc solubilizing bacillus isolates and their potential to influence zinc assimilation in soybean seeds. J Microbiol Biotechnol 22:352–359. https://doi.org/10.4014/jmb.1106.05063

Sharma V, Salwan R, Sharma PN (2017) The comparative mechanistic aspects of Trichoderma and probiotics: scope for future research. Physiol Mol Plant Path 100:84–96. https://doi.org/10.1016/j.pmpp.2017.07.005

Shi H, Wang L, Li X, Liu X, Hao T, He X, Chen S (2016) Genome-wide transcriptome profiling of nitrogen fixation in *Paenibacillus* sp. WLY78. BMC Microbiol 16:25. https://doi.org/10.1186/s12866-016-0642-6

Sibponkrung S, Kondo T, Tanaka K, Tittabutr P, Boonkerd N, Yoshida K, Teaumroong N (2020) Co-inoculation of *Bacillus velezensis* strain S141 and *Bradyrhizobium* strains promotes nodule growth and nitrogen fixation. Microorganisms 8:678. https://doi.org/10.3390/microorganisms8050678

Siddiqui ZA, Mahmood I (1999) Role of bacteria in the management of plant parasitic nematodes: a review. Bioresour Technol 69(2):167–179. https://doi.org/10.1016/S0960-8524(98)00122-9

Siddiqui IA, Haas D, Heeb S (2005) Extracellular protease of *Pseudomonas fluorescens* CHA0, a biocontrol factor with activity against the root-knot nematode, *Meloidogyne incognita*. Appl Environ Microbiol 71:5646–5649. https://doi.org/10.1128/AEM.71.9.5646-5649.2005

Sindhu SS, Parmar P, Phour M, Sehrawat A (2016) Potassium-solubilizing microorganisms (KSMs) and its effect on plant growth improvement. In: Meena VS et al (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India. https://doi.org/10.1007/978-81-322-2776-2_13

Sindhu GM, Murali M, Thriveni MC, Anupama N, Amruthesh KN (2018) Growth promotion and disease resistance in muskmelon induced by crude proteins of *Penicillium verruculosum* against gummy stem blight disease. Asian J Crop Sci 10:160–167. https://doi.org/10.3923/ajcs.2018.160.167

Singh BN, Singh A, Singh BR, Singh HB (2014) *Trichoderma harzianum* elicits induced resistance in sunflower challenged by *Rhizoctonia solani*. J Appl Microbiol 116(3):654–666. https://doi.org/10.1111/jam.12387

Singh RP, Shelke GM, Kumar A, Jha PN (2015) Biochemistry and genetics of ACC deaminase: a weapon to “stress ethylene” produced in plants. Front Microbiol 6:937. https://doi.org/10.3389/fmicb.2015.00937

Singh H, Jaiswal V, Singh B, Singh S, Tiwari SP, Katiyar D (2017) Antagonistic compounds producing plant growth promoting rhizobacteria: a tool for management of plant disease. J Adv Microbiol 3(4):1–12. https://doi.org/10.9734/JAMB/2017/33368

Singh A, Shukla N, Kabadwal BC, Tewari AK, Kumar J (2018) Review on plant-*Trichoderma*-pathogen interaction. Int J Curr Microbiol App Sci 7(2):2382–2397. https://doi.org/10.20546/ijcmas.2018.702.291

Singh RK, Tripathi R, Ranjan A, Srivastava AK (2019) Fungi as potential candidates for bioremediation. In: Singh P, Kumar A, Borthakur A (eds) Abatement of environmental pollutants. Trends and strategies. Elsevier. https://doi.org/10.1016/B978-0-12-818095-2.00009-6

Singh A, Kumar R, Yadav AN, Mishra S, Sachan S, Sachan SG (2020) Tiny microbes, big yields: microorganisms for enhancing food crop production sustainable development. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 1–15. https://doi.org/10.1016/B978-0-12-820526-6.00001-4

Solanki MK, Yandigeri MS, Kumar S, Singh RK, Srivastava AK (2019) Co-inoculation of different antagonists can enhance the biocontrol activity against *Rhizoctonia solani* in tomato. Antonie van Leeuwenhoek 112:1633–1644. https://doi.org/10.1007/s10482-019-01290-8

Som NF, Heine D, Holmes N, Knowles F, Chandra G, Seipke RF, Hoskisson PA, Wilkinson B, Hutchings MI (2017) The MtrAB two-component system controls antibiotic production in

*Streptomyces coelicolor* A3 (2). Microbiology 163(10):1415–1419. https://doi.org/10.1099/mic.0.000524

Song Z, Bi Y, Zhang J, Gong Y, Yang H (2020) Arbuscular mycorrhizal fungi promote the growth of plants in the mining associated clay. Sci Rep 10:1–9. https://doi.org/10.1038/s41598-020-59447-9

Sonowal S, Nava AR, Joshi SJ, Borah SN, Islam NF, Pandit S, Prasad R, Sarma H (2022) Biosurfactants assisted heavy metals phytoremediation: green technology for the United Nations sustainable development goals. Pedosphere 2(1):198–210. https://doi.org/10.1016/S1002-0160(21)60067-X

Sousa JAJ, Olivares FL (2016) Plant growth promotion by *Streptomycetes*: ecophysiology, mechanisms and applications. Chem Biol Technol Agric 3(1):24. https://doi.org/10.1186/s40538-016-0073-5

Srivastava S, Bist V, Srivastava S, Singh PC, Trivedi PK, Asif MH, Chauhan PS, Nautiyal CS (2016) Unraveling aspects of *Bacillus amyloliquefaciens* mediated enhanced production of rice under biotic stress of *Rhizoctonia solani*. Front Plant Sci 7:587. https://doi.org/10.3389/fpls.2016.00587

Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: endophytism defined. In: Bacon CW, White JF (eds) Microbial endophytes, vol 3. Marcel Dekker, New York, pp 29–33. https://doi.org/10.1201/9781482277302-1

Subramanian K, Santhanakrishnan P, Balasubramanian P (2006) Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci Hortic 107:245–253. https://doi.org/10.1016/j.scienta.2005.07.006

Subramanian S, Fu Y, Sunkar R, Barbazuk WB, Zhu JK, Yu O (2008) Novel and nodulation-regulated microRNAs in soybean roots. BMC Genomics 9:160. https://doi.org/10.1186/1471-2164-9-160

Surya SH, Yuwati TW (2020) The use of fungal endophyte *Penicillium citrinum* on tree seedling: applicability and limitation. Bio Web of Conferences 20:03005. https://doi.org/10.1051/bioconf/20202003005

Suryaminarsih P, Kusriningrum, Ni'matuzaroh, Surtiningsih T (2015) Antagonistic compatibility of *Streptomyces griseorubens*, *Gliocladium virens*, and *Trichoderma harzianum* against *Fusarium oxysporum* cause of tomato wilt diseases. Int J Plant Soil Sci 5(2):82–89. https://doi.org/10.9734/IJPSS/2015/11026

Swarnalakshmi K, Yadav V, Tyagi D, Wattal Dhar D, Kannepalli A, Kumar S (2020) Significance of plant growth promoting rhizobacteria in grain legumes: growth promotion and crop production. Plants 9:1596. https://doi.org/10.3390/plants9111596

Syamsia S, Idhan A, Firmansyah AP, Noerfitryani N, Rahim I, Kesaulya H, Armus R (2021) Combination on endophytic fungal as the plant growth-promoting fungi (PGPF) on Cucumber (*Cucumis sativus*). Biodiversitas 22:1194–1202. https://doi.org/10.13057/biodiv/d220315

Tahir HAS, Gu Q, Wu H, Raza W, Hanif A, Wu L, Colman MV, Gao X (2017) Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. Front Microbiol 8:171. https://doi.org/10.3389/fmicb.2017.00171

Talaat NB, Shawky B (2014) Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. Environ Exp Bot 98:20–31. https://doi.org/10.1016/j.envexpbot.2013.10.005

Tamura K, Sakazaki R, Kosako Y, Yoshizaki E (1986) *Leclerciaadecarboxylata* Gen. Nov., Comb. Nov., formerly known as *Escherichia adecarboxylata*. Curr Microbiol 13(4):179–184. https://doi.org/10.1007/BF01568943

Tenorio S, Tinoco R, Vázquez R, Caballero J, Pérez E (2013) Identification of volatile compounds produced by the bacterium *Burkholderia tropica* that inhibit the growth of fungal pathogens. Bioengineered 4(4):236–243. https://doi.org/10.4161/bioe.23808

Timmusk S, Paalme V, Pavlicek T, Bergquist J, Vangala A, Danilas T, Nevo E (2011) Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. PLoS One 6(3): e17968. https://doi.org/10.1371/journal.pone.0017968

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kännaste A, Behers L, Nevo E, Seisenbaeva G, Stenström E, Niinemets Ü (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:e96086. https://doi.org/10.1371/journal.pone.0096086

Tóth K, Stacey G (2015) Does plant immunity play a critical role during initiation of the legume-*Rhizobium* symbiosis? Front Plant Sci 6:401. https://doi.org/10.3389/fpls.2015.00401

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant microbiome interactions: from community assembly to plant health. Nat Rev Microbiol 18:607–621. https://doi.org/10.1038/s41579-020-0412-1

Tsikou D, Yan Z, Holt DB, Abel NB, Reid DE, Madsen LH, Bhasin H, Sexauer M, Stougaard J, Markmann K (2018) Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA. Science 362(6411):233–236. https://doi.org/10.1126/science.aat6907

Tucci M, Ruocco M, De Masi L, De Palma M, Lorito M (2011) The beneficial effect of *Trichoderma* spp. on tomato is modulated by plant genotype. Mol Plant Pathol 12(4):341–354. https://doi.org/10.1111/j.1364-3703.2010.00674.x

Uribe D, Sánchez-Nieves J, Vanegas J (2010) Role of microbial biofertilizers in the development of a sustainable agriculture in the tropics. In: Dion P (ed) Soil biology and agriculture in the tropics. Springer, Berlin, pp 235–250. https://doi.org/10.1007/978-3-642-05076-3_11

Van Delm T, Van Beneden S, Mommaerts V, Melis P, Stoffels K, Wackers F, Baets W (2015) Control of *Botrytis cinerea* in strawberries with *Gliocladium catenulatum* vectored by bumblebees. J Berry Res 5(1):23–28. https://doi.org/10.3233/JBR-140087

Van Nguyen T, Pawlowski K (2017) *Frankia* and actinorhizal plants: symbiotic nitrogen fixation. In: Mehnaz S (ed) Rhizotrophs: plant growth promotion to bioremediation, Microorganisms for sustainability, vol 2. Springer, Singapore. https://doi.org/10.1007/978-981-10-4862-3_12

Varma A, Kharkwal A, Bains KS, Agarwal A, Bajaj R, Prasad R (2012) *Piriformospora indica*: the model microbes for organic green revolution. Biofertilizer Newsl 20:3–8

Varma A, Swati T, Prasad R (2020) Plant microbe symbiosis. Springer International Publishing (ISBN: 978-3-030-36247-8). https://www.springer.com/gp/book/9783030362478

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586. https://doi.org/10.1023/A:1026037216893

Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016) Streptomyces as a plant's best friend? FEMS Microbiol Ecol 92:fiw119. https://doi.org/10.1093/femsec/fiw119

Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M, Sivasithamparam K (2006) Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett Appl Microbiol 43(2):143–148. https://doi.org/10.1111/j.1472-765X.2006.01939.x

Vinale F, Nigro M, Sivasithamparam K, Flematti G, Ghisalberti E, Ruocco M, Varlese R, Marra R, Lanzuise S, Eid A, Woo SL, Lorito M (2013) Harzianic acid: a novel siderophore from *Trichoderma harzianum*. FEMS Microbiol Lett 347:123–129. https://doi.org/10.1111/1574-6968.12231

Vives-Peris V, de Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep. 39:3–17. https://doi.org/10.1007/s00299-019-02447-5

Vurukonda SS, Giovanardi D, Stefani E (2018) Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Int J Mol Sci 19:952. https://doi.org/10.3390/ijms19040952

Wang Y, Li K, Chen L, Zou Y, Liu H, Tian Y, Li D, Wang R, Zhao F, Ferguson BJ, Gresshoff PM (2015) MicroRNA167-directed regulation of the auxin response factors GmARF8a and GmARF8b is required for soybean nodulation and lateral root development. Plant Physiol 168(3):984–999. https://doi.org/10.1104/pp.15.00265

Wang J, Zhang Y, Jin J, Li Q, Zhao C, Nan W, Wang X, Ma R, Bi Y (2018) An intact cytokinin-signaling pathway is required for *Bacillus* sp. LZR216-promoted plant growth and root system architecture alteration in *Arabidopsis thaliana* seedlings. Plant Growth Regul 84:507–518. https://doi.org/10.1007/s10725-017-0357-1

Wang X, Fang L, Beiyuan J, Cui Y, Peng Q, Zhu S, Wang M, Zhang X (2021) Improvement of alfalfa resistance against Cd stress through rhizobia and arbuscular mycorrhiza fungi co-inoculation in Cd-contaminated soil. Environ Pollut 277:116758. https://doi.org/10.1016/j.envpol.2021.116758

Wardle DA (2002) Communities and ecosystems: linking the aboveground and belowground components (MPB-34). Princeton University Press, Princeton, p 408. https://doi.org/10.1515/9781400847297

Warren RM, Chng SF, Butler RC (2016) Functional characteristics of New Zealand wheat rhizosphere *Pseudomonas fluorescens* isolates and their potential to inhibit in-vitro growth of *Gaeumannomycesgraminis* var. *tritici*. N Z Plant Prot 69:48–56. https://doi.org/10.30843/nzpp.2016.69.5914

Wolters V (2001) Biodiversity of soil animals and its function. Eur J Soil Biol 37(4):221–227. https://doi.org/10.1016/S1164-5563(01)01088-3

Wu G, Liu Y, Xu Y, Zhang G, Shen Q, Zhang R (2018a) Exploring elicitors of the beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 to induce plant systemic resistance and their interactions with plant signaling pathways. MPMI 31(5):560–567. https://doi.org/10.1094/MPMI-11-17-0273-R

Wu G, Liu Y, Xu Y, Zhang G, Shen Q, Zhang R (2018b) Exploring elicitors of the beneficial rhizobacterium Bacillus amyloliquefaciens SQR9 to induce plant systemic resistance and their interactions with plant signaling pathways. Mol Plant Microbe Interact 31:560–567. https://doi.org/10.1094/MPMI-11-17-0273-R

Xiang N, Lawrence K, Donald P (2018) Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: a review. J Phytopathol 166:449–458. https://doi.org/10.1111/jph.12712

Xing R, Yan HY, Gao QB, Zhang FQ, Wang JL, Chen SL (2018) Microbial communities inhabiting the fairy ring of *Floccularia luteovirens* and isolation of potential mycorrhiza helper bacteria. J Basic Microbiol 58:554–563. https://doi.org/10.1002/jobm.201700579

Yadav SK (2010) Cold stress tolerance mechanisms in plants. A review. Agron Sustain Dev 30: 515–527. https://doi.org/10.1051/agro/2009050

Yadav S, Kaushik R, Saxena AK, Arora DK (2011) Diversity and phylogeny of plant growth-promoting bacilli from moderately acidic soil. J Basic Microbiol 51(1):98–106. https://doi.org/10.1002/jobm.201000098

Yamato M, Ikeda S, Iwase K (2008) Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions. Mycorrhiza 18:241–249. https://doi.org/10.1007/s00572-008-0177-2

Yan Z, Hossain MS, Valdés-López O, Hoang NT, Zhai J, Wang J, Libault M, Brechenmacher L, Findley S, Joshi T, Qiu L, Sherrier DJ, Ji T, Meyers BC, Xu D, Stacey G (2016) Identification and functional characterization of soybean root hair microRNAs expressed in response to *Bradyrhizobium japonicum* infection. Plant Biot J 14(1):332–341. https://doi.org/10.1111/pbi.12387

Yang X, Fang S (2015) Practices, perceptions, and implications of fertilizer use in East-Central China. Ambio 44:647–652. https://doi.org/10.1007/s13280-015-0639-7

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4. https://doi.org/10.1016/j.tplants.2008.10.004

Yedidia I, Srivastva AK, Kapulnik Y, Chet I (2001) Effect of *Trichoderma harzianum* on micro-element concentrations and increased growth of cucumber plants. Plant Soil 235:235–242. https://doi.org/10.1023/A:1011990013955

Yoo SJ, Shin DJ, Won HY, Song J, Sang MK (2018) *Aspergillus terreus* JF27 promotes the growth of tomato plants and induces resistance against *Pseudomonas syringae*pv. tomato. Mycobiology 46(2):147–153. https://doi.org/10.1080/12298093.2018.1475370

Yoshioka Y, Ichikawa H, Naznin HA, Kogure A, Hyakumachi M (2012) Systemic resistance induced in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1, a microbial pesticide of seedborne diseases of rice. Pest Manag Sci 68(1):60–66. https://doi.org/10.1002/ps.2220

Yousuf J, Thajudeen J, Rahiman M, Krishnankutty S, Alikunj AP, Abdulla MHA (2017) Nitrogen fixing potential of various heterotrophic *Bacillus* strains from a tropical estuary and adjacent coastal regions. J Basic Microbiol 57(11):922–932. https://doi.org/10.1002/jobm.201700072

Zeilinger S, Gruber S, Bansal R, Mukherjee PK (2016) Secondary metabolism in chemistry meets genomics. Fungal Biol Rev 30(2):74–90. https://doi.org/10.1016/j.fbr.2016.05.001

Zhang Y, Chen FS, Wu XQ, Luan FG, Zhang LP, Fang XM, Ye JR (2018) Isolation and characterization of two phosphate-solubilizing fungi from rhizosphere soil of moso bamboo and their functional capacities when exposed to different phosphorus sources and pH environments. PLoS One 13:e0199625. https://doi.org/10.1371/journal.pone.0199625

Zohora US, Ano T, Rahman MS (2016) Biocontrol of *Rhizoctonia solani* K1 by Iturin A producer *Bacillus subtilis* RB14 seed treatment in tomato plants. Adv Microbiol 6:424–431. https://doi.org/10.4236/aim.2016.66042

# Chapter 3
# Microbes-Mediated Approaches for Improving Plant Productivity and Quality

**Jyoti Srivastava, Shulbhi Verma, and Krishna Srivastava**

**Abstract** Most of the microbes present in soils are beneficial to the plant and the environment. Soil microbes assist plants in their development and growth and vice versa plants provide nutrition and shelter to the microbes for their development. Plant and microbe interaction enrich the soil in their texture and quality. Soil improvement reduces the dependency of plant on chemical fertilizers and provides many benefits to the plants. Microbes are natural organisms; their processes are slow. Genetic engineering and biotechnology tools may hasten the microbial process and could convert less utilized microbes into more utilization. In today's scenario, utilizing the microbial approaches in enhancing the productivity of plant is more progressive movement in the direction of sustainable agriculture and clean environment.

**Keywords** Soil improvement · Environment · Sustainable agriculture · Microbial approaches · PGPRs · Mycorrhiza

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_3

## 3.1 Introduction

### *3.1.1 The Soil*

Soil and the associated biodiversity harbors is a supplier to many ecosystem services which are of paramount significance to not only agriculture, but also the environment. The top layer of the earth's crust lithosphere which consists of soil is accountable for a myriad of functions such as shaping the local climate, relief features of the earth surface, water resources, the ecological circulation of the biogenic elements, and organic matter and their retention along with the creation of suitable conditions to sustain various life-forms like microbes, animals, and plants. Soil also protects and counteracts any changes in the environment through sorption properties besides providing an economic platform to the humans to work on the land as farmers. Thus, soil is a very dynamic entity entertaining over 30% of the species existing on earth. The soil organisms are a crucial aspect of soils and can be referred to as biological engine of the earth (Haygarth and Ritz 2009). Soil microbes are the chief part of the "working class" of the soil community profoundly affecting the functions of the soil in diverse ways. The soil microbial diversity is hugely responsible for the sustainable agricultural practices and in improving better usage of the natural resources (Bagde and Prasad 2016).

### *3.1.2 Soil Microbes*

The soil microbes are invariably associated with the primary production of the organic matter and nutrient recycling (Basu et al. 2021). They promote the growth of the plants, either by suppressing the plant diseases or enhancing their root mass, water uptake, and retention capacities in rhizosphere, or help in the secretion of the plant hormones. They profusely contribute to climate changes through the synchronization of the C and N fluxes as well as modulating several greenhouse gases like $CO_2$, $CH_4$, and $N_2O$. They are also enlisted with the control of pest and diseases in humans, animals, and plants and the subsequent decontamination of the environment. The avalanche in the global food demand, scarcity of arable lands, and the concomitant environmental pressure call for a judicious and sustainable approach in modern agriculture. Soil microbial biodiversity is the linchpin in letting us achieve both our economic as well as ecological sustainable issues (Barea 2015). The amelioration in the soil texture and quality, plant nutrition, and health is the elemental role of the soil microorganism in agriculture.

### 3.1.3 Significance of Soil Microbial Consortium

The imperative complex natural processes occurring in the environment are largely controlled by the soil microbes. The soil microbiome in close relationship with plants is responsible for:

- Supplying essential nutrients (phosphorous and nitrogen etc.)
- Uptake of various nutrients
- Promoting plant protection
- Stimulating plant growth (through the production of plant hormones)
- Improving soil quality and texture
- Bioaccumulation or microbial leaching of inorganics
- Significant role in the bioremediation of contaminated soils (Brierley 1985; Ehrlich 1990; Middledrop et al. 1990).

The soil microbial biome consists of bacteria, fungi, actinomycetes, protozoa, and viruses. These exert positive and harmful effects depending on their positions. Organic farming hugely relies on the natural soil microbial flora. Microorganisms like *Pseudomonas, Flavobacterium, Bacillus, Micrococcus, Fusarium, Sclerotium, Aspergillus*, and *Penicillium* facilitate the solubilization of phosphorous for their own use which in turn is available to the plants. Sixty-five percent of the nitrogen requirement in agriculture is met through biological nitrogen fixation. Mutualist symbionts like the arbuscular mycorrhiza (AM) fungi and bacteria of the genera *Rhizobia* fix nitrogen in symbiosis with legume crops (Nihorimbere et al. 2011; De-Bruijn 2015a, b). Beneficial rhizospheric microbes boost plant growth via diverse regulatory pathways which can be intuited into direct and indirect mechanisms. These involve the manipulation of the plant hormonal signaling facilitating the bioavailability of the soil-borne nutrients and repelling the pathogenic microbial strains (Bargaz et al. 2018; Grover et al. 2021). Direct mechanism enables resource acquisition of macro- (N, P, K) and micronutrients. They articulate plant hormone biosynthesis and a varied other molecules either extracellularly in the proximity of the rhizosphere (i.e., siderophores) or intracellularly like aminocyclopropane-1-carboxylate deaminase which assists in the plant growth and development by lowering the ethylene concentrations and increasing the osmotic stress in plants (Nadeem et al. 2007; Zahir et al. 2008). Indirect mechanism employed by the soil microbes to enhance plant growth is by diminishing the inhibitory effects of the phytopathogens as they act as a biocontrol agent. They stimulate competition for nutrients, antimicrobial metabolite biosynthesis (such as HCN, hydrogen cyanate, phenazines, pyrrolnitrin 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide, tensin, etc.), and elicit induced systemic resistance to pathogen in the plant which can probably occur because of a beneficial interaction of the rhizobacteria with the plant root (Lugtenberg and Kamilova 2009; Planchamp et al. 2015).

### *3.1.4 Diversity of Microbial Interactions*

The interactions of plants with the microbes are multifarious such as epiphytic (on plant surfaces), endophytic (within the plants), rhizospheric, and the soil microbes associated with the subsurface of the plant organs and soil interfaces. Plants achieve microbial interaction which can be competitive, exploitative, neutral, commensal, or mutualistic on an ecological scale. Although much of the research has focused around the pathogenic effects such as herbivory and infections, lately positive ecological microbial interactions enhancing the plant growth have taken precedence. A vast body of research has focused on the molecular mechanisms that elicit species-specific symbiotic collaboration of the legume plants with the soil rhizobia (Pinto et al. 2014). Flavonoids secreted in the root exudate are responsible for the legume host and the rhizobial interaction (Amit et al. 2021; Basile and Lepek 2021). A large group of soil microbes can trigger a systemic response in the plants, thereby activating the plant defense mechanisms. ISR or the induced systemic resistance can be activated by inoculating the plant with nonpathogenic root zone bacteria which elicits signaling pathways to provide a higher pathogen resistance to the host. Under abiotic stress conditions, species such as *Bacillus* induce ISR response. Endophytic bacterial species commonly employed as a biocontrol agent against various plant diseases might have a cutting advantage as they are protected from the relative competition in the soil environment besides usually growing in the same plant tissue where the plant pathogen usually resides (Heil 2001).

## 3.2 Guise of Beneficial Rhizospheric Microbes in Sustainable Agriculture

The main classes of the rhizospheric microbe which compliment plant growth, development and foster sustainable crop production can be discussed under the following categories:

1. **Decomposers/detrius:** The bacterial group actinomycetes decompose a wide array of substrates; they are predominantly important in degrading recalcitrant compounds such as chitin and cellulose and are active at high pH while fungi are prominent in degrading these compounds at low pH.
2. **Antagonists /biocontrol agent:** Most of the soil microfauna which act as biocontrol agents are competitive saprophytes, facultative plant symbionts, or facultative hyperparasites. Bacterial species such as *Streptomyces, Bacillus, Burkholderia, Lysobacter, Pantoea, Pseudomonas*, and fungal (*Ampelomyces, Coniothyrium, Dactylella, Gliocladium, Paecilomyces*, and *Trichoderma*) are some of the successful biocontrol agents. Other micro- and mesofauna predators like collembolan, mites, nematodes, annelids, and insect larvae activities reduce

pathogen biomass and often stimulate plant host defense by virtue of their herbivorous activities.

3. **PGPR**: Plant growth-promoting bacteria are profusely ascertained with a wide variety of ecosystem processes such as in biocontrol of plant pathogen, nutrient recycling etc. The $N_2$-fixing bacteria and the arbuscular mycorrhizal (AM) fungi are an example of beneficial mutualistic plant symbionts. Bacterial genera "rhizobia" have the capacity to fix atmospheric nitrogen in symbiosis with the legume plants. The nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia and nitrate which is readily used by plants. The microbial consortium in agricultural soil interacts favorably to boost plant growth, which is often complex to predict (Prasad et al. 2020) (Table 3.1).

### 3.2.1 Plant Growth-Promoting Rhizobacteria

Plants have coevolved with soil microbes facilitating their growth and development in a symbiotic manner. PGPR are immensely exploited commercially and in scientific applications helping in making the soil ecosystem sustainable for crop production (Prasad et al. 2015). The PGPR associations have been investigated in oat, canola peas, tomato, lentil, barley, cucumber (Gray and Smith 2005). PGPR colonize plant root and enhance plant growth by diverse mechanisms involving various mechanisms such as: phosphate solubilization, nitrogen fixation, indole acetic acid (IAA), siderophore, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase, and hydrogen cyanate production (Liu et al. 2016). PGPR are also involved in the degradation of environmental pollutants, heavy metal detoxification, salinity tolerance, and as an antagonist to plant pathogens and insects (Egamberdieva and Lugtenberg 2014).

### 3.2.2 Different Forms of PGPR

PGPR can be organized into two distinct classes: the extracellular plant growth-promoting rhizobacteria (ePGPR) and the intracellular plant growth-promoting rhizobacteria (iPGPR) (Martinez-Viveros et al. 2010). **ePGPR** colonize the rhizosphere (on the rhizoplane) or in the spaces between the cells of the root cortex and include the following genera: *Azotobacter*, *Serratia*, *Azospirillum, Bacillus*, *Caulobacter*, *Chromobacterium*, *Agrobacterium*, *Erwinia*, *Flavobacterium*, *Arthrobacter*, *Micrococcous*, *Pseudomonas*, and *Burkholderia*. Specialized nodules in the root cells are colonized by **iPGPR** which include the endophytic microbes such as *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, as well as *Frankia* species, which harbor the ability to fix atmospheric nitrogen specifically for higher plants (Bhattacharyya and Jha 2012).

**Table 3.1** Relevant of some beneficial microbes

| S. No. | Microbial species | Plant | Function | References |
|---|---|---|---|---|
| 1 | *Achromobacter xylosoxidans* | *Vigna radiata* | Affects plant homeostasis | Ma et al. (2009) |
| 2 | *Azospirillum brasilense* | *Zea mays* | Indole acetic acid synthesis induces plant growth | Orlandini et al. (2014) |
| 3 | *Bradyrhizobium japonicum* | *Glycine max* | Phosphate solubilization | Rathore (2015) |
| 3 | *Azotobacter aceae* | *Fagopyrum esculentum* | Fixation of nitrogen | Bhattacharyya and Jha (2012) |
| 4 | *Bacillus circulans, Cladosporium herbarum* | *Vigna radiata* | Phosphate solubilization | Oteino et al. (2015) |
| 5 | *Bacillus licheniformis* | *Piper nigrum* | Protection from *Myzus persicae* | Kumar et al. (2015) |
| 6 | *Bacillus megaterium* | *Zea mays* | Phosphate solubilization | Ibarra-Galeana et al. (2017) |
| 7 | *Bacillus mucilaginosus* | *Piper nigrum, Cucumis* | Enhanced potassium intake capacity | Liu et al. (2012) |
| 8 | *Bacillus cereus* | *Gossypium hirsutum* | Prevents from *Meloidogyne incognita* and *M. javanica* | Gao et al. (2016) |
| 9 | *Brevibacterium frigoritolerans* YSP40; *Bacillus paralicheniformis* YSP151 | *Brassica juncia* | Uptake lead in metal-contaminated soil | Yahaghi et al. (2018) |
| 10 | *Burkholderia* spp. | Most of the fruit plants | Induces more ethylene production | Islam et al. (2016) |
| 11 | *Enterobacter agglomerans* | *Solanum lycopersicum* | Phosphate solubilization | Oteino et al. (2015) |
| 12 | *Flavimonas oryzihabitans INR* | *Cucumis sativus* | Protects from stripped cucumber beetle | Oteino et al. (2015), Bhattacharyya and Jha (2012) |
| 13 | *Paenibacillus polymyxa* | *Sesamum indicum* | Prevents fungal disease | Ngumbi and Kloepper (2016) |
| 14 | *Pseudomonas aeruginosa* | *Cicer arietinum* | Stimulates potassium and phosphorus uptake | Ahemad and Kibret (2014) |
| 15 | *Pseudomonas aeruginosa, Bacillus subtilis* | *Vigna radiata* | Prevents root knot formation | Ngumbi and Kloepper (2016), Ahemad and Kibret (2014) |
| 16 | *Pseudomonas fluorescens* | *Triticum aestivum* | Helps prevent *Fusarium culmorum* | Santoro et al. (2016) |

(continued)

**Table 3.1** (continued)

| S. No. | Microbial species | Plant | Function | References |
|---|---|---|---|---|
| 17 | *Pseudomonas putida* | *Arabidopsis thaliana* | Improves utilization of plant secondary metabolites | Ahemad and Khan (2012) |
| 18 | *Pseudomonas* sp. | *Dianthus caryophyllus* | Prevents Fusarium wilt | Rathore (2015), Ahemad and Khan (2012) |
| 19 | *Rhizobium leguminosarum* | *Phaseolus vulgaris* | Phosphate solubilization | Ahemad and Kibret (2014) |

## 3.2.3 Role of PGPR in Enhancing Plant Growth

Specialized traits enable the PGPR to enhance and stimulate plant growth and development through various direct and indirect mechanisms involving plant physiology and resistance to phytopathogens (Gupta et al. 2015). These includes nutrient fixation, neutralizing abiotic and biotic stress, and producing enzymes and other volatile compounds to prevent disease. The mode of operation depends upon:

- The type of host plant
- The biotic factors such as plant genotypes, development stage of the plant, and its subsequent defense mechanism and the other members of the soil microbe consortium
- Abiotic factors limiting the action of PGPR comprise of soil composition, soil management, and climatic conditions (Vacheron et al. 2013).

### 3.2.3.1 Nutrient Fixation by PGPR

PGPR have the propensity to increase the availability and concentration of nutrients by locking or fixing their supply for plant growth. Plants cannot utilize nitrogen directly; they quench their nitrogen requirement by absorbing nitrate ($NO_3^-$) and ammonium ($NH_4^+$) from the soil which are essential nutrients for the plant growth. In aerobic soils, nitrogen is predominantly available in the form of nitrates where the PGPR converts the atmospheric nitrogen into the nitrate. PGPR also possess the capacity to solubilize phosphate, resulting in an increased number of phosphate ions available in the soil and thus can be easily taken up by the plants. Species such as *Klebsiella pneumoniae* Fr1, *Bacillus pumilus* S1r1, *Acinetobacter* sp. S3r2, and *Bacillus subtilis* UPMB10 have been reported to fix atmospheric nitrogen and delay N remobilization. The microbe *Kocuria Turkanensis* 2 M4 isolated from the soil rhizosphere has been potent as a phosphate solubilizer, a siderophore producer, and IAA producer for many different plant species (Paredes and Lebeis 2016; Goswami et al. 2016).

### 3.2.3.2 Nitrogen Fixation

Symbiotic PGPR, documented to fix atmospheric $N_2$, include *Rhizobium* sp., *Azoarcus* sp., *Beijerinckia* sp., *Pantoea agglomerans,* and *K. pneumoniae* (Ahemad and Kibret 2014). Soil inoculation with a combination of rhizobacterial species improves soil quality and tremendously enhances nodule formation (Unkovich and Baldock 2010). Primarily *Nif* gene is responsible for the $N_2$ fixation, and other structural genes also involved in activating the iron protein, electron donation, biosynthesis of the iron molybdenum cofactor, and activity of the enzyme.

### 3.2.3.3 Phytohormone Production

PGPR have the capability to induce production of phytohormone like gibberellins, cytokinins, abscisic acid, ethylene, and auxin. PGPR help in root and shoot invigoration, such as *Rhizobium leguminosarum*, *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Pseudomonas* sp., and *Azotobacter* sp. through the induction of phytohormones (Umesha et al. 2018). We can thus elucidate that PGPR manifest plant growth by invoking drastic changes in the soil microbial consortium in the rhizosphere. They assist in plant growth directly by either encouraging resource/nutrient procurement (nitrogen, phosphorus, potassium, and other essential minerals) or by altering plant hormone levels, or indirectly by diminishing the inhibitory effects of different phytopathogens in the forms of biocontrol agents. The general mechanisms of plant nutrient management by microorganisms include associative nitrogen fixation, lowering of ethylene levels, production of siderophores, production of growth regulators, VOCs, solubilization of nutrients, and promotion of mycorrhizal functioning (Fig. 3.1).

**Phosphate Solubilizing Microbes (PSM)**: The second most essential macronutrient for plant growth is phosphorous. It plays cardinal role in all the metabolic processes such as energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis. Since most of the phosphorous in soil is immobilized and is either insoluble or in precipitated forms, plants cannot directly absorb it. Plants absorb phosphate only as monobasic $H_2PO_4^+$ and $HPO_4^{++}$ dibasic ions. Many bacterial genera (i.e., *Azotobacter*, *Bradyrhizobium Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Microbacterium, Pseudomonas*, *Erwinia*, *Rhizobium*, *Mesorhizobium*, *Flavobacterium*, *Rhodococcus*, and *Serratia*), fungi (i.e., *Penicillium* and *Aspergillus*), actinomycetes (i.e., *Streptomyces*), and algae have the potency of solubilizing P-metal complex to release P in bioaccessible form such as orthophosphate through specific mechanisms generally involving organic acids, siderophore production, and phosphatase enzymes which efficiently hydrolyze organic P forms. Thus, PSM significantly contribute towards plant growth by enhancing the efficiency of P utilization through exudation of organic acids or by P-hydrolyzing phosphatase enzymes which in turn enhances the bioavailable P pool directly, or indirectly via the production of other high-value bioactive molecules like

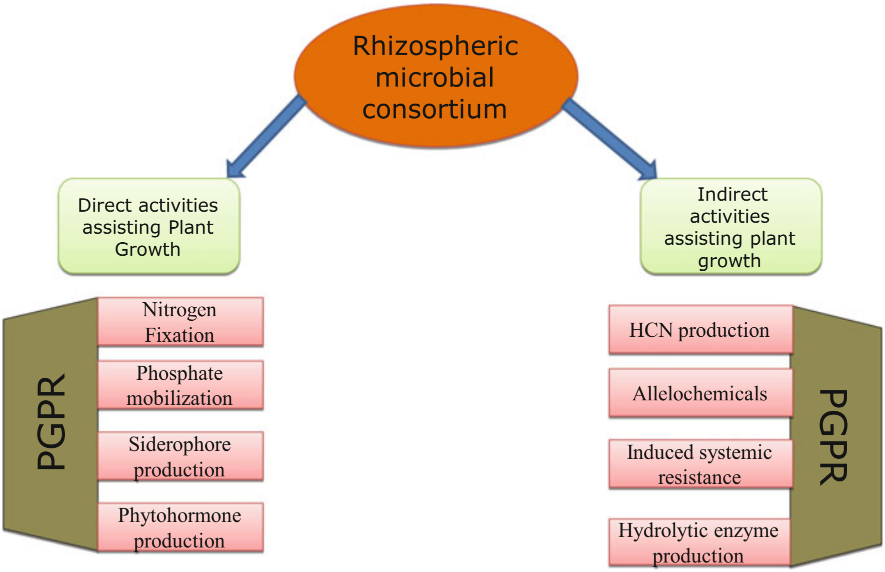

**Fig. 3.1** Significance of PGPR

phytohormones, antifungal compounds, toxin-resistance compounds, which assist in building and strengthening robust shoot/rooting system, specially under biotic and abiotic constraints. The PSM have been implicated with the production of a number of organic acids such as acetic acid, gluconic acid, glucuronic acid, butyric acid, fumaric acid, citric acid, lactic acid, propionic acid, succinic acid, oxalic acid, and valeric acid out of which 2-keto gluconic acid and gluconic acid are the most common acids produced by gram-negative bacteria (Krishnaraj and Dahale 2014). The organic acids are efficacious in solubilizing P. The decrease in pH and the cation chelating properties have been attributed as the principal reason for the solubilization of P by organic acids. The concomitant acidification in the vicinity of microbial cell leads to the substitution of $H^+$ and $Ca^{+2}$ (Zeroual et al. 2012; Behera et al. 2017).

#### 3.2.3.4 Potassium Solubilizing Microbes

Potassium chiefly exist in the form of insoluble rocks and silicate minerals and thus are not available to plants in soluble form as their concentration is extremely low in soil. Low potassium concentrations results in poor seed production, slower growth rate, and stunted roots. PGPR are promising candidates in providing the required concentration of soluble potassium in soil and thus to plants as well. They solubilize potassium rocks by secreting organic acids; *Acidothiobacillus* sp., *Bacillus edaphicus*, *Ferrooxidans* sp., *Bacillus mucilaginosus*, *Pseudomonas* sp., *Burkholderia* sp., and *Paenibacillus* sp., have been reported to release potassium in accessible form from potassium-bearing minerals in soils (Liu et al. 2012). Thus, applying potassium-solubilizing PGP microbes as biofertilizer to improve agriculture can reduce the use of agrochemicals and support eco-friendly crop production.

#### 3.2.3.5 Biological Nitrogen-Fixing Microbes

BNF implies a microbially mediated process where in the presence of an enzyme nitrogenase, atmospheric $N_2$ is reduced into ammonia ($NH_3$). Diazotrophs are the group of microbes which support such an enzymatic conversion. The process is carried out biologically either by symbiotic or nonsymbiotic interactions between microbes and plants. The legumes associate with certain soil rhizobial bacteria like *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, and *Allorhizobium*. They utilize root nodules to sequester atmospheric nitrogen as ammonia, which can be easily utilized by the plants and further be incorporated into biomolecules including proteins and nucleic acids. In symbiotic nitrogen fixation, NF microbes transfer biologically fixed N directly from the bacteria to the host plant along with a significant transfer of photosynthetically fixed plant carbon to the NF bacteria. Some noteworthy illustrations of symbiosis between NF bacteria and eukaryotes include the associations of cyanobacteria with fungi that occur in lichens, cycads, and gunnera; the association of actinomycetes (i.e., Frankia) with a variety of angiosperms like Alnus and Casuarina are also significant (Varma et al. 2020). Nonlegume plants such as grasses have been extensively investigated for their propensity to fix $N_2$. Several nonsymbiotic NF bacteria of grass species, especially cereals, also exhibit PGP properties where they have been reported to significantly increase plant vegetative growth and grain yield. Species such as *Beijerinckia*, *Azotobacter*, *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter*, *Burkholderia*, *Clostridium*, *Methanosarcina*, and *Paenibacillus* are well-known examples which help in promoting the plant growth. Unlike in the rhizobial association that lead to the formation of root nodules within their legume hosts, in nonsymbiotic NF bacteria reside either in the rhizosphere as free-living or live inside the living tissue (endophytic). They proliferate on account of the energy and nutrients derived from the plant roots. A cardinal feature of importance is that a direct controlled exchange of N and C between bacteria and plant hosts is not involved in associative $N_2$ fixation or the nonsymbiotic NF as in the symbiotic NF. Inoculation of biological $N_2$-fixing PGP microbes on crops and farm fields revitalizes growth-promoting activity, disease management, and maintains the nitrogen level in agricultural soil (Pankievicz et al. 2015).

## 3.3 Application of Soil Microbes as Inoculant to Facilitate Sustainable Agriculture

In the past decade, a comprehensive thrust has been given on formulating practical applications of high-quality microbial inoculants to sustain better crop yield production and improve soil health. Successful inoculation with rhizobia and other PGP microbes is globally recommended. However, the practical application still has many features to consider like (1) disseminating knowledge about different inoculant types and their proper applications on seed on soil or the plants etc., (2) standardizing quality control protocols, and (3) minimizing the fluctuations in field result.

### 3.3.1 *Biofertilizers*

The biofertilizers are progressive microbial inoculants containing live/dormant cells of efficient strains of nitrogen-fixing, phosphate-solubilizing, and cellulolytic microorganisms. They are not the source of nutrients but help plants in accessing nutrients in the soil. As compared to chemical fertilizers which are deleterious to the environment and soil, they help in improving the soil quality and texture and thus pave a way for sustainable production of the crops. The microorganisms which are generally used as biofertilizers include nitrogen-fixing soil bacteria (*Azotobacter, Rhizobium*), nitrogen-fixing cyanobacteria (*Anabaena*), phosphate-solubilizing bacteria (*Pseudomonas* sp.), and AM fungi. Similarly, microorganisms involved with the phytohormone (auxin) production and cellulolytic enzymes are also efficiently used as biofertilizer formulations. These organisms help in increasing the accessibility of nutrients to the plants by mediating certain biochemical processes.

Biofertilizers are one-stop shop for getting low-cost, renewable sources of plant nutrients. The efficient strains of the microbes are cultured and packed in suitable carrier (such as peat, lignite powder, vermiculite, clay, talc, rice bran, seed, charcoal, soil, rock phosphate pellet, paddy straw compost, wheat bran, or a mixture of such materials, etc. which provides better shelf life to biofertilizer formulation) in laboratory. The rapid momentum in the use of biofertilizer in recent times is because of its tremendous advantages: (1) it improves soil health, (2) increases crop yield and productivity, (3) controls soil-borne diseases, (4) diminishes the environmental pollutants by reducing the use of chemical fertilizers (Giri et al. 2019).

Currently, a variety of commercial biofertilizers formulations are available which ensure maximum viability of the microbes employed in such formulations. The above feat is achieved through various strategies which include: (1) optimization of the biofertilizer formulation, (2) application of the liquid biofertilizer, (3) application of biotic stress tolerant such as temperature and drought-tolerant genetically modified strains. The vast array of soil microbe association with the crop plants are exploited in the production of the biofertilizers. Table 3.2 enlists some of the microbial groups used as biofertilizers on the basis of their nature and function.

**Table 3.2** Some common microbes utilized as biofertilizers

| S. no. | Type of organisms | Function | Example | Reference |
|---|---|---|---|---|
| 1 | Free living | N2-fixing biofertilizers | *Azatobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena,* and *Nostoc* | Choudhary and Kennedy (2004) |
| 2 | Associated symbiotic | N2-fixing biofertilizers | *Azospirillum* | Latef et al. (2020) |
| 3 | Symbiotic | $N_2$-fixing biofertilizers | *Rhizobium, Frankia, and Anabaena azollae* | Soumare et al. (2020) |
| 4 | Bacteria | P-solubilizing biofertilizers | *Bacillus megatherium var phosphaticum, Bacillus subtilis, Bacillus circulans, and Pseudomonas striata* | Khan et al. (2016), Igiehon et al. (2019) |
| | Bacteria | High $AlPO_4$ and $FePO_4$ | *Burkholderiaceae* | |
| 5 | Fungi | P-solublizing biofertilizers | *Penicillium sp. and Aspergillus awamori* | Adhikari and Pandey (2019), Qiao et al. (2019) |
| 6 | Arbuscular mycorrhiza | P-mobilizing biofertilizers | *Glomus sp., Gigaspora sp., Acaulospora sp., Scutellospora sp., and Sclerocystis sp* | Etesami et al. (2021) |
| 7 | Orchid mycorrhiza | P-mobilizing biofertilizers | *Rhizoctonia solani* | Mosquera-Espinosa et al. (2013) |
| 8 | Pseudomonas | Plant-growth-promoting rhizobacteria | *Pseudomonas fluroscence* | Nguyen et al. (2017) |
| 9 | Silicate and zinc solubilizers | Biofertilizers for micronutrients | *Bacillus* sp. | Maleva et al. (2017) |

## 3.3.2 *Mycorrhiza*

Mycorrhiza are one of the most distinguished association of fungus with the roots of higher plant (Prasad et al. 2017). Although the system is complex to comprehend, it serves as basic model in understanding the mechanism behind stimulation of growth in the root cells because of the mycorrhizal intrusion an intricate signaling pathway ensures the formation of nodule-like structure and the penetration apparatus. The chief bioligands exuded by mycorrhiza and rhizobium are the Myc factors and the Nod factors which are seized by the host roots to incite an array of signal transduction pathways through unknown receptors (SYMRK and NORK) which activate the release of $Ca^{+2}$ in the cytosol. The majority of the receptors implicated in this pathway are kinases-related proteins like DM1 and SYM71, which phosphorylate their substrate. Nuclear core complex and its associated proteins (NUP) incite calcium spiking. A DM1 protein helps in the frequent movement of calcium ions inside and outside the nucleus. Channel proteins like $Ca^{++}$ along with certain transporters also corroborate in this process. The calcium calmodulin-dependent protein kinase CCAMK phosphorylates the product of CYCLOPS proteins which elicits the activation of multiple genes involved in the formation of penetration apparatus and nodule-like structure formation (Table 3.2) (Umesha et al. 2018).

### *3.3.3 Biopesticides*

The United States Environmental Protection Agency (EPA) defines biopesticides as pesticides procured from natural materials (e.g., animals, plants, bacteria, etc.) and certain minerals (Kachhawa 2017). Biopesticides encompass a variety of different matter which may be living organisms (natural harmful pests), phytochemicals, microbial products, or other by-products, which can be used for pest management. The biopesticides are promising eco-friendly tool against the menace caused by phytopathogen in crop, alleviating the use of chemical pesticides which pose a serious threat to soil microbiome. Some common biopesticides include bioinsecticides (*Bacillus thuringiensis*), biofungicides (*Trichoderma* spp.), bioherbicides (*Phytopthora*), etc. (Table 3.3). These biopesticides are less harmful for agriculture as well as for animals and human beings.

Microbial biopesticides encompass a diverse group of organisms like bacteria, fungus, virus, protozoan, or alga as active agents (Pandey et al. 2010). One of the most notable examples of biopesticide is the bacterium *Bacillus thuringiensis* which possesses insecticidal properties. The *B. thuringiensis* produces a protein harmful to a specific insect pest (Dipteran). Besides the *B. thuringiensis*, other bacteria and fungus such as *Bacillus sphaericus*, *Trichoderma viride*, *T. harzianum* are also successful in controlling the phytopathogens. The efficacy of biopesticide bacteria such as *Bacillus circulans*, *Agrobacterium radiobacter*, *Bacillus pumilus*, and *Pseudomonas aureofaciens* and fungi such as *Ampelomyces quisqualis*, *Fusarium oxysporum*, *Gliocladium virens*, *Trichoderma harzianum*, and *Pythium oligandrum* has been utilized to support sustainable growth and development of agriculture in various countries (Hynes and Boyetchko 2006). *Pseudomonas fluoresens*, *Beauveria bassiana* have also been successfully employed for the pest management against different targets. Bioinsecticides are gaining widespread popularity (Table 3.3). They have shorter shelf lives, a low dose quantity results in higher efficacies, and are harmless towards animals and human beings in comparison to their synthetic counterparts (insecticides). They are target-specific with discrete mode of action. They mostly affect a single species of insect, and are often slow in action; however, the timing of their application is relatively crucial for their success. Besides bacteria and fungus, viruses have also been reported to possess bioinsecticidal potential (Fig. 3.2). Baculoviruses affect insect pests like corn borers, potato beetles, flea beetles, and aphids. A particular strain is being used as a control agent for Bertha army worms, which attack canola, flax, and vegetable crops (Kachhawa 2017). Conventional insecticides do not affect the worm until after it has reached this stage and by then much of the damage has been occurred. Now the scientists are

**Table 3.3** Some commonly employed biopesticides

| S. No | Type | Microbial species | Mode of action | Target organism/ pest | References |
|---|---|---|---|---|---|
| 1. | Bactericide | *Agrobacterium radiobacter* | Antagonism and antibiosis | Crown gall (*Agrobacterium tumefaciens*) | Kawaguchi (2013) |
| | | *Bacillus velezensis* | Antagonism and antibiosis | Crown gall | Gharsa et al. (2021) |
| | | *Bacillus subtilis* | Colonization on plant root and competition | Bacterial pathogen | Hashem et al. (2019) |
| | | *Pseudomonas fluorescens* | Overpopulates and controls the growth of plant pathogens | Several bacterial diseases such as frost-forming bacteria | Jain and Das (2016) |
| 2 | Fungicide | *Bacillus subtilis* | Colonization on plant root, competition, and antibiosis | Soil foliage, fungal pathogens such as *Rhizoctonia, Fusarium, Aspergillus,* and others | Hashem et al. (2019) |
| | | *Bacillus pumilus* | Colonization on plant root, competition, and antibiosis | Seedling disease | Zhu et al. (2020) |
| | | *Burkholderia cepacia* | Controls fungi via seed treatment | Fungal pathogens | Jung et al. (2018) |
| | | *Candida oleophila* | Colonization of diseased tissues | Postharvest pathogens | Hernandez et al. (2019) |
| | | *Gliocladium catenulatum* | Enzymatic mechanism | Seed-borne and soil-borne diseases | Pertit et al. (2019) |
| | | *Pseudomonas fluorescens* | Seed and root exudates help in colonization and produce a diverse array of bioactive metabolites | Plant soil-borne diseases, fireblight | |
| | | *Pseudomonas syringae* | Utilizes seed exudates, produces a wide spectrum of bioactive metabolites | Postharvest disease | |
| | | *Streptomyces* | Mycoparasitism, antagonism, and antibiosis | Fungi-causing damping off, stem, and crown rots | |
| | | *Trichoderma viride/ Harzianum* | Mycoparasitism, antagonism, and antibiosis | Soil-borne fungal disease | |

(continued)

**Table 3.3** (continued)

| S. No | Type | Microbial species | Mode of action | Target organism/pest | References |
|---|---|---|---|---|---|
| 3 | Insecticide | *Bacillus thuringiensis* (Bt) | Digestive system | Butterfly and moths (Lepidoptera) | Voirol et al. (2018) |
| | | *Metarhizium anisopliae* | Penetration of the insect exoskeleton and grows directly through the cuticle to the inner body of their host | Coleoptera and lepidoptera, termites, mosquitoes, leafhoppers, beetles, and grubs | Sharma and Sharma (2021) |
| | | *Paecilomyces fumosoroseus* | Parasitic | Whitefly and thrips | Gavira et al. (2020) |
| | | *Verticillium lecanii* | Grows directly through the cuticle to the inner body of their host | Whitefly, coffee green bug, and homopteran pests | Sani et al. (2020) |
| 4 | Herbicide | *Alternaria destruens* | | Dodder | Harding and Raizada (2015) |
| | | *Chondrostereum purpureum* | | Stump sprout inhibitor | Hamberg et al. (2020) |
| | | *Colletotrichum gloeosporioides* | | Northern jointvetch | Boyette et al. (2019) |
| | | *Phytophthora palmivora* | | Strangler vine | Harding and Raizada (2015) |
| 5 | Nematicide | *Bacillus firmus* | Competition, antibiosis | Nematodes | Huang et al. (2021) |
| | | *Paecilomyces lilacinus* | Infection and destruction of nematode's eggs | Nematodes | Monjil and Ahmed (2020) |

paying their attention to the development of sustainable agriculture in which the high productivities of plants are ensured using their natural adaptive potentials with a minimal environmental harm. The most promising strategy to reach this goal is to use alternative to the hazardous agrochemicals with environment-friendly preparations of symbiotic microbes, which could increase the nutrition of crops and livestock as well as their protection from biotic and abiotic stresses.

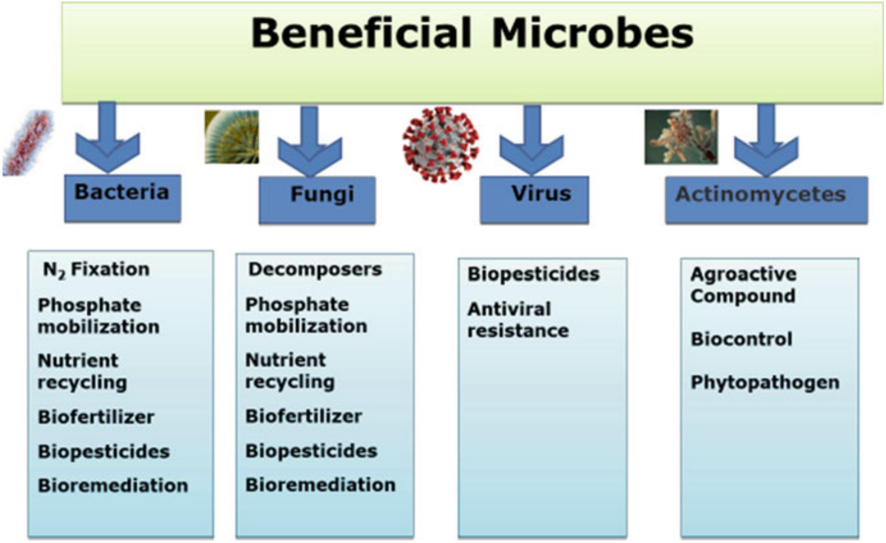

**Fig. 3.2** Beneficial microbes and their uses

## 3.4 Role of Biotechnology in Microbes for Enhancing the Plant Productivity

Biotechnology has opened many fronts in agriculture for plant growth and development. Biotechnology approaches in the microbes assist in food security for increasing population. Productivity of crop depends not only on plants but also microbes present in soils. They are equivalent important for the crop yield and quality. Soil microbes participate in plant growth by many ways such as protection from the diseases, several biotic and abiotic stresses, assisting in nitrogen fixation, protecting from weed and from bioremediation (Lugtenberg 2015).

Plant rhizosphere have abundant amount of root exudates which consists of several chemical compounds for mediating the communication between the soil and plants through signaling (Verma and Verma 2021). Rhizosphere signaling is generally based on host patterns recognition receptors (PRR) and nod-like receptor (NLR) and microbial effector protein which alters the communication and affects the plant health and growth. In this contest, PRR on plant have the capacity to identify pathogen or beneficial microbes using the conserved pattern (Bukhat et al. 2020). Rhizosphere communication can be through quorum sensing molecules, volatile organic compounds, root exudates, flavonoids, rhizobia *nod* genes. PGPR inoculation also assist plants in their immunity and growth through signaling pathways. Plant receives the stimuli either from environment or PGPR which triggers local immune defense response at root zone and then translates into a systemic defensive response regulated by hormonal signaling pathways of salicylic acid, ethylene/ jasmonic acid, etc. In this way, phytohormone plays important role in plant defense

(Denancé et al. 2013). Plants recognize microbes, pathogen, damage-associated molecular pattern (MAMPs, PAMPs, DAMPs) for the activation of signaling cascade for defense (Boller and Felix 2009). The mitogen-activated protein kinase and calcium-dependent protein kinases transduce primary signal PTI which is the (PAMPs triggered immunity) into several intracellular defensive responses. Activation of PTI followed the stimulation of ethylene signaling, stomatal closure, callose deposition, production of ROS, and secondary metabolite accumulation, particularly, antimicrobials (Zipfel and Oldroyd 2017; Li et al. 2016). Pathogens prevent the PTI signaling detection by producing the effector protein which leads to ETS (effector-triggered susceptibility) (Gimenez-Ibanez et al. 2016); in response plants have effector-triggered immunity (ETI) system which increases the resistance with the assistance of NB-LRR (nucleotide binding–leucine rich repeat receptor protein) (Pieterse et al. 2014). DNA methylation, histone acetylation, chromatin modification, translation inhibition, degradation and silencing phenomenon at the stage of transcription and posttranscription level also regulate the defense-related gene (Zhang et al. 2011a). The miRNAs and histone deacetylases also assist in plant immunity (Zhang et al. 2011b). After recognition of microbes-associated molecular patterns (MAMPs), plant activates SA, methyl jasmonate, brassinosteroid, abscisic acid, gibberellins, auxins, and cytokinin for defense signal (Pieterse et al. 2009; Shah and Zeier 2013). Abiotic stresses signal is initiated in plant by receptors and senses present on the cell membrane. These signals stimulate the intracellular chemicals such as ROS, inositol phosphate, calcium ion, nitric oxide, and sugars (Bhargava and Sawant 2013). Hormonal signaling, CDPKs, and MAPKs involved in abiotic stress signaling either repress or activate the transcription factor such as *bZIP, WRKY, NAC, MYB,* and *EREBP/AP2* (Danquah et al. 2014). There are different level of modifications at posttranscription stage apart from TFs in transcription stage such as sumoylation, ubiquitination which assist in the formation of complex regulatory signaling network for alteration in gene expression related to physiological and metabolic responses (Mizoi et al. 2013).

Rhizosphere engineering of PGPR microbes is another section in biotechnology which assists in the plant growth and development. PGPR assists in inducing the stress-responsive genes for tolerating the stress in plants (Tiwari et al. 2017). Rhizosphere zone comprises of plant root and soil microbes and modification of either or both the components changes the rhizosphere. Several studies have investigated the modification of plants and rhizosphere microbes but manipulating and engineering the microbiome is quite effective in terms of plant growth and development (Shrivastava et al. 2014; Bhatt et al. 2020). Genetic engineering technology in microbes assists plants in the development such as in genetically engineered bacteria *Pseudomonas syringae* which prevent frost damage in plants, genetically engineered *Rhizobium* which possess more nitrogen-fixation capacity from natural bacteria (De-Bruijn 2015a, b), another strain of bacteria *Pseudomonas fluorescens* genetically changed to produce more endotoxin for more insecticidal capability; the series of insecticidal *Bacillus thuringiensis* is also considered as biological insecticidal, genetic engineering in Baculoviruses infects only the insect (Kamita et al. 2017). *Bacillus* spp. could be engineered with NifH gene from *Paenibacillus* to

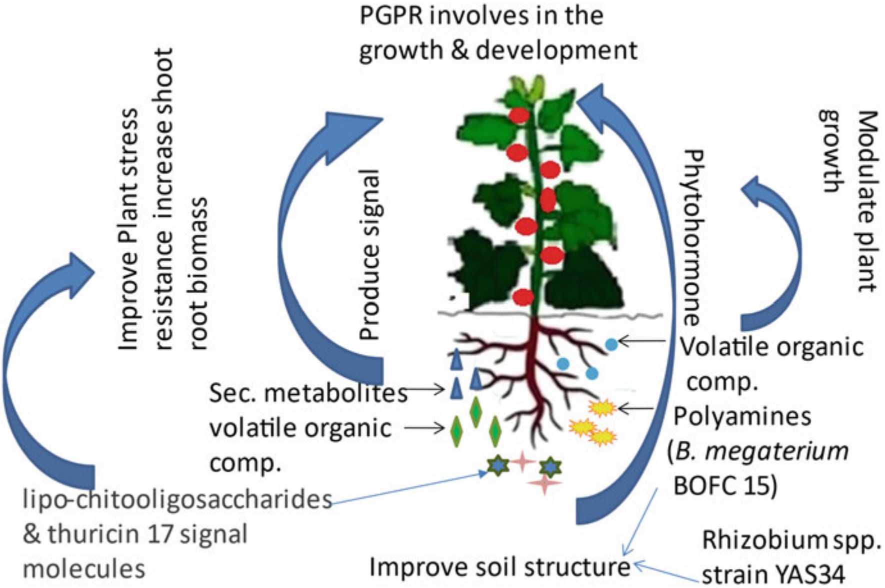

**Fig. 3.3** Role of PGPR in enhancing the plants growth and productivity

contain a $N_2$-fixing machinery (Kim and Timmusk 2013). Many fungi could be utilized as insecticides such as *Metarhizium* and *Beauveria* belong to *Ascomycetes* genera (Lovett and Leger 2017). Plant phytohormones such as auxin, gibberellin, ethylene, cytokinin etc. are important in plant growth and development. So the modification in biosynthesis of plant hormone in microbes (Hedden and Phillips 2000). The field of microbes engineering paves the path for plant growth and development through rhizosphere (Fig. 3.3) (Table 3.4).

## 3.5 Conclusion

The emphasis on exploiting microbes to provide a holistic approach to sustain agriculture and improve yields has gained momentum during the past decade. The soil microbes open a plethora of opportunities to conserve our environment while catering to our nutritional demands and requirement in sustainable manner. The microbial rhizospheric activities such as BNF, P solubilization, dynamic nutrient recycling through the crops such as legume cereals foster a key role in making amicable approaches to meet the surplus nutritional demand which is all set to soar in the coming years whilst saving our environment and ensuring a better health for the living biome.

**Table 3.4** Biotechnology approaches in rhizosphere

| S. no. | Type of organisms | Approach | Effect | Reference |
|---|---|---|---|---|
| 1. | Transgenic lotus | Engineering in root exudates which produces two opines (mannopine and nopaline) in rhizosphere to characterize different microbial community | Microbes present in transgenic lotus rhizosphere: Rhizobium and Duganella spp., Duganella, Afipia, Phyllobacterium, Arthrobacter, and Bosea spp., Proteobacteria, Rhizobiaceae family | Oger et al. (2004) |
| 2. | Rhizosphere pseudomonas | Alteration in root exudates confirmed by RNA-seq profiling | Change in expression of genes encoding numerous catabolic and anabolic enzymes, transporter, transcriptional regulators, stress response | Mavrodi et al. (2021) |
| 3. | *Populus trichocarpa* | Overexpression of *PtVP1.1* pyrophosphatase | Induces more acidic rhizosphere which upregulates the activity of the plasma membrane $H^+$-ATPase for auxin transport | Yang et al. (2015) |
| 4. | Transgenic tobacco | Citrate synthase gene from *Pseudomonas aeruginosa in tobacco root* | Increased citrate efflux which results in improved aluminum tolerance | Delhaize et al. (2001) |
| 5. | Soyabean | Engineered plant growth-promoting *Azospirillum brasilense* strains Ab-V5 used as biofertilizers | Impressive results of increases in root growth, biomass production, grain yield, uptake of nutrients and water, and increased tolerance to abiotic stresses | Santos et al. (2021) |
| 6. | *Allium cepa* L. | Synthetic microbial community(*Azospirillum brasilense, Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae*, and *Burkholderia ambifaria)* | Increased crop productivity | Pellegrini et al. (2021) |
| 7. | Rhizosphere *Klebsiella oxytoca* | Modification in nitrogen-fixating gene cluster | For more nitrogen in soil for plants | Temme et al. (2012) |
| 8. | Grape vine | Plant engineering and rhizosphere engineering | For more sustainability, reduce the use of pesticide | Dries et al. (2021) |
| 9. | Mosses | Bioprospecting of plant microbiomes | Enhanced richness in secondary metabolites, enzymes for the microbes | Muller et al. (2016) |
| 10. | *P. fluorescens* SBW25 | *merA* gene introduction | Mercury resistance | Hall et al. (2020) |

## 3.6 Future Perspectives

As we face the global environmental issues affecting our biome, the incessant deterioration of forest, the constant rise in the pollutants, and global warming all endangering the nutritional demand of the global population, a direct need to shift our concerns towards innovative agri-input methodologies is required which can foster a healthy solution. We need to enable our agricultural system to adapt to the current environmental constraints while trying to find a remedial solution all the while. Exploiting microbial resources ensures to meet most of our current demands while offering us a promising approach to save our environment and help in sustainable agriculture. Biostimulants, a subcategory of bioinoculants, are among the beacons of hope which can become one of the major microbial inoculants involved in sustainable intensification of agriculture and ecosystem. They have shown profound result in fostering soil fertility and crop productivity in major cropping systems (Du Jardin 2015). Reproducibility of results is a major concern with the biostimulants as a lot of abiotic and biotic factors; the native soil microbiome all directly or indirectly affects its successful implementation. Rapid advancement in this area is dependent on broadening our understanding of all the associated factors to ensure successful manipulation of the beneficial microbes, their commercialization, and widespread use.

## References

Adhikari P, Pandey A (2019) Phosphate solubilization potential of endophytic fungi isolated from *Taxus wallichiana* Zucc. roots. Rhizosphere 9:2–9

Ahemad M, Khan MS (2012) Evaluation of plant-growth promoting activities of rhizobacterium *Pseudomonas putida* under herbicide stress. Ann Microbiol 62:1531–1540

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Amit V, Harish M, Parteek P, Shulbhi V (2021) Flavonoid infochemicals: unravelling insights of rhizomicrobiome interactions. In: Nath M, Bhatt D, Bhargava P, Choudhary DK (eds) Microbial metatranscriptomics belowground. Springer, Singapore, pp 163–177

Bagde US, Prasad R (2016) Role of microbes in recycling elements in natural environment. In: Bagde US (ed) Fundamentals of environmental biotechnology. LAP LAMBERT Academic Publishing, Saarbrucken, pp 28–41

Barea JM (2015) Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J Soil Sci Plant Nutr 15(2):261–282

Bargaz A, Lyamlouli K, Chtouki M, Zeroual Y, Dhiba D (2018) Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Front Microbiol 9:1606. https://doi.org/10.3389/fmicb.2018.01606

Basile L, Lepek VC (2021) Legume- rhizobium dance: an agricultural tool that could be improved. Microb Biotechnol 14(5):1897–1917

Basu S, Kumar G, Chhabra S, Prasad R (2021) Role of soil microbes in biogeochemical cycle for enhancing soil fertility. In: Verma JP, Macdonald C, Gupta VK, Podile AR (eds) New and future developments in microbial biotechnology and bioengineering: phytomicrobiome for sustainable agriculture. Elsevier, pp 149–157

Behera BC, Yadav H, Singh SK, Mishra RR, Sethid BK, Dutta SK et al (2017) Phosphate solubilization and acid phosphatase activity of Serratia sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. J Genet Eng Biotechnol 15:169–178. https://doi.org/10.1016/j.jgeb.2017.01.003

Bhargava S, Sawant K (2013) Drought stress adaptation: metabolic adjustment and regulation of gene expression. Plant Breed 132:21–32

Bhatt P, Verma A, Verma S et al (2020) Understanding phytomicrobiome: a potential reservoir for better crop management. Sustainability 12:5446

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

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Boyette CD, Hoagland RE, Stetina KC (2019) Extending the host range of the bioherbicidal fungus *Colletotrichum gloeosporioides* f. sp. Aeschynomene. Biocontrol Sci Technol 29(7):720–726. https://doi.org/10.1080/09583157.2019.158113

Brierley JA (1985) Use of microorganisms for mining metals. In: Halvorson HO, Pramer D, Rogul M (eds) Engineered organisms in the environment: scientific issues. ASM Press, Washington, pp 141–146, D5A Sci. Rep 6: 26710

Bukhat S, Imran A, Javaid S, Shahid M, Majeed A, Naqqash T (2020) Communication of plants with microbial world: Exploring the regulatory networks for PGPR mediated defense signaling. Microbiol Res 238:126486

Choudhary A, Kennedy I (2004) Prospects and potential for system of biological nitrogen fixation in sustainable rice production. Biol Fertil Soils 39:219–227

Danquah A, deZelicourt A, Colcombet J, Hirt H (2014) The role of ABA and MAPK signaling pathways in plant abiotic stress responses. Biotechnol Adv 32:40–52

De-Bruijn FJ (2015a) Biological nitrogen fixation. In: Lugtenberg B (ed) Principles of plant microbes interactions. Springer International Publishing, Heidelberg, pp 215–224

De-Bruijn FJ (2015b) Biological nitrogen fixation. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer International Publishing, Heidelberg, pp 215–224

Delhaize E, Hebb DM, Ryan PR (2001) Expression of a *Pseudomonas aeruginosa* citrate synthase gene in tobacco is not associated with either enhanced citrate accumulation pr efflux. Plant Physiol 125(4):2059–2067

Denancé N, Sánchez-Vallet A, Goffner D, Molina A (2013) Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front Plant Sci 4:155

Dries L, Hendgen M, Schnell S, Lohnertz O, Vortkamp A (2021) Rhizosphere engineering: leading towards a sustainable viticulture. OENO One 55(2). https://doi.org/10.20870/oeno-one.2021.55.2.4534

Du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Sci Hortic 196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021

Egamberdieva D, Lugtenberg B (2014) Use of plant growth-promoting rhizobacteria to alleviate salinity stress in plants. PGPR to alleviate salinity stress on plant growth. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses. Springer, pp 73–96

Ehrlich HL (1990) Geomicrobiology, 2nd edn. Dekker, New York, p 646

Etesami H, Jeong BR, Glick BR (2021) Contribution of arbuscular mycorrhizal fungi, phosphate solubilizing bacteria, and silicon to P uptake by plant. Front Plant Sci 12:699618. https://doi.org/10.3389/fpls.2021.699618

Gao H, Qi G, Yin R, Zhang H, Li C, Zhao X (2016) *Bacillus cereus* strain S2 shows high nematicidal activity against *Meloidogyne incognita* by producing sphingosine. Sci Rep 6:28756

Gavira AM, Huertas V, Dianez F, Montesins NS, Santos M (2020) Paecilomyces and its importance in the biological control of agricultural pests and diseases. Plants 9:1746. https://doi.org/10.3390/plants912174

Gharsa HB, Bouri M, Hamdane AM, Schuster C, Leclerque A, Rhouma A et al (2021) Bacillus velezensis strain MBY2, a potential agent for the management of crown gall disease. PLoS One 16(6):e0252823. https://doi.org/10.1371/journal.pone.0252823

Gimenez-Ibanez S, Chini A, Solano R (2016) How microbes twist jasmonate signaling around their little fingers. Plants 5:9

Giri B, Prasad R, Wu Q-S, Varma A (2019) Biofertilizers for sustainable agriculture and environment. Springer International Publishing. isbn:978-3-030-18932-7. https://www.springer.com/gp/book/9783030189327

Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric 2:1–19

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR:Commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Grover M, Bodhankar S, Sharma A, Sharma P, Singh J, Nain J (2021) PGPR Mediated alternations in root traits: way towards sustainable crop production. Front Sustain Food Syst 4:618230. https://doi.org/10.3389/fsufs.2020.618230

Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V (2015) Plant growth promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microbiol Biochem 7:96–102

Hall JPJ, Harrison E, Parnanen K, Virta M, Brockhurst MA (2020) The impact of mercury selection and conjugative genetic elements on community structure and resistance gene transfer. Front Microbiol 11:1846. https://doi.org/10.3389/fmicb.2020.01846

Hamberg L, Saksa T, Hantula J (2020) Role and function of *Chondrostereum purpureum* in biocontrol of trees. Appl Microbiol Biotechnol 105:431–440

Harding DP, Raizada M (2015) Controlling weeds with fungi, bacteria and viruses:a review. Front Plant Sci 6:659

Hashem A, Tabassum B, Fathi EAA (2019) *Bacillus subtilis*: a plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi J Biol Sci 26(6):1291–1297

Haygarth PM, Ritz K (2009) The future of soils and land use in the UK: soil systems for the provision of land-based ecosystem services. Land Use Policy 26:187–197

Hedden P, Phillips AL (2000) Manipulation of hormone biosynthetic genes in transgenic plants. Curr Opin Biotechnol 11(2):130–137

Heil B (2001) Induced systemic resistance (ISR) against pathogens—a promising field for ecological research. Persp Plant Ecol Evol Syst 4(2):65–79

Hernandez SC, Perez JJR et al (2019) Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: a review. Agronomy 9:121. https://doi.org/10.3390/agronomy9030121

Huang M, Bulut A, Shrstha B, Matera C et al (2021) Bacillus firmus I-1582 promotes plant growth and impairs infection and development of the cyst nematode *Heterodera schachtii* over two generations. Sci Rep 11:14114

Hynes RK, Boyetchko SM (2006) Research initiatives in the art and science of biopesticide formulations. Soil Biol Biochem 38(4):845–849

Ibarra-Galeana JA, Castro-Martinez C, Fierro-Coronado RA et al (2017) Characterization of phosphate-solubilizing bacteria exhibiting the potential for growth promotion and phosphorus nutrition improvement in maize (*Zea mays* L.) in calcareous soils of Sinaloa, Mexico. Ann Microbiol 67:801–811

Igiehon NO, Babalola OO, Aremu BR (2019) Genomics insights into plant growth promoting rhizobia capable of enhancing soyabean germination under drought stress. BMC Microbiol 19: 159

Islam S, Akanda AM, Prova A, Md. Islam T, Md. Hossain M (2016) Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol 6(1360):1–12

Jain A, Das S (2016) Insight into the interaction between plants and associated fluorescent *Pseudomonas* spp. https://doi.org/10.1155/2016/4269010

Jung BK, Hong SJ, Park GS, Kim MC, Shin JH (2018) Isolation of *Burkholderia cepacia* JBK9 with plant growth-promoting activity while producing pyrrolnitrin antagonistic to plant fungal diseases. Appl Biol Chem 61:173–180

Kachhawa D (2017) Microorganisms as a biopesticides. J Entomol Zool Stud 5:468–473

Kamita SG, Kang KD, Inceoglu B (2017) In: Gilbert LI (ed) Comprehensive molecular insect science, vol. 6. Elsevier. pp 271–322

Kawaguchi A (2013) Biological control of crown gall on grapevine and root colonization by nonpathogenic *Rhizobium vitis* strain ARK-1. Microbes Environ 28(3):306–311

Khan AL, Halo BA, Elyassi A, Ali S, Al-Hosni K, Hussin J (2016) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum* electron. J Biotechnol 21:58–64

Kim SB, Timmusk S (2013) A simplified method for gene knockout and direct screening of recombinant clones of application in *Paenibacillus polymyxa*. PLoS One 8:e68092

Krishnaraj PU, Dahale S (2014) Mineral phosphate solubilization: concepts and prospects in sustainable agriculture. Proc Ind Natl Sci Acad 80:389–405. https://doi.org/10.16943/ptinsa/2014/v80i2/55116

Kumar ADS, Vidhya AK, Ragunathan R, Johney J (2015) Production and purification and characterization of streptokinase using *Bacillus licheniformis* under solid state fermentation. J Global Biosci 4(7):2703–2712

Latef HA, Alhmad MFA, Zakir A (2020) Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions. J Plant Growth Regul 39:1293–1308

Li B, Meng X, Shan L, He P (2016) Transcriptional regulation of pattern-triggered immunity in plants. Cell Host Microbe 19:641–650

Liu D, Lian B, Dong H (2012) Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. J Geomicrobiol 29:413–421

Liu W, Wang Q, Hou J, Tu C, Luo Y, Christie P (2016) Whole genome analysis of halotolerant and alkalotolerant plant growth-promoting rhizobacterium Klebsiella sp. D5A. Sci Rep 6:26710. https://doi.org/10.1038/srep26710

Lovett B, Leger RJ (2017) Genetically engineering better fungal biopesticides. Pest Manag Sci 74(4):781–789. https://doi.org/10.1002/ps.4734

Lugtenberg B (2015) Life of microbes in the rhizosphere. In: Lugtenberg B (ed) Principal of plant-microbes interactions. Springer International Publishing, Heidelberg, pp 7–15

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63: 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918

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

Maleva M, Borisova G, Koshcheeva O, Sinenko O (2017) Biofertilizers based on silicate solubilizing bacteria improves photosynthetic function of *Brassica juncea*. AGROFOR Int J 2(3): 13–19

Martinez-Viveros O et al (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10:293–319. https://doi.org/10.4067/S0718-95162010000100006

Mavrodi OV, Mcwilliams JR, Peter JO et al (2021) Root exudates alter the expression of diverse metabolic, transport, regulatory, and stress response genes in rhizosphere *Pseudomonas*. Front Microbiol 12:651282. https://doi.org/10.3389/fmicb.2021.651282

Middledrop PJM, Briglia M, Salkinoja-Salonen M (1990) Biodegradation of pentachlorophenol in natural polluted soil by inoculated Rhodococcus chlorophenolicus. Microb Ecol 20:123–139

Mizoi J, Ohori T, Moriwaki T, Kidokoro S, Todaka D, Maruyama K et al (2013) GmDREB2A; 2, a canonical dehydration-responsive element-binding protein2-type transcription factor in soybean, is post translationally regulated and mediates dehydration-responsive element-dependent gene expression. Plant Physiol 161:346–361

Monjil M, Ahmed S (2020) *Paecilomyces lilacinus* on egg hatching and larval population of meloidogyne sp. Bangladesh J Plant Pathol 33(1 & 2):71–78

Mosquera-Espinosa AT, Bayman P, Prado GA et al (2013) The double life of Ceratobasidium: orchid mycorrhizal fungi and their potential for biocontrol of Rhizoctonia solani sheath blight of rice. Mycologia 105(1):141–150

Muller CA, Obermeier MM, Berg G (2016) Bioprospecting plant-associated microbiomes. J Biotechnol 10(235):171–180

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbial 53:1141–1149. https://doi.org/10.1139/W07-081

Ngumbi E, Kloepper J (2016) Bacterial-mediated drought tolerance: current and future prospects. Appl Soil Ecol 105:109–125

Nguyen TH, Phan TC, Choudhury ATMA, Rose MT, Deaker RJ, Kennedy IR (2017) BioGrow: a plant growth promoting biofertilizer validated by 15 years research from laboratory selection to rice farmer's fields of the Mekong Delta. In: Agro-environmental sustainability, pp 237–257

Nihorimbere V, Ongena M, Smargiassi M, Thonart P (2011) Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol Agron Soc Environ 2:327–337

Oger PM, Mansouri H, Nesme X, Dessaux Y (2004) Engineering root exudation of lotus toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. Micrbiol Ecol 47(1):96–103

Orlandini V, Emiliani G, Fondi M, Maida E, Perrin E, Fani R (2014) Network analysis of Plasmidomes: the *Azospirillum Brasilense* sp. 245 case. Int J Evol Biol 2014:1–14. https://doi.org/10.1155/2014/951035

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front Microbiol 6:745

Pandey RK, Prasad R, Mangunath VG, Goswami BK (2010) Biotechnology of biocontrol based biopesticides: Core component of biological deterrents. In: Gupta VK, Tuohy M, Gaur RK (eds) Fungal biochemistry and biotechnology. LAP LAMBERT Academic Publishing AG & Co. KG, Saarbrucken, pp 214–244

Pankievicz VCS, do Amaral FP, Santos KFDN, Agtuca B, Xu Y, Schueller MJ et al (2015) Robust biological nitrogen fixation in a model grass-bacterial association. Plant J 81:907–919. https://doi.org/10.1111/tpj.12777

Paredes SH, Lebeis SL (2016) Giving back to the community: microbial mechanisms of plant–soil interactions. Funct Ecol 30(7):1–10

Pellegrini M, Spera DM, Ercole C, Gallo MD (2021) *Allium cepa* L. inoculation with a consortium of plant growth-promoting bacteria: effects on plants, soil, and the autochthonous microbial community. Microorganisms 9(3):639

Pertit I, Alabouvette C et al (2019) Mini-paper—the use of microbial biocontrol agents against soil-borne diseases. Eip-agri focus group soil-borne diseases, pp 1–11

Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecules hormones in plant immunity. Nat Chem Biol 5:308–316

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375

Pinto C, Pinho D, Sousa S, Pinheiro M, Egas C, Gomes AC (2014) Unravelling the diversity of grapevine microbiome. PLoS One 9(1):85622

Planchamp C, Glauser G, Mauch-Mani B (2015) Root inoculation with *Pseudomonas* putida KT2440 induces transcriptional and metabolic changes and systemic resistance in maize plants. Front Plant Sci 5:719. https://doi.org/10.3389/fpls.2014.00719

Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S and Varma A (eds) Plant growth-promoting Rhizobacteria (PGPR) and medicinal plants. Springer International Publishing Switzerland, pp 247–260

Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 1–7

Prasad R, Chhabra S, Gill SS, Singh PK, Tuteja N (2020) The microbial symbionts: potential for the crop improvement in changing environments. In: Tuteja N, Tuteja R, Passricha N, Saifi SK (eds) Advancement in crop improvement techniques. Elsevier, Amsterdam, pp 233–240

Qiao H, Sun XR, Wu XQ, Li GE, Wang Z, Li DW (2019) The phosphate solubilizing ability of *Penicillium guanacastense* and its effects on the growth of *Pinus massoniana* in phosphate limiting condition. Biol Open 8:bio046797

Rathore R (2015) A review on approaches to develop plant growth promoting rhizobacteria. Int J Recent Sci Res 5(2):403–407

Sani I, Ismail SI, Abdulla S, Jalinas J, Jamian S, Saad N (2020) A review of the biology and control of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), with special reference to biological control using entomopathogenic fungi. Insects 11(9):619. https://doi.org/10.3390/insects11090619

Santoro MV, Bogino PC, Nocelli N, Cappellari LR, Giordano WF, Banchio E (2016) Analysis of plant growth promoting effects of fluorescent pseudomonas strains isolated from *Mentha piperita* rhizosphere and effects of their volatile organic compounds on essential oil composition. Front Microbiol 7(1085):1–17

Santos MS, Nogueria MA, Hungria M (2021) Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: lessons that farmers are receptive to adopt new microbial inoculants. Rev Bras Cienc Solo 45. https://doi.org/10.36783/18069657rbcs20200128

Shah J, Zeier J (2013) Long-distance communication and signal amplification in systemic acquired resistance. Front Plant Sci 4:30

Sharma R, Sharma P (2021) Fungal entomopathogens:a systematic review. Egypt J Biol Pest Control 31:57

Shrivastava S, Prasad R, Varma A (2014) Anatomy of root from eyes of a microbiologist. In: Morte A, Varma A (eds) Root engineering, vol 40. Springer, Berlin, pp 3–22

Soumare A, Diedhiou AG, Thuita M, Hafidi M et al (2020) Exploiting biological nitrogen fixation: a route towards a sustainable agricultural. Plants 9:1011

Temme K, Zhao D, Voigt CA (2012) Refactoring the nitrogen fixation gene cluster from *Klebsiella oxytoca*. PNAS 109(18):7085–7090

Tiwari S, Prasad V, Chauhan PS, Lata C (2017) *Bacillus amyloliquefaciens* confers tolerance to various abiotic stresses and modulates plant response to phytohormones through osmoprotection and gene expression regulation in rice. Front Plant Sci 8:1510

Umesha S, Singh PK, Singh RP (2018) Microbial biotechnology and sustainable agriculture biotechnology for sustainable agriculture, vol. 6, pp 185–204. https://doi.org/10.1016/B978-0-12-812160-3.00006-4

Unkovich MJ, Baldock J (2010) Measurement of a symbiotic $N_2$ fixation in Australian agriculture. Soil Biol Biochem 329:75–89. https://doi.org/10.1016/j.soilbio.2008.08.021

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Combaret CP (2013) Plant growth promoting rhizobacteria and root system functioning Front. Plant Sci 4(356):1–19

Varma A, Swati T, Prasad R (2020) Plant microbe symbiosis. Springer International Publishing (ISBN: 978-3-030-36247-8). https://www.springer.com/gp/book/9783030362478

Verma S, Verma A (2021) Plant root exudate analysis. In: Verma A, Saini JK, Hesham AEL, Singh HB (eds) Phytomicrobiome interaction and sustainable agriculture. Wiley, pp 1–14

Voirol LRP, Frago E, Kaltenpoth M, Hilker M, Fatouros NE (2018) Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. Front Microbiol 9:556. https://doi.org/10.3389/fmicb.2018.00556

Yahaghi Z, Shirvani M, Nourbakhsh F, Coba T et al (2018) Isolation and characterization of pb-solubilizing bacteria and their effects on Pb uptake by *Brassica juncea*: implications for microbe-assisted phytoremediation. J Microbiol Biotechnol 28(7):1156–1167. https://doi.org/10.4014/jmb.1712.12038

Yang Y, Tang RJ, Li B, Wang HH, Jin CM, Bao Y, Su HY, Zhao N, Ma XJ (2015) Overexpression of a *Populus trichocarpa* $H^+$-pyrophosphatase gene *PtVP1.1* confers salt tolerance on transgenic poplar. Tree Physiol 35(6):663–677

Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of pea (*Pisum sativum*) under drought conditions. J Microbiol Biotechnol 18:958–963

Zeroual Y, Chadghan R, Hakam A, Kossir A (2012) Biosolubilization of mineral insoluble phosphates by immobilized fungi (*Aspergillus niger*) in fluidized bed bioreactor. J Biotechnol Biomater S6:e004. https://doi.org/10.4172/2155-952X.S6-004

Zhang W, Gao S, Zhou X, Chellappan P, Chen Z, Zhang X et al (2011a) Bacteria-responsive microRNAs regulate plant innate immunity by modulating plant hormone networks. Plant Mol Biol 75:93–105

Zhang X, Zhao H, Gao S, Wang W, Katiyar-Agarwal S, Huang H et al (2011b) *Arabidopsis* argonaute 2 regulates innate immunity via miRNA393*-mediated silencing of a golgi-localized SNARE gene, MEMB12 Mol. Cell 42:356–366

Zhu ML, Wu XQ, Wang YH, Dai Y (2020) Role of biofilm formation by *Bacillus pumilus* HR10 in biocontrol against pine seedling damping-off disease caused by *Rhizoctonia solani*. Forests 11: 652. https://doi.org/10.3390/f11060652

Zipfel C, Oldroyd GE (2017) Plant signalling in symbiosis and immunity. Nature 543:328–336

# Chapter 4
# Microbial Fertilizer as an Alternative to Chemical Fertilizer in Modern Agriculture

**Kiran Bala**

**Abstract** The continuous decline of earth's natural resources and increased use of harmful chemical fertilizers pose a great threat to the health of soil. Exploitation of microbes as biofertilizers are considered to some extent an alternative of chemical fertilizers. Many promising species of bacteria, algae, fungi have fertilizer-like activities and are beneficial in agricultural sector. They have extensive potentiality in enhancing crop production, food safety and maintaining long-term soil fertility which is essential for meeting global food demand. Microbes interact with the crop plants and enhance their immunity, development and yield. Many essential nutrients are required for the proper growth of crops, which is present in insoluble form. Microbes by their action convert them in useable form. Different types of biofertilizers and advancement in the field of microfertilizers are discussed in this chapter. Microbial fertilizers are not only cost-effective, nontoxic and eco-friendly but also serve a good substitute for expensive and harmful chemical fertilizers. The aim of this chapter is to discuss microfertilizers' important roles, pros and cons of the techniques and their advancement.

**Keywords** Biofertilizers · Sustainable agriculture · Chemical fertilizers · Encapsulation · Lyophilization · Nanoencapsulation · Biofilm microbial fertilizers · Nanobiofertilizer

## 4.1 Introduction

Conventional agriculture has an important role in fulfilling the food demands of a growing human population, which has also led to an increasing dependence on chemical fertilizers and pesticides (Subha Rao 2015). Chemical fertilizers are industrially synthesized with substances composed of known quantities of nitrogen, phosphorus, potassium and their exploitation causes air and groundwater pollution

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_4

by eutrophication of water bodies (Serpil 2012). In past few decades, the use of chemical fertilizers was a common practice, whereas microbial fertilizers were neglected (Adesemoye et al. 2009). Due to irregular application of chemical fertilizers and their harmful effects on human and environmental health, a lot of importance is being paid on organisms to provide nutrition requirement of plants (Astarai and Kochaki 1996; Singh et al. 2011). Soil microorganisms also known as microbial fertilizers have emerged as one of the alternatives to application of chemical inputs for needs of fertilizers. They are a large population of a specific or a group of beneficial microorganisms for enhancing the productivity of soil. The use of microbial fertilizer plays a pivotal role both in the improvement of soil texture and stimulating plant growth (Gahukar 2005; Giri et al. 2019). Their use in agriculture in preference to chemical fertilizers offers economic and ecological benefits by maintaining soil health and fertility (Venkatramani 1996). The use of microbial fertilizers in place of chemicals is likely to reduce the impact on air, water and also has the potential to improve human health (Miransari 2010; Bhardwaj et al. 2014). The chemical fertilizer enhances the crop production but it is not completely absorbed. Therefore, the soil surface as well as soil water is polluted. The use of chemical fertilizers especially in fields may affect the growth-inhabiting microorganisms (Youseff and Eissa 2014) therefore destroying the ecology of soil. Moreover, they are costly with adverse effect on soil as compared to microbial fertilizers.

## 4.2 Microbial Fertilizers

Microbial fertilizer is a substance of living cells especially beneficial microflora which when applied to the soil colonizes the rhizosphere and promotes growth by increasing the supply or availability of nutrient to the host plant (Vessey 2003). According to Hari and Permural (2010), microfertilizers are commonly referred to as selected strains of beneficial soil microorganisms cultured in the laboratory and packed in suitable carriers. Commonly used microorganisms are mycorrhizal fungi, blue green algae, potassium- and phosphorus-solubilizing bacteria, growth-promoting rhizobacteria (PGPR) (Fig. 4.1).

Microfertilizers interact in the rhizosphere when applied through the seeds or directly in soil where they multiply and participate in nutrient cycling and increase crop yield (Singh et al. 2011). They accelerate different microbial processes in the soil which result in availability of nutrients that are assimilated easily by the plants (Saxena and Joshi 2002; Rai and Shukla 2020). The term microbial fertilizer denotes all the nutrient inputs of biological origin for plant growth (Subha Rao 1982). They are also known as microbial inoculants by NS Subha Rao. Some of them are known to fix atmospheric nitrogen. Both bacteria and cyanobacteria are widely used as nitrogen-providing microbial fertilizers (Ray and Handerson 2001). Bacteria such as rhizobium, azospirillum and azotobacter are known for their nitrogen-metabolizing capacity. Plant growth-promoting rhizobacteria produce IAA (auxin) which induce another secondary molecule nitric oxide (NO) further involved in developmental

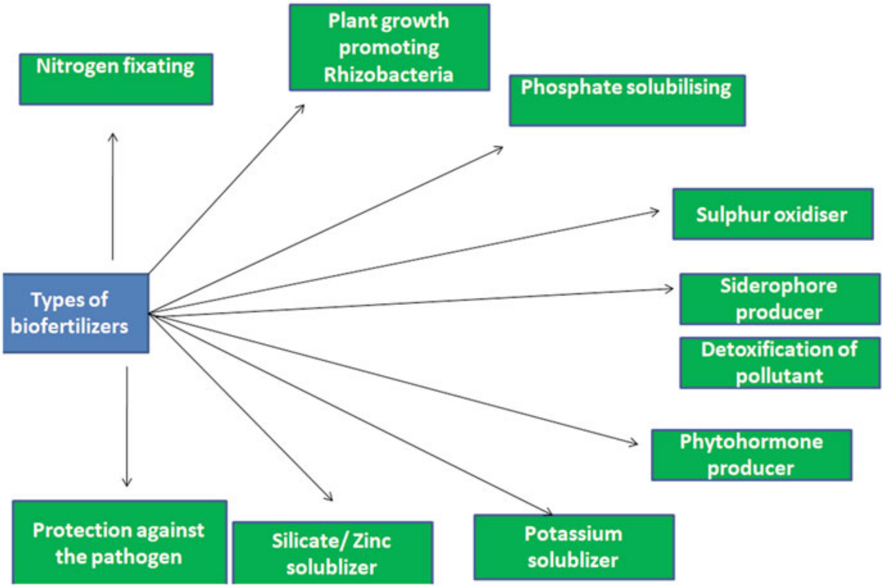


**Fig. 4.1** Types of microbial fertilizers

processes in plants (Molina-Favero et al. 2007). Living cells of soil such as *Bacillus megaterium, Aspergillus awamori, Penicillium digitatum*, etc. can also solubilize insoluble salts like phosphate and can produce fertilizing substance (Mazid and Khan 2014).

Besides benefitting nitrogen and phosphorous nutrition, the microbes like cyanobacteria, rhizobium, Bacillus and Pseudomonas increase plant growth, affect seed germination and crop yield by secretion of phytohormones like auxin, gibberellins, cytokinin. Studies have suggested the role of *Azospirillum* to secrete gibberellins, ethylene and auxin (Perrig et al. 2007; Bent et al. 2001). They also play considerable role in decomposition of organic materials (Zemrany et al. 2006). Enrichment of compost rhizobium and bacillus were reported to synthesize IAA at different cultural conditions from agrowaste (Sudha et al. 2012). Some microbes are known to control plant pathogen (Jurgen et al. 2019). Biofertilizers keep the soil environment rich in all kinds of micro- and macronutrients (Sinha et al. 2014). Mycorrhizal fungi withdraw minerals from organic matter (Dwivedi and Sangeeta 2015; Komala et al. 2017). Free-living mycelium can take nitrate and ammonium from the soil (Bago et al. 1996; Finlay et al. 1989). Subsequently, these compounds reach the mantle and Hartig net and transfer to plants. There are studies which suggest role of mycorrhiza in auxin biosynthesis (Ansari et al. 2013). Arbuscular mycorrhizal fungi (AMF) are another well-known microfertilizers that represent a key link between plants and soil mineral nutrients. AMF are obligate symbionts with almost 80% of land plant species including several agricultural crops (Prasad et al. 2017a). They are known to provide the host plant with mineral nutrients and water in exchange of photosynthetic products. AMF inoculums that emerge from the root system can acquire nutrients from soil (Allen 2011; Kumar et al. 2017; Teotia et al. 2017).

## 4.3 Advantage of Microbial Fertilizers

Chemical fertilizers have become popular worldwide because they are easily affordable and have advantage of quick action. However, there are many demerits of chemical fertilizers which cannot be overlooked. These findings have led to the need for the provision of an environment-friendly fertilizer known as microbial fertilizer (Table 4.1). Microbial inoculums are inoculated in the field for the improvement and supply of nutrients such as nitrogen, phosphorus, potassium and other essential elements which are necessary for the proper growth of plants (Varma et al. 2012; Digambar 2019). Microbial fertilizers are an alternative to the conventional approach because of their lower cost than the chemical fertilizers. When they are required in bulk, they can be generated at the farm itself; therefore these are economically attractive for the farmers (Venkatramani 1996) and renewable source of nutrients (Berg et al. 2013). They play a vital role in improving soil fertility and ensure maintaining long-term sustainability (Venkatramani 1996). Microbial fertilizer colonizes around the root and maintains the availability of nutrient therefore increasing the growth in plants. Several other beneficial functions like enhancing seed germinations and advancing the root architecture (Gholami et al. 2008), inhibition of pathogenic microorganisms (Mali and Bodhkar 2009; Prasad et al. 2017b), improvement of water status of plants, increase in yield (Sharma et al. 2017), enhancement of plant hormone (Wong et al. 2015) and defence of plants against pathogens and herbivore (Finkal et al. 2017; Thamer et al. 2011)), production of antibiotics and biodegradation of organic matter in the soil (Sinha et al. 2014) were reported. They help in regaining the soil's natural nutrient cycle and establish soil matter

**Table 4.1** Comparison of chemical and microbial fertilizer

| Chemical fertilizer | Microbial fertilizer |
|---|---|
| Chemical fertilizer consist of synthetic chemicals of known compositions | Microbial fertilizer consist of living organisms i.e. bacteria, algae, and fungi |
| Harmful effects after application | No harmful effects after application or eco-friendly |
| Deplete soil quality | Improve soil quality |
| They are costly | They are low in cost |
| Easy to use | Difficult to use |
| Hazards to living organism | Non-hazards to living organism |
| Exp—Ammonium sulphate, ammonium phosphate | Exp—Rhizobium, blue green algae, mycorrhizal fungi, Azobacterium |
| Crop cultivated is less of flavour, test, and aroma | Crop cultivated in micro fertilizer are with more flavour, test, and aroma |

(Venkatramani 1996). As they are responsible for the good yield, they can be used in place of chemical fertilizer (Digambar Shelke 2019). They are available in solid as well as in liquid forms. Microorganisms produce a wide range of extracellular enzymes which have the potential to mediate utilization of organic sources of nitrogen and phosphorus in soil (Reed and Glick 2013). Many workers have reported that the uses of biofertilizers are beneficial to both soil and crops (Sharma et al. 2011). They could be applied to the soil directly with seeds and seedlings. They increase crop yield and fix nitrogen (Sharma et al. 2011). After using 3–4 years continuously, there is no need of application of microbial fertilizers because parental inoculums are sufficient for growth and multiplication. They improve soil texture, pH and other properties of soil (Olanrewaju et al. 2019). Microfertilizers not only ensure food safety but also add to the biodiversity of soil (Raja 2013). They also covert immobilized chemical into soluble forms and make them accessible to the plants. Application of microbial inoculants in the fields started almost 50–60 years back and now it is apparent that these beneficial microbes can also enhance plant resistance to adverse conditions. Therefore, microbial fertilizers have been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable way.

## 4.4 Disadvantage of Chemical Fertilizer

Chemical fertilizer usually affects the soil. However, it is not evident immediately due to buffering capacity of the soil (Ding et al. 2016). In general, 60–90% of the total applied fertilizer is lost and remaining part is used by the plants (Bhardwaj et al. 2014). They disturb pH and acidity of the soil (Ajmal et al. 2018). Acidity destroys the habitat of the microbes in the soil; hence, many beneficial microbes, worms etc. become extinct from the soil. They cause nutrient imbalance in the soil. These fertilizers contain heavy metals which are harmful to the environment (Sönmez et al. 2007; Aoun et al. 2010). These heavy metals enter the food chain, water and later in groundwater (Järup 2003; Savci 2012). Intensive use of chemical fertilizers can cause eutrophication where biological oxygen demand gets increased due to increase in algal growth (Sönmez et al. 2007) and result into water pollution. As a result of eutrophication, phosphate algal concentration in water increases and makes it unusable. Drainage, leaching and flow in water are responsible for nitrogen pollution. Leaching is mostly reported by use of nitrogenous fertilizers during cultivation. Nitrogen fertilizer reaches down to the soil and gets converted into nitrates by microbes in the nitrification process (Divya and Belagali 2014). Nitrates again penetrate deep in the soil and dissolve in water. When this water is consumed by the human beings, it results in several inflammatory diseases in digestive and urinary tracts. It also has potential to cause infant diseases such as methemoglobinemia, also known as blue baby syndrome in infants. It occurs due to nitrate poisoning in human beings (Lorna 2004). Different forms of nitrogen also have been reported for their cancerous nature (Khalid 2017). Excessive use of fertilizers produce oxides of nitrogen which are emitted in the atmosphere resulting in air pollution (Robert 2012). In atmosphere, they react with other compounds and result in harmful chemicals such as carbon dioxide, hydrogen sulphide (Byrnes 1990). The foods produced by the use of chemical fertilizers have very adverse effect on the health of humans as well as animals. Evaporation of $NH_3$ occurs from alkaline soils that have been treated with ammonium fertilizers (Ross 2018). This can cause acid rain eventually after some chemical transformation which can cause damage to animals and vegetation (Shaviv 2000; Sommer et al. 2004). The contributions of environmental pollution by chemical fertilizers are both manufacturing as well as application site (Tomkins and Bird 2002). They also weaken the roots of plants thereby making them to be susceptible to unwanted diseases (Ritika and Uptal 2014). Crops cultivated with chemical fertilizer have less flavour, taste and aroma than those cultivated without them (Itelima et al. 2018).

## 4.5 Negative Impacts of Biofertilizer

Though microbial fertilizers are beneficial in many respects, that is, low cost and eco-friendly, there are many restrictions that limit the use of microbial fertilizers. In contrast to the microfertilizers, the nutrient-based fertilizers give better results and are reliable as well (Elsayed et al. 2020). When compared with chemical fertilizers, they are low in nutrient content and result in deficiency symptoms. Additionally, the core effectiveness of the procedure depends upon the biological, chemical interactions and many physical factors like pH, moisture, temperature and other environmental variables (Ajmal et al. 2018). Microbial fertilizers multiply only when outer conditions are feasible. Otherwise, they diminish gradually with time and result in wastage of time and money. The lack of effective strains is one of the most important fault that make the biofertilizers unfit for the crops and soil. The selected strains have to be better and competitive than others. They should have affinity to different environmental conditions and should be able to survive in broths and inoculants carriers. Mutation during fermentation results in quality loss. Therefore, there is a need to give more attention to this aspect in order to eliminate such unwanted conditions. The storage of microbial fertilizer affects its efficacy. Even though they have many positive effects, sometime their use not lead to expected positive results. It could be because of exposure of high temperature and hostile conditions before use. Biofertilizers should be stored at cold temperatures. The shelf life of microbial fertilizer is confined if proper culture is not used. Carrier used for microbial inoculants should have 75% carbon, carbon-holding capacity, free from toxic substances and adjustable pH. Different carriers like charcoal, peat lignite, charcoal powder, etc. are used for this purpose, However, quantity of carbon in carrier above than 75% is rare. Finding such carrier is not easy task (Youseff and Eissa 2014). Local population of microbes around the rhizosphere sometime inhibits the efficiency of inoculants and reduces the establishment of microbial fertilizer (Ajmal et al. 2018). Since these products contain living organisms, their handling, transport and storage is not very easy to manage. The personnel dealing with the sale of microbial fertilizer should be aware about inoculation techniques. Otherwise, the lack of expertise and the level of uncertainty in this field give rise to complications. The fertility of soil, climatic conditions, high nitrate level, high temperature, unfavourable pH, drought, deficiency of phosphorus, copper, cobalt, molybdenum and other toxic substances affect microbial growth and crop responses.

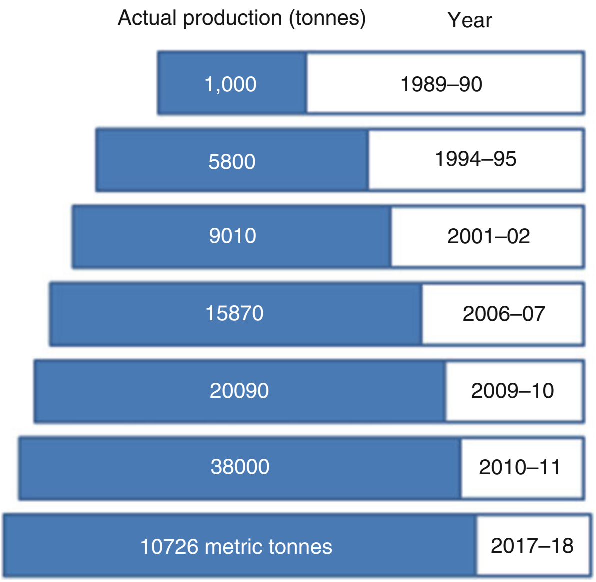
## 4.6 Recent Technology for Biofertilizers Commercialization and Post-covid Impacts

Commercialization of technology is a key factor in accelerating national economy and growth. India is the second most populous country in the world and has high demand of essential food items (Tawate et al. 2018). Agriculture yield in India accounts approximately 2.4 tons per hectare, which is very low agriculture productivity in comparison to China (4.7 tons per hectare) and Brazil (3.6 tonnes per hectare) (Raghavan 2014). As India is agriculture-based economy, the agricultural productivity can contribute towards enhancing India's gross domestic product (GDP). The current contribution of India is only 16% (Tawate et al. 2018). Therefore, the Indian government is focusing on development of different areas of agriculture including microfertilizers. The Indian soil has low level of carbon and nitrogen content which presents the need to use microfertilizer in agriculture. Excessive use of chemical fertilizer in India has resulted in degradation of the soil. Therefore, initiative has been taken by the Indian government to use microfertilizers in the country. In this time of rapidly changing business environment, it is essential for the organization to enhance technology commercialization for sustaining in global competition. Technology commercialization plays an important role in production, competitive market advantage and opportunity for trade (Chen et al. 2011). Literature survey shows lack of studies in biofertilizer commercialization. There are many technological and market-related challenges associated with the commercialization of biofertilizer. Commercialization of biofertilizers started in 1895 when 'Nitrazin' using *Rhizobium sp.* was introduced by Nobbe and Hilter in 1895. In 1950, several studies on fungi reported positive plant growth effect (Koide and Mosse 2004). However, despite many beneficial effects, commercialization of biofertilizer in not widespread. Some reasons limiting their use are unstable response in various soils, environment conditions, shelf life etc. (Debnath et al. 2019). The production of biofertilizer is based on technologies on inoculation, fermentation. Production needs huge investment on equipment. The Indian government has implemented the scheme for the production of biofertilizers since seventh five-year plan. Under this scheme, one national centre—NCOF (National Centre for Organic Farming) and six regional centres—RCOF (Regional Centre for organic farming) have been established. The main function of these centres includes the promotion of biofertilizer through the training, demonstration and supply of efficient culture for production of biofertilizers. The Indian Council of Agricultural Research (ICAR) under 'Network Project on Soil Biodiversity-Biofertilizers' has developed improved and efficient strains of biofertilizers specific to different crops and soil types. Liquid biofertilizers technology with higher shelf life has been developed. The government under capital investment subsidy scheme (CISS) of soil health management (SHM) of national mission of sustainable agriculture (NMSA) is providing assistance for setting up of state-of-art liquid-/carrier-based fertilizer units with 200 ton per annum capacity (data is based upon response of agriculture minister given in Lok Sabha). Hundred percent assistance is provided to state government and government agencies. For individual/ private agencies, assistance up to 25% as capital investment is provided through NABARD (National Bank for Agriculture and Rural Development). As per data provided by the NABARD, private fertilizer companies and non-government organisations are also involved in commercial production. Sixty-one biofertilizers and fruit vegetable compost production units were established under capital investment subsidy scheme in different states. At present, there are more than 150 biofertilizer companies engaged in production and sale of various products. The funding for 10% biofertilizer production units and 16 biofertilizer testing labs was done through soil health management scheme since 2015. Besides there are other schemes such as the National Food Security Mission, Rashtriya Krishi Vikash Yojana, National Horticultural Mission which support biofertilizer production. In India, during the 1990s, the production of biofertilizer was almost 10,000 tonnes mainly of rhizobium. Later, it was enhanced up to 20,090 tonnes (2009–2010). The estimation production for 2010–2011 is 38,000 tonnes (Table 4.2) and now it is of 10,726 metric tonnes during 2017–2018 (NCOF 2018) which have been increased further (Indian biofertilizer scenario 2012–2013; Dolmani et al. 2020). However, in spite of best efforts, use of microfertilizer production in less compared to chemical fertilizers. Very few reports have addressed about the biofertilizer commercialization (Twate et al. 2018, Pandey and Chandra 2016). These studies emphasize developing technology commercialization models for bio-agricultural segment to enhance the chances of technology commercialization process (Tawate et al. 2018). Therefore, more attempts are needed to understand technology commercialization.

There are many programmes such as NPOP (National Programme for Organic Production) under Ministry of Commerce and Industry under Government of India to promote organic farming. Organic agricultural statistics for the year 2020–2021 shows total organic farming and organic production (Fig. 4.2). Table 4.3 represents contribution of different states in organic farming.

The global biofertilizer market size is estimated to be valued at USD 2.6 billion and expected to increase two times more by 2026. Factors such as adoption of precision farming and protected agriculture along with increase in the environment concerns are some factors driving the growth of biofertilizer market. The global market has witnessed a relatively stable market growth post-COVID-19. The outbreak of COVID-19 has severely impacted several industries including biofertilizer industry across the world. Due to the COVID-19 pandemic, there has been a supply chain disruption because of which the agriculture sector has faced problems such as

**Table 4.2** Micro fertilizer production in India (Source: Department of Agriculture and Farmers Welfare 2021)



| Actual production (tonnes) | Year |
|---|---|
| 1,000 | 1989–90 |
| 5800 | 1994–95 |
| 9010 | 2001–02 |
| 15870 | 2006–07 |
| 20090 | 2009–10 |
| 38000 | 2010–11 |
| 10726 metric tonnes | 2017–18 |

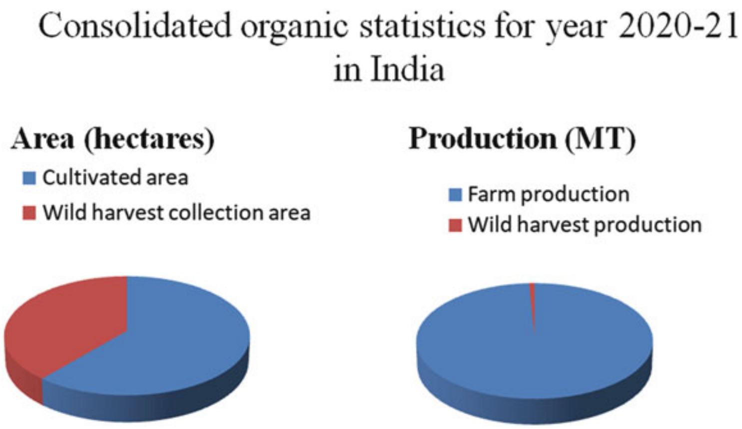


**Fig. 4.2** Cultivation and production of organic farming (Source: Website of Ministry of commerce and industry, Govt. of India 2021)

labour unavailability, transportation barriers, restriction for market access and lack of inventories in some regions which slightly affects the growth of biofertilizer market. For most of the industries, the revenue has gone down in 2020 and has resumed an uptrend gradually from 2021.

**Table 4.3** Stepwise cultivation farm in different states in India (Source: Website of Ministry of commerce and industry, Govt. of India 2021)

| States | Organic area (ha) | Conversion area (ha) | Total area (ha) |
|---|---|---|---|
| Madhya Pradesh | 540993.98 | 479,024 | 1020017.98 |
| Maharashtra | 219659.41 | 152063.21 | 371722.62 |
| Rajasthan | 177599.56 | 121086.74 | 298686.29 |
| Gujarat | 72,318 | 75,548 | 147866.41 |
| Karnataka | 61115.97 | 33934.11 | 95050.08 |
| Odessa | 78,148 | 14546.81 | 92694.81 |
| Sikkim | 74647.31 | 1082.34 | 75729.66 |
| Uttarakhand | 31556.80 | 432696.60 | 74826.40 |
| Uttar Pradesh | 53194.79 | 14247.83 | 67442.61 |
| Jharkhand | 0.0 | 53,261 | 53261.70 |
| Kerala | 256,565 | 19413.78 | 45070.38 |
| Meghalaya | 34816.30 | 3560.09 | 38376.39 |
| Andhra Pradesh | 22343.07 | 14458.29 | 36801.36 |
| Tamil Nadu | 14086.32 | 17542.74 | 31629.06 |
| Jammu & Kashmir | 19028.70 | 11591.12 | 30619.82 |
| Bihar | 4.9 | 29897.64 | 29902.54 |
| Chhattisgarh | 14744.46 | 8465.06 | 23209.52 |
| Assam | 6719.27 | 11751.57 | 18470.84 |
| Nagaland | 7384.96 | 7505.42 | 14790.38 |
| Arunachal Pradesh | 265.37 | 12848.74 | 13114.12 |
| Mizoram | 40.45 | 12998.44 | 13038.89 |
| Manipur | 4419.25 | 8305.67 | 12724.92 |
| Goa | 9243.79 | 3388.53 | 12632.32 |
| Himachal Pradesh | 9108.87 | 2745.12 | 11854.00 |
| Telangana | 4723.74 | 2141.82 | 6865.56 |
| Tripura | 203.56 | 6317.75 | 6521.31 |
| West Bengal | 5462.79 | 839.82 | 6302.61 |
| Haryana | 3345.85 | 1557.22 | 4903.06 |
| Punjab | 879.87 | 1141.63 | 2021.50 |
| Lakshadweep | 895.91 | 0.0 | 895.51 |
| Ladakh | 0.0 | 817.85 | 817.85 |
| Pondicherry | 2.84 | 20.81 | 23.65 |
| New Delhi | 0.72 | 4.45 | 5.17 |
| Total | 1492611.02 | 1165278.31 | 2657889.33 |

## 4.7 Combined Use of Biofertilizers

The use of mixture of bacterial strains instead of a single one is considered an important approach as different mechanisms of action provide more potential for the use of microfertilizer. In recent years, microfertilizer application has shifted from single strain to microbial consortia inoculation. Co-inoculants of microbial species not only allows the wide range of microfertilizer efficiency but also increases reliability for the fixation of nitrogen, phosphate solubilisation, siderophore production, balanced growth and nutrition as compared to a single inoculant (Lebin and Ishwer 2020). Microbial consortia consist of two or more strains that are related (Kyei-Boahen et al. 2005; Jin et al. 2013) or distant (Ramírez-López et al. 2019; El Maaloum et al. 2020) which further gives an overall additive or symbiotic biofertilization effect, that is, association of rhizobia and AMF on legumes. Mixed inoculants contain microbial essential phytohormones that control the stress conditions. Consortia instead of single microbial strain facilitates combined microfertilization effect and helps plants to promote better uptake of nutrients. A number of studies have reported a synergistic effect on plant growth promotion (Xavier and Germida 2003; Ashrafi et al. 2014; Ruth et al. 2017; Kavitha et al. 2013). Some studies show negative effects of AMF on nodule development or nonsignificant effects on crop yield (Antunes et al. 2009; Menéndez and Paço 2020). Microfertilizers developed from novel strain of *Mesorhizobium ciceri* and PGPR (Bacillus and Pseudomonas) have ability to improve the growth and nodulation of chickpea, thus enabling it to withstand the period of drought. Inoculation with multi-strain biofertilizer containing *Mesorhizobium ciceri*, *Pseudomonas* sp. and *Bacillus* sp. significantly improved the nodulation of chickpea at all experimental sites (Maqshoof Ahamd et al. 2017). Recent studies explored that the ability of a N-fixing bacteria consortium *Azospirillum, Azpcarcus, Azorhizobium* when applied as foliar-spraying inoculums during tillering improves root growth and nitrogen accumulation in common wheat (Cortivo et al. 2017). Similarly, AMF-bacteria consortium of *Rhizophagus irregularis, Azotobacter vinelandii* was found to enhance root growth and mineral uptake in this crop (Cortivo et al. 2018). Different combination of microfertilizers enhances plant growth, nitrogen accumulation, but not significant effect on grain yield when applied by seed. The growth could be due to increase in the rhizobial microbial mass and activity of several enzymes involved in organic decomposition when applied in wheat. In another report, combined biofertilizers give highest protein percentage and yield in soyabean cultivar (Zarei et al. 2012). Recent studies have investigated a combined effect of co-inoculant containing AMF and rhizobacteria for the enhanced growth of the leguminous plants (Igiehon and Olubukola 2017). Despite the many beneficial effects of developing biofertilizers consisting of microbial consortia, it is unknown how these inoculants would establish across a range of agricultural field settings (Finkel et al. 2017). Moreover, even if inoculated microbes colonize their new environment initially, their persistence over time is not guaranteed. Therefore, there is a need to develop new approaches to develop suitable bioinoculants at commercial scale for screening potential candidate microorganisms, designing the inoculants and optimizing formulations (Bagde et al. 2010).

A recent advancement is the development of culturomics technique in which we can identify bacterial species by developing multiple culture states. Culturomics is a culture method that uses a variety of culture conditions and used to make more than one combinations of culturing conditions, incubation rate, different growth media and atmospheric condition for the development of microbiome that is associated by plant and soil (Mitter et al. 2021; Faizaa 2021). Researchers have suggested a plant-dependent culturomics method in which plant-associated media is combined with culturomics. Furthermore, there is an online database for media preparation called as KOMODO. In this database, there are more than 18,000 mixtures of strains and up to 3300 combinations of microbial variants that are helpful for the development of effective media preparation in lab to obtain suitable and desired species for inoculum (Oberhardt et al. 2015).

## 4.8 Advancement in Microbial Fertilizer Technique

Biofertilizers have made higher achievements for the sustainable production of crops and establish more favourable conditions for the growth of microbes. But several pathogenic problems and reduced viability of microbial species during field application is observed (Çakmakçı 2019). Therefore, advanced techniques are needed for making a bioinoculant which is beneficial to indigenous soil population. For that propose, we need effective strategies that protect microbes (Stamenkovic et al. 2018). Formulation needs to be stable for longer period of time and should remain viable during the process of synthesis, packaging, transport and on field application.

### *4.8.1 Nanobiofertilizer*

Nowadays combined use of nanotechnology and biofertilizer is used for efficient and enhanced productivity of crops. Both nanomaterial and microbial fertilizers are helpful to maintain soil moisture and uptake of nutrients for plant. Problems such as instability in the field due to fluctuation in environmental condition, change of pH, poor shelf life, short-term efficiency and need of large amount of fertilizer to cover wide area result in poor growth and provide less yield (Çakmakçı 2019). The use of nanobiofertilizer provides essential benefit to farmers in term of good field performance, low cost, less expenses, and more yield (Mala et al. 2017; Prasad et al. 2014, 2017c). Nanofertilizer offers benefits in nutrition management through their strong potential to increase nutrient use efficiency. Nutrients are applied either alone or in combination with nano-dimensional adsorbents which release nutrients slowly compared to conventional fertilizers. This approach not only increases nutrient use efficiency but also minimizes nutrient leaching into groundwater. Nanobiofertilizers are not only eco-friendly with the environment but also produce best quality crops by enhancing the efficiency of indigenous microbial population through utilization of essential nutrients such as potassium, nitrogen and phosphorus. This leads to stimulating the activity of microbial enzymes which contributes to the fertility of soil. Furthermore, nanofertilizers may also be used for enhancing abiotic stress tolerance (Faizal et al. 2019). Although nanofertilizers use in agriculture is promising to improve plant nutrition and stress tolerance, not all nanomaterials are equally safe; therefore, the risk of using nanofertilizer should be carefully examined before use. Further studies are needed for the safe application of nanomaterials in agriculture.

### *4.8.2 Encapsulation and Lyophilisation Technique*

The wide application of plant-beneficial microorganisms is accepted as effective alternative that other form of fertilizers. Two main problems can be noticed in their production and application. One is economical competitiveness based on the overall upstream and downstream operational cost. The second problem is development of commercial products with a high soil plant colonization potential in controlled condition but not able to effectively mobilize soil nutrient or combat plant pathogens in the field (Nikolay et al. 2020). To solve these problems, microbe-based formulations produced by immobilization method such as encapsulation are gaining attention. Encapsulation technology is one of the current emerging techniques which allow the controlled release of biofertilizer into the environment (Nikolay et al. 2020). In this technology, microbial species having regulating composition and structure characteristics are used widely (Schoebitz et al. 2013). An advanced subfield of microencapsulation works on the mechanism of constructing a protective covering such as capsule around active molecule that has the ability to increase crop productivity and shelf life of biofertilizer. This type of formulation is more advantageous in terms of slow and sustained release into soil and also provides protection against environmental stress to microfertilizer (Çakmakçı 2019). Lyophilisation is the preservation and storage of microorganisms by freeze-drying method. This technique helps to make laboratory formulations of microorganisms. By this method survival rate of bacteria and storage for longer period can be enhanced further. The lyophilized microbial organisms can be used directly or in combination with a suitable carrier in the field (Faizaa 2021).

### *4.8.3 Nanoencapsulation Technology*

The use of soil microbes as microfertilizers has developed over decades. Nowadays, microfertilizers are used in different formulations for different agricultural products and their value is increasing every day. Encapsulation refers to any technological process that allows one or more active compounds to be enclosed within inert material, and in case of microfertilizer it is referred to coating of microfertilizer at a nanoscale with the help of a nanomaterial. One of most attractive advantage associated with nanoencapsulation is attributed to its ability to protect material from degradation. This is a versatile technology which facilitates increased shelf life, provides controlled release of microfertilizer and also allows controlled diffusion by microfertilizer. Studies have shown that PGPR-containing biofertilizers can be protected by using nanoencapsulation technology which allows its controlled release (Golbashy et al. 2017). The use of *Pseudomonas fluorescens* and *Bacillus subtilis* nanocapsules significantly enhanced the root length and proliferation in rootstock of pistachio in plant tissue culture (Mojdee et al. 2019). Further, nanoformulation of the bacterial metabolite led to the highest rootlets and largest shoot in same. Therefore, nowadays biofertilizers along with nanofertilizers are one of the most important tools in modern agriculture (El-Ramady et al. 2018). Also, microfertilizers and nanofertilizers play as promising methods for increasing use efficiency of different water and land resources reducing environmental pollution as well. So, eco-friendly biotechnological approaches may offer alternative to chemical fertilizers. Many microfertilizers such as rhizobium, cyanobacteria, azotobacter, acetobacter, and P, K and Zn solubilizer microbes play important role in soil biogeochemical cycle and plant growth promotion. The biosynthesis of nanomaterials using all these microbes has led to new area of research for the formation of inorganic nanoparticles as eco-friendly fertilizers.

### 4.8.4 Biofilm Microbial Fertilizers

Biofilm fertilizers are microbial fertilizers where thin layer of microbes are formed when applied on the surface of the soil. Microbes can be either biotic or abiotic and fixed with some biological component that facilitates sustainability and structural characteristic to the biofilm (Junaid and Khan 2018). It is used to improve fertilizers' efficiency and increase crop yield. The biofilm is a complex community of various species of microbes attached to plant roots and resistive to environmental stress and antagonists. They have ability to increase crop yield. Recent studies have shown that biofilm is the next generation of microfertilizer showing good resistance to abiotic as well as biotic stress (Mondol and Chakraborhy 2020). They have been prepared by community of microorganisms that accumulated together with adhesive forces embedded in a matrix can secrete extracellular polymeric substances for self-protection (Donlan Rodney 2002; Velmourougane and Saxena 2017). Compared to a biofilm comprising of single microbial species, multiple microbial strains in a biofilm is investigated to be more resistant and sustainable approach. For instance, the uptake of nutrient and tolerance to environmental stress is significantly increased for bacterial-fungal biofilm as compared to those microorganisms that are single or do not form biofilm (Velmourougane and Saxena 2017). So many positive impacts on modern agriculture such as increase in yield, increase in availability of phosphorous increase the soil organic content associated with it (Mondol and Chakraborhy 2020).

## 4.9 Conclusions

The reduced load of chemical fertilizer into the crop fields without causing production loss is feasible but a big challenge. Current soil management strategies are mainly dependent on inorganic chemical-based fertilizers which causes a serious threat to human and environment. The use of beneficial microbes as biofertilizers has become important in agriculture for their potential role in food safety and sustainable crop production. It is important to realize the useful aspects of microbial fertilizer and implement its application to modern agriculture. However, there is lack of awareness regarding the protocol of biofertilizer application to this field. The success of biofertilizer depends upon inventions of new strategies. The major challenge in this area lies in the understanding of its functioning. Short shelf life, lack of suitable carrier, temperature sensitivity, difficulty in transportation and storage are the areas that still need to be solved in order to get effective inoculums. Studies should be conducted on proper strain and optimum growth conditions are needed. The efficacy of microbial fertilizers can be improved by detail studies on genetic diversity of different microbial inoculants. Further studies are needed to make these microbial inoculants more compatible. A better understanding of genetic and biochemical mechanism that governs the plant-microbe interactions is essential for using them in large-scale applications.

## References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929. https://doi.org/10.1007/s00248-009-9531-y

Ajmal M, HafizaIqra A, Rashid S, Akhtar A, Muniba T, Muhammad ZM, Aneesa A (2018) Biofertilizer as an alternative for chemical fertilizer. Res Rev 7(1):2

Allen MF (2011) Linking water and nutrient through the vadose zone: a fungi interfere between the soil and plant systems. J Arid Land 3:155–163. https://doi.org/10.3724/SP.J.1227.2011.00155

Ansari MW, Trividi DK, Sahoo RK, Gill SS, Tueja N (2013) A critical review on fungi mediated plant response with special emphasis to *Piriformospora indica* on improved production and protection of crops. Plant Physiol Biochem 70:403–410. https://doi.org/10.1016/j.plaphy.2013.06.005

Antunes PM, Koch AM, Dunfield KE, Hart MM, Downing A, Rillig MC, Klironomos J (2009) Influence of commercial inoculation with *Glomus intraradices* on the structure and functioning of an AM fungal community from an agricultural site. Plant Soil 317:257–266. https://doi.org/10.1007/s11104-008-9806-y

Aoun M, Samrani AG, Lartiges BS, Kazpard V, Saad Z (2010) Releases of phosphate fertilizer industry in the surrounding environment: investigation on heavy metals and polonium-210 in soil. J Environ Sci 22:1387–1397. https://doi.org/10.1016/s1001-0742(09)60247-3

Ashrafi E, Zahedi M, Razmjoo J (2014) Co-inoculations of arbuscular mycorrhizal fungi and rhizobia under salinity in alfalfa. Soil Sci Plant Nutr 60:619–629. https://doi.org/10.1080/00380768.2014.936037

Astarai AR, Kochaki A (1996) Application of permanent biological fertilizers. Ferdosi Mashhad University, Mashhad

Bagde US, Prasad R, Varma A (2010) Mass cultivation of *Piriformopsora indica* in new Brunswick fermenter and its formulation as biofertilizer. Asian J Microbiol Biotechnol Environ Sci 12:911–916

Bago B, Vierheilig PY, Azcon-Aguilar C (1996) Nitrate depletion and pH changes induced by the extraradial mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. New Phytol 133(2):273–280

Bent E, Tuzun S, Chanway CP, Enebak S (2001) Alteration in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. Can J Microbiol 2(47):793–800. https://doi.org/10.1139/w01-080

Berg G, Zachow C, Muller H, Phillips J, Tilcher R (2013) Next generation bio- products sowing the seeds of success for sustainable agriculture. Agronomy 3:648–656. https://doi.org/10.3390/agronomy3040648

Bhardwaj D, Mohannad WA, Sahoo RK, Tutreja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13:66–72

Byrnes BH (1990) Environment effects of N-fertilizer use—An overview. Fertil Res 26:209–215. https://doi.org/10.1007/BF01048758

Çakmakçı R (2019) A review of biological fertilizers current use, new approaches, and future perspectives. Int J Innov Stud Sci Eng Technol 5(7):83–92

Chen CJ, Chang CC, Hung SW (2011) Influence of technological attributes and environmental factors on technology commercialization. J Bus Ethics 104:525–535

Cortivo C, Barion G, Visioli G, Mattarozzi M, Mosca G, Vamerali T (2017) Increased root growth and nitrogen accumulation in common wheat following PGPR inoculums: assessment of plant-microbe interactions by ESEM. Agric Ecosyt Environ 247:396–408. https://doi.org/10.1016/j.agree.2017.07.006

Cortivo DC, Barion G, Visioli FM, Visioli G, Dramis L, Panozzo A et al (2018) Effect of field inoculation with VAM and bacteria consortia on root growth and nutrient uptake in common wheat. Sustainability 10:3286. https://doi.org/10.3390/su10093286

Debnath S, Rawat D, Mukherjee A, Adhikary S (2019) Application and constraints of plant beneficial micro-organism in agriculture. In: Rhizosphere and soil microbes—Utilization in agriculture and industry under current scenario. https://doi.org/10.5772/INTECHOPEN.89190

Digambar S (2019) Rejuvenation of bio-fertilizer: an alternative source. London Journal Press, 19(3):1.0

Ding H, Zhang YS, Li WH, Zheng XZ, Wang LN, Tang DL, Chen D (2016), Nutrient release from a novel gel based slow/controlled release fertilizer. Appl Environ Soil Sci. https://doi.org/10.1155/2016/2013463

Divya J, Belagali SL (2014) Assessment of seasonal variations of chemical fertilizers residues in agricultural areas of Najangud Taluk, Mysore District. Int J Innov Res Sci Eng Technol 3:8639–8646. http://inpressco.com/category/ijcet

Dolmani A, Thakur JK, Mandal A, Sahu A, Reddy KK (2020) Production and utilization of legume inoculants in India. Soil Health Manag 3(2):28–31

Donlan Rodney M (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890. https://doi.org/10.3201/eid0809.020063

Dwivedi SK, Sangeeta (2015) Role of mycorrhizae as biofertilizer and bioprotectant. Int J Pharm Biosci 6(2):B1014–B1026

El Maaloum S, Elabed A, Alaoui-Talibi Z, El Meddich A, Filali-Maltouf A, Douira A et al (2020) Effect of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria consortia associated with phospho-compost on phosphorus solubilization and growth of tomato seedlings (*Solanum lycopersicum* L.). Commun Soil Sci Plant Anal 51:622–634. https://doi.org/10.1080/00103624.2020.1729376

El-Ramady HR, El-Ghamry MA, Mosa AA, Alshaal T (2018) Nanofertilizers vs biofertilizers new insights. Environ Biodiver Soil Security 2(1):40–50. https://doi.org/10.21608/jenvbs.2018.3880.1029

Elsayed SIM, Glala AA, Darwish MA (2020) Effect of biofertilizer and organic fertilizer on growth, nutrient contents and fresh yield of dill (*Anethum graveolens*). Bull Natl Res Centre 44:122

Faizaa Q (2021) Recent advances in biofertilizers. Research gate, pp 3–8. https://www.researchgate.net/publication/35215209

Faizal Z, Mirium N, Ashraf M, Akram NA, Munne S, Munne BS (2019) Nanofertilizer use for sustainable agriculture: advantages and limitations. Plant Sci 289:110270

Finkel OM, Castrillo G, Paredes SH, Dangl JL, Herrera Paredes S, Salas González I et al (2017) Understanding and exploiting plant beneficial microbes. Curr Opin Plant Biol 38:155–163. https://doi.org/10.1016/j.pbi.2017.04.018

Finlay RD, Ek H, Odham G, Soderstorm B (1989) Uptake, translocation and assimilation of nitrogen from 15 N-labelled ammonium and nitrate sources by intact ectomycorrhizal systems of fagussylvation infected with *Paxillus involutus*. New Phytol 113(1):47–55. https://doi.org/10.1111/j.1469-8137.1989.tb02394.x

Gahukar RT (2005–2006) Potential and use of bio-fertilizers in India everyman's science, XL(5): 354–361

Gholami A, Shahsavani S, Nezarat S (2008) The effect of plant growth promoting rhizobia (PGPR) on germination seedling growth and yield of maize. Eng Technol 49:1

Giri B, Prasad R, Wu QS, Varma A (2019) Biofertilizers for sustainable agriculture and environment. Springer International Publishing. isbn:978-3-030-18932-7. https://www.springer.com/gp/book/9783030189327

Golbashy M, Sabahi H, Allahdadi I, Nazokdast H, Hosseini M (2017) Synthesis of highly intercalated urea-clay nanocomposite via domestic montmorillonite as eco-friendly slow-release fertilizer. Arch Agron Soil Sci 63(1):84–95

Hari M, Permural K (2010) Booklet on biofertilizer (phosphobacteria). Shri Annm Murugapa Chettiar Research Centre, Taramani Chennai, pp 1–6

Igiehon N, Olubukola OB (2017) Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhiza fungi. Appl Microbiol Biotechnol 101:4871–4881

Itelima JU, Bang WJ, Sila OE (2018) A review: biofertilizer-s as key player in enhancing soil fertility and crop productivity. J Microbiol Rep 2(1):22–28

Järup L (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182. https://doi.org/10.1093/bmb/ldg032

Jin H, Germida JJ, Walley FL (2013) Impact of arbuscular mycorrhizal fungal inoculants on subsequent arbuscular mycorrhizal fungi colonization in pot-cultured field pea (*Pisum sativum* L.). Mycorrhiza 23:45–59. https://doi.org/10.1007/s00572-012-0448-9

Junaid PM, Khan F (2018) Biofilm: a future generation biofertilizer. Indian J Appl Pure Biol 33(2): 131–134

Jurgen K, Kolnaar R, Ravensberg WJ (2019) Mode of action of microbial biological control agents against plant disease: relevance beyond efficacy. Front Plant Sci 10:845

Kavitha, Srinivasan S, Ranjini (2013) Individual and combine effect of biofertilizer, chemical fertilizer and vermicompost on *Amaranthus tristis*. Int J Pharm Sci Rev 20:190–195

Khalid RH (2017) Erratum to: nitrate and nitrogen oxides: sources, health effects and their remediation. Rev Environ Contam Toxicol 242:183–217. https://doi.org/10.1007/398_2016_11

Koide RT, Mosse B (2004) A history of research on arbuscular mycorrhiza. Mycorrhiza 14:145–163. https://doi.org/10.1007/s00572-004-0307-4

Komala NT, Gurumurthy R, Surendra P (2017) Role of mycorrhiza in cereals. Bull Environ Pharmacol Life Sci 6(4):1–5

Kumar V, Kumar M, Prasad R, Tuteja N, Varma A (2017) Mycorrhizas in forest tree health. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 177–185

Kyei-Boahen S, Nleya T, Hynes R, Walley FL (2005) Single and multistrain rhizobial inocula for pinto and black bean cultivars. J Plant Nutr 28:1679–1692. https://doi.org/10.1080/01904160500250664

Lebin T, Ishwer S (2020) Microbial biofertilizers: types and applications. In: Biofertilizers for sustainable agriculture and environment, pp 1–19. https://doi.org/10.1007/978-3-030-18933-4-1

Lorna F (2004) Drinking-water nitrate, methemoglobinemia and global burden of disease—a discussion. Environ Health Perspect 112:14. https://doi.org/10.1289/ehp.7216

Mala R, Celsia ASR, Barathi SV, Blessina S, Shanmuga RR, Maheswari GU (2017) Evaluation of nano structured slow release fertilizer on the soil fertility, yield and nutritional profile of *Vigna radiata*. Recent Pat Nanotechnol 11(1):50–62

Mali GV, Bodhkar M (2009) Antifungal and phytohormone production potential of *Azobacter chroococcum* isolates from groundnut (*Arachis hypogea* L.) rhizosphere. Res J Chem Environ 15(2):1–7

Mazid M, Khan TA (2014) Future of bio-fertilizers in Indian agriculture: an overview. Int J Agric Food Res 3:10–23

Menéndez E, Paço A (2020) Is the application of plant probiotic bacterial consortia always beneficial for plants? Exploring synergies between rhizobial and non-rhizobial bacteria and their effects on agro-economically valuable crops. Life 10:1–18. https://doi.org/10.3390/life10030024

Miransari M (2010) Biological fertilization. In: Méndez-Villa A (ed) Current research and education topics in applied microbiology and microbial biotechnology

Mitter EK, Tosi M, Obregón D, Dunfield KE, Germida JJ (2021) Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies. Front Sustain Food Syst 5:29

Mojdee MP, Roohallah SR, Mohammadinejad R, Hosseini A (2019) Nano-Encapsulation of plant growth-promoting rhizobacteria and their metabolite using alginate-silica nanoparticle and carbon nanotube improves UCB1 *Pistachio* micropropagation. J Microbiol Biotechnol 29(7): 1096–1103. https://doi.org/10.4014/jmb.1903.03022

Molina-Favero C, Monica Creus C, Luciana Lanteri M, Correa- Aragunde N, Lombardo MC, Barassi AC, Lamattina L (2007) Nitric oxide and plant growth promoting rhizobacteria: Common Features influencing root growth and development. Adv Bot Res 46:1–33. https://doi.org/10.1016/S0065-2296(07)46001-3

Mondol K, Chakraborhy A (2020) Biofilm—a next generation bio-fertilizers. Krishi Sci eMagazine Agric Sci 1(3):6–9

Nikolay VM, Vassilev VM, Luis F, Garciadel MJ, Kowalska BT, Malsue E (2020) Formulation of microbial inoculants by encapsulation in natural polysaccharides: focus on beneficial properties of carrier additives and derivatives. Front Plant Sci 11:270. https://doi.org/10.3389/fpls.2020.00270

Oberhardt MA, Zarecki R, Gronow S, Lang E, Klenk HP, Gophna U et al (2015) Harnessing the landscape of microbial culture media to predict new organism—media pairing. Nat Commun 6: 8493. https://doi.org/10.1038/ncomms.9493

Olanrewaju OS, Ayangbenro AS, Glick BR, Babalola OO (2019) Plant health: feedback effect of root exudates rhizobiome interactions. Appl Microbiol Biotechnol 103:1155–1166. https://doi.org/10.1007/s00253-018-9556-6

Pandey V, Chandra K (2016) Agriculturally important micro-organisms as biofertilizers: commercialization and regulatory requirements in Asia. In: Agriculturally important microorganisms, pp 133–145. https://doi.org/10.1007/978-981-10-2576-1_8

Perrig D, Boiero ML, Masciarelli OA, Penna C, Ruiz OA, Casssan FD, Luna MV (2007) Plant growth promoting compounds produced by two agronomically important strains of *Azospirillum brasilense* and implication for inoculants formation. Appl Microbiol Biotechnol 75:1143–1150. https://doi.org/10.1007/s00253-007-0909-9

Prasad R, Kumar V, Prasad KS (2014) Nanotechnology in sustainable agriculture: present concerns and future aspects. Afr J Biotechnol 13(6):705–713

Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017a) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 1–7

Prasad R, Gupta N, Satti U, Wang S, Ahmed ISA, Varma A (2017b) Management of fungal pathogens by mycorrhiza. In: Varma A, Prasad R, Tuteja N. Mycorrhiza. Springer International Publishing AG, pp 179–194

Prasad R, Bhattacharyya A, Nguyen QD (2017c) Nanotechnology in sustainable agriculture: recent developments, challenges and perspectives. Front Microbiol 8:1014. https://doi.org/10.3389/fmicb.2017.01014

Raghavan S (2014) India's agricultural yield suffers from low productivity. http://www.livemint.com/Opinion/nw9JKiPrDPpqCuWfmoibPN/Indias-agricultural-yield-suffers-from-lowproductivity.html

Rai S, Shukla N (2020) Biofertilizer: an alternative of synthetic fertilizer. Plant Arch 20(2): 1374–1379

Raja N (2013) Biopesticides and biofertilizers: ecofriendly sources for sustainable agriculture. J Biofertilizer 3:112–115. https://doi.org/10.4172/2155-6202.1000e112

Ramírez-López C, Esparza-García FJ, Ferrera-Cerrato R, Alarcón A, Cañizares-Villanueva RO (2019) Short-term effects of a photosynthetic microbial consortium and nitrogen fertilization on soil chemical properties, growth, and yield of wheat under greenhouse conditions. J Appl Phycol 31:3617–3624. https://doi.org/10.1007/s10811-019-01861-2

Ray RN, Handerson G (2001) Endophytic nitrogen fixation in sugarcane: present knowledge and future applications, technical expert meeting on increasing the use of biological nitrogen fixation (BNF) in agriculture. FAO, Rome

Reed M, Glick BR (2013) Introduction of plant growth promoting bacteria for plant and soil system. In: Application of microbial engineering, vol 2. Taylor and Francis, Enfield, pp 181–229

Ritika B, Uptal D (2014) Biofertilizers a way towards organic agriculture: a review. Acad J 8(24): 2332–2342. https://doi.org/10.5897/AJMR.2013.637a

Robert S (2012) Fertilizers use responsible for increase in nitrous oxide in atmosphere. In: Science and environment. http://newscenter.berkeley.edu/2012/04/02/fertilizer-use-responsible-for-increase-in-nitrous-oxide-in-atmosphere

Ross HM (2018) Understanding how fertilizers reacts and behave in soil can be key to a healthy crop. In: Agronomy fertility and nutrients. http://www.topcropmanager.com/fertilizers-reactions-and-behaviour-reactions-and behaviour-in-soil-21129

Ruth WM, John B, Tumuhairwe PE, Abdel H, Moses AG, Thuita CM (2017) Front Plant Sci. 13. https://doi.org/10.3389/fpls.2017.00219

Savci S (2012) Investigation of effect of chemical fertilizers on environment. PCBEE Procedia 1: 287–292. https://doi.org/10.1016/j.apcbee.2012.03.047

Saxena P, Joshi N (2002) In: Role of microorganisms in the decomposition of organic wastes matter. Ph.D. thesis, Gurukul Kangri University, Haridwar

Schoebitz M, López MD, Roldán A (2013) Bio encapsulation of microbial inoculants for better soil-plant fertilization: a review. Agron Sustain Dev 33(4):751–765

Serpil S (2012) An agricultural pollutant: chemical fertilizer. Int J Environ Sci Dev 3:1. https://doi.org/10.7763/IJESD.2012.V3.191

Sharma S, Sharma AK, Prasad R, Varma A (2017) Arbuscular mycorrhiza: a tool for enhancing crop production. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 235–250

Shaviv A (2000) Advances in controlled release of fertilizers. Adv Agron 71:1–49. https://doi.org/10.1016/S0065-2113(01)71011-5

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. https://doi.org/10.1016/j.agee.2011.01.017

Sinha RK, Valani D, Cheuhan K, Agarwal S (2014) Embarking on a second free revolution for sustainable agriculture by vermiculture biotechnology using earthworms: reviving the dreams of Sir Charles Darwin. Int J Agric Health Saf 1:50–64. http://www.academicjournals.org/jabsd

Sommer SG, Schjoerring JK, Denmead OT (2004) Ammonia emission from mineral fertilizers and fertilized crops. Adv Agron 82:557–622

Sönmez İ, Kaplan M, Sonmez Z (2007) An investigation of seasonal changes in nitrate contents of soils and irrigation waters in greenhouses located in Antalya-Demre region. Asian J Chem 19: 5639–5646

Stamenkovic S, Beskoski V, Karabegovic I, Lazic M, Nikolic N (2018) Microbial fertilizers: a comprehensive review of current findings and future perspectives. Spanish J Agric Res 16:1–18. https://doi.org/10.5424/sjar/2018161-12117

Subha Rao NS (1982) Advances in agricultural microbiology. In: Subha Rao NS (ed) Studies in the agricultural and food sciences. Butterworth Scientific, London, pp 295–303

Subha Rao NS (2015) Soil microbiology. In: Soil microorganisms and plant growth, 4th edn, Oxford, Oxford IBH Publishing Co. Pvt. Ltd

Sudha M, Gowri RS, Prabhavati P, Astapriya P, Devi SY, Saranya A (2012) Production and optimization of indole acetic acid by indigenous microflora using agrowaste as substrate. Pakistan J Biol Sci 15(1):39–43. https://doi.org/10.3923/pjbs.2012.39.43

Tawate S, Gupta R, Jain K (2018) Technology commercialization in bio-fertilizer firm: an Indian case. Int J Global Business Competitiveness 13(1):65–74

Teotia P, Kumar M, Prasad R, Kumar V, Tuteja N, Varma A (2017) Mobilization of micronutrients by mycorrhizal fungi. In: Varma A, Prasad R and Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 9–26

Tomkins P, Bird C (2002) Chemicals, plants and man: the organic farming residue. In: Secret life of plants, pp 240–258

Varma A, Kharkwal A, Bains KS, Agarwal A, Bajaj R, Prasad R (2012) *Piriformospora indica*: the model microbes for organic green revolution. Biofertilizer Newsletter 20:3–8

Velmourougane K, Saxena A (2017) Agriculturally important microbial biofilms: present status and future prospectus. J Basic Microbiol 57:548–573. https://doi.org/10.1002/jobm.201700046

Venkatramani G (1996) In: Hindus Survey of Indian Agriculture, p 29

Vessey JK (2003) Plant growth promoting rhizobacteria as bio-fertilizer. J Plant Soil 225(43): 571–586. https://doi.org/10.1023/A:1026037216893

Wong SW, Swee NT, Ge L (2015) The importance of phytohormone and microbes in biofertilizers. http://www.researchgate.net/publication/300238542

Xavier LJC, Germida JJ (2003) Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. viciae enhance pea yield and nutrition. Biol Fertil Soils 37:261–267. https://doi.org/10.1007/s00374-003-0605-6

Youseff MMA, Eissa MFM (2014) Biofertilizer and their role in management of plant parasitic nematodes: a review. E3 J Biotechnol Pharm Res 5:1–6. http://www.e3journals.org

Zarei I, Sohrabi Y, Heidari GR, Jalilian A, Mohammadi K (2012) Effects of biofertilizers on grain yield and protein content of two soyabean cultivars. Afr J Biotechnol 11(27):7028–7037. https://doi.org/10.5897/AJB11.3194

Zemrany HEI, Corter J, Lutz MP, Chabert A, Baudoin E, Haurat J, Maughan N, Felix D, Defago G, Belly R, Moënne-Locoz Y (2006) Field survival of the phytostimulator *Azospirillum lipoferum* CRT1 and functional impacts on maize crop, biodegradation of crop residue and soil faunal indicators in a context of decreasing nitrogen fertilisation. Soil Biol Biochem 38:1712–1726

# Chapter 5
# Phosphate Solubilizing Microorganisms: Potential Bioinoculants for Sustainable Agriculture

**Sonal Bhardwaj, Rajesh Kaushal, Prakriti Jhilta, Anchal Rana, and Bhawna Dipta**

**Abstract** Despite its availability in both organic and inorganic forms, phosphorus (P) is typically inaccessible for plant uptake due to its complexation with metal ions in the soil. The use of phosphatic fertilizers to fulfil P demand to improve crop yield has resulted in a decline in ecosystem and soil health as well as a microbial imbalance. Phosphate solubilizing microorganisms (PSMs) play an important role in solubilizing insoluble phosphates through a variety of mechanisms. PSMs also promote plant growth and shield plants against phytopathogens via the production of phytohormones, ACC deaminase, siderophores, exopolysaccharides (EPSs), lytic enzymes, and antibacterial/antifungal metabolites. In this chapter, an attempt has been made to focus on the role of PSMs in solubilization and mineralization of P, crop response to PSM bioinoculants, and genetic engineering of PSM.

**Keywords** ACC deaminase · Gluconic acid · Indole acetic acid · Organic P · Pyrroloquinoline quinone · Siderophore

## 5.1 Introduction

Phosphorus (P) ranks second only to nitrogen as one of the most important macronutrients for plant growth and development. It is involved in the synthesis of nucleic acids, phospholipids, ATP, nucleotides, and enzymes. Phosphorus also regulates vital functions including photosynthesis, respiration, and energy generation. The leaf

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_5

shape may be distorted with thin stems, and purplish pigmentation appears in older leaves due to more anthocyanin synthesis under limited P conditions. P-deficiency results in delayed plant maturity. These changes, however, differ depending on the crop species. The deficiency of P also affects nodule formation, growth as well as the amount of energy available to sustain nodule metabolic activity. An adequate supply of P stimulates seed germination, seedling establishment, root formation, seed development, flower initiation, fruit production, and crop yield (Malhotra et al. 2018). Increased soil P availability enhances nodule number, weight, and volume, resulting in increased nitrogen fixation and crop growth (Bashir et al. 2011).

The available forms of P that plants can take up are monobasic phosphate ion ($H_2PO_4^{-}$), dibasic phosphate ion ($HPO_4^{2}$), and phosphate ion ($PO_4^{3-}$) (Hanyabui et al. 2020). Phosphorus makes up 0.2–0.8% of the plant's dry weight; however, only 0.1% of that P is available to plants (Zou et al. 1992). To obliterate P deficiency, large amounts of phosphatic fertilizers are frequently applied, particularly in conventional and intensive agricultural soils. However, plants absorb a relatively small amount, and about 80–90% of the soil P is unavailable to the plant due to its fixation as aluminum/iron hydroxides in acidic soils or calcium phosphates in calcareous soils, rendering it unavailable for uptake by plants. Moreover, most of the applied fertilizers leach into groundwater and surface water, leading to eutrophication and groundwater contamination (Kleinman et al. 2009). The regular application of phosphatic fertilizers is expensive as well as damaging to the environment. To produce adequate yields of crops, there is an essential need to increase the plant availability of P in soils. The application of phosphate solubilizing microorganisms (PSMs) has shown potential in the transformation of unavailable forms of P into available forms. This chapter focuses on the potential role of PSMs as bioinoculants, their mechanism in P-solubilization, P-mineralization, and the genetic engineering of PSMs.

## 5.2 Phosphorus Availability in Soil

Phosphorus is abundant in the soil in both inorganic and organic forms (Rawat et al. 2020). Inorganic P occurs mostly in insoluble mineral complexes such as apatite, hydroxyapatite, oxyapatite, mono-, di-, and tricalcium phosphates, accounting for 35–70% of the total soil P. Inorganic P is associated with Al and Fe compounds in acidic soil, whereas calcium phosphate predominates in alkaline soil (Khan et al. 2009). Organic matter is a major source of immobilized P that accounts for 20–80% of total soil P in most soils. Nucleic acids, inositol phosphates, phospholipids, sugar phosphates, polyphosphates, and phosphonates are the most common organic P sources.

## 5.3 Phosphate Solubilizing Microorganisms (PSMs)

The rhizosphere is the nutrient-rich area of soil surrounding the roots due to the accumulation of a variety of plant exudates such as sugars, organic acids, amino acids, vitamins, enzymes, and organic or inorganic ions. It is inhabited by a diverse range of microorganisms which can be classified into beneficial, deleterious, and neutral groups based on their effects on plant growth. Phosphate solubilizing microorganisms (PSMs) are beneficial microorganisms that increase the accessibility of P in the soil by making unavailable forms of P available to plants through secretion of organic acids or release of protons, production of chelating substances, and secretion of phosphatases, resulting in better growth and higher yield (Chittora et al. 2020). A considerable number of PSMs with phosphate solubilization and mineralization potential have been isolated, characterized, and tested for their efficacy in plant growth promotion and soil characteristics. These microorganisms are abundant in soil and play an important role in the biogeochemistry of soil P cycling, involving processes like weathering, mineralization, solubilization, dissolution, and immobilization (Tian et al. 2021). PSMs play an important role in the enhancement of soil fertility as they are capable of releasing P from soil minerals in conditions when the requirement of P is high but availability is low (Wakelin et al. 2012). With the application of PSM and plant growth-promoting rhizobacteria (PGPR) together, it is feasible to reduce the rate of soluble P-fertilizer by 50% without any significant reduction in grain yield (Yazdani et al. 2009). However, the PSMs exert their growth-promoting traits under certain environmental conditions, such as soil pH, moisture, texture, organic matter content, and enzymatic activities. Besides, PSMs must be able to compete with native soil microflora and successfully colonize the crop rhizosphere upon introduction into the environment. In this regard, the application of native microorganisms in the soil as efficient bioinoculants could prove to be significant.

PSMs are ubiquitous but their number varies from soil to soil and most of these have been isolated from the plant rhizosphere as they are metabolically most active in those particular sites (Reyes et al. 2006). The efficient bacterial phosphate solubilizers include species from the genera *Pseudomonas* (Parani and Saha 2012), *Bacillus* (Ramesh et al. 2014; Matos et al. 2017), *Rhizobium* (Halder et al. 1990), and *Enterobacter* (Shahid et al. 2012). The solubilization of inorganic phosphate and plant growth promotion by actinobacteria is also well known (Anwar et al. 2016). Sreevidya et al. (2016) have reported increased shoot and root length in chickpeas after inoculation with *Streptomyces* sp. strain VAI-7. Actinobacteria belonging to genera *Streptomyces*, *Micromonospora*, *Actinobispora*, *Saccharomonospora*, *Saccharopolyspora*, *Streptoverticillium*, and *Thermonospora* have been reported as P-solubilizers (Kaviyarasi et al. 2011; Hamdali et al. 2012). Mixed cultures of PSMs are found to be more efficient in organic phosphate mineralization (Molla et al. 1984). The positive effect of two native phosphate solubilizing bacteria (PSB) namely, *Bacillus aryabhattai* (JX285) and *Pseudomonas auricularis* (HN038) on plant growth, photosynthetic capacity, NP content of the leaves of *Camellia oleifera*, and available NPK content of the rhizosphere soil was recorded by Wu et al. (2019). Xu et al. (2019) elucidated that PSB *Pantoea ananatis* HCR2 and *Bacillus thuringiensis* GL-1 can effectively release soluble P by solubilizing phosphate rock. The solubilized phosphate reacted rapidly with $Pb^{2+}$ to form insoluble lead compounds that caused soil remediation by significantly reducing the phytoavailability of $Pb^{2+}$ and improving plant growth and net photosynthetic rate. Mendoza-Arroyo et al. (2020) also concluded in their studies that P-solubilizing *Enterobacter* sp. ITCB-09 in immobilized form promoted growth of *Capsicum Chinese* Jacq. seedlings besides contributing to soil health and fertility.

Fungi are more active than bacteria in solubilizing insoluble phosphate (Cunningham and Kuiack 1992; Sanjotha et al. 2011). Kucey (1983) observed that in terms of solubilizing calcium phosphate and Idaho rock phosphate, the fungi were superior to bacteria. This capacity was also preserved by fungi after several subculturing. Moreover, soil fungi can navigate long distances in the soil region as compared to bacteria. The predominant P-solubilizing fungal genera include *Trichoderma* (Garcia-Lopez et al. 2015; Franca et al. 2017), *Aspergillus* (Chuang et al. 2007; Li et al. 2015), and *Penicillium* (Asea et al. 1988; Pandey et al. 2008). Meena et al. (2010) observed in their pot experiments that inoculation of *Pseudomonas striata* along with endophytic fungi *Piriformospora indica* resulted in significant increased dry plant biomass of the chickpea crop, besides leading to an increased population of *Pseudomonas striata* in the rhizosphere region. Ram et al. (2015) also reported a significant increase in wheat grain yield by 12.6% over uninoculated control with the application of *Penicillium bilaii*. Kumari and Nanayakkara (2017) screened *Aspergillus* sp., *Penicillium oxalicum*, and *Trichoderma virens* for their potential in solubilizing inorganic phosphates under in vitro conditions. The isolates expressed significant solubilization of tricalcium phosphate (TCP) and Eppawala rock phosphate (ERP). Further, a synergism by *Aspergillus* sp. and *Penicillium oxalicum* towards P-solubilization recorded significant solubilization of TCP and ERP, making them potential candidates for biofertilizer production. In another study, Qiao et al. (2019) reported that PSF *Penicillium guanacastense* isolated from pine tree rhizosphere soil promoted the growth of *Pinus massoniana*. A diverse range of PSMs are presented in Table 5.1.

## 5.4 Role of Mycorrhizae in Phosphate Mobilization

Mycorrhizae are defined as a symbiotic relationship between fungi and plants. Mycorrhizal fungi are ubiquitous, facilitating plants with more intimate access to water and nutrients and thus boosting plant growth, particularly under stress (Kaushal 2019; Begum et al. 2019). Even in P-deficient soils, mycorrhizal roots can absorb a sufficient amount of P. Mycorrhizal fungus easily colonizes P-deficient plants due to higher carbohydrate content in the roots and increased exudation of

**Table 5.1** Diversity of phosphate solubilizing microorganisms (PSMs)

| Genus | PSMs | Reference |
|---|---|---|
| Bacteria | *Chryseobacterium*, *Gordonia*, *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis*, and *Delftia* sp. | Chen et al. (2006) |
| | *Pseudomonas aeruginosa*, *Pseudomonas mosselii*, *Pseudomonas monteilii*, *Pseudomonas plecoglossicida*, *Pseudomonas putida*, *Pseudomonas fulva*, and *Pseudomonas fluorescens* | Naik et al. (2008) |
| | *Exiguobacterium acetylicum* | Selvakumar et al. (2010) |
| | *Erwinia rhapontici* and *Pseudomonas chlororaphis* | Muleta et al. (2013) |
| | *Pantoea agglomerans* and *Burkholderia anthina* | Walpola and Yoon (2013) |
| | *Pantoea cypripedii* and *Pseudomonas plecoglossicida* | Kaur and Reddy (2013) |
| | *Acinetobacter* sp., *Klebsiella* sp., *Enterobacter* sp., *Bacillus* sp., *Bacillus megaterium*, *Paenibacillus taichungensis*, *Ochrobactrum* sp., and *Sphingobacterium* sp. | Zhang et al. (2017) |
| | *Burkholderia fungorum*, *Paenibacillus* sp., *Paenebacillus kribbensis*, *Pseudomonas* sp., and *Acinetobacter* sp. | de Amaral et al. (2020) |
| | *Sphingobacterium thalpophilum*, *Klebsiella variicola*, *Ochrobactrum pseudogrignonense*, *Burkholderia tropica*, *Achromobacter xylosoxidans*, and *Pseudomonas aeruginosa* | Nacoon et al. (2020) |
| Actinobacteria | *Actinobispora yunnanensis* | Kaviyarasi et al. (2011) |
| | *Streptomyces rochei*, *Streptomyces carpinensis*, and *Streptomyces thermolilacinus* | Jog et al. (2012) |
| | *Streptomyces* sp., *Streptomyces djakartensis*, *Streptomyces enissocaesilis*, *Streptomyces nobilis*, *Streptomyces mutabilis*, and *Streptomyces kunmingensis* | Anwar et al. (2016) |
| | *Streptomyces* sp., *Nocardioides* sp., *Saccharomonospora* sp., *Actinomadura* sp., and *Prauserella* sp. | Nafis et al. (2019) |
| | *Streptomyces roseocinereus* and *Streptomyces natalensis* | Chouyia et al. (2020) |
| | *Streptomyces alboviridis*, *Streptomyces griseorubens*, *Streptomyces microflavus*, and *Nocardiopsis alba* | Boubekri et al. (2021) |
| Fungi | *Arthrobotrys oligospora* | Duponnois et al. (2006) |
| | *Aspergillus aculeatus* | Narsian and Patel (2000) |
| | *Penicillium expansum*, *Mucor ramosissimus*, and *Candida krissii* | Xiao et al. (2009) |
| | *Trichoderma harzianum*, *Trichoderma viride*, *Aspergillus parasiticus*, *Aspergillus terreus*, *Aspergillus candidus*, *Aspergillus niger*, *Aspergillus rugulosus*, *Penicillium zonatum*, *Penicillium simplicissimum*, and *Penicillium rubrum* | Aseri et al. (2009) |

(continued)

**Table 5.1** (continued)

| Genus | PSMs | Reference |
|---|---|---|
| | *Aspergillus niger*, *Penicillium canescens*, *Eupenicillium ludwigii*, and *Penicillium islandicum* | de Oliveira et al. (2014) |
| | *Chaetomium globosum, Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus sydawi*, *Aspergillus terreus,* and *Aspergillus versicolor* | Selvi et al. (2017) |
| | *Aspergillus hydei* | Doilom et al. (2020) |

sugars and amino acids. Mycorrhizal roots have a higher P absorption rate per unit root length than non-mycorrhizal roots due to an expanded absorption surface as extended extramatricular hyphae reach up to 7 cm out from the roots to feed in a larger region or have an apparent stronger affinity for P. Phosphatase activity is also greater in mycorrhizal roots than in non-mycorrhizal roots. In comparison to the bulk soil, the activity of acid phosphatase was found to be 2- to 2.5-fold higher in the mycorrhizal rhizoplane soil of Norway spruce (Haussling and Marschner 1989). Dense cluster roots formed during symbiosis between plants and arbuscular mycorrhizal fungi (AMF) secrete organic anions/$H^+$ ions that release phosphates from P compounds, thereby enhancing the uptake of soil phosphates by AMF and plants (Smith et al. 2011).

In both low and high P soils, mycorrhizal colonization increased the shoot P content, suggesting that mycorrhization might be a valuable criterion for breeding maize varieties with increased mycorrhizal responsiveness for P absorption in modern agriculture (Chu et al. 2013). AMF can boost the uptake of P in plants by enriching soil PSMs in close vicinity to the extensive hyphae under nutrient-limited conditions, thereby exploiting P-rich soil horizons. Zhang et al. (2016) also reported in their studies that the addition of P to increase soil-available P caused PSBs to enhance the hyphal growth of AMF and, in return, the fungus stimulated the activity of these PSBs. According to Mackay et al. (2017) AMF *Rhizophagus irregularis* plays a major role in wheat P acquisition from dried sewage sludge and hence can aid in the recycling of P in waste. Jangandi et al. (2017) reported that the combined application of *Bacillus polymyxa* and *Rhizophagus fasciculatus* increased plant growth, dry matter, and P uptake in *Terminalia paniculata* and *T. tomentosa* seedlings as compared to uninoculated seedlings and sole inoculants. Wahid et al. (2020) also reported that conjoint application of PSB along with AMF and ground rock phosphate had significant potential for enhancing maize-wheat yields and P uptake as compared to treatment consisting of expensive P-fertilizers in P-deficient soils.

## 5.5 Plant Growth Attributes of PSMs

Nitrogen is an essential nutrient limiting plant growth. PSMs enhance plant growth by increasing the availability of nitrogen to plants (Hassan and Abdelgani 2009). PSMs also promote plant growth by synthesizing various phytohormones. Phytohormones are an intrinsic pathway that controls the metabolic activities in different plant tissues and is elicited by rhizospherice microorganisms (Boivin et al. 2016). Many PSMs are known to secrete phytohormones that are readily taken up by the plant roots and regulate root/shoot growth besides playing a role in stress management (Arkhipova et al. 2005; Zhao and Zhang 2015). PSMs produce ACC deaminase, which hydrolyses 1-aminocyclopropane-1-carboxylate (ACC) to α-ketobutyrate and ammonia, thus reducing the level of ethylene, thereby increasing the root length and growth (Singh et al. 2015). PSMs protect plants by suppressing the growth and proliferation of phytopathogens, typically by producing antibiotics, phytohormones, siderophores, and lytic enzymes (Vassilev et al. 2006; Ali et al. 2020). Moreover, EPS produced by PSMs have an important role in desiccation resistance, microbial aggregation, plant-microbe interaction, surface adhesion, and bioremediation (Naseem et al. 2018). Figure 5.1 depicts multifarious plant growth-promoting traits exhibited by PSM.

Auxins, cytokinins, ethylene, gibberellic acid (GA), and abscisic acid (ABA) are the major classes of hormones involved in plant growth promotion. PGPRs produce a variety of auxins like indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), indole-3-pyruvic acid (IPA), tryptophol (TOL), and indole lactic acid (ILA). Out of these auxins, IAA is the most common phytohormone and its production is an important characteristic of rhizospheric bacteria that promotes plant growth and

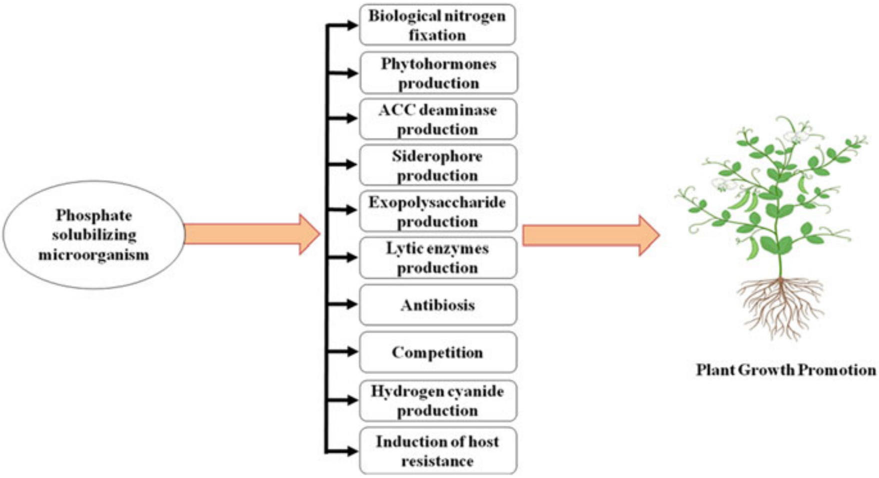

**Fig. 5.1** Phosphate solubilizing microorganism showing multifarious plant growth-promoting traits

development. Cytokinins play an important role in plant development by regulating cell division and differentiation. Regulation of plant organogenesis and root architecture depends upon the equilibrium between auxins and cytokinin levels (Kudoyarova et al. 2019). Synthesis of cytokinins by various PSMs increases root exudates production by the plants and hence increases interactions between the PGPR and the plant (Kudoyarova et al. 2014). Several studies have described the potential of PSMs in the production and regulation of ABA or GA hormones in plants. ABA phytohormone is well known for its significance in drought stress conditions. GA stimulates primary root elongation and lateral root extension with the aid of other phytohormones and extra-regulatory mechanisms. Kang et al. (2009) isolated *Acinetobacter calcoaceticus* SE370 from soil and screened it for the production of extracellular GA and phosphate solubilization. The isolate was capable of producing ten different gibberellins, namely, $GA_1$, $GA_3$, $GA_4$, $GA_9$, $GA_{12}$, $GA_{15}$, $GA_{19}$, $GA_{20}$, $GA_{24}$, and $GA_{53}$. Bioactive $GA_3$ and $GA_4$ were produced at the rate of 6.25 ng/100 mL and 2.83 ng/100 mL respectively, whereas bioactive $GA_1$ was produced at the rate of 0.45 ng/100 mL of culture filtrate. The isolate solubilized TCP and lowered the pH of the medium. Culture filtrates significantly promoted the growth of cucumber, Chinese cabbage, and crown daisy as compared to control. Zhao and Zhang (2015) conducted a study to show that *Trichoderma asperellum* Q1 isolate could solubilize P in the culture supernatant. This strain also possessed the capability of producing GA, IAA, and ABA. Moreover, inoculation of cucumber seedlings with *T. asperellum* Q1 also enhanced the root growth and root activities. In another study, three rhizobacterial strains viz. *Pseudomonas stutzeri* MTP40, *Stenotrophomonas maltophilia* MTP42, and *Pseudomonas putida* MTP50 were characterized for their phytohormone-producing ability. IAA was detected in all three isolates, wherein highest production was found in *S. maltophilia* MTP42 (240 μg/mL) followed by *P. stutzeri* MTP40 (250 μg/mL) and *P. putida* MTP50 (233 μg/mL). The production of GA was recorded to be maximum in isolate MTP40 (34 μg/mL), followed by isolate MTP42 (31 μg/mL) and MTP50 (27 μg/mL). Whereas, cytokinin production by the isolates viz., MTP40, MTP42, and MTP50 were recorded to be 13, 11, and 7.5 μg/mL, respectively (Patel and Saraf 2017).

Exopolysaccharides (EPSs) help the bacteria to readily colonize the plant rhizosphere, stick to the surface of roots, and maintain moisture content thereby contributing to soil structure and stability. EPS have immense agro-economical importance including the survivability and maintenance of microbial communities in their habitat (Gauri et al. 2012). It has been reported that plants inoculated with EPS-producing microbes are more tolerant to drought stress due to better soil-aggregation capability and water-holding capacity by such strains which in turn promotes plant growth (Kaushal and Wani 2016). The application of microbial antagonists as biocontrol agents against phytopathogens is achieved via the synthesis of antibacterial and antifungal compounds, extracellular lytic enzymes, siderophore production, intra-rhizosphere competition, hydrogen cyanide (HCN) production, and induction of host resistance. The class of antibiotics produced by microorganisms to control root diseases in plants include pyrrolnitrin, phenazine-1-carboxyclic acid, 2,4-diacetyl phloroglucinol, oomycin, pyoluteorin, kanosamine, zwittermycin-A, and pantocin (Hamid et al. 2021). The important hydrolytic enzymes used as defense systems against phytopathogenic agents are chitinases, lipases, cellulases, proteases, and glucanases. Then the siderophores produced also act as biocontrol agents by preventing phytopathogens from the acquisition of sufficient amount of iron thereby hindering the growth ability of such pathogens. Competition for substrate in the rhizosphere is mediated by this feature. Siderophores are iron-chelating low-molecular-weight compounds with a high affinity for Fe produced by bacteria and fungi living under low iron conditions (Das et al. 2007; Schalk et al. 2011). Siderophores can be classified into three main functional groups, that is, hydroxamates, catecholates, and carboxylates depending upon the oxygen ligands for Fe coordination (Miethke and Marahiel 2007). Kuzyk et al. (2021) reported for the first time that aerobic anoxygenic phototrophs produced highly diffusible secondary metabolites, that is, siderophores, which might lead to metal(loid) chelation and detoxification in their surroundings.

The production of HCN by various rhizobacteria also acts as broad-spectrum biocontrol agents by hindering the growth and proliferation of pathogenic microbes. HCN efficiently inhibits the cytochrome oxidase pathway and is extremely toxic to all aerobic microorganisms at picomolar concentrations. Certain fluorescent pseudomonads are thought to be involved in the control of root infections by producing HCN (Voisard et al. 1989). Agbodjato et al. (2015) screened *Bacillus* sp., *Pseudomonas* sp., and *Serratia* sp. for HCN production and revealed that all strains (100%) were able to produce HCN with the appearance of red color on the soaked filter paper. Rijavec and Lapanje (2016) proposed a new concept where HCN was involved in the chelation of metals thereby indirectly enhancing the availability of phosphate which is beneficial for plant growth.

Another effective strategy for disease control in plants induced by microorganisms is induced systemic resistance (ISR) and systemic acquired resistance (SAR). ISR is induced by soil-borne microbes that colonize the plant roots and SAR is mediated by pathogens (Romera et al. 2019). ISR is mediated by jasmonic acid and ethylene, whereas salicylic acid is required for SAR response. Multiple plant growth-promoting traits associated with PSMs are shown in Table 5.2.

## 5.6 Plant Response to the Inoculation of PSMs

Biofertilizers are preparations containing latent cells of selected and beneficial living microorganisms which, when applied to the seeds or plant surfaces adjacent to soil, can colonize the rhizosphere or the interior parts of the plants and thereby promote root growth that not only helps in the uptake of nutrients by plants but also reduces the quantum of inorganic nutrients or organic manures to be applied. Amendment of soil with efficient PSM in P-deficient soil plays a vital role in better crop productivity, greater yield performance, and maintenance of the soil's nutrient status. Inoculation of PSMs in soil or seeds has been shown to improve the solubilization of

**Table 5.2** Multifarious plant growth-promoting traits of phosphate solubilizing microorganisms (PSMs)

| PSMs | Plant growth promoting traits | Reference |
|---|---|---|
| *Rhizobium leguminosarum* bv. *Phaseoli* P31 | IAA, siderophore, and HCN producer | Chabot et al. (1996) |
| *Burkholderia* sp. J62 | IAA and siderophore producer. Also showed ACC deaminase activity | Jiang et al. (2008) |
| *Exiguobacterium acetylicum* 1P (MTCC 8707) | IAA, siderophore, and HCN producer | Selvakumar et al. (2010) |
| *Klebsiella* SN 1.1 | IAA producer | Chaiharn and Lumyong (2011) |
| *Burkholderia* sp. (MTCC 8369) | IAA, siderophore, HCN, protease, and EPS producer. Also showed 48% growth inhibition against *Fusarium oxysporum* and 50% inhibition against *Phytophthora* sp. | Stephen and Jisha (2011) |
| *Streptomyces rochei* IDWR19, *Streptomyces carpinensis* IDWR53, and *Streptomyces thermolilacinus* IDWR81 | IAA and siderophore producer | Jog et al. (2012) |
| *Pantoea cypripedii* and *Pseudomonas plecoglossicida* | IAA and siderophore producer | Kaur and Reddy (2013) |
| *Ochrobactrum haematophilum* HB36 | IAA and siderophore producer | Gao et al. (2016) |
| *Bacillus megaterium* M08 | IAA and siderophore producer | Zhang et al. (2017) |
| *Pseudomonas koreensis* MS16 and *Enterobacter cloacae* MS32 | IAA and GA producer, solubilized zinc compounds, and showed nitrogenase and ACC deaminase activity | Suleman et al. (2018) |
| *Pseudomonas fluorescens* and *Bacillus cereus* | Siderophore producer | Abbas et al. (2019) |
| *Streptomyces* sp. (MNC-1, MNT-1, MNB-2, and KNC-5), *Saccharomonospora* sp. LNS-1, and *Nocardioides* sp. KNC-3 | IAA and siderophore producer | Nafis et al. (2019) |
| *Pseudomonas aeruginosa* PS2 and *Pseudomonas aeruginosa* PS3 | IAA, cytokinin, siderophore, and HCN producer and showed ACC deaminase activity | Linu et al. (2019) |
| *Pseudomonas* sp. (UFPI B5-8A) and *Burkholderia fungorum* (UFLA 04-155) | IAA producer | de Amaral et al. 2020 |
| *Streptomyces roseocinereus* MS1B15 | Siderophore and IAA producer, showed ACC deaminase activity and exhibited antimicrobial activity against *Fusarium oxysporum*, *Botrytis cinerea*, *Phytophthora cactorum*, and *Phytophthora cryptogea* | Chouyia et al. (2020) |

(continued)

**Table 5.2** (continued)

| PSMs | Plant growth promoting traits | Reference |
|---|---|---|
| *Enterobacter* sp. ITCB-09 | Siderophore and EPS producer | Mendoza-Arroyo et al. (2020) |
| *Klebsiella variicola* | IAA producer | Nacoon et al. (2020) |
| *Rhizobium* sp. V3E1 | IAA producer | Lebrazi et al. (2020) |
| *Enterobacter* 64S1, *Pseudomonas* 42P4, *Cellulosimicrobium* 60I1, and *Ochrobactrum* 53F | IAA and siderophore producer, and nitrogen fixer | Perez-Rodriguez et al. (2020) |
| *Pseudomonas grimontii* (CFML97-514) | IAA, siderophore producer, and showed nitrogenase and ACC deaminase activity | Chen et al. (2021) |

applied and fixed phosphates, resulting in a better crop yield. By establishing an expanded network around the root system, PSM is thought to help in the absorption of P from a broader region. Increased fruit and foliage nutrient contents (N, P, K, Ca, and Fe) were observed after inoculation with *Bacillus* FS3 and *Aspergillus* FS9. These PSMs showed significant potential as yield-enhancing soil supplements in Turkey's P-deficient calcareous soils (Gunes et al. 2009). A significant increase in growth parameters, grain yield, total P uptake, and soil fertility was observed when inoculated with two PSB, namely, *Pantoea cypripedii* and *Pseudomonas plecoglossicida* along with RP (Kaur and Reddy 2013). Walpola and Yoon (2013) reported enhanced shoot and root length, shoot and root dry matter, and P uptake in mung bean plants when co-inoculated with *Pantoea agglomerans* and *Burkholderia anthina*. Rafique et al. (2017) studied that inoculation of PSB *Lysinibacillus fusiformis* strain 31MZR with sawdust biochar showed positive effects on maize plant height, root and shoot length, and nutrient concentration.

Ahmad et al. (2018) reported a significant increase in shoot length, root length, and root fresh weight of cotton on inoculation with *Bacillus subtilis* strain Q3. However, the maximum increase in the shoot's fresh weight was observed with *Paenibacillus* sp. strain Q6. Blanco-Vargas et al. (2020) observed >90% germination of *Allium cepa* L. seeds after co-inoculation with phosphate solubilizing *Pseudomonas* sp. (A18) and *Serratia* sp. (C7). Further, they recorded an increase in total dry weight (69 ± 13 mg) compared to the total dry weight (38 ± 5.0 mg) of control with water. Qarni et al. (2021) reported that both bacterial and fungal strains showed the potential of increasing P uptake by plants as well as increased soil available P after harvest. The studies thereby suggested that the application of such P-solubilizers could prove a better option for the utilization of indigenous soil phosphate reserves for sustainable agriculture. The phosphate solubilizing microbial communities have shown considerable outcomes for plants when employed singly or in combination with other beneficial microorganisms as presented in Table 5.3.

**Table 5.3** Beneficial effects of phosphate solubilizing microorganisms (PSMs) on host plants

| PSMs | Host plant | Beneficial effects on growth parameters | Reference |
|---|---|---|---|
| *Penicillium bilaji* | Wheat | Plant dry matter yield and total plant P uptake was significantly increased by 16% and 14%, respectively | Asea et al. (1988) |
| *Bacillus subtilis* ($TT_0$) | Mung bean | Improved nodulation, the available $P_2O_5$ content of the alluvial soil, root and shoot biomass, straw and grain yield, as well as P and N uptake of the crop | Gaind and Gaur (1991) |
| *Penicillium pinophilum* | Faba bean | Increased the yield of faba bean seeds by 14.7% and 29.4% in the soil treated with rock phosphate and superphosphate. The uptake of P by crop was also significantly increased | Wahid and Mehana (2000) |
| *Aspergillus niger* and *Penicillium italicum* | Soybean | Significantly increased plant height, number of pods/plant, dry matter, and yield. A significant increment in the percentage of protein and oil was also recorded. The physiochemical properties of the soil were also improved | El-Azouni (2008) |
| *Aspergillus niger* and *Penicillium* sp. | Mung bean | Significantly increased growth, seed yield, and P uptake as well as improved nodulation status | Saber et al. (2009) |
| *Rhizobium* strain TAL 169 and *Bacillus megaterium* var. *phosphaticum* | Lablab bean | Increased nodulation, nodule dry weight, shoot dry weight, and N and P content in the shoot | Hassan and Abdelgani (2009) |
| *Candida krissii* HB-3 | Wheat | Increased shoot length, root length, shoot and root dry weight, available P content in the soil. Also recorded an increase of 27.6% in P uptake and 19.3% in N uptake over the control | Xiao et al. (2009) |
| *Exiguobacterium acetylicum* 1P (MTCC 8707) | Wheat | Recorded 16.54 and 8.16% higher root and shoot lengths, respectively. An increase of 51.9, 38.5, and 45.4% in the uptake of N, P, and K were also recorded | Selvakumar et al. (2010) |
| *Bacillus* sp. | Bell pepper | Enhanced the plant emergence, root and shoot length, biomass, fruit yield, and available NPK content | Mandyal et al. (2012) |
| *Pseudomonas* sp. and *Rhizobium* sp. | Faba bean | Increased the percentage of seed germination, vigor index, radical and plumule length. Plant height, root length, P content, P uptake, nodule number, and nodule weight were also improved | Demissie et al. (2013) |
| *Trichoderma asperellum* | Cherry tomato | Promoted leaf number, total leaf area, leaf dry mass, and shoot dry mass | Franca et al. (2017) |

(continued)

**Table 5.3** (continued)

| PSMs | Host plant | Beneficial effects on growth parameters | Reference |
|---|---|---|---|
| *Azotobacter* (SR-4) and *Aspergillus niger* | Bottle gourd and okra | Showed significantly increased plant height, leaf length/width, fruit size, and the number of fruits per plant | Din et al. (2019) |
| *Streptomyces roseocinereus* MS1B15 | Barley | Significantly increased shoot and ear length as well as the number of ears. Also increased available P in ears and leaves and P and N contents in the soil | Chouyia et al. (2020) |
| *Nocardiopsis alba* BC11 | Wheat | Significantly improved root length, root volume, root dry weight, shoot length, and shoot dry weight | Boubekri et al. (2021) |
| *Funneliformis mosseae* and *Apophysomyces spartima* | Beach palm | Increased growth parameters, photosynthetic efficiency, and the concentration of photosynthetic pigments under saline conditions by enhancing N, P, and K uptake | Zai et al. (2021) |

## 5.7 Screening of PSMs

Pikovskaya (1948) was the first to describe a reliable method for preliminary screening and isolation of potential PSM. It works by plating 0.1 mL of serially diluted rhizospheric soil suspension onto a sterilized Pikovaskaya's (PVK) medium supplemented with TCP as a P source. Colonies forming a clear halo zone after incubation at an appropriate temperature are screened as P-solubilizers. P-solubilizing ability can be assessed in terms of the solubilization index (SI) and solubilization efficiency (%).

$$\text{Solubilization index (SI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

$$\text{Solubilization efficiency (\%)} = \frac{\text{Halozne diameter} - \text{Colony diameter}}{\text{Colony diameter}} \times 100$$

## 5.8 Mechanisms Employed by PSMs for Phosphate Solubilization and Mineralization

PSMs employ different mechanisms for solubilizing and mineralizing P based on the organic and inorganic types of P-source present in the soil.

### 5.8.1 Mechanism of Phosphate Solubilization

Mineral phosphate solubilization is the conversion of an unavailable form of inorganic P to a P-accessible form. The major mechanism of inorganic P-solubilization involves the secretion of organic acids in the soil solution, which results in acidification of the surrounding soil, releasing soluble orthophosphate ions from insoluble sources which are readily taken up by plants. These organic acids chelate with the cations associated with phosphate and release dissolvable forms of phosphate. In vitro gluconic acid production and phosphate solubilization by *Azospirillium brasilens* (Cd and 8-I) and *A. lipoferum* JA4 were observed by Rodriguez et al. (2004). Stephen and Jisha (2011) identified gluconic acid as the principal organic acid via HPLC analysis of the culture filtrate of *Burkholderia* sp. (MTCC 8369). HPLC of cell-free supernatant of *Enterobacter* sp. Fs-11 produced gluconic acid (16.64 μg/mL) and malic acid (2.43 μg/mL) in Pikovskaya's broth (Shahid et al. 2012). Jog et al. (2014) reported that *Streptomyces* mhcr0816 produced a high amount of malic acid (RT 13.1 min, 50–55 mmol/L). In another study, the amount of succinic acid produced by *Bacillus megaterium* Y924 was strongly linearly correlated with the amount of P released, suggesting that organic acid may mobilize microbial P (Zheng et al. 2018).

Further, the secretion of organic acids by the P-solubilizing fungi has also been well documented. Akintokun et al. (2007) observed that *Aspergillus niger* produced the highest amount of malic acid (18.20 mg/100 mL) in the rock phosphate medium, whereas *Aspergillus terreus* was found to produce the highest amount of fumaric acid (264.45 mg/100 mL) in the TCP medium. Rinu and Pandey (2011) isolated a psychrotolerant P-solubilizing fungus, *Paecilomyces hepiali* (MTCC 9621), from the rock soil of a cold desert site in the Indian Himalayas. The tested fungus was found to be more efficient at producing gluconic acid (4.77 μg/mL) than the other four acids, namely, malic, succinic, α-keto glutaric, and citric. *Penicillium oxalicum* P4 released complex mixtures of organic acids. The dominant organic acids were tartaric and citric acids, followed by lesser amounts of succinic, acetic, lactic, and oxalic acids whereas, malic, formic, and fumaric acids were detected in traces (Yin et al. 2015). The production of various organic acids from PSMs is depicted in Table 5.4.

Inorganic acids such as sulfuric acid, hydrochloric acid, carbonic acid, and nitric acid also help in P-solubilization. Acidophilic and sulfur-oxidizing bacteria create $H_2S$, which interacts with ferric phosphate to form ferrous sulfate, releasing the bound P (Florentino et al. 2016). The sulfur-oxidizing bacterium *Delftia* sp. strain SR4 converted elemental sulfur and thiosulfate converted to sulfate. This strain exhibited up to 116% higher P-solubilizing efficiency in *Brassica juncea* plants as compared to the uninoculated plants (Roy and Roy 2019). Pumping out of protons from the cell is also one of the major aspects responsible for P-solubilization. Illmer et al. (1995) reported that the most probable explanation for microbial solubilization without acid production is thought to be proton ($H^+$) excretion accompanying $NH_4^+$ assimilation. Additionally, the production of siderophores and EPSs has also been

**Table 5.4** Organic acids produced by phosphate solubilizing microorganisms (PSMs)

| Organic acid produced | PSMs | Reference |
|---|---|---|
| Citric acid and 2-ketogluconic acid | *Penicillium* sp. LAF$_2$ | Banik and Dey (1983) |
| 2-ketogluconic acid | *Rhizobium leguminosarum* biovar viceae BICC635 | Halder et al. (1990) |
| Citric acid and oxalic acid | *Penicillium bilaii* | Cunningham and Kuiack (1992) |
| 2-ketogluconic acid | *Enterobacter intermedium* | Hwangbo et al. (2003) |
| Citric acid and oxalic acid | *Penicillium bilaiae* | Takeda and Knight (2006) |
| Gluconic acid and oxalic acid | *Aspergillus niger* isolates 1B and 6A | Chuang et al. (2007) |
| Gluconic acid and citric acid | *Serratia marcescens* CTM 50650 | Farhat et al. (2009) |
| 2-ketogluconic acid and gluconic acid | *Erwinia rhapontici* (AUEY28) and *Pseudomonas chlororaphis* (AUPY10) | Muleta et al. (2013) |
| Oxalic acid | *Penicillium oxalicum* I1 | Gong et al. (2014) |
| Gluconic acid | *Penicillium canescens* FS23, *Eupenicillium ludwigii* FS27, and *Penicillium islandicum* FS30 | de Oliveira et al. (2014) |
| Gluconic acid | *Pseudomonas fluorescens* strain L228 | Oteino et al. (2015) |
| Citric acid and oxalic acid | *Aspergillus tubingensis* SANRU | Jamshidi et al. (2016) |
| Acetic acid, gluconic acid, formic acid, and propionic acid | *Pantoea* sp. Pot1 | Sharon et al. (2016) |
| Gluconic acid, tartaric acid, and acetic acid | *Psychrobacter alimentarius* HB15 | Gao et al. (2016) |
| Malic acid, lactic acid, and acetic acid | *Serratia* sp. | Behera et al. (2017) |
| Citric acid, succinic acid, fumaric acid, and gluconic acid | *Azospirillum* sp. | Selvi et al. (2017) |
| Gluconic acid, acetic acid, oxalic acid, and succinic acid | *Pseudomonas koreensis* MS16 | Suleman et al. (2018) |
| Oxalic acid, tartaric acid, and citric acid | *Aspergillus niger* CS-1 | Wang et al. (2018) |
| Gluconic acid, lactic acid, acetic acid, and succinic acid | *Bacillus megaterium* | Saeid et al. (2018) |
| Gluconic acid, citrate acid, succinic acid, α-ketoglutaric acid, and pyruvic acid | *Pantoea ananatis* HCR2 | Xu et al. (2019) |
| Oxalic acid, quinic acid, and lactic acid | *Burkholderia fungorum* (UFLA 04-155) | de Amaral et al. 2020 |
| Gluconic acid, oxalic acid, lactic acid, and acetic acid | *Klebsiella variicola* | Nacoon et al. (2020) |

correlated with phosphate solubilization. Siderophores are iron-chelating agents that selectively bind with ferric ion ($Fe^{3+}$) and actively transport it to the microbial cells. Aallam et al. (2021) reported acidification of the medium and excretion of siderophores responsible for solubilization of RP and TCP by actinomycete strains. EPSs are high molecular weight polymers that mostly provide cell adhesion and protection against negative environmental conditions. Yi et al. (2008) found that three phosphate solubilizing bacteria, namely, *Enterobacter* sp. EnHy-401, *Arthrobacter* sp. ArHy-505, and *Azotobacter* sp. AzHy-510 producing EPS showed a stronger ability for P-solubilization than *Enterobacter* sp. EnHy-402 that does not show any EPS production. *Enterobacter* sp. EnHy-401 exhibited a stronger capacity for P-solubilization with the highest EPS production (3.18 g/L) compared to the other strains. Goldstein (1995) proposed that the extracellular oxidation pathway by microorganisms dissolves insoluble phosphates present in the soil. Glucose is transformed to gluconic acid by glucose dehydrogenase, which is then oxidized to 2-ketogluconic acid by gluconate dehydrogenase in the direct oxidation process. Minerals that are phosphate-bound are chelated by these acids (Krishnaraj and Goldstein 2001).

### 5.8.2 Mechanism of Phosphate Mineralization

Soil microorganisms are effective in releasing P from the organic pools of P by mineralization with the help of different enzymes. Phosphatase enzymes mineralize organic P compounds through two distinct enzymes, namely, phosphodiesterase and phosphomonoesterase. Phosphodiesterase catalyzes the hydrolysis of complex organic P compounds into phosphomonoesters. Phosphomonoesterase further mineralizes these compounds into orthophosphate, which can be directly absorbed by plants. Phosphatases vary widely in metal ion requirements, pH ranges, and substrate specificities.

Phytic acid (myo-inositol hexakisphosphate) is a major reservoir of phosphate found in mature seeds of both monocot and dicot plants. Phytases cleave the phosphomonoester bonds of phytic acid and liberate myo-inositol, inositol phosphate, and inorganic phosphate. Phytases were first identified in rice bran. Phytases are widespread and can be produced by a wide range of plant and animal tissues and microorganisms such as bacteria, yeast, and fungi. The phytase producing microbial genera include *Aspergillus* (Neira-Vielma et al. 2018), *Bacillus* (Shimizu 1992; Liu et al. 2018), *Penicillium* (Tseng et al. 2000), *Rhizopus* (Sabu et al. 2002), *Emericella* (Yadav and Tarafdar 2007), *Streptomyces* (Aly et al. 2015), *Enterobacter* and *Serratia* (Yoon et al. 1996; Kalsi et al. 2016). Phosphonatases and Carbon-Phosphorus (C-P) lyases play an important role in the mineralization of organic P. The role of PSM in P-solubilization and mineralization are depicted in Fig. 5.2.

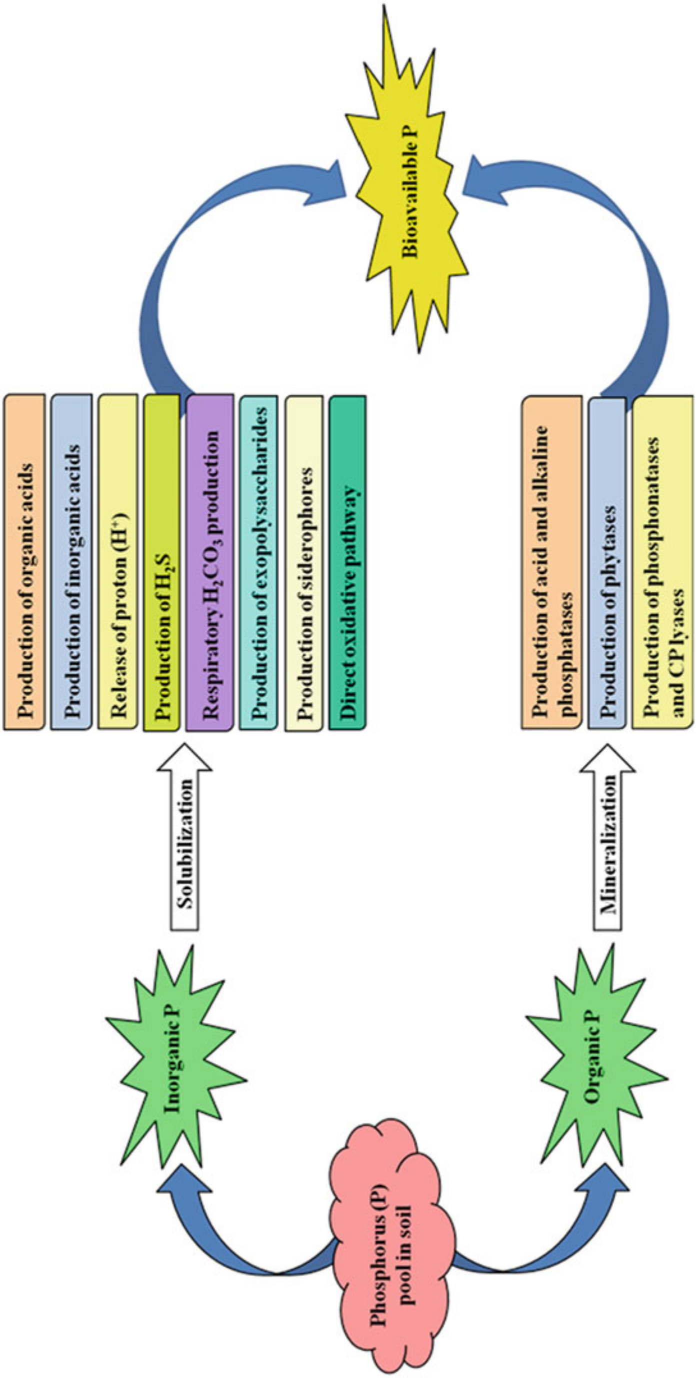

**Fig. 5.2** Solubilization and mineralization of P by phosphate solubilizing microorganism

## 5.9 Obtainment of Improved Phosphate Solubilizing Activity by Genetic Engineering

The mineral phosphate solubilization (MPS) involves the synthesis of gluconic acid, which is produced from glucose involving a glucose dehydrogenase (GDH) enzyme which requires the cofactor pyrroloquinoline quinone (PQQ). PQQ is the non-covalently bound prosthetic group of quinoproteins. It serves as the redox cofactor for several bacterial dehydrogenases such as glucose dehydrogenase and methanol dehydrogenase. The *pqq* genes involved in PQQ synthesis have been characterized in several bacteria such as *Acinetobacter calcoaceticus* (Goosen et al. 1989), *Methylobacterium organophilum* (Biville et al. 1989), *Klebsiella pneumoniae* (Meulenberg et al. 1992), *Methylobacterium extorquens* (Springer et al. 1996; Toyama et al. 1997), *Gluconobacter oxydans* (Felder et al. 2000), and *Pseudomonas aeruginosa* (Gliese et al. 2004). Goldstein and Liu (1987) cloned a gene engaged in MPS from *Erwinia herbicola*. The cloned gene resulted in the generation of gluconic acid and conferred MPS activity in *Escherichia coli* HB101. Liu et al. (1992) did a sequence analysis of the gene and discovered that it was involved in the synthesis of PQQ synthase. PQQ synthase causes the synthesis of the cofactor PQQ, which is involved in the generation of the holoenzyme glucose dehydrogenase (GDH). Babu-Khan et al. (1995) isolated another MPS gene, *gabY*, from *Pseudomonas cepacia*, which conferred MPS activity to *E. coli* JM109 but showed homology with histidine permease system membrane protein, differing from previously cloned PQQ synthetase gene. Kim et al. (1998) have cloned the genes that confer the MPS trait from *Rahnella aquatilis* into *E. coli* strains HB101 and DH5α. *E. coli* strains confer the ability to solubilize hydroxyapatite and produce gluconic acid. Rodriguez et al. (2000) transformed and expressed the *Erwinia herbicola* PQQ gene in *E. coli* MC1061. The recombinant plasmids were introduced to *Burkholderia cepacia* IS-16 and *Pseudomonas* sp. PSS cells by conjugation. Clones carrying recombinant plasmids developed higher clearing halos on plates containing insoluble phosphate as the P source.

In another attempt, the napA phosphatase gene isolated from *Morganella morganii* was cloned into *Burkholderia cepacia* IS-16 using a vector pRK293. The recombinant strain displayed higher extracellular phosphatase activity (Fraga et al. 2001). In another study, *E. coli* DH5α expressing the *pqq* gene cluster of *Enterobacter intermedium* (60-2G) activated an endogenous glucose dehydrogenase to permit gluconic acid secretion that solubilized the phosphate from hydroxyapatite with a drop in pH to 4.0 (Kim et al. 2003). A substantial increase in oxaloacetate, a precursor in the synthesis of organic acids involved in P-solubilization was observed due to overexpression of *Synechococcus elongatus* PCC 6301 phosphoenolpyruvate carboxylase (*ppc*) gene in *Pseudomonas fluorescens* ATCC 13525 (Buch et al. 2010). Miller et al. (2010) screened a transposon mutant library of *Pseudomonas fluorescens* F113 for TCP solubilization ability. P-solubilization activity was lowered as a result of mutations in the *gcd* and *pqqE* genes. However, it was moderately reduced due to mutations in the *pqqB* gene. It was shown that

P-solubilization is affected by the modifications in the *pqq* biosynthetic genes. Farhat et al. (2013) investigated the co-expression of *gdh* (glucose dehydrogenase) and *pqqABCDE* (pyrroloquinoline quinone cofactor) genes cloned from *Serratia marcescens* CTM 50650 in *E. coli* on MPS ability. It was observed that *E. coli* solubilized TCP (574 mg/L), hydroxyapatite (426 mg/L), and Gafsa rock phosphate (217 mg/L). In another study, *pqqE* of *Erwinia herbicola* and *pqq* gene clusters of *Pseudomonas fluorescens* B16 and *Acinetobacter calcoaceticus* were over-expressed in *Herbaspirillum seropedicae* Z67 (ATCC 35892). Transformants Hs (pSS2) and Hs (pOK53) liberated 125.47 μM and 168.07 μM P, respectively, in a minimal medium containing 50 mM glucose under aerobic conditions. Moreover, under the N-free minimal medium, Hs (pSS2) and Hs (pOK53) not only released significant P but also showed enhanced growth, biofilm formation, and EPS secretion (Wagh et al. 2014).

## 5.10 Conclusion

Modern agriculture is facing the challenge of increasing food production when farmland area is shrinking and phosphate rock supplies are rapidly dwindling. The regular and excessive use of phosphatic fertilizers pose an adverse effect on crop productivity and soil health. PSM enhances plant growth by improving the P acquisition efficiency of plants by converting insoluble forms of P to orthophosphates. The use of PSMs as bioinoculants is an appealing, environmentally friendly, and low-cost alternative strategy to exploit soil native P while minimizing the use of chemical fertilizers.

## References

Aallam Y, Dhiba D, Lemriss S et al (2021) Isolation and characterization of phosphate solubilizing *Streptomyces* sp. endemic from sugar beet fields of the Beni-Mellal region in Morocco. Microorganisms 9:914. https://doi.org/10.3390/microorganisms9050914

Abbas ZR, Al-Ezee AMM, Authman SH (2019) Siderophore production and phosphate solubilization by *Bacillus cereus* and *Pseudomonas fluorescens* isolated from Iraqi soils and soil characterization. Int J Pharm Qual Assur 10:74–79. https://doi.org/10.25258/ijpqa.v9i4.14545

Agbodjato NA, Noumavo PA, Baba-Moussa F et al (2015) Characterization of plant growth promoting rhizobacteria isolated from maize (*Zea mays* L.) in Central and Northern Benin (West Africa). Appl Environ Soil Sci 2015:901656. https://doi.org/10.1155/2015/901656

Ahmad M, Ahmad I, Hilger TH et al (2018) Preliminary study on phosphate solubilizing *Bacillus subtilis* strain Q3 and *Paenibacillus* sp. strain Q6 for improving cotton growth under alkaline conditions. PeerJ 6:5122. https://doi.org/10.7717/peerj.5122

Akintokun AK, Akande GA, Akintokun PO et al (2007) Solubilization of insoluble phosphate by organic acid producing fungi isolated from Nigerian soil. Int J Soil Sci 2:301–307. https://doi.org/10.3923/ijss.2007.301.307

Ali S, Hameed S, Shahid M et al (2020) Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. Microbiol Res 232:126389. https://doi.org/10.1016/j.micres.2019.126389

Aly MM, Tork S, Al-Garni SM, Kabli SA (2015) Production and characterization of phytase from *Streptomyces luteogriseus* R10 isolated from decaying wood samples. Int J Agric Biol 17:515–522. https://doi.org/10.17957/IJAB/17.3.14.453

Anwar S, Ali B, Sajid I (2016) Screening of rhizospheric actinomycetes for various *in-vitro* and *in-vivo* plant growth promoting (PGP) traits and for agroactive compounds. Front Microbiol 17: 1334. https://doi.org/10.3389/fmicb.2016.01334

Arkhipova TN, Veselov SU, Melentiev AI et al (2005) Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 272:201–209. https://doi.org/10.1007/s11104-004-5047-x

Asea PEA, Kucey RMN, Stewart JWB (1988) Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Soil Biol Biochem 20:459–464. https://doi.org/10.1016/0038-0717(88)90058-2

Aseri GK, Jain N, Tarafdar JC (2009) Hydrolysis of organic phosphate forms by phosphatases and phytase producing fungi of arid and semi arid soils of India. Am Eurasian J Agric Environ Sci 5: 564–570

Babu-Khan S, Yeo TC, Martin WL et al (1995) Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia*. Appl Environ Microbiol 61:972–978. https://doi.org/10.1128/AEM.61.3.972-978.1995

Banik S, Dey BK (1983) Phosphate-solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate as a sole phosphate source. Zbl Mikrobiol 138:17–23. https://doi.org/10.1016/S0232-4393(83)80060-2

Bashir K, Ali S, Umair A (2011) Effect of different phosphorus levels on xylem sap components and their correlation with growth variables of mash bean. Sarhad J Agric 27:595–601

Begum N, Qin C, Ahanger MA et al (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 10:1068. https://doi.org/10.3389/fpls.2019.01068

Behera BC, Yadav H, Singh SK et al (2017) Phosphate solubilization and acid phosphatase activity of *Serratia* sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. J Genet Eng Biotechnol 15:169–178. https://doi.org/10.1016/j.jgeb.2017.01.003

Biville F, Turlin E, Gasser F (1989) Cloning and genetic analysis of six pyrroloquinoline quinone biosynthesis genes in *Methylobacterium organophilum* DSM 760. J Gen Microbiol 135:2917–2929. https://doi.org/10.1099/00221287-135-11-2917

Blanco-Vargas A, Rodriguez-Gacha LM, Sanchez-Castro N et al (2020) Phosphate solubilizing *Pseudomonas* sp., and *Serratia* sp., co-culture for *Allium cepa* L. growth promotion. Heliyon 6: 05218. https://doi.org/10.1016/j.heliyon.2020.e05218

Boivin S, Fonouni-Farde C, Frugier F (2016) How auxin and cytokinin phytohormones modulate root microbe interactions. Front Plant Sci 7:1240. https://doi.org/10.3389/fpls.2016.01240

Boubekri K, Soumare A, Mardad I et al (2021) The screening of potassium- and phosphate-solubilizing actinobacteria and the assessment of their ability to promote wheat growth parameters. Microorganisms 9:470. https://doi.org/10.3390/microorganisms9030470

Buch A, Archana G, Nareshkumar G (2010) Heterologous expression of phosphoenolpyruvate carboxylase enhances the phosphate solubilizing ability of fluorescent pseudomonads by altering the glucose catabolism to improve biomass yield. Bioresour Technol 101:679–687. https://doi.org/10.1016/j.biortech.2009.08.075

Chabot R, Antoun H, Cescas MP (1996) Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar, *phaseoli*. Plant Soil 184:311–321. https://doi.org/10.1007/BF00010460

Chaiharn M, Lumyong S (2011) Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. Curr Microbiol 62:173–181. https://doi.org/10.1007/s00284-010-9674-6

Chen YP, Rekha PD, Arun AB et al (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41. https://doi.org/10.1016/j.apsoil.2005.12.002

Chen J, Zhao G, Wei Y et al (2021) Isolation and screening of multifunctional phosphate solubilizing bacteria and its growth-promoting effect on Chinese fir seedlings. Sci Rep 11: 9081. https://doi.org/10.1038/s41598-021-88635-4

Chittora P, Sharma D, Aseri GK et al (2020) Fungi as phosphate-solubilizing microorganisms in arid soil: current perspective. In: Singh J, Gehlot P (eds) New and future developments in microbial biotechnology and bioengineering: recent advances in application of fungi and fungal metabolites: environmental and industrial aspects. Elsevier, pp 99–108. https://doi.org/10.1016/B978-0-12-821007-9.00009-7

Chouyia FE, Romano I, Fechtali T et al (2020) P-solubilizing *Streptomyces roseocinereus* MS1B15 with multiple plant growth-promoting traits enhance barley development and regulate rhizosphere microbial population. Front Plant Sci 11:1137. https://doi.org/10.3389/fpls.2020.01137

Chu Q, Wang X, Yang Y et al (2013) Mycorrhizal responsiveness of maize (*Zea mays* L.) genotypes as related to releasing date and available P content in soil. Mycorrhiza 23:497–505. https://doi.org/10.1007/s00572-013-0492-0

Chuang CC, Kuo YL, Chao CC, Chao WL (2007) Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger*. Biol Fertil Soils 43:575–584. https://doi.org/10.1007/s00374-006-0140-3

Cunningham JE, Kuiack C (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. Appl Environ Microbiol 58:1451–1458. https://doi.org/10.1128/aem.58.5.1451-1458.1992

Das A, Prasad R, Srivastava A, Giang PH, Bhatnagar K, Varma A (2007) Fungal siderophores: structure, functions and regulations. In: Varma A, Chincholkar SB (eds) Microbial siderophores, vol 12. Springer-Verlag, Berlin, pp 1–42

de Amaral LA, de Souza Cardoso AA, de Almeida LR et al (2020) Selected bacterial strains enhance phosphorus availability from biochar-based rock phosphate fertilizer. Ann Microbiol 70:6. https://doi.org/10.1186/s13213-020-01550-3

de Oliveira MG, de Freitas ALM, Pereira OL et al (2014) Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Ann Microbiol 64:239–249. https://doi.org/10.1007/s13213-013-0656-3

Demissie S, Muleta D, Berecha G (2013) Effect of phosphate solubilizing bacteria on seed germination and seedling growth of faba bean (*Vicia faba* L.). Int J Agric Res 8:123–136. https://doi.org/10.3923/ijar.2013.123.136

Din M, Nelofer R, Salman M et al (2019) Production of nitrogen fixing *Azotobacter* (SR-4) and phosphorus solubilizing *Aspergillus niger* and their evaluation on *Lagenaria siceraria* and *Abelmoschus esculentus*. Biotechnol Rep 20:00323. https://doi.org/10.1016/j.btre.2019.e00323

Doilom M, Guo JW, Phookamsak R et al (2020) Screening of phosphate-solubilizing fungi from air and soil in Yunnan, China: four novel species in *Aspergillus*, *Gongronella, Penicillium* and *Talaromyces*. Front Microbiol 11:585215. https://doi.org/10.3389/fmicb.2020.585215

Duponnois R, Kisa M, Plenchette C (2006) Phosphate-solubilizing potential of the nematophagous fungus *Arthrobotrys oligospora*. J Plant Nutr Soil Sci 169:280–282. https://doi.org/10.1002/jpln.200520551

El-Azouni IM (2008) Effect of phosphate solubilizing fungi on growth and nutrient uptake of soybean (*Glycine max* L.) plants. J Appl Sci Res 4:592–598

Farhat MB, Farhat A, Bejar W et al (2009) Characterization of the mineral phosphate solubilizing activity of *Serratia marcescens* CTM 50650 isolated from the phosphate mine of Gafsa. Arch Microbiol 191:815–824. https://doi.org/10.1007/s00203-009-0513-8

Farhat MB, Fourati A, Chouayekh H (2013) Coexpression of the pyrroloquinoline quinone and glucose dehydrogenase genes from *Serratia marcescens* CTM 50650 conferred high mineral phosphate-solubilizing ability to *Escherichia coli*. Appl Biochem Biotechnol 170:1738–1750. https://doi.org/10.1007/s12010-013-0305-0

Felder M, Gupta A, Verma V et al (2000) The pyrroloquinoline quinone synthesis genes of *Gluconobacter oxydans*. FEMS Microbiol Lett 193:231–236. https://doi.org/10.1111/j.1574-6968.2000.tb09429.x

Florentino AP, Weijma J, Stams AJ, Sanchez-Andrea I (2016) Ecophysiology and application of acidophilic sulfur-reducing microorganisms. In: Rampelotto H (ed) Biotechnology of extremophiles. Springer, Cham, pp 141–715. https://doi.org/10.1007/978-3-319-13521-2-5

Fraga R, Rodriguez H, Gonzalez T (2001) Transfer of the gene encoding the napA acid phosphatase from *Morganella morganii* to a *Burkholderia cepacia* strain. Acta Biotechnol 21:359–369. https://doi.org/10.1002/1521-3846(200111)21:4<359::aid-abio359>3.0.co;2-b

Franca DVC, Kupper KC, Magri MMR et al (2017) *Trichoderma* spp. isolates with potential of phosphate solubilization and growth promotion in cherry tomato. Pesq Agropec Trop 47:360–368. https://doi.org/10.1590/1983-40632017v4746447

Gaind S, Gaur AC (1991) Thermotolerant phosphate solubilizing microorganisms and their interaction with mung bean. Plant Soil 133:141–149. https://doi.org/10.1007/BF00011908

Gao L, Kong F, Feng C et al (2016) Isolation, characterization, and growth promotion of phosphate-solubilizing bacteria associated with *Nicotiana tabacum* (tobacco). Pol J Environ Stud 25:993–1003. https://doi.org/10.15244/pjoes//61820

Garcia-Lopez AM, Aviles M, Delgado A (2015) Plant uptake of phosphorus from sparingly available P-sources as affected by *Trichoderma asperellum* T34. Agric Food Sci 24:249–260. https://doi.org/10.23986/afsci.49532

Gauri SS, Mandal SM, Pati BR (2012) Impact of *Azotobacter* exopolysaccharides on sustainable agriculture. Appl Microbiol Biotechnol 95:331–338. https://doi.org/10.1007/s00253-012-4159-0

Gliese N, Khodaverdi V, Schobert M, Gorisch H (2004) AgmR controls transcription of a regulon with several operons essential for ethanol oxidation in *Pseudomonas aeruginosa* ATCC 17933. Microbiology 150:1851–1857. https://doi.org/10.1099/mic.0.26882-0

Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol Agric Hortic 12:185–193. https://doi.org/10.1080/01448765.1995.9754736

Goldstein AH, Liu ST (1987) Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Nat Biotechnol 5:72–74. https://doi.org/10.1038/nbt0187-72

Gong M, Du P, Liu X, Zhu C (2014) Transformation of inorganic P fractions of soil and plant growth promotion by phosphate-solubilizing ability of *Penicillium oxalicum* I1. J Microbiol 52: 1012–1019. https://doi.org/10.1007/s12275-014-4406-4

Goosen N, Horsman HPA, Huinen RGM, van de Putte P (1989) *Acinetobacter calcoaceticus* genes involved in biosynthesis of the coenzyme pyrrolo-quinoline-quinone: nucleotide sequence and expression in *Escherichia coli* K-12. J Bacteriol 171:447–455

Gunes A, Ataoglu N, Turan M et al (2009) Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. J Plant Nutr Soil Sci 172:385–392. https://doi.org/10.1002/jpln.200800121

Halder AK, Mishra AK, Bhattacharyya P, Chakrabartty PK (1990) Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. J Genet Appl Microbiol 36:81–92. https://doi.org/10.2323/jgam.36.81

Hamdali H, Moursalou K, Tchangbedji G et al (2012) Isolation and characterization of rock phosphate solubilizing actinobacteria from a Togolese phosphatemine. Afr J Biotechnol 11: 312–320

Hamid S, Lone R, Mohamed HI (2021) Production of antibiotics from PGPR and their role in biocontrol of plant diseases. In: Mohamed HI, El-Beltagi HES, Abd-Elsalam KA (eds) Plant growth-promoting microbes for sustainable biotic and abiotic stress management. Springer, Cham, pp 441–461. https://doi.org/10.1007/978-3-030-66587-6-16

Hanyabui E, Apori SO, Frimpong KA et al (2020) Phosphorus sorption in tropical soils. AIMS Agric Food 5:599–616. https://doi.org/10.3934/agrfood.2020.4.599

Hassan MA, Abdelgani ME (2009) Effect of microbial biofertilization on nodulation, nitrogen and phosphorus content and forage yield of lablab bean (*Lablab purpureus* L.). Am Eurasian J Sustain Agric 3:829–835

Haussling M, Marschner H (1989) Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [*Picea abies* (L.) Karst.] trees. Biol Fertil Soils 8:128–133. https://doi.org/10.1007/BF00257756

Hwangbo H, Park RD, Kim YW et al (2003) 2-ketogluconic acid production and phosphate solubilization by *Enterobacter intermedium*. Curr Microbiol 47:87–92. https://doi.org/10.1007/s00284-002-3951-y

Illmer P, Barbato A, Schinner F (1995) Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganisms. Soil Biol Biochem 27:265–270. https://doi.org/10.1016/0038-0717(94)00205-F

Jamshidi R, Jalili B, Bahmanyar MA, Salek-Gilani S (2016) Isolation and identification of a phosphate solubilising fungus from soil of a phosphate mine in Chaluse, Iran. Mycology 7: 134–142. https://doi.org/10.1080/21501203.2016.1221863

Jangandi S, Negalur CB, Narayan LHC (2017) Effect of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi with and without rock phosphate on four forest tree seedlings. Int J Bioassays 6:5204–5207. https://doi.org/10.21746/ijbio.2017.01.003

Jiang CY, Sheng XF, Qian M, Wang QY (2008) Isolation and characterization of a heavy metal-resistance *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72: 157–164. https://doi.org/10.1016/j.chemosphere.2008.02.006

Jog R, Nareshkumar G, Rajkumar S (2012) Plant growth promoting potential and soil enzyme production of the most abundant *Streptomyces* spp. from wheat rhizosphere. J Appl Microbiol 113:1154–1164. https://doi.org/10.1111/j.1365-2672.2012.05417.x

Jog R, Pandya M, Nareshkumar G, Rajkumar S (2014) Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology 160:778–788. https://doi.org/10.1099/mic.0.074146-0

Kalsi HK, Singh R, Dhaliwal HS, Kumar V (2016) Phytases from *Enterobacter* and *Serratia* species with desirable characteristics for food and feed applications. 3 Biotech 6:64. https://doi.org/10.1007/s13205-016-0378-x

Kang SM, Joo GJ, Humayun M et al (2009) Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. Biotechnol Lett 31:277–281. https://doi.org/10.1007/s10529-008-9867-2

Kaur G, Reddy MS (2013) Role of phosphate-solubilizing bacteria in improving the soil fertility and crop productivity in organic farming. Arch Agron Soil Sci 60:549–564. https://doi.org/10.1080/03650340.2013.817667

Kaushal M (2019) Microbes in cahoots with plants: MIST to hit the jackpot of agricultural productivity during drought. Int J Mol Sci 20:1769. https://doi.org/10.3390/ijms20071769

Kaushal M, Wani SP (2016) Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. Ann Microbiol 66:35–42. https://doi.org/10.1007/s13213-015-1112-3

Kaviyarasi K, Kanimozhi K, Madhanraj P et al (2011) Isolation, identification and molecular characterization of phosphate solubilizing actinomycetes isolated from the coastal region of Manora, Thanjavur (Dt.). Asian J Pharm Tech 1:119–122

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

Kim KY, Jordan D, Krishnan HB (1998) Expression of genes from *Rahnella aquatilis* that are necessary for mineral phosphate solubilization in *Escherichia coli*. FEMS Microbiol Lett 159: 121–127. https://doi.org/10.1111/j.1574-6968.1998.tb12850.x

Kim CH, Han SH, Kim KY et al (2003) Cloning and expression of pyrroloquinoline quinone (PQQ) genes from a phosphate-solubilizing bacterium *Enterobacter intermedium*. Curr Microbiol 47: 457–461. https://doi.org/10.1007/s00284-003-4068-7

Kleinman PJA, Sharpley AN, Saporito LS et al (2009) Application of manure to no-till soils: phosphorus losses by subsurface and surface pathways. Nutr Cycl Agroecosyst 84:215–227. https://doi.org/10.1007/s10705-008-9238-3

Krishnaraj PU, Goldstein AH (2001) Cloning of a *Serratia marcescens* DNA fragment that induces quinoprotein glucose dehydrogenase-mediated gluconic acid production in *Escherichia coli* in the presence of stationary phase *Serratia marcescens*. FEMS Microbiol Lett 205:215–220. https://doi.org/10.1111/j.1574-6968.2001.tb10950.x

Kucey RMN (1983) Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678. https://doi.org/10.4141/cjss83-068

Kudoyarova GR, Melentiev AI, Martynenko EV et al (2014) Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. Plant Physiol Biochem 83:285–291. https://doi.org/10.1016/j.plaphy.2014.08.015

Kudoyarova G, Arkhipova T, Korshunova T et al (2019) Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. Front Plant Sci 10:1368. https://doi.org/10.3389/fpls.2019.01368

Kumari PDSU, Nanayakkara CM (2017) Phosphate-solubilizing fungi for efficient soil phosphorus management. SLJFA 3:1–9. https://doi.org/10.4038/sljfa.v3i2.46

Kuzyk SB, Hughes E, Yurkov V (2021) Discovery of siderophore and metallophore production in the aerobic anoxygenic phototrophs. Microorganisms 9:959. https://doi.org/10.3390/microorganisms9050959

Lebrazi S, Niehaus K, Bednarz H et al (2020) Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. J Genet Eng Biotechnol 18:71. https://doi.org/10.1186/s43141-020-00090-2

Li X, Luo L, Yang J et al (2015) Mechanisms for solubilization of various insoluble phosphates and activation of immobilized phosphates in different soils by an efficient and salinity-tolerant *Aspergillus niger* strain An2. Appl Biochem Biotechnol 175:2755–2768. https://doi.org/10.1007/s12010-014-1465-2

Linu MS, Asok AK, Thampi M et al (2019) Plant growth promoting traits of indigenous phosphate solubilizing *Pseudomonas aeruginosa* isolates from chilli (*Capsicum annuum* L.) rhizosphere. Commun Soil Sci Plant Anal 50:444–457. https://doi.org/10.1080/00103624.2019.1566469

Liu ST, Lee LY, Tai CY et al (1992) Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101: nucleotide sequence and probable involvement in biosynthesis of the coenzyme pyrroloquinoline quinone. J Bacteriol 174:5814–5819. https://doi.org/10.1128/jb.174.18.5814-5819.1992

Liu L, Li A, Chen J et al (2018) Isolation of a phytase-producing bacterial strain from agricultural soil and its characterization and application as an effective eco-friendly phosphate solubilizing bioinoculant. Commun Soil Sci Plant Anal 49:984–994. https://doi.org/10.1080/00103624.2018.1448863

Mackay JE, Cavagnaro TR, Muller Stover DS et al (2017) A key role for arbuscular mycorrhiza in plant acquisition of P from sewage sludge recycled to soil. Soil Biol Biochem 115:11–20. https://doi.org/10.1016/j.soilbio.2017.08.004

Malhotra H, Vandana SS, Pandey R (2018) Phosphorus nutrition: plant growth in response to deficiency and excess. In: Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B (eds) Plant nutrients and abiotic stress tolerance. Springer, Singapore, pp 171–190. https://doi.org/10.1007/978-981-10-9044-8-7

Mandyal P, Kaushal R, Sharma K, Kaushal M (2012) Evaluation of native PGPR isolates in bell pepper for enhanced growth, yield and fruit quality. Int J Farm Sci 2:28–35

Matos ADM, Gomes ICP, Nietsche S et al (2017) Phosphate solubilization by endophytic bacteria isolated from banana trees. Ann Braz Acad Sci 89:2945–2954. https://doi.org/10.1590/0001-3765201720160111

Meena KK, Mesapogu S, Kumar M et al (2010) Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea. Biol Fertil Soils 46:169–174. https://doi.org/10.1007/s00374-009-0421-8

Mendoza-Arroyo GE, Chan-Bacab MJ, Aguila-Ramirez RN et al (2020) Inorganic phosphate solubilization by a novel isolated bacterial strain *Enterobacter* sp. ITCB-09 and its application potential as biofertilizer. Agriculture 10:383. https://doi.org/10.3390/agriculture10090383

Meulenberg JJM, Sellink E, Riegman NH, Postma PW (1992) Nucleotide sequence and structure of the *Klebsiella pneumoniae pqq* operon. Mol Gen Genet 232:284–294. https://doi.org/10.1007/BF00280008

Miethke M, Marahiel MA (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71:413–451. https://doi.org/10.1128/MMBR.00012-07

Miller SH, Browne P, Prigent-Combaret C et al (2010) Biochemical and genomic comparison of inorganic phosphate solubilization in *Pseudomonas* species. Environ Microbiol Rep 2:403–411. https://doi.org/10.1111/j.1758-2229.2009.00105.x

Molla MAZ, Chowdhury AA, Islam A, Hoque S (1984) Microbial mineralization of organic phosphate in soil. Plant Soil 78:393–399. https://doi.org/10.1007/BF02450372

Muleta D, Assefa F, Borjesson E, Granhall U (2013) Phosphate-solubilizing rhizobacteria associated with *Coffea arabica* L. in natural coffee forests of southwestern Ethiopia. J Saudi Soc Agric Sci 12:73–84. https://doi.org/10.1016/j.jssas.2012.07.002

Nacoon S, Jogloy S, Riddech N et al (2020) Interaction between phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on growth promotion and tuber inulin content of *Helianthus tuberosus* L. Sci Rep 10:4916. https://doi.org/10.1038/s41598-020-61846-x

Nafis A, Raklami A, Bechtaoui N et al (2019) Actinobacteria from extreme niches in Morocco and their plant growth-promoting potentials. Diversity 11:139. https://doi.org/10.3390/d11080139

Naik PR, Raman G, Narayanan KB, Sakthivel N (2008) Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. BMC Microbiol 8:230. https://doi.org/10.1186/1471-2180-8-230

Narsian V, Patel HH (2000) *Aspergillus aculeatus* as a rock phosphate solubilizer. Soil Biol Biochem 32:559–565

Naseem H, Ahsan M, Shahid MA, Khan N (2018) Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. J Basic Microbiol 58:1009–1022. https://doi.org/10.1002/jobm.201800309

Neira-Vielma AA, Aguilar CN, Ilyina A et al (2018) Purification and biochemical characterization of an *Aspergillus niger* phytase produced by solid-state fermentation using triticale residues as substrate. Biotechnol Rep 17:49–54. https://doi.org/10.1016/j.btre.2017.12.004

Oteino N, Lally RD, Kiwanuka S et al (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front Microbiol 6:745. https://doi.org/10.3389/fmicb.2015.00745

Pandey A, Das N, Kumar B et al (2008) Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. World J Microbiol Biotechnol 24:97–102. https://doi.org/10.1007/s11274-007-9444-1

Parani K, Saha BK (2012) Prospects of using phosphate solubilizing *Pseudomonas* as biofertilizer. EJBS 4:40–44. https://doi.org/10.5829/idosi.ejbs.2012.4.2.63117

Patel T, Saraf M (2017) Biosynthesis of phytohormones from novel rhizobacterial isolates and their in vitro plant growth-promoting efficacy. J Plant Interact 12:480–487. https://doi.org/10.1080/17429145.2017.1392625

Perez-Rodriguez MM, Piccoli P, Anzuay MS et al (2020) Native bacteria isolated from roots and rhizosphere of *Solanum lycopersicum* L. increase tomato seedling growth under a reduced fertilization regime. Sci Rep 10:15642. https://doi.org/10.1038/s41598-020-72507-4

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

Qarni A, Billah M, Hussain K et al (2021) Isolation and characterization of phosphate solubilizing microbes from rock phosphate mines and their potential effect for sustainable agriculture. Sustainability 13:2151. https://doi.org/10.3390/su13042151

Qiao H, Sun XR, Wu XQ et al (2019) The phosphate-solubilizing ability of *Penicillium guanacastense* and its effects on the growth of *Pinus massoniana* in phosphate-limiting conditions. Biol Open 8:046797. https://doi.org/10.1242/bio.046797

Rafique M, Sultan T, Ortas I, Chaudhary HJ (2017) Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil. Soil Sci Plant Nutr 63:460–469. https://doi.org/10.1080/00380768.2017.1373599

Ram H, Malik SS, Dhaliwal SS et al (2015) Growth and productivity of wheat affected by phosphorus-solubilizing fungi and phosphorus levels. Plant Soil Environ 61:122–126. https://doi.org/10.17221/982/2014-PSE

Ramesh A, Sharma SK, Yadav N, Joshi OP (2014) Phosphorus mobilization from native soil P-pool upon inoculation with phytate-mineralizing and phosphate-solubilizing isolates for improved P-acquisition and growth of soybean and wheat crops in microcosm conditions. Agric Res 3: 118–127. https://doi.org/10.1007/s40003-014-0105-y

Rawat P, Das S, Shankhdhar D, Shankhdhar SC (2020) Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-020-00342-7

Reyes I, Valery A, Valduz Z (2006) Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. Plant Soil 287:69–75. https://doi.org/10.1007/s11104-006-9061-z

Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785. https://doi.org/10.3389/fmicb.2016.01785

Rinu K, Pandey A (2011) Slow and steady phosphate solubilization by a psychrotolerant strain of *Paecilomyces hepiali* (MTCC 9621). World J Microbiol Biotechnol 27:1055–1062. https://doi.org/10.1007/s11274-010-0550-0

Rodriguez H, Gonzalez T, Selman G (2000) Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. J Biotechnol 84:155–161. https://doi.org/10.1016/s0168-1656(00)00347-3

Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91:552–555. https://doi.org/10.1007/s00114-004-0566-0

Romera FJ, Garcia MJ, Lucena C et al (2019) Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. Front Plant Sci 10:287. https://doi.org/10.3389/fpls.2019.00287

Roy S, Roy M (2019) Characterization of plant growth promoting feature of a neutromesophilic, facultatively chemolithoautotrophic, sulphur oxidizing bacterium *Delftia* sp. strain SR4 isolated from coal mine spoil. Int J Phytoremediation 21:531–540. https://doi.org/10.1080/15226514.2018.1537238

Saber WIA, Ghanem KM, El-Hersh MS (2009) Rock phosphate solubilization of by two isolates of *Aspergillus niger* and *Penicillium* sp. and their promotion to mung bean plants. Res J Microbiol 4:235–250. https://doi.org/10.3923/jm.2009.235.250

Sabu A, Sarita S, Pandey A et al (2002) Solid-state fermentation for production of phytase by *Rhizopus oligosporus*. Appl Biochem Biotechnol 102–103:251–260. https://doi.org/10.1385/ABAB:102-103:1-6:251

Saeid A, Prochownik E, Dobrowolska-Iwanek J (2018) Phosphorus solubilization by *Bacillus* species. Molecules 23:2897. https://doi.org/10.3390/molecules23112897

Sanjotha P, Mahantesh P, Patil CS (2011) Isolation and screening of efficiency of phosphate solubilizing microbes. Int J Microb Res 3:56–58. https://doi.org/10.9735/0975-5276.3.1.56-58

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854. https://doi.org/10.1111/j.1462-2920.2011.02556.x

Selvakumar G, Kundu S, Joshi P et al (2010) Growth promotion of wheat seedlings by *Exiguobacterium acetylicum* 1P (MTCC 8707) a cold tolerant bacterial strain from the Uttarakhand Himalayas. Indian J Microbiol 50:50–56. https://doi.org/10.1007/s12088-009-0024-y

Selvi KB, Paul JJA, Vijaya V, Saraswathi K (2017) Analyzing the efficacy of phosphate solubilizing microorganisms by enrichment culture techniques. Biochem Mol Biol 3:1. https://doi.org/10.21767/2471-8084.100029

Shahid M, Hameed S, Imran A et al (2012) Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11. World J Microbiol Biotechnol 28:2749–2758. https://doi.org/10.1007/s11274-012-1086-2

Sharon JA, Hathwaik LT, Glenn GM et al (2016) Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. J Soil Sci Plant Nutr 16:525–536. https://doi.org/10.4067/S0718-95162016005000043

Shimizu M (1992) Purification and characterization of phytase from *Bacillus subtilis* (*natto*) N-77. Biosci Biotech Biochem 56:1266–1269. https://doi.org/10.1271/bbb.56.1266

Singh RP, Shelke GM, Kumar A, Jha PN (2015) Biochemistry and genetics of ACC deaminase: a weapon to “stress ethylene” produced in plants. Front Microbiol 6:937. https://doi.org/10.3389/fmicb.2015.00937

Smith SE, Jakobsen I, Gronlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understandings and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057. https://doi.org/10.1104/pp.111.174581

Springer AL, Ramamoorthi R, Lidstrom ME (1996) Characterization and nucleotide sequence of *pqqE* and *pqqF* in *Methylobacterium extorquens* AM1. J Bacteriol 178:2154–2157. https://doi.org/10.1128/jb.178.7.2154-2157.1996

Sreevidya M, Gopalakrishnan S, Kudapa H, Varshney RK (2016) Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. Braz J Microbiol 47:85–95. https://doi.org/10.1016/j.bjm.2015.11.030

Stephen J, Jisha MS (2011) Gluconic acid production as the principal mechanism of mineral phosphate solubilization by *Burkholderia* sp. (MTCC 8369). J Trop Agric 49:99–103

Suleman M, Yasmin S, Rasul M et al (2018) Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. PLoS One 13:0204408. https://doi.org/10.1371/journal.pone.0204408

Takeda M, Knight JD (2006) Enhanced solubilization of rock phosphate by *Penicillium bilaiae* in pH-buffered solution culture. Can J Microbiol 52:1121–1129. https://doi.org/10.1139/W06-074

Tian J, Ge F, Zhang D et al (2021) Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. Biology 10:158. https://doi.org/10.3390/biology10020158

Toyama H, Chistoserdova L, Lidstrom ME (1997) Sequence analysis of *pqq* genes required for biosynthesis of pyrroloquinoline quinone in *Methylobacterium extorquens* AM1 and the purification of a biosynthetic intermediate. Microbiology 143:595–602. https://doi.org/10.1099/00221287-143-2-595

Tseng YH, Fang TJ, Tseng SM (2000) Isolation and characterization of a novel phytase from *Penicillium simplicissimum*. Folia Microbiol 45:121–127. https://doi.org/10.1007/BF02817409

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. https://doi.org/10.1007/s00253-006-0380-z

Voisard C, Keel C, Haas D, Defago G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351–358

Wagh J, Shah S, Bhandari P et al (2014) Heterologous expression of pyrroloquinoline quinone (*pqq*) gene cluster confers mineral phosphate solubilization ability to *Herbaspirillum seropedicae* Z67. Appl Microbiol Biotechnol 98:5117–5129. https://doi.org/10.1007/s00253-014-5610-1

Wahid OAA, Mehana TA (2000) Impact of phosphate-solubilizing fungi on the yield and phosphorus-uptake by wheat and faba bean plants. Microbiol Res 155:221–227. https://doi.org/10.1016/s0944-5013(00)80036-1

Wahid F, Fahad S, Danish S et al (2020) Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. Agriculture 10:334. https://doi.org/10.3390/agriculture10080334

Wakelin S, Mander C, Gerard E et al (2012) Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. Appl Soil Ecol 61:40–48. https://doi.org/10.1016/j.apsoil.2012.06.002

Walpola BC, Yoon MH (2013) Phosphate solubilizing bacteria: assessment of their effect on growth promotion and phosphorous uptake of mung bean (*Vigna radiata* [L.] R. Wilczek). Chilean J Agric Res 73:275–281. https://doi.org/10.4067/S0718-58392013000300010

Wang X, Wang C, Sui J et al (2018) Isolation and characterization of phosphofungi, and screening of their plant growth-promoting activities. AMB Express 8:63. https://doi.org/10.1186/s13568-018-0593-4

Wu F, Li J, Chen Y et al (2019) Effects of phosphate solubilizing bacteria on the growth, photosynthesis, and nutrient uptake of *Camellia oleifera* Abel. Forests 10:348. https://doi.org/10.3390/f10040348

Xiao C, Chi R, He H et al (2009) Isolation of phosphate-solubilizing fungi from phosphate mines and their effect on wheat seedling growth. Appl Biochem Biotechnol 159:330–342. https://doi.org/10.1007/s12010-009-8590-3

Xu JC, Huang LM, Chen C et al (2019) Effective lead immobilization by phosphate rock solubilization mediated by phosphate rock amendment and phosphate solubilizing bacteria. Chemosphere 237:124540. https://doi.org/10.1016/j.chemosphere.2019.124540

Yadav BK, Tarafdar JC (2007) Ability of *Emericella rugulosa* to mobilize unavailable P compounds during Pearl millet [*Pennisetum glaucum* (L.) R. Br.] crop under arid condition. Indian J Microbiol 47:57–63. https://doi.org/10.1007/s12088-007-0011-0

Yazdani M, Bahmanyar MA, Pirdashti H, Esmaili MA (2009) Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (*Zea mays* L.). World Acad Sci Eng Technol 3:50–52. https://doi.org/10.5281/zenodo.1080014

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. https://doi.org/10.1007/s11274-007-9575-4

Yin Z, Shi F, Jiang H et al (2015) Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareous soil. Can J Microbiol 61:1–11. https://doi.org/10.1139/cjm-2015-0358

Yoon SJ, Choi YJ, Min HK et al (1996) Isolation and identification of phytase-producing bacterium, *Enterobacter* sp. 4, and enzymatic properties of phytase enzyme. Enzyme Microb Technol l8:449–454

Zai XM, Fan JJ, Hao ZP et al (2021) Effect of co-inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing fungi on nutrient uptake and photosynthesis of beach palm under salt stress environment. Sci Rep 11:5761. https://doi.org/10.1038/s41598-021-84284-9

Zhang L, Xu M, Liu Y et al (2016) Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytol 210:1022–1032. https://doi.org/10.1111/nph.13838

Zhang J, Wang P, Fang L et al (2017) Isolation and characterization of phosphate-solubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. Pol J Microbiol 66:57–65. https://doi.org/10.5604/17331331.1234993

Zhao L, Zhang YQ (2015) Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. J Integr Agric 14: 1588–1597. https://doi.org/10.1016/S2095-3119(14)60966-7

Zheng BX, Ibrahim M, Zhang DP et al (2018) Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. AMB Express 8:47. https://doi.org/10.1186/s13568-018-0575-6

Zou K, Binkley D, Doxtader KG (1992) A new method for estimating gross phosphorus mineralization and immobilization rates in soils. Plant Soil 147:243–250. https://doi.org/10.1007/BF00029076

# Chapter 6
# Arbuscular Mycorrhizal Fungi: A Next-Generation Biofertilizer for Sustainable Agriculture

**Arti Sharma, Neelam P. Negi, Parul Narwal, Punam Kumari, and Deepak Kumar**

**Abstract** Climate change has a significant impact on environmental conditions, which affects the growth and productivity of plants. As a result, sustainable crop production continues to be a major global challenge, attracting increasing attention from the scientific community in order to feed the world's growing population while reducing the use of conventional chemical fertilizers and pesticides. Arbuscular mycorrhizal fungi (AMF) are widely used to build symbiotic relationships with over 80% of the species of the land, including most of the cultivated plants. These fungi are of great interest because of their biofertilizer potential (microbial inoculants) in low-input and organic agriculture, which represents an adequate alternative tool for chemical fertilizers. Using AMF as biofertilizer enables plants to use mineral elements such as nitrogen and phosphorous effectively. In addition to an improvement in plant nutrition, AMF plays an important role in improving soil structure, fertility and heavy metal remediation. In conclusion, AMF can be used as a potential biofertilizer for control of environmental stress and may open new strategies to support agriculture and increase global food safety.

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_6

**Keywords** Arbuscular mycorrhizal fungi (AMF) · Biofertilizer · Sustainable agriculture · Plant nutrition · Abiotic stress · Biotic stress

## 6.1 Introduction

Food production needs to be doubled by 2050 in order to meet the demands of a growing population. The rising costs and adverse effects of chemical fertilizers on the environment and human health have pushed the agrarian community to look for substitutes for these chemical fertilizers (Srivastava et al. 2018). Biofertilizers are suitable alternatives to artificially synthesized fertilizers as they are less harmful to the environment, improve soil health and promote the quality and quantity of crop yield (Suhag 2016). Biofertilizers are "microbial inoculants" that allow effective intake of mineral elements such as nitrogen and phosphorus and enhance drought tolerance, salt tolerance and improve plant health (Alori et al. 2017; Igiehon and Olubukola 2017). Most farmers in the world widely use living organisms like bacteria, fungi and cyanobacteria etc. as biofertilizers that are inoculated with seed or in soil to colonize the rhizosphere to increase the availability of nutrients (Sadhana 2014). The use of symbiotic mycorrhizal fungi, particularly arbuscular mycorrhizal fungi (AMF), as biofertilizer has been adopted in agriculture systems because of their potential for improving soil quality, water stress tolerance, altering root architecture and pathogen resistance (Abbot and Robson 1991). The mycorrhiza is an obligatory symbiotic association between fungi and the roots of higher plants (Sieverding 1995). The German Forest pathologist Frank invented the name mycorrhiza in 1885, which comes from two terms, the Greek word "mycos" meaning fungus, and the Latin word "rhiza" referring to fungal roots (Frank 1885). The AMF are ubiquitous endomycorrhiza that can inhabit a variety of ecosystems and form symbiotic association with roots of angiosperms and other plants (Gerdemann 1968) with more than 80% of land plant species including crops (Wang and Qiu 2006). AMF also gives protection against abiotic stress (Auge 2001; Javaid 2007) and biotic stress to their host plants (Khaosaad et al. 2007). Mycorrhiza also increase the fixation of nitrogen in nodule plants. Plants which receive good nutrition can withstand infections, and this is one strategy to combat diseases that are transmitted to the soil (Linderman and Davis 2004). The barrier created by Ectomycorrhizae when they cover the exterior surface of the root is the most obvious mechanism for protecting against illnesses (Castellano and Molina 1989). The current chapter focuses on the significance of AMF as biofertilizers for sustainable agriculture, highlighting the importance of AMF and achievements in research related to their agricultural applications.

## 6.2 Development of Mycorrhizal Network

The AM fungi are classed as a separate phylum termed glomeromycota, which has roughly 150 species with considerable genetic and functional diversity (Smith and Read 2008; Bucking et al. 2012). A study by Hosny et al. (1998) indicates that asexually reproducing fungi have coenocytic hyphae and spores. The mycorrhizal fungi is not strictly a biofertilizer as it does not add mineral nutrition to soil like nitrogen-fixing bacteria but it improves the uptake of soil nutrients through arbuscules and improves plant development and soil health (Garg and Manchanda 2007; Solaiman 2014). An arbuscular is a tiny tree-shaped fungal structure that grows in the intercellular and intracellular regions of roots and is a key site for the exchange of nutrients between the two symbiotic partners (He and Nara 2007). A variety of genes and hormones initiate the symbiotic interaction between plant roots and the fungi. Strigolactones and lipochito-oligosaccharides generated by fungi are important in the development of the association (Mohanta and Bae 2015; Sharma et al. 2021).

The symbiosis is established through a series of morphological and physiological interactions between the two hosts (Amalero et al. 2003). The various developmental stages of the AM colony in the plant roots are as follows.

### *6.2.1 Pre-symbiotic Stage*

AM fungi are obligatory biotrophs; they rely on their autotrophic host to complete their life cycle in a symbiotic association and generate the next generation of spores (Fig. 6.1). Germination of fungal spores in a soil is the only plant-independent phase in the life cycle of mycorrhizal fungi (Bonfante and Bianciotto 1995). The spores germinate and grow into an extended mycelium for 2–3 weeks into extended mycelium, displaying apical dominance. The mycelium growth ceases after 2–4 weeks in the absence of an appropriate host. The presence of host root exudate stimulates intense hyphal growth and branching to increase the probability of contact with host roots (Paszkowski 2006).

### *6.2.2 Early Symbiotic Phase*

Between the fungus and plant root epidermis AMF forms a cell-to-cell contact called appressorium (hypophodium). The formation of appressorium is the first morphological sign of symbiosis. The AM fungi penetrate into the roots of the host plant by penetrating the hyphae emerging from the appressorium. The hyphae successfully penetrates the cell wall using both mechanical and enzymatic catalysed mechanisms (Garcia-Garrido and Ocampo 2002).

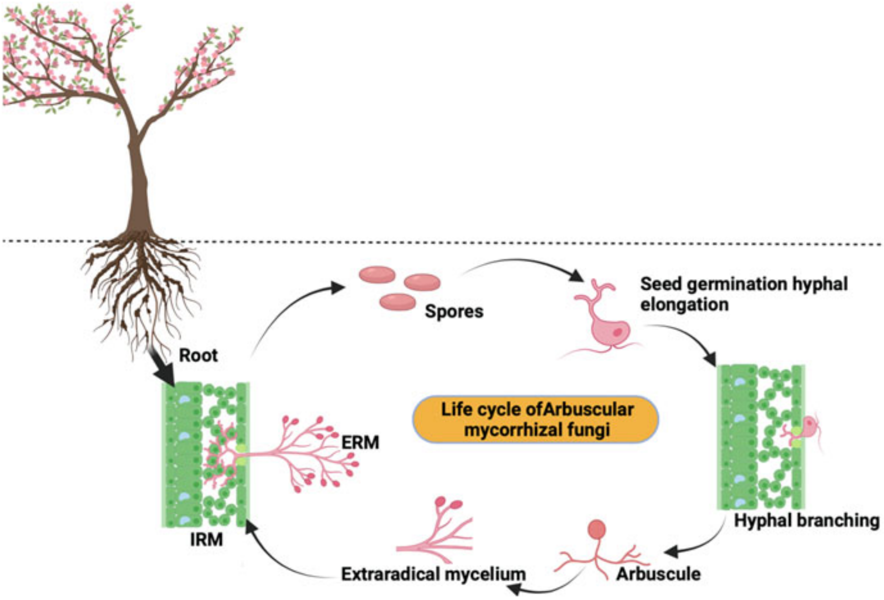

**Fig. 6.1** Life cycle of arbuscular mycorrhizal fungi

### 6.2.3 *Mature Symbiotic Phase*

The mycorrhiza colonizes roots by arbuscule, the tree-like fungal structures formed intracellularly subtended by intercellular hyphae in the cortical region. The structures are key sites for exchange of nutrients between two hosts (Dickson et al. 2007). The periarbuscular membrane (PAM), a key interface for symbiotic interaction, keeps the fungus excluded from host cytoplasm. The exchange of nutrients between the two partners is mediated by membrane transport proteins such as P-type $H^+$ ATPase and phosphate transport (Bucher 2007).

The AM fungi can make a network of mycelia in plant roots and in the soil. Extraradical mycelium (ERM) grows in the soil (Fig. 6.2). They draw nutrients from the soil and deliver them to the plant's roots. The mycelium formed within the roots is called intraradical mycelium (IRM). The IRM releases nutrients at the interface and absorbs carbon from the plant roots in exchange. The absorbed carbon is utilized for expansion and spore formation by ERM. The spores can initiate colonization of nearby plants (Bucking et al. 2012).

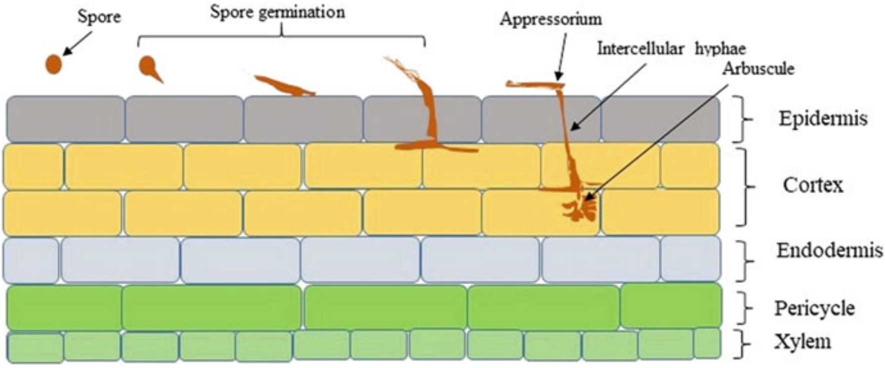

**Fig. 6.2** Development of mycorrhizal network

## 6.3 Arbuscular Fungi as a Potential Biofertilizer

The AM fungi act as an important link between plants and the soil to achieve the goal of sustainable agriculture. They mediate nutrient transfer and therefore contribute to the maintenance of soil structure, soil nutrition and plant nutrition (Gentili and Jumpponen 2006). The high metabolic rate and efficient translocation of micronutrients and macronutrients from soil to plant mediated by fungi improve plant growth and yield in chick pea, custard apple and olive plantlets (Kumar et al. 2002; Briccoli et al. 2015). Fungi can mobilize important nutrients like phosphorus (P), nitrogen (N) and act as a carbon sink in the soil (Bonfante and Genre 2010). As a result, the fungi have the potential to act as biofertilizer for sustainable agriculture (Giri et al. 2019).

## 6.4 The Role of AMF in Improving Soil Health and Fertility

By enhancing soil nitrogen intake by the plant, fungal hyphae stabilize soil aggregates. Extracellular polysaccharide and glomalin exudates aid in the formation of network hyphae in the soil. The polysaccharide glomalin is the main contributor to soil formation because it promotes the development of organic matter and attachment of the hyphae to the soil (Adetunji et al. 2019). Soil aggregation improves soil health and quality by improving soil porosity, water-holding capacity, gaseous exchange, protecting organic carbon and promoting the growth of beneficial micro-fauna (Srivastava et al. 2018). Several studies show that AMF reduces the harmful impact of heavy metal contamination in soil caused by anthropogenic activities and the usage of agrochemical products (Schützendübel and Polle 2002; Dong et al. 2008). The fungi can absorb calcium, aluminium, cadmium, selenium and arsenic acid (Khan et al. 2000; Al-Agel et al. 2005). It mitigates the effects of these heavy metals by immobilization, adsorption on the hyphal wall. The AMF also causes metal resistance in plants by altering metabolic processes such as phenylpropanoid pathway (Janeeshma and Puthur 2020).

## 6.4.1 Role of AMF in Plant Nutrition

Many studies have shown that AMF colonizes plant roots containing essential plant nutrients such as nitrogen (N), phosphorus (P), sulphur (S), potassium (K), calcium (Ca), copper (Cu), iron (Fe), and magnesium (Mg), among others. (Marschner and Dell 1994). AMF facilitates dissolution, transportation of immobile nutrients bound to rocks and mineralization of organic matter (Parihar et al. 2019).

### 6.4.1.1 Phosphorus (P) Absorption

Phosphorus is an essential nutrient for plants but difficult to absorb from soil due to low diffusion rate. Mycorrhizal fungi release an enzyme phosphatase that mobilizes organic P and increases its absorption by plants (Shen et al. 2011; Malla et al. 2004; Nath et al. 2018). Phosphorus deficiency in plants inhibits photosynthesis, respiration and cell division, which subsequently reduces the yield (Baas and Kuiper 1989). A study by Walder et al. (2015) indicates that the symbiotic interaction between the two hosts induces the expression of the Pi transporter in sorghum and flex plants. The fungal hyphae also reduce phosphorus leaching by different mechanisms involving extensive adsorption on the hyphae surface, storage of orthophosphate and polyphosphate in the hyphae and chelation of P with fungi exuded glycoprotein (Parihar et al. 2019).

### 6.4.1.2 Nitrogen (N) Absorption

The AMF can absorb N in both organic forms as amino acids (Whiteside et al. 2012) and inorganic form as nitrate and ammonium (Govindarajulu et al. 2005). The extraradical mycelium of the fungi absorbs the inorganic forms. In the soil, the hyphae can take ammonium at a lower quantity than the roots (Johansen et al. 1994). Ammonium transporters are found in arbuscules which provide nutrients to the host plant. The AM fungi-inducible nitrate and ammonium transporters have been identified in tomato and soyabean plants that facilitate the absorption of nutrients by the plants (Kobae et al. 2010). Several amino acids such as glycine, cysteine, serine, arginine, aspartic acid, glutamine acids and cysteine etc. are absorbed by the fungi and then converted to ammonium for translocation at the symbiotic interface with the plant (Smith and Smith 2011).

#### 6.4.1.3 Sulphur Absorption

Because of its redox characteristics and capacity to form disulphide bonds between cysteine amino acids, sulphur plays an important role in the biological function of many substances. Although plants absorb inorganic sulphate as their primary source of sulphur, 95% of soil sulphur is bonded in organic molecules. The form is not directly available to plants. The mycorrhizal fungi have sulphur transporters that make the element available to the plants (Giovannetti et al. 2014). The mycorrhizal plants can obtain sulphur from organic sources. Allen and Shachar-Hill (2009) observed 25% more sulphur content in plant roots with mycorrhizal association at moderate sulphur concentration as compared to nonmycorrhizal plant roots.

#### 6.4.1.4 Potassium Absorption

Potassium is considered an important macronutrient for plants responsible for enzyme activation, regulation of stomatal opening and serving as an osmolyte in plant cells (Morgan and Connolly 2013; Kumar et al. 2020). Although potassium is abundant in soil, it is not readily available to plants. The role of AMF in potassium uptake by host plants has received less attention. A study by Jianjian et al. (2019) observed the overexpression of potassium transporter protein in the roots of *Lotus japonicas* plants infected with AMF. Furthermore, AM symbiosis associated with potassium nutrition is correlated to alleviating abiotic stresses including salinity, drought, heavy metals and temperature stress (Berruti et al. 2016).

### *6.4.2 Role of AMF in Plant Biotic Stress Tolerance*

More than 90% of total mycorrhizal roots colonize the fungus in intercellular and intracellular tissues. It is proposed that plants can tolerate the intense mycorrhizal network by suppression of plant defence mechanisms against the AMF (Chen et al. 2018). However, the general disease resistance of the plant is not attenuated. Indeed, plants show increased disease resistance against rhizospheric pathogens, pests and parasitic plants either by secreting repulsive exudates from mycorrhizal roots (Kwak et al. 2018). The AMF provides an effective way to control the biotic stress by improved nutrition and induction of plant defence process called as systemic acquired resistance (SAR). Additionally, plants exhibit fast and strong reactions against pathogens by a phenomenon called priming or induced systemic resistance (IRS) (Conrath et al. 2006). The AMF can directly interfere with plant pathogens either by release of antimicrobial substances or by competing with the pathogens for space and resources (Jacott et al. 2017). AMF-induced alleviated plant defence response against various biotic stress is as follows.

#### 6.4.2.1 AMF and Parasite Tolerance

Plants colonized with AMF increase tolerance against parasitic *Meloidogyne* species of nematode. The AMF competes directly with the nematode for root space and reduces the process of reproduction (Dar and Reshi 2017). A study by López-Ráez et al. (2009) indicates that the AMF inhibit the growth of parasitic plants like *Striga hermonthica* in maize and *Striga* and *Orobanche* in sorghum. Thus, the presence of fungi in plant roots can act as biocontrol agents for sustainable agriculture.

#### 6.4.2.2 AMF and Soil-Borne Pathogens

A number of reports have explained the positive effect of AMF-induced plant tolerance to biotic stress triggered by soil-borne pathogens. The symbiosis suppresses growth of fungi *Fusarium, Macrophomina, Rhizoctonia*, *Verticillum*, and oomycetes like *Pythium* and *Phytophthora* responsible for wilting and root rot disease (Hao et al. 2009; Harrier and Watson 2004; Whipps 2004).

#### 6.4.2.3 AMF and Insects

Rhizophagous insects are a common biotic stress for many plants. Hartley and Gange (2009) explained that the mycorrhiza can strongly influence the insect's growth by enhancing insect resistance of plant, but the effects may vary with the feeding mechanisms and lifestyle of the insects. Additionally, AMF-associated plant defence against insects is closely associated with levels of flavonoids and phenolic compounds in host plants (Wang et al. 2020).

### *6.4.3 Role in Plant Abiotic Stress Tolerance*

The plants confront abiotic stress like drought, salinity, extreme temperature, and heavy metals which show harmful effects on their growth and yield (Kumar et al. 2017; Nath et al. 2017). Abiotic stress can negatively affect plant survival and productivity. Therefore, it can act as a foremost threat to global food security (Kumar and Verma 2018). The AMF improves plants' tolerance to these abiotic stresses by various metabolic and physiological changes in plants (Malhi et al. 2021). The role of AMF to combat various abiotic stresses is as discussed below:

#### 6.4.3.1 AMF and Drought Stress Tolerance

Drought is a condition when water is unavailable to plants for its physiological functions. The environmental condition is also known as water stress (Subramanian and Charest 1998). The fluctuated transpiration rate generates reactive oxygen species (ROS), and consequently accelerates oxidative stress in plants (Auge 2001; Barzana et al. 2012). Mycorrhiza can progress plant development and growth by enhancing root network and thickness, plant biomass and nutrient absorption and transport during drought conditions (Davies et al. 2002). The mycorrhizal inoculation facilitates synthesis of more dense hyphal networks and excretes glumalin which augment more water and nutrients absorption, which in turn improves soil quality (Gholamhoseini et al. 2013).

The AMF symbiosis influences numerous biochemical and physiological processes such as (1) augmented osmotic regulation, (2) enhanced gas exchange, (3) absorption and transport of water and nutrients, and (4) better defence against oxidative stress (Marulanda et al. 2007). A study on *Zea mays* plants colonized with mycorrhiza *Glomus intraradices* reported expression of two aquaporin genes (Gint AQPF1 and Gint AQPF2) in root cortical cells holding arbuscules under drought stress (Moussa and Abdel-Aziz 2008; Li et al. 2013). AMF-mediated enhancement in drought resistance has been demonstrated by (Li et al. 2019) in C3 plant (*Leymus chinensis*) and C4 plant (*Hemarthria altissima*) observed because of alteration in expression of antioxidants enzyme.

#### 6.4.3.2 AMF and Salt Stress Tolerance

Salinity in soil is a prime problem for many plants growing in arid and semiarid regions (Giri et al. 2003; Al-Karaki 2006). The high accumulation of salt in soil decreases aeration and porosity of soil and therefore affects water translocation, which results in drought-like stress (Mahajan and Tuteja 2005). Plants under salt stress show decrease in rate of photosynthesis, reduction in activities of antioxidant enzyme, less stomatal conductance, decreased membrane stability, and low relative water content of the plants (Talaat and Shawky 2012). Salinity also causes oxidative stress in the plant by producing more reactive oxygen species (ROS) (Ahmad et al. 2010). AMF inoculated plants develop strategies to enhance the antioxidant system which protects the plant cells from oxidative damage (Rai et al. 2011). The defence system develops superoxide dismutase (SOD) antioxidant enzyme that converts superoxide molecules to oxygen and hydrogen peroxide ($H_2O_2$). Besides SOD, catalase (CAT) enzyme clears $H_2O_2$ by decomposing it to less reactive water and oxygen. These enzymes are continuously generated in the mitochondria, peroxisome and cytoplasm of the plant.

Several research investigations have reported on AMF's efficiency to promote growth, yield and development in plants subjected to salinity stress. AMF is effective for plants' response under different salt concentrations. *Allium sativum* plant inoculated with AMF showed expanded leaf area, more fresh and dry weights under high NaCl concentration (100 mM) as compared to the plant without mycorrhizal association (Borde et al. 2010). A study of Ghazi and Al-Karaki (2001) on tomato plant inoculated with fungi *Glomus mosseae* observed increase in biomass under moderate saline conditions. Under salt stress, AMF-inoculated rice plants preferably absorb more $K^+$ ion and avoid intake of $Na^+$ ions compared with control rice plants. The crop showed AMF induced more salt tolerance and crop yield (Mohsin et al. 2020). El-Nashar (2017) observed that the *Antirrhinum majus* plants improved their growth rate, their feed-water potential and their water efficiency. The favourable benefits of AMF association on biological parameters like photosynthetic rate, stomatal conductivity, and leaf water relationships under salt stress have been described by Ait-El-Mokhtar et al. (2019).

#### 6.4.3.3 Heavy Metal Tolerance

Heavy metals like Cu, Co, Fe, Mn, and Zn are essential for plant growth. However, increased concentrations of these metals are hazardous to the plants due to the production of reactive oxygen species (ROS) by the plants (Palmer and Guerinot 2009; Puig and Penarrubia 2009). The AMF shows positive effects on plant growth under cadmium stress by lowering the levels of hydrogen peroxide and malonaldehyde (Hashem et al. 2016). A study by Yong et al. (2014) reported effective removal of heavy metals from polluted environments in clone of *Schizosaccharomyces pombe*. Metal dilution in plant tissues is also thought to be caused by increased growth or chelation in the rhizospheric soil (Kapoor et al. 2013; Audet 2014). AMF would have reportedly bind Cd and Zn in the cortical cells and mental hyphae to restrict their intake by plant and increase growth, yield and nutrient status of plants (Andrade and Silveira 2008; Garg and Chandel 2012).

#### 6.4.3.4 Thermal Stress Tolerance

Thermal fluctuations lead to reduced germination, low rate of photosynthesis, retarded plant growth, yield and biomass production (Wahid et al. 2007; Hasanuzzaman et al. 2013). A study by Maya and Matsubara (2013) reported the beneficial effects of AMF on plant growth and yield under thermal stress in *Glomus fasciculatum*. A number of reports suggest AMF improves growth rates in plants grown under low temperatures when compared to plants without mycorrhizal association (Zhu et al. 2010a, 2010b). The AMF symbiosis supports the plants' survival under low temperature along with improved plant growth and development (Gamalero et al. 2009; Birhane et al. 2012). AMF strengthens the plant defence system, leading to more synthesis of various secondary metabolites and proteins (Abdel Latef and Chaoxing 2011b). It also facilitates the plant in efficient moisture retention, improved chlorophyll production and better osmotic adjustment capacity (Abdel Latef and Chaoxing 2011a; Zhu et al. 2010a, 2010b). In addition, the AMF can maintain host plant moisture (Zhu et al. 2010a), boost secondary plant metabolites leading towards strengthening plant immune systems and enhance plant protein in support of cold stress conditions (Abdel Latef and Chaoxing 2011b).

## 6.5 Conclusion and Future Challenges

There are many factors, such as compatibility with the environment, competition with other soil organisms, and timing of the inoculation, that can affect the success of establishment of symbiosis. The use of AM fungi in agriculture requires the knowledge of its adaption in the target ecosystem and the establishment of a functional symbiosis in different types of soils.

The use of AM fungi as a biofertilizer is an economical, effective and eco-friendly approach toward the attainment of low-input farming. The symbiotic fungi develop an intensive network of mycelium that improves soil structure, fertility and plant health by efficient absorption of micro- and macronutrients from the soil. Fungi have a number of genes and molecular pathways that facilitate more effective nutrient uptake and transport to the plant roots as compared to plants without a mycorrhizal association. AMF association in plant roots is an effective tool to combat biotic and abiotic stresses responsible for loss of crop productivity and yield. Therefore, the AM fungi have potential biofertilizer to act as sustainable agriculture.

**Acknowledgements** The work in DK laboratory is supported by by the “Seed Grant" under Dev. Scheme 6031 (B) of Institutions of Eminence (IoE) scheme of Banaras Hindu University. DK also acknowledges Science and Engineering Research Board (Grant No- EEQ/2016/000487 and EEQ/2021/000593), India, for providing financial support to the laboratory and Centre of Advanced study in Botany, Institute of Science, Banaras Hindu University for providing necessary infrastructure facilities. NPN acknowledges the DST-SERB grant no EEQ/ 2018/001236 for financial support.

## References

Abbot LK, Robson AD (1991) Factors influencing the occurrence of arbuscular mycorrhizae. Agric Ecosyst Environ 35:121–150

Abdel Latef AA, Chaoxing H (2011a) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Sci Hortic 127:228–233

Abdel Latef AA, Chaoxing H (2011b) Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol Plant 33:1217–1225

Adetunji CO, Kumar D, Raina M, Arogundade O, Sarin NB (2019) Endophytic microorganisms as biological control agents for plant pathogens: a panacea for sustainable agriculture. In: Varma A, Tripathi S, Prasad R (eds) Plant biotic interaction-state of the art. Springer Nature, Switzerland. isbn:978-3-030-26656-1

Ahmad P, Jaleel CA, Sharma S (2010) Antioxidative defense system, lipid peroxidation, proline metabolizing enzymes and biochemical activity in two *Morus alba* genotypes subjected to NaCl stress. Russian J Plant Physiol 57:509–517

Ait-El-Mokhtar M, Laouane RB, Anli M, Boutasknit A, Wahbi S, Meddich A (2019) Use of mycorrhizal fungi in improving tolerance of the date palm (*Phoenix dactylifera* L.) seedlings to salt stress. Sci Hortic 253:429–438

Al-Agel A, Sylvia DM, Ma LQ (2005) Mycorrhizae increase arsenic uptake by the hyperaccumulator Chinese brake fern (Pteris vittata L.). J Environ Qual 34:2181–2186

Al-Karaki GN (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci Hortic 109:1–7

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549–560

Alori ET, Dare MO, Babalola OO (2017) Microbial inoculants for soil quality and plant health. In: Sustainable agriculture reviews, pp 281–307

Amalero EG, Guido LI, Graziella BE, Philippe LE (2003) Methods for studying root colonization by introduced beneficial bacteria. Agronomie 23:407–418

Andrade SAL, Silveira APD (2008) Mycorrhiza influence on maize development under Cd stress and P supply. Braz J Plant Physiol 20(1):39–50

Audet P (2014) Arbuscular mycorrhizal fungi and metal phytoremediation: ecophysiological complementarity in relation to environmental stress. In: Ahmad P, Rasool S (eds) Emerging technologies and management of crop stress tolerance. Academic, San Diego, pp 133–160

Auge RM (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Baas R, Kuiper D (1989) Effects of vesicular-arbuscular mycorrhizal infection and phosphate on *Plantago major* ssp. pleiosperma in relation to internal cytokinin concentrations. Physiol Plant 76:211–215

Barzana G, Aroca R, Paz JA (2012) Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Ann Bot 109:1009–1017

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol 6:1–13. https://doi.org/10.3389/fmicb.2015.01559

Birhane E, Sterck F, Fetene M, Bongers F, Kuyper T (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904

Bonfante P, Bianciotto V (1995) Presymbiotic versus symbiotic phase in arbuscular endomycorrhizal fungi: morphology and cytology. In: Vanna A, Hock B (eds) Mycorrhiza: structure, function, molecular biology, and biotechnology, pp 229–247

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48

Borde MY, Dudhane MP, Jite PK (2010) AM fungi influences the photosynthetic activity, growth and antioxidant enzymes in *Allium sativum* L. under salinity condition. Notulae Sci Biol 2(4): 64–71

Briccoli BC, Santilli E, Lombardo L (2015) Effect of arbuscular mycorrhizal fungi on growth and on micronutrient and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels. Mycorrhiza 25:97–108

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol 173(1):11–26

Bucking H, Liepold E, Ambilwade P (2012) The role of the mycorrhizal symbiosis in nutrient uptake of plants and the regulatory mechanisms underlying these transport processes. In: Dhal NK, Sahu SC (eds) Plant science. IntechOpen. https://doi.org/10.5772/52570

Castellano MA, Molina R (1989) Mycorrhizae. In: Landis TD, Tinus RW, McDonald SE, Barnett JP (eds) The container tree nursery manual, Agriculture handbook. 674, vol 5. U.S. Department of Agriculture, Forest Service, Washington, DC, pp 101–167

Chen M, Arato M, Lorenzo B, Eva N, Didier R (2018) Beneficial services of arbuscular mycorrhizal fungi—from ecology to application. Front Plant Sci 9:1–14

Conrath U, Beckers GJM, Flors V, Garcia-Agustin P, Jakab G, Mauch F (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062–1071

Dar MH, Reshi ZA (2017) Vesicular arbuscular mycorrhizal (VAM) fungi—as a major biocontrol agent in modern sustainable agriculture system. Russ Agric Sci 43(2):138–143

Davies FT Jr, Olalde-Portugal V, Aguilera-Gomez L, Alvarado MJ, Ferrera-Cerrato RC, Boutton TW (2002) Alleviation of drought stress of Chile ancho pepper (Capsicum annuum L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. Sci Hortic 92(3–4):347–359

Dickson S, Smith FA, Smith SE (2007) Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? Mycorrhiza 17:375–393

Dong Y, Zhu YG, Smith FA et al (2008) Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (*Trifolium repens* Linn.) and ryegrass (*Lolium perenne* L.) plants in an arsenic-contaminated soil. Environ Pollut 155(1):174–181

El-Nashar YI (2017) Response of snapdragon Antirrhinum majus L. to blended water irrigation and arbuscular mycorrhizal fungi inoculation: uptake of minerals and leaf water relations. Photosynthetica 55(2):201–209

Frank B (1885) Über die auf Wurzelsymbiosenberuhende Ernährunggewisser Bäumedurchunterirdische Pilze. Berichte der DeutschenBotanischen Gesellschaft 3:128–145

Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514

Garcia-Garrido JM, Ocampo JA (2002) Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J Exp Bot 53(373):1377–1386

Garg N, Chandel S (2012) Role of arbuscular mycorrhizal (AM) fungi on growth, cadmium uptake, osmolyte, and phytochelatin synthesis in *Cajanus cajan* (L.) Millsp. under NaCl and Cd stresses. J Plant Growth Regul 31(3):292–308

Garg N, Manchanda G (2007) Symbiotic nitrogen fixation in legume nodules: process and signaling: a review. Agron Sustain Dev 27:59–68

Gentili F, Jumpponen A (2006) Potential and possible uses of bacterial and fungal biofertilizers. In: Rai MK (ed) Handbook of microbial biofertilizers. Food Products Press, New York, pp 1–28

Gerdemann JW (1968) Vesicular-arbuscular mycorrhizae and plant growth. Annu Rev Phytopathol 6:397–418

Ghazi J, Al-Karaki GN (2001) Mycorrhizal influence on fruit yield and mineral content of tomato grown under salt stress. J Plant Nutr 24:1311–1323

Gholamhoseini M, Ghalavand A, Dolatabadian A (2013) Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. Agric Water Manag 117:106–114

Giovannetti M, Tolosano M, Volpe V, Kopriva S, Bonfante P (2014) Identification and functional characterization of a sulfate transporter induced by both sulfur starvation and mycorrhiza formation in *Lotus japonicus*. New Phytol 204:609–619

Giri B, Kapoor R, Mukerji KG (2003) Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. Biol Fertil Soils 38:170–175

Giri B, Prasad R, Wu Q-S, Varma A (2019) Biofertilizers for sustainable agriculture and environment. Springer International Publishing (ISBN: 978-3-030-18932-7). https://www.springer.com/gp/book/9783030189327

Govindarajulu M, Pfeffer P, Jin H (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Hao Z, Fayolle L, Van TD, Gianinazzi-Pearson V, Gianinazzi S (2009) Mycorrhiza reduce development of nematode vector of grapevine fanleaf virus in soils and root systems. In: Boudon-Padfieu E (ed) Extended abstract 16th meeting of ICVG, Dijon, France, pp 100–1001

Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Manag Sci 60:149–157

Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342

Hasanuzzaman M, Gill SS, Fujita M (2013) Physiological role of nitric oxide in plants grown under adverse environmental conditions. In: Tuteja N, Gill SS (eds) Plant acclimation to environmental stress. Springer, New York, pp 269–322

Hashem A et al (2016) Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. Saudi J Biol Sci 23:272–281

He XH, Nara K (2007) Element biofortification: can mycorrhizas potentially offer a more effective and sustainable pathway to curb human malnutrition? Trends Plant Sci 12:331–333

Hosny M, Gianinazzi-Pearson V, Dulieu H (1998) Nuclear DNA contents of 11 fungal species in Glomales. Genome 41:422–429

Igiehon NO, Olubukola OB (2017) Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. Appl Microbiol Biotechnol 101(12):4871–4881

Jacott CN, Murray JD, Ridout CJ (2017) Trade-offs in arbuscular mycorrhizal symbiosis: disease resistance, growth responses and perspectives for crop breeding. Agronomy 7:75

Janeeshma E, Puthur JT (2020) Direct and indirect influence of arbuscular mycorrhizae on enhancing metal tolerance of plants. Arch Microbiol 202:1–16

Javaid A (2007) Allelopathic interactions in mycorrhizal associations. Allelopath J 20:29–42

Jianjian L, Junli L, Jinhui L, Miaomiao C, Yujuan H, Yuan T, Aiqun C, Guohua X (2019) The potassium transporter SlHAK10 is involved in mycorrhizal potassium uptake. Plant Physiol 80(1):465–479

Johansen A, Jakobsen I, Jensen ES (1994) Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. Plant Soil 160:1–9

Kapoor R, Evelin H, Mathur P, Giri B (2013) Arbuscular mycorrhiza: approaches for abiotic stress tolerance in crop plants for sustainable agriculture. In: Tuteja N, Singh Gill S (eds) Plant acclimation to environmental stress. Springer, pp 359–401

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take all diseases is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39:727–734

Kobae Y, Tamura Y, Takai S, Banba M, Hata S (2010) Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. Plant Cell Physiol 51:1411–1415

Kumar A, Verma JP (2018) Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52

Kumar R, Jalali BL, Chand H (2002) Influence of vesicular arbuscular mycorrhizal fungi on growth and nutrient uptake in chickpea. J Mycol Plant Pathol 32(1):11–15

Kumar M, Prasad R, Kumar V, Tuteja N, Varma A (2017) Mycorrhizal fungi under biotic and abiotic stress. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 57–70

Kumar P, Tapan Kumar T, Singh S, Tuteja N, Prasad R, Singh J (2020) Potassium: a key modulator for cell homeostasis. J Biotechnol 324:198–210. https://doi.org/10.1016/j.jbiotec.2020.10.018

Kwak MJ, Kong HG, Choi K, Kwon SK, Song JY, Lee J, Lee PA, Choi SY, Seo M, Lee HJ, Jung EJ (2018) Rhizosphere microbiome structure alters to enable wilt resistance in tomato. Nat Biotechnol 36(11):1100–1109

Li T, Hu Y, Hao Z, Li H, Wang Y, Chen B (2013) First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 197:617–630

Li J, Meng B, Chai H, Yang X, Song W, Li S, Sun W (2019) Arbuscular mycorrhizal fungi alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses via altering antioxidant enzyme activities and photosynthesis. Front Plant Sci 10:499

Linderman RG, Davis EA (2004) Evaluation of commercial inorganic and organic fertilizer effects on arbuscular mycorrhizae formed by *Glomus intraradices*. Hortic Technol 14(2):196–202

López-Ráez JA, Matusova R, Cardoso C, Jamil M, Charnikhova T, Kohlen W, Verstappen F, Ruyter-Spira C, Bouwmeester HJ (2009) Strigolactones: ecological significance and use as a target for parasitic plant control. Pest Manag Sci 64:471–477

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Malhi GS, Kaur M, Kaushik P, Alyemeni MN, Alsahli AA, Ahmad P (2021) Arbuscular mycorrhiza in combating abiotic stresses in vegetables: an eco-friendly approach. Saudi J Biol Sci 28(2):1465–1476

Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmueller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus *Piriformospora indica*. Endocytobiosis Cell Res 15(2):579–600

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Marulanda A, Porcel R, Barea JM, Azcón R (2007) Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought sensitive Glomus species. Microb Ecol 54:543–552

Maya MA, Matsubara Y (2013) Influence of arbuscular mycorrhiza on the growth and antioxidative activity in Cyclamen under heat stress. Mycorrhiza 23(5):381–390

Mohanta TK, Bae H (2015) Functional genomics and signaling events in mycorrhizal symbiosis. J Plant Interact 10(1):21–40

Mohsin SM, Hasanuzzaman M, Nahar K, Hossain M, Bhuyan MH, Parvin K, Fujita M (2020) Tebuconazole and trifloxystrobin regulate the physiology, antioxidant defense and methylglyoxal detoxification systems in conferring salt stress tolerance in Triticum aestivum L. Physiol Mol Biol Plants 26(6):1139–1154

Morgan JB, Connolly EL (2013) Plant-soil interactions: nutrient uptake. Nat Educ Knowl 4:2

Moussa HR, Abdel-Aziz SM (2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust J Crop Sci 1:31–36

Nath M, Bhatt D, Prasad R, Tuteja N (2017) Reactive oxygen species (ROS) metabolism and signaling in plant-mycorrhizal association under biotic and abiotic stress conditions. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 223–232

Nath M, Bhatt D, Bhatt MD, Prasad R, Tuteja N (2018) Microbe-mediated enhancement of nitrogen and phosphorus content for crop improvement. In: Prasad R, Gill SS, Tuteja N (eds) Crop improvement through microbial biotechnology. Elsevier, pp 291–301

Palmer C, Guerinot ML (2009) A question of balance: facing the challenges of Cu, Fe and Zn homeostasis. Nat Chem Biol 5:333–340

Parihar M, Meena VS, Mishra PK, Rakshit A, Choudhary M, Yadav RP, Rana K, Bisht JK (2019) Arbuscular mycorrhiza: a viable strategy for soil nutrient loss reduction. Arch Microbiol 201(6): 723–735

Paszkowski U (2006) A journey through signalling in arbuscular mycorrhizal symbioses. New Phytol 172(1):35–46

Puig S, Penarrubia L (2009) Placing metal micronutrients in context: transport and distribution in plants. Curr Opin Plant Biol 12:299–306

Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK (2011) Developing stress tolerant plants through in vitro selection—an overview of the recent progress. Environ Exp Bot 71:89–98

Sadhana B (2014) Review article arbuscular mycorrhizal fungi (AMF) as a biofertilizer—a review. Int J Curr Microbiol App Sci 3(4):384–400

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53(372):1351–1365

Sharma A, Singh A, Raina M, Kumar D (2021) Plant-fungal association: an ideal contrivance for combating plant stress tolerance. In: Prasad R, Nayak SC, Kharwar RN, Dubey NK (eds) Mycoremediation and environmental sustainability. Springer Nature, Singapore

Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang F (2011) Phosphorus dynamics: from soil to plant. Plant Physiol 156(3):997–1005

Sieverding E (1995) Vesicular-arbuscular mycorrhiza management in tropical agrosystems. AGRIS, GTZ No. 224. Eschborn 371

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, Amsterdam

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

Solaiman ZM (2014) Contribution of arbuscular mycorrhizal fungi to soil carbon sequestration. In: Mycorrhizal fungi: use in sustainable agriculture and land restoration. Springer, Berlin, pp 287–296

Srivastava P, Saxena B, Giri B (2018) Arbuscular mycorrhizal fungi: green approach/technology for sustainable agriculture and environment. In: Mycorrhiza—nutrient uptake, biocontrol, eco restoration, 4th edn. https://doi.org/10.1007/978-3-319-68867-1_20

Subramanian KS, Charest C (1998) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. Physiol Plant 102:285–296

Suhag M (2016) Potential of biofertilizers to replace chemical fertilizers. Int J Adv Res Sci Eng Technol 3(5):163–167

Talaat NB, Shawky BT (2012) 24-Epibrassinolide ameliorates the saline stress and improves the productivity of wheat (*Triticum aestivum* L.). Environ Exp Bot 82:80–88

Wagg C, Pautler M, Massicotte HB, Peterson RL (2008) The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. Mycorrhiza 18(2):103–110

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223

Walder F, Brule D, Koegel S, Wiemken A, Boller T, Courty PE (2015) Plant phosphorus acquisition in a common mycorrhizal network: regulation of phosphate transporter genes of the Pht1 family in sorghum and flax. New Phytol 205:1632–1645

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Wang C, Tian B, Yu Z, Ding J (2020) Effect of different combinations of phosphorus and nitrogen fertilization on arbuscular mycorrhizal fungi and aphids in wheat. Insects 11(6):365

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

Whiteside MD, Gercia MO, Treseder KK (2012) Amino acid uptake in arbuscular mycorrhizal plants. PLoS One 7(10):e47643

Yong X et al (2014) Enhanced cadmium resistance and accumulation in Pseudomonas putida KT2440 expressing the phytochelatin synthase gene of Schizosaccharomyces pombe. Lett Appl Microbiol 58:255–226

Zhu XC, Song FB, Xu HW (2010a) Arbuscular mycorrhizae improve low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil 331:129–137

Zhu XC, Song FB, Xu HW (2010b) Effects of arbuscular mycorrhizal fungi on photosynthetic characteristics of maize under low temperature stress. Acta Ecol Sin 21:470–475

# Chapter 7
# Fungal Endophytes: Potential Benefits of Their Future Use in Plant Stress Tolerance and Agriculture

**Deepak Bhaskar Shelke, Mahadev R. Chambhare, and Hiralal Sonawane**

**Abstract** Global climate change, improper land use, overuse of chemical fertilizers and urban sprawl lead to radical impact on the agricultural production. It also increases abiotic and biotic stresses on crop plants which in turn causes decline in crop yield. This increased the concern of food security worldwide. To provide the food for increasing global population, there is need to increase crop production through sustainable route. The endophytes are group of microorganisms found in plant tissues. It may be beneficial, non-pathogenic, commensal and pathogenic. The beneficial endophytes are the living microorganisms mutually associated with a specific plant and help them to survive under adverse climate conditions. The plant diversity in different climatic zones also leads to endophyte diversity due to their host specificity and growing environment. Among the microorganisms some group of fungi also reside inside the plant body which is referred to as a fungal endophyte. The beneficial fungal endophytes help plant for their growth and development and protect them from adverse climate conditions for their successful survival. Moreover, it produces various metabolites which help plant to defence against abiotic and biotic stresses. The metabolites produced from fungal endophytes recently also gain popularity for their use in various biological fields. Therefore, this chapter provides information on the fungal endophytes, their characteristics, potential use to improve plant abiotic and biotic stress tolerance, weed control, avoiding post-harvest loss and utilization of their bioactive metabolites as prerequisite for crop improvement and sustainable agriculture.

**Keywords** Fungal endophytes · Plant · Stress tolerance · Sustainable agriculture · Weed control

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_7

## 7.1 Introduction

The uncertainty in environmental conditions due to global climate change affects agricultural land which in turn causes decline in crop yields. The improper agricultural practices, overutilization of chemical fertilizers, contamination of irrigation water, intrusion of seawater and urbanization are also major factors influencing on agricultural production. In other side continuous increase in world's population raises the problem of global food security in the near future. It is estimated that in 2050 we need to provide food for 9.1 billion people (Liu et al. 2017). To mitigate the increasing food demand, there is need for continuous increase in agricultural productions. The scientist is utilizing the various ways like breeding of high-yielding varieties, development of transgenic plants, reclamation of contaminated soil, utilization of growth-promoting agents, etc. to increase crop production. However, there is still need to find the sustainable ways to meet this demand. However, utilization of microorganisms for enhancing plant growth of crop plants has a sustainable and eco-friendly approach (Fadiji and Babalola 2020). The many plants have natural association to microorganisms and endophytes among them. Endophytes are the group of microorganisms that reside inside the tissues of plant (Fadiji and Babalola 2020). They could not cause disease symptom to host plant. Therefore, they are beneficial and showing obligate or facultative and mutualistic association (Nair and Padmavathy 2014). However, they may cause disease symptoms when tested to other plants. The endophytes have wide distribution. It associated with different climatic zones terrestrial as well as aquatic plants. The Bacteria and Fungi that fall under the category of endophytes have symbiotic association to plants (Gautam and Avasthi 2019). However mutualistic behaviour of endophytes is beneficial to plants. The endophytes established association to plant by synthesizing the metabolites, and these metabolites act as plant growth promoter (Dai et al. 2008), elevate plant stress tolerance and protect them from pathogens. However, endophytes improve the plant strength and enable them to adapt under various abiotic and biotic stress conditions.

The fungal endophytes majorly contribute to earth fungal diversity (Hyde and Soytong 2008; Gautam and Avasthi 2019) and play an important role in their ecological niche (Mishra et al. 2015). They have wide distribution and gain popularity as an important source for pant growth promotion and bioactive metabolites (Gangadevi and Muthumary 2008; Sonaimuthu et al. 2010; Bhardwaj and Agrawal 2014; Gautam and Avasthi 2019; Sonawane et al. 2020). Some of the fungal endophytes associated with specific plant species (Hardoim et al. 2015), while some have wide range of host. In addition, tissue specificity is also reported in host plant. However, the endophytes relationship to plants is still meagerly understood. The metabolites produced due to fungal association to host regulate plant growth and subsequently enhance biotic and abiotic tolerance in plants (Murphy et al. 2018). Also, the diverse metabolite produced from fungal endophytes has importance to control various ailments of humans and animals. Noteworthy the fungal endophytes emerge as one of the important metabolite synthesizers (Gautam and Avasthi 2019). Recently emphasis is given on the discovery of novel metabolites from fungal endophytes for agricultural, medicinal and industrial use. However, such compounds discovery and their use as a biocontrol agent on crop plants is notable (Mane and Vedamurthy 2018). The endophytic fungi have attracted the attention of biologist for its potential benefits in agriculture, pharmaceutical and industrial sector (Chadha et al. 2015; Prasad et al. 2017).

The occurrence of biotic and abiotic stresses due to number of factors hampers agricultural productivity. The abiotic factors such as scarcity of water, salinity, cold, heat, nutrient deficiency, water logged, toxic metals and pollutant and biotic factors like fungal, bacterial, nematodes, viruses and mycoplasma pathogens affects the crop yield (Pandey et al. 2017). The severe exposure leads to death of the plant. It was estimated that 96.5% of global rural land area get affected due to abiotic stress and cause up to 70% major crop yield loss (Waqas et al. 2019). From 1990 to 2013, there was 37% increase in salinity of irrigated land (Waqas et al. 2019). Global climate change and global warming increased fluctuation in temperature and precipitation. High temperature causes evapotranspiration which increased harshness of drought stress, while direct exposure to plant causes heat stress (Dai 2011). The uncertainty in precipitation also leads to drought stress, while heavy precipitation causes flooding stress to plants. Increase in urbanization and industrialization contaminated the water resources and arable land with toxic metals and salts. This not only limits crop yield but influences human health (Waqas et al. 2019; Rehman et al. 2018). The change in abiotic factors simultaneously influences crop plant by increasing spread of weeds, pathogens, insects and pests (Ziska et al. 2010; Peters et al. 2014). The biotic factors globally contribute to decline major staple food crop production up to 30% (Savary et al. 2019). However, impacts of abiotic and biotic factors on plant physiological, biochemical and molecular mechanisms are well understood. Therefore, various strategies were employed to nullify abiotic and biotic stress impact on crop plants. However, still most eco-friendly and sustainable way is in need to overcome it.

Currently agricultural scientist focused to endophytic fungi globally for their potential benefit in host plant growth, development and defence (Sharma et al. 2017). The endophytic fungi also synthesized the secondary metabolites of prospective interest due to their importance in biotechnological, pharmaceutical and various agriculture allied fields. The utilization of these endophytes to control pathogens has gained popularity. However, yet more research is in need to explore many things. The present chapter summarizes the information of fungal endophytes characteristics, their potential to enhance abiotic and biotic stress tolerance in crop plants and their utilization in various allied fields. This chapter reduced the hindrance and helps researchers to point out the possible area of research in future importance.

## 7.2 Fungal Endophytes

A fungus is a separate group of eukaryotic organisms. Some group of fungi includes microorganisms because of their microscopic nature. Some of the fungal groups live inside the plants termed as endophytes (Gautam and Avasthi 2019). Endophytes reside in various parts of plant without or with some symptoms. The beneficial endophyte has symbiotic association to plant (Dudeja et al. 2012). However, fungal endophytes are extensively studied for their occurrence, symbiosis, reproduction, host specificity, relationship and applications (Dai et al. 2008; Gangadevi and Muthumary 2008; Sonaimuthu et al. 2010; Bhardwaj and Agrawal 2014). It obligately or facultatively associated with various plants (Gautam and Avasthi 2019). The biological features of fungal endophytes mainly depend on growing environment, nutrition, population density, reproduction, transmission, host range and host growth stage (Patle et al. 2018). The many members of endophytes belong to *Ascomycota*. However, some members of Oomycota, *Zygomycota* and *Basidiomycota* are also endophytes (Stone et al. 2004; Rajamanikyam et al. 2017). The endophytes' mode of transmission and type of host are many times taken into consideration for classification. Based on mode of nutrition, reproduction, host range, colonization in host, mode of transmission and symptoms of host plant, endophytes are mainly categorized as clavicipitaceous and nonclavicipitaceous endophytes (Purahong and Hyde 2011; Mane and Vedamurthy 2018). The clavicipitaceous endophytes mainly associated with grasses, while nonclavicipitaceous with vascular and nonvascular plants (Bamisile et al. 2018; Gautam and Avasthi 2019).

All the plants with fungal endophytes reveals wide distribution and diversity (Gautam and Avasthi 2019). The many fungal endophytes were reported from plants of various climatic zones (Zhang et al. 2006). Almost 3 lakhs land plant species have associations of one or more fungal species (Arnold 2008). The study showed that every plant from nature has association of fungal endophytes either they may be mycorrhiza or other fungi (Khiralla et al. 2017). Therefore, there is still need to screen plants to explore more fungal endophytes. The host specific diversity of fungal endophytes is listed in Table 7.1. The endophytes showed their specific characteristic to colonize a particular tissue. The diverse fungal nature was observed in roots than aboveground parts (Vandenkoornhuyse et al. 2002). It has been reported that the successful colonization of endophytes also depends on habitat and tissue type (Agostinelli et al. 2018). However, all these are the basis of endophyte diversity. The many plants have association of the same fungal endophytes (Sharma and Gautam 2018). However, the broad host range endophytes are in demand for sustainable agriculture. Therefore, further screening of plants for endophytes and their host range needs to be investigated. The transmission is also one of the important factors in case of endophytes and also contributes for fungal diversity (Dudeja and Giri 2014). The endophytic fungi transmitted in host plant through other plant is referred to as horizontal transmission, while if it is transmitted through infected seeds, it is referred to as a vertical transmission. Both ways of transmission are important; however time period of ascospores formation is a limiting factor for their successful transmission (Saikkonen et al. 2002). The definite way for endophytes transmission is still poorly known. The soil, water and wind are still the important medium for transmission of endophytic fungi. Endophytic fungi showed the different mode of penetration and colonization than pathogenic fungi (Gautam and Avasthi 2019). The successful penetration of fungal endophyte showed

**Table 7.1** Fungal endophytes associated with host plants

| Fungal endophytes | Host plant | Reference |
|---|---|---|
| *Alternaria alternata*, *A. tenuissima*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *Cladosporium cladosporioides*, *C. herbarum*, *Curvularia siddiquii*, *C. verruculosa*, *Drechslera* sp., *Epicoccum nigrum*, *Fusarium moniliforme*, *F. solani*, *Helminthosporium* sp., *Humicola grisea*, *Penicillium citrinum*, *P. notatum*, *Rhizopus nigricans* | *Withania somnifera* | Alwadi and Baka (2001), Gautam (2014) |
| *Gloeosporium musae*, *Myxosporium* spp., *Deightoniella torulosa*, *Alternaria tenuis*, *Sphaceloma* spp., *Aureobasidium* spp., *Melida* spp., *Uncinula* spp., *Penicillium* spp., *Aspergillus* spp., *Sarcinella* spp., *Cladosporium* sp., *Cephalosporium* sp. | *Musa acuminata* | Cao et al. (2002) |
| *Balansia* sp., *Pestalotiopsis versicolor*, *Aspergillus aculeatus*, *A. carbonarius*, *A. flavus*, *A. japonicas*, *A. niger*, *A. pulverulentus*, *F. moniliforme*, *Gilmaniella* sp., *Nigrospora* sp., *Penicillium citrinum*, *P. herquei*, *P. janthinellum*, *P. rubrum*, *P. rugulosum*, *P. simplicissimum*, *P. implicatum*, *Trichoderma koningii*, *T. nivale* | *Melia azedarach* | Geris dos Santos et al. (2003) |
| *Sporidiobolus* sp., *Rhodotorula* sp., *Pilidium concavum*, *Corynespora cassiicola*, *Neodeightonia subglobosa*, *Aspergillus awamori*, *Aspergillus* sp. | *Fragaria x ananassa* | Ezra et al. (2004) |
| *Muscodor albus* | *Cinnamomum zeylanicum* | Ezra et al. (2004) |
| *Alternaria* sp., *Cladosporium* sp., *Chaetomium* sp., *Curvularia* sp., *Drechslera* sp., *Scopulariopsis* sp., *Acremonium* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Paecilomyces* sp., *Penicillium* sp. | *Glycine max* | Pimentel et al. (2006) |
| *Alternaria* sp., *Colletotrichum* sp., *Nigrospora* sp., *Phomopsis* sp., *Fusarium* sp., *Penicillium* sp., *Schizophyllum commune* | *Tectona grandis*, *Samanea saman* | Chareprasert et al. (2006) |
| *Alternaria* sp., *Colletotrichum* sp., *Phomopsis* sp., *Xylaria* sp. | *Artemisia capillaris*, *Azadirachta indica*, *A. lactiflora* | Huang et al. (2009) |
| *Glomus mosseae*, *Glomus intraradices*, *Glomus claroideum* | *Olea europaea* | Porras-Sorianoa et al. (2009) |

(continued)

**Table 7.1** (continued)

| Fungal endophytes | Host plant | Reference |
|---|---|---|
| *Cladosporium* sp., *Acremonium* sp., *Trichoderma* sp., *Monilia* sp., *Fusarium* sp., *Spicaria* sp., *Humicola* sp., *Rhizoctonia* sp., *Cephalosporium* sp., *Botrytis* sp., *Penicillium* sp., *Chalaropsis* sp., *Geotrichum* sp. | *Cephalotaxus mannii* | Saithong et al. (2010) |
| *Fusarium oxysporum*, *Fusarium solani*, *Emericella nidulans* | *Ipomea batatas*, *Taxus baccata* | Hipol (2012), Tayung et al. (2011), Mirjalili et al. (2012) |
| *Fusarium oxysporum*, *F. solani*, *F. proliferatum* | *Cajanus cajan* | Zhao et al. (2012) |
| *Aspergillus flavus*, *Chaetomium globosum*, *Cochliobolus lunatus*, *Fusarium dimerum*, *F. oxysporum*, *P. chrysogenum* | *Calotropis procera* | Gherbawy and Gashgari (2013) |
| *Phomopsis* sp., *Alternaria raphani*, *M. hiemalis*, *Monodictys paradoxa*, *Aspergillus fumigates*, *A. japonicas*, *A. niger*, *Fusarium semitectum* | *Vitex negundo* | Monali and Bodhankar (2013) |
| *Fusarium* sp., *Phaeoacremonium* sp., *Acremonium* sp., *Cladosporium* sp., *C. gloeosporioides Penz.*, *Phomopsis archeri*, *A. flavus*, *Nigrospora sphaerica* | *Sesbania grandiflora* | Powthong et al. (2013) |
| *Piriformospora indica* | *Bacopa monnieri*, *Arabidopsis thaliana*, *Aloe vera*, *Hordeum vulgare* | Prasad et al. (2013), Sharma et al. (2016), Ghaffari et al. (2016) |
| *Aspergillus niger*, *A. flavus*, *A. nidulans*, *Penicillium chrysogenum*, *P. citrinum*, *Phoma* sp., *Rhizopus* sp., *Colletotrichum* sp., *Cladosporium* sp., *Curvularia* sp. | *Cannabis sativa* | Gautam et al. (2013), Meenatchi et al. (2016) |
| *Acremonium* sp., *Colletotrichum* sp., *Cochliobolus* sp., *Fusarium* sp., *Hypocrea* sp., *Nemania* sp. | *Lycium chinense* | Paul et al. (2014) |
| *Glomerella* spp., *Diaporthe/ Phomopsis* sp., *Alternaria* spp., *Cochliobolus* sp., *Cladosporium* sp., *Emericella* sp. | *Aegle marmelos*, *Coccinia indica*, *Moringa oleifera* | Gokul Raj et al. (2014) |
| *Rhizopus stolonifer*, *Drechslera*, *Cladosporium*, *Curvularia lunata*, *Chaetomium*, *Penicillium* spp., *Fusarium*, *Ulocladium consortiale*, *Mucor hiemalis*, *Scytalidium thermophilum*, *Phoma solani*, *Taeniolella exilis*, *Botryodiplodia theobromae* | *Boswellia sacra* | El-Nagerabi et al. (2014) |

(continued)

**Table 7.1** (continued)

| Fungal endophytes | Host plant | Reference |
|---|---|---|
| *Aspergillus* sp., *Penicillium* sp., *Eurotiomycetes* sp., *Acremonium* sp., *Colletotrichum* sp., *Fusarium* sp., *Nodulisporium* sp., *Pestalotiopsis* sp. | *Marchantia polymorpha* | Hipol et al. (2015) |
| *Aspergillus niger*, *Bipolaris maydis*, *Meyerozyma guilliermondii*, *Fusarium verticillioides* | *Ocimum sanctum* | Chowdhary and Kaushik (2015) |
| *Funneliformis mosseae*, *Rhizophagus intraradices*, *Claroideoglomus etunicatum* | *Sesbania sesban* | Abd-Allah et al. (2015) |
| *Fusarium oxysporum*, *Fusarium solani* and *Fusarium* sp., *F. graminearum* | *Glycine max* | Fernandes et al. (2015) |
| *Penicillium chrysogenum*, *P. chrysogenum*, *Fusarium oxysporum*, *F. nygamai* | *Tamarix nilotica*, *Cressa cretica* | Gashgari et al. (2016) |
| *Fusarium proliferatum*, *Fusarium* sp., *F. solani*, *C. lunata*, *Trichoderma atroviride*, *Calonectria gracilis*, *Rhizoctonia solani*, *Bionectria ochroleuca* | *Musa acuminata* | Zakaria et al. (2016) |
| *Pythium ultimum*, *Sclerotium oryzae*, *Rhizoctonia solani*, *Pyricularia oryzae* | *Zea mays*, *Oryza sativa* | Potshangbam et al. (2017) |
| *Glomerellaacutata*, *Epicoccum nigrum*, *Diaporthe* spp., *Penicillium chloroleucon*, *Diaporthe endophytica*, *Mucor circinelloides* | *Vitex negundo* | Sibanda et al. (2018) |
| *Clonostachys* sp., *Colletotrichum* sp., *Trichoderma* sp. | *Hevea brasiliensis* | Vaz et al. (2018) |
| *Aspergillus fumigatus*, *Colletotrichum gloeosporioides*, *Diaporthe discoidispora*, *Diaporthe pseudomangiferae*, *Nodulisporium* sp., *Penicillium* sp., *Pestalotiopsis* sp., *Phyllosticta capitalensis*, *Xylaria* sp. | *Mangroves* | Rajamani et al. (2018) |
| *Alternaria alternata (Fr.) Keissl.*, *Aspergillus flavus*, *A. niger*, *Chaetomium globosum*, *Chaetomium* sp., *Chloridium* sp., *Cochlonema* sp., *Colletotrichum* sp., *Curvularia* sp., *Drechslera* sp., *Fusarium* spp., *Penicillium* spp., *Gliomastix* sp., *Humicola* sp., *Nigrospora* sp., *Pestalotiopsis* spp., *Phoma eupyrena*, *Phoma* sp., *Phomopsis* sp., *Phyllosticta* sp., *Scytalidium* sp., *Trichoderma* sp., *Trichoderma* spp., *Verticillium* sp. | *Azadirachta indica* | Chutulo and Chalannavar (2018) |
| *Cladosporium* sp., *Penicillium* sp., *Trichoderma* sp. | *Populus trichocarpa* | Huang et al. (2018) |
| *Cladosporium omanense* | *Zygophyllum coccineum* | Halo et al. (2019) |

successful symbiosis in plant. This symbiotic association is important to recycle nutrient, stimulate plant growth and improve plant biotic and abiotic stress tolerance (Sturz and Nowak 2000; Varma et al. 2020). But the penetration and colonization into plant is a difficult task for endophytes. However pathogenic fungi transmission, penetration and colonization route may emerge as an effective way for beneficial endophytes delivery to plant.

## 7.3 Fungal Endophytes for Stress Tolerance in Crop Plant

The abiotic and biotic stresses are serious limiting factors for crop plants (Pandey et al. 2017). Globally they effect on crop productivity and sustainability. The researchers are in search of most sustainable way to overcome it. However fungal endophytes are important associator of plants which showed their importance in agriculture field as a potential growth promoter to support plant under adverse environmental conditions. It is well known to enhance tolerance of abiotic and biotic stress (Murphy et al. 2018).

### *7.3.1 Abiotic Stresses*

Abiotic stresses are the most limiting factors that cause serious yield loss of crop plants (Waqas et al. 2019). The drought, salinity, higher and lower temperature, water logged, flooding and toxic metals are the important factors that affect crop plants yield (Zafar et al. 2018; Waqas et al. 2019). Globally 90% of arable lands are under serious threat of above stresses (Waqas et al. 2019). These stresses affect major crop plants and cause 70% yield loss (Waqas et al. 2019). Plants cope up with these stresses by activation of series of biochemical processes. Recently it was reported that endophytes associated with plant synthesized chemical compounds when exposure of plants to stress acts as an anti-stress agent (Kaur 2020). The experimental evidences also support that endophytic fungi can help the host plants under stress conditions and can thus increase the plant growth.

#### 7.3.1.1 Drought Stress

The unavailability of water to plant from soil causes water stress or drought stress in plants. It is one of the important abiotic stresses that majorly affects crop production (IPCC 2007). Water stress is a major constraint of arid and semiarid region of the world (Kabiri et al. 2014). The increase in drought-prone area globally due to change in global climate causes insufficient and irregular precipitation and depletion of groundwater table and ability of soil particles to retain water (Dai 2013; Kapoor et al. 2020). Depletion of water level from soil causes negative impact on plants from seed germination to seed formation stage (Zlatev and Lidon 2012). To cope up with this, plant changes their physiological, anatomical and biochemical mechanisms and metabolisms (Kapoor et al. 2020). The fungal endophytes enhance stress tolerance through activating stress tolerance response of host and by synthesizing anti-stress compounds in host (Singh et al. 2011). The osomotic adjustment is of prime importance to any plants that suffer from any stress; therefore plants synthesize osmolytes in their tissues. The stress plants significantly accumulate solutes in their tissues which help plant to improve leaf water and stomatal conductance (Malinowski and Belesky 2000). Stomatal conductance and osmotic balance were regulated by endophyte *Neotyphodium* spp. which protect grass plant under drought stress (Chhipa and Deshmukh 2019). Lavender plants challenged by drought after inoculation of *Glomus* spp. enhance tolerance through maintaining water, N and P content and increase root biomass by accumulating osmolytes in their tissues (Porcel et al. 2006; Marulanda et al. 2009). The *Chaetomium globosum* and *P. resedanum* associated with *Capsicum annuum* plants report increase in biomass and shoot length under drought (Khan et al. 2012a, 2014a). The plants with endophytic fungi requires less water and increase in biomass as compared to non-endophytic fungi. Rice plant inoculated with *Fusarium culmorum* and *Curvularia protuberata* showed increase in biomass under drought stress (Redman et al. 2011). Increases in chlorophyll and leaf area were also reported in fungal endophytes associated with plants than non-associated plants. The increase in photosynthetic rate under drought stress in *C. annuum* plants is reported after colonization of *C. globosum* (Khan et al. 2012b) and *P. resedanum* (Khan et al. 2014b). However, increase in chlorophyll and leaf area positively correlates to photosynthetic rate. The osmotic protection is mostly preferred by endophytes under drought stress condition. The many endophytes synthesized loline alkaloids which act as an osmolyte agent under drought stress (Singh et al. 2011), but further study is in need to know its accountable contribution. Along with this, soluble sugars and sugar alcohols are synthesized by the plant or endophyte that act as important osmoregulators (Singh et al. 2011). Some osmolytes also act as antioxidant to neutralize a ROS generated in plant due to drought stress. Many studies on endophytes to different plants under drought stress suggested that endophytes regulate plant growth by maintaining nutrients, water and photosynthetic balance (Dastogeer and Wylie 2017). Some endophytes involved to enhance drought stress tolerance in plants are listed in Table 7.2.

#### 7.3.1.2 Salt Stress

Salinity stress is another abiotic factor drastically affecting crop yield and production. Salinity worldwide affects 0.8 billion hectares of land, which is almost 6% of total land area (Shelke et al. 2019a). It includes 20% of cultivated land and 33% of irrigated land, decline 50% of major crop yield (Shelke et al. 2017). Poor-quality irrigation water, rock erosion, improper agricultural practices and overutilization of chemical fertilizers are the major factors causing soil salinity. Salinity causes osmotic and ionic stress to plant. Plant faces osmotic stress instantly upon salt

**Table 7.2** Fungal endophytes to elevate abiotic stress tolerance in plants

| Fungal endophytes | Host plant | Abiotic stress | Reference |
|---|---|---|---|
| *Neotyphodium* sp. | *Festuca pratensis*, *Perennial Ryegrass*, *F. arizonica* | Drought | Malinowski et al. (1997), Barker et al. (1997), Morse et al. (2002) |
| *N. lolii* | Perennial ryegrass | Drought | Latch et al. (1985), Ravel et al. (1997) |
| *N. coenophialum* | Tall fescue | Drought | Belesky et al. (1989), de Battista et al. (1990) |
| *N. uncinatum* | Meadow fescue | Drought | Malinowski (1995) |
| *Acremonium* sp. | Tall fescue | Drought | White et al. (1992) |
| *Phialophora* sp. | *F. pratensis* | Drought | Malinowski et al. (1997) |
| *C. protuberata* (Cp4666D) (CpMH206), *Fusarium culmorum* (Fc18), *F. culmorum* (FcRed1) | *D. lanuginosum*, *Leymus mollis*, *Oryza sativa*, *Lycopersicon esculentum* | Drought | Rodriguez et al. (2008) |
| *C. protuberata* (Cp4666D) | *Triticum aestivum*, watermelon | Drought | Rodriguez et al. (2008) |
| *Curvularia* sp., *Alternaria* sp. | *L. esculentum* | Heat/ drought | Rodriguez and Redman (2008) |
| *Colletotrichum magna* (path-1), *C. magna* (L2.5), *C. musae* (927), *C. orbiculare* (683), *C. gloeosporioides*, *C. gloeosporioides* (95-41A) | *L. esculentum*, *Capsicum annuum* | Drought | Redman et al. (2001) |
| *Colletotrichum* sp., *Fusarium* sp. | *L. esculentum* | Drought | |
| *C. orbiculare*, *C. magna*, *C. gloeosporioides*, *C. musae* | *L. esculentum* cv. Big Beef and Seattle's Best, *Triticum aestivum*, *C. annuum* cv. Calif. Wonder, watermelon | Drought | Rodriguez and Redman (2008) |
| *P. indica* | *Arabidopsis* sp., *Brassica campestris* ssp. *chinensis* | Drought | Sherameti et al. (2008), Sun et al. (2010) |
| *Trichoderma hamatum* (DIS 219b) | *Theobroma cacao* | Drought | Bae et al. (2009) |
| *Ampelomyces* sp. and *Penicillium sp.* | *Lycopersicon esculentum* | Drought, salinity | Morsy et al. (2020) |
| *F. culmorum* (FcRed1) | *D. lanuginosum*, *Leymus mollis*, *Oryza sativa*, *Lycopersicon esculentum* | Salinity | Rodriguez et al. (2008) |
| *Piriformospora indica* | *Hordeum vulgare*, *Hordeum vulgare* cv. Ingrid | Salinity | Waller et al. (2005), Baltruschat et al. (2008) |

(continued)

**Table 7.2** (continued)

| Fungal endophytes | Host plant | Abiotic stress | Reference |
|---|---|---|---|
| *Porostereum spadiceum* AGH786 | *Glycine max* | Salinity | Hamayun et al. (2017) |
| *Yarrowia lipolytica* | *Euphorbia milii* | Salinity | Jan et al. (2019) |
| *C. protuberata* | *L. esculentum* | Heat | Rodriguez et al. (2008) |
| *Curvularia* spp. | *Dichanthelium lanuginosum* | Heat | Stierle et al. (1993) |
| *Aspergillus japonicus* | *Euphorbia indica*, *G. Max*, *H. annuus* | Heat | Ismail et al. (2018) |
| *Thermomyces lanuginosus* | *C. plicata* | Heat, drought | Ali et al. (2019) |
| *C. protuberata* | *Oryza sativa* | Cold | Redman et al. (2011) |
| *Glomus mosseae* | *Triticum aestivum*, *Lycopersicon esculentum*, *Citrus tangerine*, *Elymus nutans* | Cold | Paradis et al. (1995), Abdel-Latef and Chaoxing (2011), Wu and Zou (2010), Chu et al. (2016) |
| *Glomus claroideum* | *Gnaphalium norvegicum* | Cold | Ruotsalainen and Kytcoviita (2004) |
| *Glomus etunicatum* | *Zea mays* | Cold | Zhu et al. (2010a, b, c) |
| *G. intraradices* | *Oryza sativa* | Cold | Liu et al. (2014) |
| *Chaetomium globosum*, *Epicoccum nigrum* and *Piriformospora indica* | *Hordeum vulgare* | Cold | Murphy et al. (2014) |
| *Epichloe* sp. | *Festuca sinensis* | Cold | Zhou et al. (2015) |
| *Rhizophagus irregularis* | *C. sativus*, *Digitaria eriantha* | Cold | Ma et al. (2015), Pedranzani et al. (2016) |
| *Rhizophagus intraradices* | *Jatropha curcas* | Cold | Pedranzani et al. (2015) |
| *Mucor* sp. | *Arabidopsis arenosa* | Heavy metal | Domka et al. (2019) |
| *Phialocephala fortinii*, *Rhizodermeaveluwensis* and *Rhizoscyphus* sp. | *C. barbinervis* | Heavy metal | |

exposure, while ionic stress after on accumulation of $Na^+$ and $Cl^-$ ion. The osmotic stress has impact on cell turgor pressure, water balance, elongation, division and root development, while ion uptake affects ion regulation of cell which in turn causes hormonal modulation, mechanisms (photosynthesis, transpiration, nutrient translocation) and metabolic processes. The increased in $Na^+$ and $Cl^-$ion content in cell causes toxicity to plants. However, both ions showed their specificity to cause toxicity in plants (Shelke et al. 2019b). Therefore, focus has been given to improve osmotic balance and ion regulation in perspective of plant salinity tolerance. The crop breeding approach was utilized to develop salt-tolerant crop plants but is time-consuming and gives a smaller number of tolerant genotypes. The genetic engineering approach could emerge as an alternative way but is cost-effective.

Therefore, utilization of fungal endophytes to enhance plant salt tolerance is a viable alternative for saline soil agriculture. The fungal endophytes not only enhance the salt tolerance but participate in plant growth promotion. These fungal endophytes maintained soil–water–plant relationships and modulate phytohormonal signalling to elicit numerous other mechanisms that work in a combined mode to enhance salt stress tolerance in plants. However, there is a little understanding of benign fungal endophytes mediated mechanisms underlying salt tolerance and plant growth enhancement that needs to be crammed. There is now rising aspect that plant salt tolerance is well connected with their associated fungal endophytes (Hardoim et al. 2015). However, some fungal endophytes fail to provide beneficial property under harsh environmental conditions. Therefore, there is need to search broad-range endophytes in context to various plant as well as environmental conditions.

The *Hordeum vulgare* inoculated with *P. indica* enhance salt tolerance by increasing glutathione reductase, catalase, dehydroascorbate reductase, ascorbate peroxidase and monodehydroascorbate reductase activity and ascorbate level while decreased in lipid peroxidation of leaf under salt stress (Waller et al. 2005; Baltruschat et al. 2008). In *Leymus mollis*, plant without *F. culmorum* association dried and severely wilted within 7 days and dies in 14 days when exposed to 500 mmol/L NaCl, while plant with symbiotic association is reported with wilting symptoms after 14 days (Rodriguez et al. 2008). However, many studies which reported endophytic fungi role to enhance salt tolerance through various ways are listed in Table 7.2. Therefore, there is urge need to study the role of fungal endophytes in salt stress tolerance and sustainable saline agriculture.

#### 7.3.1.3 Heat and Cold Temperature Stress

Global climate change raises the concern of temperature fluctuations in various agro-climatic zones. High temperature causes degradation of proteins and change in membrane permeability which in turn cause cellular damage (Hussain et al. 2018), whereas decrease in temperature stops enzyme functioning and causes cell bio-molecules interactions and membrane fluidity which in turn causes cellular damage (Andreas et al. 2012; Acuna-Rodriguez et al. 2020). However cellular damage leads to impairment of metabolic processes which causes decrease on crop yield. The *Dichanthelium lanuginosum* has association of endophytes *Curvularia* spp. which make this plant stable till soil temperatures reach up to 57 °C (Stierle et al. 1993). However, this endophyte increases heat stress tolerance in wheat, tomato and watermelon (Redman et al. 2002). There is positive correlation between endophyte *C. protuberata* and associated plant *Dichanthelium lanuginosum* for thermal tolerance (Redman et al. 2002). Moreover *C. protuberata* also enhance rice-germinating seeds cold tolerance (Redman et al. 2011). The tomato plants inoculated with *G. mosseae* enhanced cold stress tolerance by increasing soluble protein, leaf sugar and pigment in plant (Abdel-Latef and Chaoxing 2011). However, many other endophytes have a role to enhance the heat and cold tolerance of host plants which are listed in Table 7.2.

#### 7.3.1.4 Toxic Metal Stress

Utilization of heavy metals in various industries raises the concern of soil and water pollution. The heavy metal-contaminated water and soil decline the crop yield up to 25–80% by causing toxicity to root and upper plant parts (Kaur 2020). Fungal endophytes assist plant to give heavy metal tolerance through chelation, sequestration, distribution and conversion of it into non-toxic form (Likar 2011; Wang et al. 2016). The chemical compounds synthesized by fungal endophytes bind to heavy metals which restrict entry of it into cell which in turn avoids cell toxicity. *Phialocephala fortinii* endophytes synthesized melanin which bind to heavy metals and avoid cell toxicity restricting it to reach living cell (Fogarty and Tobin 1996). The fungal endophytes *P. fortinii*, *R. veluwensis* and *Rhizoscyphus* sp. associated with *Clethra barbinervis* showed increase in nutrient uptake and growth promotion by decreasing heavy metal concentration. It achieved heavy metal tolerance through synthesized siderophores which exclude heavy metals into rhizospheric soil. Noteworthy siderophores are a chemical compound synthesized by plant or endophytes which chelate heavy metals in rhizosphere soil and restrict its uptake (Kaur 2020). Domka et al. (2019) reported fungal endophytes *Mucor* sp. help *Arabidopsis arenosa* to enhance heavy metal tolerance by decreasing oxidative stress.

### *7.3.2 Biotic Stresses*

Stress in plants is considered as exterior circumstances that negatively influence growth and development and subsequently yield or productivity of plants. Stress generates a broad spectrum of plant reactions such as alteration in gene expression, cell metabolism, variations in growth index, crop productivity, etc. (Verma et al. 2013). Plant stress primarily can be categorized into abiotic and biotic stress. In biotic stress, a stress factor facilitates to the plants as a biological entity such as bacteria, fungi, mycoplasma, virus, insects, nematodes, arachnids and weeds (Zhu 2002; Verma et al. 2013). An organism causing biotic stress openly withdraws the nutrition from host which can lead to death of host plants. It could be a principal cause of pre- and post-harvest fatalities. Although there is lack of adaptive immune response, plants can adapt counteract by developing specific defence mechanisms. The biotic stress is completely differing from abiotic stress which is obligatory to the plants. In response to biotic stress, crop plants can develop self-defence system to defend against particular type of biotic stress. Several biotic stresses affect rate of reaction of photosynthesis, as leaf part consumed by insects or any kind of biotic entity. Therefore, various types of biotic agents cause particular sort of damage to the host plants as described in Table 7.3.

**Table 7.3** Fungal endophytes to elevate biotic stress tolerance in plants

| Fungal endophytes | Host plant | Biotic stress | Reference |
|---|---|---|---|
| *Aspergillus terreus* and *Penicillium citrinum* | *Helianthus annuus* | Stem rot (*Sclerotium rolfsii*) | Waqas et al. (2014) |
| *Fusarium oxysporum* | *Lycopersicon esculentum* | *Meloidogyne incognita* | Gond et al. (2010) |
| *Acremonium zeae* | *Zea mays* | *Aspergillus flavus*, *Fusarium verticillioides* | Gao et al. (2010) |
| *T. harzianum*, *Guignardia* sp. and *Phomopsis* sp. | *Coffea arabica* and *C. robusta* | *Salmonella choleraesuis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* | Gond et al. (2010) |
| *Fusarium verticillioides* and *Humicola* spp. | *Camellia sinensis* | Fungal pathogen | |
| *Acremonium sclerotigenum* | *Terminalia bellerica* | Bacterial, fungal and viral pathogens | |
| *Fusarium* spp. | *Dioscorea zingiberensis* and *F. redolens* | *B. subtilis*, *E. coli*, *S. haemolyticus* and *X. vesicatoria* | Gond et al. (2010) |
| *Muscodor vitigenus* | *Paullinia paullinioides* | Bowl fly larvae | Demain (2000), Daisy et al. (2002) |
| *Chaetomium*, *Alternaria*, *Cercophora*, *Fusarium*, *Hypoxylon*, *Nigrospora*, *Cladosporium*, *Thielavia*, *Schizophyllum*, *Gibberella* | *Cannabis sativa*, *Cedrus deodara*, *Pinus roxburghii*, *Picrorhiza kurroa*, *Withania somnifera* | Fungal and viral pathogens | |
| *C. sphaerospermum* | *Picea glauca* | Homoptera *Adelges abietis* | Gond et al. (2010) |
| *Alternaria*, *Colletotrichum*, *Aspergillus*, *Fusarium*, *Gliocladium* and *Cunninghamella* | *Malus sieboldii* | Fungal pathogen | |
| *Hypoxylon* sp. | *Persea indica* | Microbial (*Botrytis*, *Cercospora*, *Phytophthora* and *Sclerotinia*) | |
| *Aspergillus niger*, *A. flavus*, *A. nidulans*, *Colletotrichum*, *Curvularia*, *Cladosporium*, *Penicillium chrysogenum*, *P. citrinum*, *Phoma* and *Rhizopus* | *Cannabis sativa* | Fungal pathogens | Gautam et al. (2013) |

(continued)

**Table 7.3** (continued)

| Fungal endophytes | Host plant | Biotic stress | Reference |
|---|---|---|---|
| *Trichoderma*, *Nigrospora* and *Curvularia* | *Rauwolfia serpentina* | *Fusarium oxysporum* and *Phytophthora* spp. | |
| *Alternaria tenuissima*, *Aspergillus fumigatus*, *A. japonicas*, *A. niger*, *A. repens*, *Curvularia pallescens*, *Fusarium solani*, *F. semitectum*, *Phoma hedericola* and *Drechslera australien* | *Ricinus communis* | Fungal pathogens | |
| *Cladosporium* spp. | *Tinospora cordifolia* | Tobacco cutworm (*Spodoptera litura*) | |
| *Nigrospora*, *Fusarium* spp. | *Crescentia cujete* | Fungal pathogen | |
| *Phomopsis* | *Brucea javanica* | Bacterial and fungal pathogens | |
| *Acremonium*, *Aspergillus niger*, *Cladosporium*, *Curvularia lunata*, *C. brachyspora*, *Penicillium* spp. | *Urginea indica* | Bacterial pathogen | |
| *Phomopsis oblonga* | Elm tree | Dutch elm disease (*Ceratocystis ulmi*) | |
| *Piriformospora indica* | *Prosopis juliflora* and *Ziziphus nummularia* | Pathogens (growth inhibitors) | |
| *Alternaria*, *Pyrenochaeta*, *Cladosporium*, *Colletotrichum*, *Leptosphaeria*, *Fusarium*, *Paraconiothyrium*, *Stephanonectria* | *Holcoglossum rupestre* and *H. flavescens* | Fungal pathogen | |

#### 7.3.2.1 Bacterial

Biotic stress in plants is triggered by biological agents especially bacteria which hold nutrients from the host plant. Bacterial pathogens are more versatile among all living microorganisms that can invade nearly all environmental habitats especially crop plants. The plants infected with bacteria suffer various conformational changes like affecting cell osmolarity (low or high osmolarity) which leads to death of plants. *E. coli* affects potassium ($K^+$) cellular transport system which exhibits low affinity with TrkG, Kup, TrkH and TrkF transporters which hampers $K^+$ uptake (Sleator and Hill 2001). However, the adapted bacterial pathogens have modified specialized mechanisms to affect plant defence response and disease receptiveness of hosts. Currently, plant pathogenic bacteria are using type III response effector proteins, toxins and other parameters to decline host defence. The ethylene levels within plant tissues increase after bacterial incidence which exhibits various modulations in the physiological and biochemical pathways (Van Loon et al. 2006; Hao et al. 2011). In India *Citrus* canker and black arm of cotton are the two major bacteria inducing diseases which devastatingly affect the productivity of *Citrus* spp. and cotton, respectively. Fungal endophyte *Galactomyces geotrichum* has been isolated from *Trapa japonica* which is used to synthesize higher amount of jasmonic acid producing systemic resistance against fungal and bacterial pathogens in soybean (Waqas et al. 2014). The endophytic fungi *Epicoccum nigrum* identified from *Saccharum officinarum* especially exhibit bactericidal potential against phytoplasma bacteria in (apple) *Malus domestica* (de Favaro et al. 2012). Therefore, various fungal endophytes showed antibacterial activity against different pathogenic bacteria in several crop species as represented in Table 7.3.

#### 7.3.2.2 Fungal

Fungal pathogens are the very broad range or devastating biotic stress among the other biotic stress-inducing agents like bacteria, virus, nematodes, etc. There are some pre-harvesting fungal pathogens like *Alternaria*, *Aspergillus*, *Cercospora* leaf spots, *Choanephora* blight, *Fusarium* stem rot, *Fusarium* wilt, *Phytophthora* blight, *Verticillium* wilt and powdery mildew that are grown in various crops (Hussain and Usman 2019). Bebber and Gurr (2015) have reported that pre- and post-harvest crop yield decline about 30% occurs under biotic stress. Polyamines metabolism has well adapted to deform in plant cells counter to devastating variability in plants exposing with fungal or mycorrhizal pathogens (Asthir et al. 2004). The excess ethylene synthesis in plant tissues is followed by fungal infection like *Pyricularia oryzae* (Amutharaj et al. 2012) and *Pythium aphanidermatum* (El-Tarabily 2013). In Indian agriculture system, the common fungal pathogens affect various crop plants such as club rot of crucifers, downy mildew of grapes, head smut of jawar, leaf spot of turmeric and tikka disease of groundnut caused by *Cercospora*. The appearance of these pathogenic fungi on specific crop declines the crop productivity. The compound cryptocin isolated from fungus *Cryptosporiopsis quercina* exhibits antifungal activity against fungus *Pyricularia oryzae* and various fungal pathogens in plant *Tripterygium wilfordii*. The fungal endophytes *Curvularia*, *Nigrospora* and *Trichoderma* identified from *Rauwolfia serpentina* exhibit antifungal activity in opposition to *Fusarium oxysporum* and *Phytophthora*. An endophytic fungus *Fusarium oxysporum* identified in tomato exhibits induced resistance against *Fusarium* wilt disease (Gond et al. 2010). The reduction in growth rate of pathogen *Ustilago maydis* is influenced by endophytic fungus *Fusarium verticillioides* within host crop plant *Zea mays* (Lugtenberg et al. 2016). Endophytic fungi *Alternaria* spp., *A. tenuissima*, *Chaetomium* spp., *Colletotrichum truncatum*, *Thielavia subthermophila* and *Nigrospora oryzae* isolated from medicinal plant *Tylophora indica* showed antifungal activity against *Fusarium oxysporum* and *Sclerotinia sclerotiorum* by inhibiting growth. Likewise, the various fungal endophytes that possess antifungal activity against various pathogenic fungi in different crop plants are listed in Table 7.3.

#### 7.3.2.3 Viral

Recent global climate transform and emergence of unexpected rate of contagious diseases affect alarming environmental problems. In India, farming is the conventional raw material production sector among the other countries where highest part of the inhabitants lies on commercial agriculture for their earnings source (Huseynova et al. 2014). Crop plants are majorly affected by a broad range of pathogens exhibiting variable degrees and various types of signs. In plant system, to date various viral diseases are reported such as CMV, TMV, ToMV, ToCV, MNSV, TYLCV, FBNYU, BTB, etc. (Huseynova et al. 2014). The viral pathogens comprise a major portion of diseases that occurred in all plant categories, with variety of recognitions including curling of leaves, distortion in leaves, yellowing mosaic, stunting growth and decline in productivity (Yamji et al. 2013; Huseynova et al. 2014). Viral pathogens are the foremost basis of crop yield loss all over the globe, including India, and dispersion areas as well as form of viral diseases vary from year to year (Huseynova et al. 2014). For instance, cucumber mosaic virus (CMV) is one of the most frequent viral pathogens that occurs in several vegetable crops like *Cucumber* spp., and it is generally spread through aphid vectors in crop plants. As per the literature available, various strains of CMV can affect about 1287 plant spp. including cucumber, beans, lettuce, peppers, sugar beet, tobacco and tomato (Mochizuki and Ohki 2012). Likewise, tobamoviruses include tobacco and tomato mosaic virus (TMV and ToMV) correspondingly, which affect majorly *Nicotiana tabacum* and *Solanum tuberosum* spp. (Huseynova et al. 2014). Recently, papaya ring spot virus (PRSV), tobacco mosaic virus (TMV) and bunchy top of banana (BTB) are the major viral threats affecting yield potential of crop plants. The infection of various fungal endophytes in crop plants *L. perenne* and *Festuca arundinaceum* appears to be more resistant to BYDV (barley yellow dwarf virus) (Gond et al. 2010). Fungal endophyte *Chaetomium* spp. are identified in various crop plants like *Piper nigrum*, *Citrus reticulata*, *Lycopersicon esculentum* and *Fragaria* spp. which possess antiviral potential against different viral pathogens. *Piriformospora indica* fungal endophyte isolated from host plant *Hordeum vulgare* exhibits harmful effects against various viral pathogens (Murphy et al. 2013).

#### 7.3.2.4 Nematodes

Nematodes are the parasitic group of microorganisms associated with rhizospheres of various crop plants which causes different diseases to the host plants. Endophytic fungi possess nematocidal potential as they are recognized to secrete various metabolites which are harmful to nematodes (Kaur 2020). The initial report on inhibitory effect of endophytic fungi was screened against plant parasitic nematode

*Pratylenchus scribneri* in *F. arundinacea* plant (Gond et al. 2010). For instance, a fungus endophyte *Fusarium oxysporum* inhibits the growth and development of *Meloidogyne incognita* a parasitic nematode found in tomato plant (Diedhiou et al. 2003). Likewise, endophyte fungal isolates (*Fusarium oxysporum* and *Trichoderma atroviride*) exhibit potential ability to improve and enhance banana yield by inhibiting the population growth of burrowing parasitic nematode *Radopholus similis* inhabited in banana crop (Felde et al. 2006). The arbuscular mycorrhizal fungus and endophyte *Glomus coronatum* and *Fusarium oxysporum*, respectively, showed effective nematicidal activity against *Meloidogyne incognito* in tomato crop (Gond et al. 2010). Among diverse fungal species, *Fusarium oxysporum* is the dominant endophyte identified in various crop plants (banana, tomato and melons), and it has toxic effects to plant parasitic nematodes such as *Helicotylenchus multicinctus*, *Meloidogyne incognita*, *M. graminicola*, *Pratylenchus goodeyi* and *R. similis* (Menjivar et al. 2011). A fungal endophyte *Epichloe coenophiala* acts as nematicidal agent against *Helicotylenchus pseudorobustus*, *Meloidogyne marylandi* and *Pratylenchus scribneri* in the crop tall fescue (Elmi et al. 2000), whereas a fungal endophyte *Chaetomium globosum* against *Meloidogyne incognito* in cotton plant (Zhou et al. 2016; Kumar and Dara 2021). An incidence of *M. incognito* to *Lycopersicon esculentum* (tomato) is controlled by the fungal endophyte *Fusarium oxysporum.* A fungal endophyte *Fusarium* spp. isolated from fruit crop *Musa paradisiaca* (banana) exhibits nematicidal activity against *Radopholus similis*. Similarly, a fungal endophyte *Fusarium oxysporum* identified in banana crop plants showed harmful effects against various nematodes like *H. multicinctus*, *P. goodeyi* and *R. similis*. Therefore, various fungal endophytes are recognized in several crops (fruit, tuber, ornamental and fodder crops) for effective nematicidal activity as listed in Table 7.3.

#### 7.3.2.5 Insects and Pests

Endophytic fungi provide significant protection to the various crop species from highly devastating insects, pests and herbivores. Until now, various fungal endophytes are recognized to produce array of secondary metabolites which possess insecticidal and pesticidal properties (Lugtenberg et al. 2016). At the initial time, *Phomopsis oblonga* endophyte was reported as insecticidal activity against beetle *Physocnemum brevilineum* found on elm tree. Lugtenberg et al. (2016) reported the nodulisporic acid which is produced by various fungal endophytes exhibits the potential insecticidal in opposition to the larvae of blowfly. Likewise, the nodulisporic compounds are secreted by an endophyte *Nodulisporium* spp. in the host plant *Bontia daphnoides* that showed insecticidal activity (Demain 2000). A novel endophyte *Epichloe* produces peramine metabolite which provides resistance against Argentine stem weevil (*Listronotus bonariensis*) pest. Similarly, fungal endophytes *Epichloe* are symbiotically found in temperate grass species and synthesize some bioactive metabolites in the host tissues which exhibit deterrent effects to various pests and herbivores. An endophytic fungus *Muscodor vitigenus* identified from Peruvian Amazonian liana produces naphthalene volatile compound that exhibits strong insecticidal activity against *Cephus cinctus* (stem sawfly) found in wheat crop (Lugtenberg et al. 2016). In *Nicotiana tabacum* plant resistance was improved against *Rhizoctonia solani* with association of fungus *Metarhizium anisopliae* (Kannojia et al. 2017). The benzofuran compounds are produced by unrecognized fungal endophyte in host plant *Gaultheria procumbens* that exhibits harmful effect against larval and adult spruce budworm (Lugtenberg et al. 2016). Likewise, various fungal endophytes are producing different metabolites in several crop plants that possess toxic effect against various insects and pests as listed in Table 7.3.

## 7.4 Fungal Endophytes for Plant Growth Enhancement

Endophytes perform various symbiotic associations with plants and enlighten plant progression by various physiological and biochemical mechanisms. Endophytic fungi alleviate nutrition as well as shelter from the host, while enriching fixation of nutrients and providing protection to the host from biotic and abiotic stresses. In addition, endophytes also influence the growth, developments and health of plants (Hardoim et al. 2015; Fadiji and Babalola 2020). Endophytes are essential partners of sustainable agriculture in response to their ability to enhance plant growth and yield and also provide stress resistance against biotic and abiotic agents (Vega et al. 2008; Gill et al. 2016). Fouda et al. (2015) have isolated fungal isolates (*Alternaria alternate* and *Penicillium chrysogenum*) from *Asclepias sinaica* plant which are beneficial in root growth promotion by secreting extracellular enzymes like amylase, cellulase, gelatinase and tyrosinase. Khan et al. (2015) have reported adverse effects of biotic stress can be overcome by endophytic fungi associated with gibberellins production beneficial for enhancing plant growth, development and biomass production under stressed condition. Likewise, the fungus isolate *Galactomyces geotrichum* from *Trapa japonica* has been reported for indole-3-acetic acid and gibberellic acid production (Waqas et al. 2014). The root-associated fungal endophyte *Piriformospora indica* significantly improves mineral uptake and translocation as well as stimulation of phytohormones implicated in growth and development of various crop plants (Lutenberg et al. 2016). Therefore, the biotic factors influence the growth and development of crop plants and medicinal plants as illustrated in Table 7.3.

## 7.5 Source for Biocontrol Agent's Production

For sustainable agriculture there is need to utilize approach which is alternative for current chemical-based approach to control various pests and diseases of plants. The fungal endophytes are important source of bioactive metabolite to synthesize natural bioactive products for various purposes. Study on fungal endophytes demonstrates that it has a role to control the pathogen infection in host–pathogen interactions. These interactions get varied according to endophytes mechanism to control pathogens at their various developmental stages. Some interactions mechanisms include production of biocontrol agent and enhancement of plant defence. Alteration in metabolism of host plant is another important mechanism regulated by endophytes to enhance plant defence against pathogen (Prasad et al. 2017; Varma et al. 2017). Some plants keep endophytes to control the agents which cause infections to them (Strobel 2003). However, the metabolites produced from them are important to control various plant pathogens (Gautam and Avasthi 2019). The bioactive compounds listed in Table 7.4 are produced by various endophytes in host-specific interactions that have been used against many plant fungal pathogens in agriculture sector. However fungal endophytes culture extract showed their ability to inhibit many plant fungal pathogens (Kim et al. 2007; Ownley et al. 2010; Hardoim et al. 2015; Terhonen et al. 2019). The diverse metabolites produced from the fungal endophytes control animals including human fungal as well as bacterial pathogens (Suryanarayanan and Hawksworth 2005). The medicinal plants report 35% of fungal endophytes have bioactive potential, while 29% from crop plants and 18% from other plants (Yu et al. 2010). The many fungal endophytes compounds are approved as a drug because of its antibiotic potentials (Pelaez 2005). Therefore, the utilization of fungal endophytes to control various pests and diseases of plants is a promising tool for sustainable agriculture. In addition, the metabolite produced from fungal endophytes has a promising role to control various human infections.

## 7.6 Fungal Endophytes for Weed Control

In view of the high perspective of fungal endophytes in weed control management, it seems to be the presence of their secreted metabolites harmful to the weeds (Suryanarayanan et al. 2009). The utilization of herbicidal metabolites produced by fungi is the best alternative for weed management instead of the use of synthetic and biocontrol agents in the established environment. The weed control mechanism of endophyte compounds encourages chlorosis and subsequently programmed cell death in *Lemna minor* plant. The endophytic fungus *Chaetomium globosum* is identified in the leaves of *Amaranthus viridis* that secretes phytotoxic compounds azaphilone derivatives which possess effective herbicidal potential (Piyasena et al. 2015). Ahmad et al. (2020) reported the significant inhibition in the seed germination and growth of invasive weeds *Avena fatua*, *Chenopodium album* and *Parthenium hysterophorus* by the application of fungal endophytes *Alternaria* spp. and *Drechslera hawaiiensis*. An endophyte *Trichoderma* spp. effectively inhibits the growth of *Avena fatua* invasive weed in the crop fields (Javaid and Ali 2011). Similarly, an endophytic fungus *Drechslera* spp. exhibits strong weedicidal activity against *Chenopodium arvensis.* A fungal endophyte *Alternaria* spp. possesses bioactive potential in the control of invasive weed *Parthenium hysterophorus* by

**Table 7.4** Synthesis of bioactive metabolites by fungal endophytes associated with host plants

| Fungal endophytes | Bioactive compounds | Host | Reference |
|---|---|---|---|
| *Acremonium coenophialum* | Chitinases | Tall fescue | |
| *Neotyphodium* sp. | Ergonovine | Drunken horse grass | |
| *Cryptosporiopsis* cf. *quercina* | Cryptocandin | *Tripterygium wilfordii* | Strobel et al. (1999) |
| *Colletotrichum gloeosporioides* | Colletotric acid | *Artemisia mongolica* | |
| *Epichloe festucae* | IAA, IEtOH, methylindole-3-carboxylate, indole-3-carboxaldehyde, diacetamide, cyclonerodiol | *Agrostis*, *Bromus*, *Cinna*, *Elymus*, *Festuca*, *Hordyelymus*, *Lolium*, *Poa* and *Stipa* | |
| *Morinia pestalozzioides* | Moriniafungin | *Juniperus communis* | |
| *Helminthosporium* sp., *Phoma* sp., *Phomopsis* sp., *Xylaria* sp., *Hypoxylon* sp., *Chalara* sp., *Rhinocladiella* sp. | Cytochalasins | *Tripterygium wilfordii* | |
| *Pestalotiopsis jester* | Jesterone, hydroxyjesterone | *Fagraea bodenii* | |
| *Pestalotiopsis microspora* | Ambuic acid | *P. microspora* | |
| *Muscodor albus* | 1-Butano, 3-methylacetate | *Cinnamomum zeylanicum* | |
| *Periconia* sp. | Periconicin A | *Taxus cuspidata* | |
| *Aspergillus niger* | Rubrofusarin B,<br>Fonsecinone A,<br>Asperpyrone B,<br>Aurasperone A | *Cynodon dactylon* | |
| *Penicillium* sp. | Berkeleydione | *Glochidion ferdinandi* | |
| *Phomopsis phaseoli*, *Melanconium betulinum* | 3-Hydroxypropionic acid | *Mesua ferrea*, *Betula pendula* and *B. pubescens* | |
| *Chaetomium chiversii* C5-36-62 | Radicicol | *Ephedra fasciculata* | |
| *Pestalotiopsis adusta* | Pestalachlorides | – | |
| *Xylaria* sp. YX-28 | 7-Amino-4-methylcoumarin | Mangrove | |
| *Phomopsis* sp. | Terpenoid antimicrobial | *Plumeria acutifolia* | |
| *Colletotrichum gloeosporioides* | Piperine | *Piper nigrum* | |
| *Fusarium redolens* | Peimisine and imperialine-3β-D-glucoside | *Fritillaria unibracteatavar.* wabuensis | |
| *Cladosporium delicatulum* | Plumbagin (5-hydroxyl-2-methylnaptalene-1,4-dione) | *Terminalia pallida*, *Rhynchosia beddomei*, *Pterocarpus santalinus* | |

inhibiting the growth and development (Ahmad et al. 2020). Literature survey suggested that the herbicidal compounds secreted by endophytes are not harmful to the crop plants at certain concentration and harmless to the rhizospheric microbes (Thirumalai et al. 2013; Piyasena et al. 2015). A gall-forming endophytic fungus *Uromycladium tepperianum* showed potential weedicidal activity against invasive plant *Acacia saligna* in South Africa (Trognitz et al. 2016). Endophytic fungi *Colletotrichum truncatum* and *C. orbiculare* act as strong weedicidal agents against *Sesbania exaltata* and *Xanthium spinosum* (Harding and Raizada 2015).

## 7.7 Fungal Endophytes for Post-harvest Product Preservation

Currently, food wastage is a principal concern globally, particularly with an exponential growing human population and fact that about one third of total produced food for human consumption is either wasted or lost. According to the Food and Agriculture Organization of United Nations, it is estimated that 1.3 billion tons of food is wasted (Salas et al. 2017). The fungal biopreservation agents are used to control the post-harvest pathogenic diseases in stored agricultural food products, and it encourages new possibilities to control spoilage of food by biofumigation measures (Di Francesco et al. 2016; Salas et al. 2017). For instance, a fungal endophyte *Penicillium chrysogenum* recognized from dry-cured ham is beneficial in growth inhibition of *A. flavus* and *P. restrictum* by producing mycotoxic proteins (Salas et al. 2017). Likewise, to avoid black spot formation during ripening process in dry-cured ham, the bioprotective fungal endophyte *P. chrysogenum* is used. A bioactive compound 2-phenylethanol was recognized from fungal endophyte *P. expansum* that exhibits harmful effects against post-harvest fungal pathogens *P. digitatum* and *P. italicum* (Rouissi et al. 2013; Li et al. 2014; Salas et al. 2017). The fungal endophytes *Phomopsis* spp. and *Alternaria* spp. produce a cyclic tetrapeptide (L-leucyl-trans-4-hydroxy-L-prolyl-D-leucyl-trans-4-hydroxy-L-proline) metabolite which is bioactive against fungal pathogens (*Fusarium graminearum*, *Gaeumannomyces graminis*, *Helminthosporium sativum* and *Rhizoctonia cerealis*) and grows on maize food products. Likewise, 2-hydroxybenzoic acid produced by *R. glutinis* exhibits biocontrol efficiency against fungal pathogens *A. alternata* and *P. expansum* in cherry fruits (Salas et al. 2017).

## 7.8 Conclusions

The diversity of entophytic fungi mainly depends on agro-climatic zones where host plants grow. However endophytic fungi firstly adapt in ever-changing climate and make host plant tolerant to this condition by regulating various plant processes.

These fungal endophytes reside suitably inside the host plant and enhance their growth when plant faces the environmental as well as biotic challenges. Noteworthy it increases host nutrient uptake, maintaining osmotic balance, regularized metabolic processes and many other phenomenon in host plant by synthesizing various metabolites. Therefore, endophyte diversity is a promising sustainable tool for agriculture industry. The fungal endophytes controlling the plant pathogens through synthesized bioactive metabolites pave the way for their utilization in agriculture sustainability. However synthesized bioactive metabolites have also applications in various fields. There is an opportunity to utilize endophytes bioactive metabolites as an alternative for current synthetic compounds used in plant growth and disease control if it is cautiously exploited. Recently the endophytes utilization for weed control and to avoid post-harvest yield loss is also emerging vastly. However, the role and mechanisms of endophytes to control plant growth, weeds and stress tolerance and avoid post-harvest yield loss still need clear understanding (Fig. 7.1).

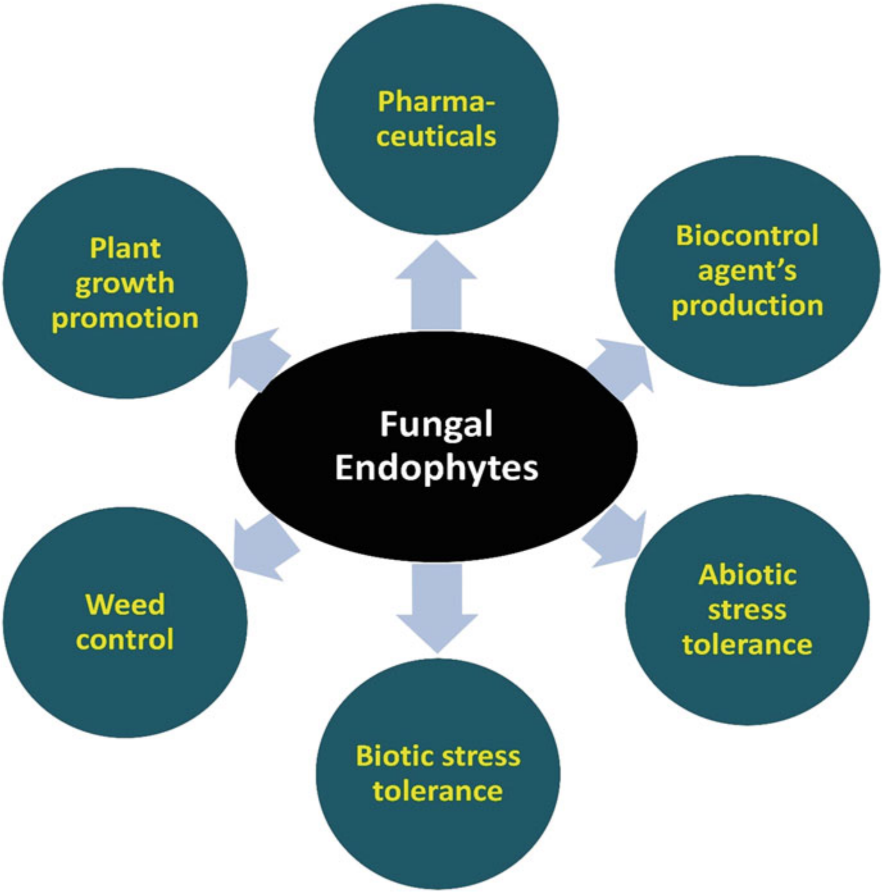

**Fig. 7.1** Potential application of endophytic fungi

## References

Abd-Allah EF, Hashem A, Alqarawi AA, Bahkali AH, Alwhibi MS (2015) Enhancing growth performance and systemic acquired resistance of medicinal plant *Sesbania sesban* (L.) Merr using arbuscular mycorrhizal fungi under salt stress. Saudi J Biol Sci 22(3):274–283

Abdel-Latef AA, Chaoxing H (2011) Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol Plant 33:1217–1225

Acuna-Rodriguez IS, Newsham KK, Gundel PE, Torres-Diaz C, Molina-Montenegro MA (2020) Functional roles of microbial symbionts in plant cold tolerance. Ecol Lett 23:1034–1048. https://doi.org/10.1111/ele.13502

Agostinelli M, Cleary M, Martin JA, Albrectsen BR, Witzell J (2018) Pedunculate Oaks (*Quercus robur* L.) differing in vitality as reservoirs for fungal biodiversity. Front Microbiol 9:1758

Ahmad Y, Ahmad MN, Zia A, Alam SS, Khan RAA, Riaz M (2020) Biocontrol of economically important weed species through endophytic fungi isolated from *Parthenium hysterophorus* (family: Asteraceae). Egypt J Biol Pest Control 30:138. https://doi.org/10.1186/s41938-020-00339-5

Ali AH, Radwan U, El-Zayat S, El-Sayed MA (2019) The role of the endophytic fungus, Thermomyces lanuginosus, on mitigation of heat stress to its host desert plant *Cullen plicata*. Biol Futura 70(1):1–7. https://doi.org/10.1556/019.70.2019.01

Alwadi HM, Baka ZAM (2001) Microorganisms associated with *Withania somnifera* leaves. Microbiol Res 156(4):303–309

Amutharaj P, Sekar C, Natheer SE (2012) Intergeneric microbial coaggregates: bioinoculation effect of ACC deaminase positive wild type strains of *Pseuodomonas* and *Paenibacillus*, as coaggregates, on the maximization of ISR against *Pyricularia oryzae* in upland rice cv. ASD-19. CIBTech. J Microbiol 1:57–66

Andreas T, Christophe C, Essaid AB (2012) Physiological and molecular changes in plants at low temperatures. Planta 235:1091–1105. https://doi.org/10.1007/s00425-012-1641-y

Arnold AE (2008) In endophytic fungi: hidden components of tropical community ecology. In: Schnitzer S, Carson W (eds) Tropical forest community ecology. Blackwell Publishing, Malden

Asthir B, Spoor W, Duffus C (2004) Involvement of polyamines, diamine oxidase and polyamine oxidase in resistance of barley. Euphytica 136:307–312

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL et al (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60:3279–3295. https://doi.org/10.1093/jxb/erp165

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G et al (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol 180:501–510

Bamisile BS, Dash CK, Akutse KS, Keppanan R, Wang L (2018) Fungal endophytes: beyond herbivore management. Front Microbiol 9:544. https://doi.org/10.3389/fmicb.2018.00544

Barker DJ, Hume DE, Quigley PE (1997) Negligible physiological responses to water deficit in endophyte-infected and uninfected perennial ryegrass. In: Bacon CW, Hill NS (eds) Neotyphodium/grass interactions. Plenum Press, New York, pp 137–139

Bebber DP, Gurr SJ (2015) Crop-destroying fungal and oomycete pathogens challenge food security. Fungal Genet Biol 74:62–64

Belesky DP, Stringer WC, Hill NS (1989) Influence of endophyte and water regime upon tall fescue accessions. I. Growth characteristics. Ann Bot 63:495–503

Bhardwaj A, Agrawal P (2014) A review fungal endophytes: as a store house of bioactive compound. World J Pharm Pharm Sci 3(9):228–237

Cao LX, You JL, Zhou SN (2002) Endophytic fungi from *Musa acuminata* leaves and roots in South China. World J Microbiol Biotechnol 18(2):169–171

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 BioSci 6(1):333–343

Chareprasert S, Piapukiew J, Thienhirun S, Whalley AJS, Sihanonth P (2006) Endophytic fungi of teak leaves *Tectona grandis* L. and rain tree leaves *Samanea saman* Merr. World J Microbiol Biotechnol 22(5):481–486

Chhipa H, Deshmukh SK (2019) Fungal endophytes: rising tools in sustainable agriculture production. In: Jha S (ed) Endophytes and secondary metabolites. Springer International Publishing, Cham, pp 1–24. https://doi.org/10.1007/978-3-319-76900-4_26-1

Chowdhary K, Kaushik N (2015) Fungal endophyte diversity and bioactivity in the Indian medicinal plant *Ocimum sanctum* Linn. PLoS One 10(11):e0141444

Chu XT, Fu JJ, Sun YF, Xu YM, Miao YJ, Xu YF et al (2016) Effect of arbuscular mycorrhizal fungi inoculation on cold stress-induced oxidative damage in leaves of *Elymus nutans* Griseb. S Afr J Bot 104:21–29

Chutulo EC, Chalannavar KC (2018) Endophytic mycoflora and their bioactive compounds from *Azadirachta indica*: a comprehensive review. J Fungi 4(2):42. https://doi.org/10.3390/jof4020042

Dai A (2011) Drought under global warming: a review. Wiley Interdiscip Rev Clim Chang 2:45–65. https://doi.org/10.1002/wcc.81

Dai A (2013) Increasing drought under global warming in observations and models. Nat Clim Chang 3:52–58

Dai CC, Yu BY, Li X (2008) Screening of endophytic fungi that promote the growth of Euphorbia pekinensis. Afr J Biotechnol 7(19):3505–3509

Dastogeer KMG, Wylie SJ (2017) Plant-microbe interactions. In: Singh DP, et al (eds) Agro-ecological perspectives. Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-10-5813-4_8

de Battista JP, Bouton JH, Bacon CW, Siegel MR (1990) Rhizome and herbage production of endophyte-removed tall fescue clones and populations. Agron J 82:651–654

de Favaro LC, de Sebastianes FL, Araujo WL (2012) Epicoccum nigrum P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. Liles MR (ed.). PLoS One 7

Demain AL (2000) Microbial natural products: a past with a future. In: Wrigley SK, Hayes MA, Thomas R et al (eds) Biodiversity: new leads for pharmaceutical and agrochemical industries. The Royal Society of Chemistry, Cambridge, pp 3–16

Di Francesco A, Martini C, Mari M (2016) Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action? Eur J Plant Pathol 145:711–717

Diedhiou PM, Hallmann J, Oerke EC, Dehne HW (2003) Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. Mycorrhiza 13:199–204. https://doi.org/10.1007/s00572-002-0215-4

Domka A, Rozpadek P, Wazny R, Turnau K (2019) *Mucor* sp.—an endophyte of Brassicaceae capable of surviving in toxic metal-rich sites. J Basic Microbiol 59:24–37

Dudeja SS, Giri R (2014) Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria (review). Afr J Microbiol Res 8(15):1562–1572

Dudeja SS, Giri R, Saini R, Suneja-Madan P, Kothe E (2012) Interaction of endophytic microbes with legumes. J Basic Microbiol 52(3):248–260

Elmi AA, West CP, Robbins RT, Kirkpatrick TL (2000) Endophyte effects on reproduction of a root-knot nematode (*Meloidogyne marylandi*) and osmotic adjustment in tall fescue. Grass Forage Sci 55:166–172

El-Nagerabi SAF, Elshafie AE, Alkhanjari SS (2014) Endophytic fungi associated with endogenous *Boswellia sacra*. Biodiversitas 15(1):24–30

El-Tarabily KA (2013) Biocontrol of damping-off and root and crown rots of cucumber caused by *Pythium aphanidermatum* by ACC-deaminase producing endophytic actinomycetes. Phytopathology 103:40

Ezra D, Hess WM, Strobel GA (2004) New endophytic isolates of *Muscodor albus*, avolatile-antibiotic-producing fungus. Microbiology 150(Pt 12):40234031

Fadiji AE, Babalola OO (2020) Exploring the potentialities of beneficial endophytes for improved plant growth. Saudi J Biol Sci 27:3622–3633

Felde ZA, Pocasangre LE, Carnizares Monteros CA, Sikora RA, Rosales FE, Riveros AS (2006) Effect of combined inoculations of endophytic fungi on the biocontrol of *Radopholus similis*. InfoMusa 15:12–18

Fernandes EG, Pereira OL, da Silva CC, Bento CB, de Queiroz MV (2015) Diversity of endophytic fungi in *Glycine max*. Microbiol Res 181:84–92. https://doi.org/10.1016/j.micres.2015.05.010

Fogarty RV, Tobin JM (1996) Fungal melanins and their interaction with metals. Enzyme Microb Technol 19:311–317. https://doi.org/10.1016/0141-0229(96)00002-6

Fouda AH, Hassan SED, Eid AM, Ewais EED (2015) Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). Ann Agric Sci 60:95–104

Gangadevi V, Muthumary J (2008) Taxol, an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides* Tassi, isolated from medicinal plant, *Aegle marmelos* Correa ex Roxb. World J Microbiol Biotechnol 24(717):717–724

Gao F, Dai C, Liu X (2010) Mechanisms of fungal endophytes in plant protection against pathogens. Afr J Microbiol Res 4(13):1346–1351

Gashgari R, Gherbawy Y, Ameen F, Alsharari S (2016) Molecular characterization and analysis of antimicrobial activity of endophytic fungi from medicinal plants in Saudi Arabia. Jundishapur J Microbiol 9(1):e26157

Gautam AK (2014) Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India. Arch Phytopathol Plant Protect 47(5):537544

Gautam AK, Avasthi S (2019) Role of plant growth promoting microorganisms in sustainable agriculture and nanotechnology. https://doi.org/10.1016/B978-0-12-817004-5.00014-2

Gautam AK, Kant M, Thakur Y (2013) Isolation of endophytic fungi from *Cannabis sativa* and study their antifungal potential. Arch Phytopathol Plant Protect 46(6):627–635

Geris dos Santos RM, Rodrigues-Fo E, Rocha WC, Teixeira MFS (2003) Endophytic fungi from Melia azedarach. World J Microbiol Biotechnol 19(8):767770

Ghaffari M, Mehdi K, Behnam H, Mohammad S, Patrick S, Ghasem H (2016) Metabolic and transcriptional response of central metabolism affected by root endophytic fungus *Piriformospora indica* under salinity in barley. Plant Mol Biol 90(10):1007

Gherbawy YA, Gashgari RM (2013) Molecular characterization of fungal endophytes from *Calotropis procera* plants in Taif region (Saudi Arabia) and their antifungal activities. Plant Biosyst 148(6):10851092

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:332. https://doi.org/10.3389/fmicb.2016.00332

Gokul Raj K, Sundaresan N, Ganeshan EJ, Rajapriya P, Muthumary J, Sridhar J et al (2014) Phylogenetic reconstruction of endophytic fungal isolates using internal transcribed spacer 2 (ITS2) region. Bioinformation 10(6):320–328

Gond SK, Verma VC, Mishra A, Kumar A, Kharwar RN (2010) Role of fungal endophytes in plant protection. In: Management of fungal plant pathogens, pp 183–197

Halo BA, Maharachchikumbura S, Al-Yahyai R, Al-Sadi AM (2019) *Cladosporium omanense*, a new endophytic species from *Zygophyllum coccineum* in Oman. Phytotaxa 388(1):145

Hamayun M, Hussain A, Khan SA, Kim H-Y, Khan AL, Waqas M, Irshad M, Iqbal A, Rehman G, Jan S (2017) Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Front Microbiol 8:686

Hao Y, Charles TC, Glick BR (2011) An ACC deaminase containing *A. tumefaciens* strain D3 shows biocontrol activity to crown gall disease. Can J Microbiol 57:278–286

Harding DP, Raizada MN (2015) Controlling weeds with fungi, bacteria, and viruses: a review. Front Plant Sci 6:659. https://doi.org/10.3389/fpls.2015.00659

Hardoim PR, Van Overbeek LS, Berg G, Pirttila AM, Compant S, Campisano A et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Hipol RM (2012) Molecular identification and phylogenetic affinity of two growth promoting fungal endophytes of sweet potato (*Ipomea batatas* (L.) Lam.) from Baguio City, Philippines. Electron J Biol 8(3):5761

Hipol RM, Tamang SMA, Gargabite BF, Bronola-Hipol RLC (2015) Diversity of fungal endophytes isolated from *Marchantia polymorpha* populations from Baguio City, Philippines. Bull Environ Pharmacol Life Sci 4(3):87–91

Huang WY, Cai YZ, Surveswaran S, Hyde KD, Corke H, Sun M (2009) Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species. Fungal Divers 36:69–88

Huang Y, Zimmerman NB, Arnold AE (2018) Observations on the early establishment of foliar endophytic fungi in leaf discs and living leaves of a model woody angiosperm, *Populus trichocarpa* (Salicaceae). J Fungi 4(2):E58. https://doi.org/10.3390/jof4020058

Huseynova I, Sultanova N, Mammadov A, Suleymanov S, Aliyev JA (2014) Biotic stress and crop improvement. In: Improvement of crops in the era of climatic changes, vol. 2. https://doi.org/10.1007/978-1-4614-8824-8_4, © Springer Science+Business Media New York

Hussain F, Usman F (2019) Fungal biotic stresses in plants and its control strategy. https://doi.org/10.5772/intechopen.83406

Hussain SS, Mehnaz S, Siddique KHM (2018) Harnessing the plant microbiome for improved abiotic stress tolerance. In: Egamberdieva D, Ahmad P (eds) Plant microbiome: stress response. Springer Nature, Singapore, pp 21–43. https://doi.org/10.1007/978-981-10-5514-0_2

Hyde KD, Soytong K (2008) The fungal endophyte dilemma. Fungal Divers 33:163–173

IPCC (2007) Climate change 2007: synthesis report. In: Pachauri RK, Reisinger A (eds) Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. IPCC, Geneva, p 104

Ismail, Hamayun M, Hussain A, Iqbal A, Khan SA, Lee IJ (2018) Endophytic fungus *Aspergillus japonicus* mediates host plant growth under normal and heat stress conditions. Biomed Res Int 2018:7696831. https://doi.org/10.1155/2018/7696831

Jan FG, Hamayun M, Hussain A, Jan G, Iqbal A, Khan A, Lee IJ (2019) An endophytic isolate of the fungus *Yarrowia lipolytica* produces metabolites that ameliorate the negative impact of salt stress on the physiology of maize. BMC Microbiol 19:3

Javaid A, Ali S (2011) Alternative management of a problematic weed of wheat *Avena fatua* L. by metabolites of *Trichoderma*. China J Agri Res 71:205

Kabiri R, Nasibi F, Farahbakhsh H (2014) Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. Plant Protect Sci 50:43–51

Kannojia P, Sharma PK, Kashyap AK, Manzar N, Singh UB, Chaudhary K, Malviya D, Singh S, Sharma SK (2017) Microbe-mediated biotic stress management in plants. In: Singh DP et al (eds) Plant-microbe interactions in agro-ecological perspectives. https://doi.org/10.1007/978-981-10-6593-4_26

Kapoor D, Bhardwaj S, Landi M, Sharma A, Ramakrishnan M, Sharma A (2020) The impact of drought in plant metabolism: how to exploit tolerance mechanisms to increase crop production. Appl Sci 10:5692. https://doi.org/10.3390/app10165692

Kaur T (2020) Fungal endophyte-host plant interactions: role in sustainable agriculture. https://doi.org/10.5772/intechopen.92367

Khan AL, Hamayun M, Hussain J, Kang SM, Lee IJ (2012a) The newly isolated endophytic fungus *Paraconiothyrium* sp. LK1 produces ascotoxin. Molecules 17:1103–1112. https://doi.org/10.3390/molecules17011103

Khan AL, Shinwari ZK, Kim Y, Waqas M, Hamayun M, Kamran M et al (2012b) Role of endophyte *Chaetomium globosum* lk4 in growth of *Capsicum annuum* by production of gibberellins and indole acetic acid. Pak J Bot 44:1601–1607

Khan AL, Waqas M, Hussain J, Al-Harrasi A, Lee IJ (2014a) Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (Sitiens and Rhe). Biol Fertil Soils 50:75–85. https://doi.org/10.1007/s11274-013-1378-1

Khan AL, Waqas M, Lee IJ (2014b) Resilience of *Penicillium resedanum* LK6and exogenous gibberellin in improving *Capsicum annuum* growth under abiotic stresses. J Plant Res 128:259–268. https://doi.org/10.1007/s10265-014-0688-1

Khan AL, Hussain J, Al-Harrasi A, Al-Rawahi A, Lee IJ (2015) Endophytic fungi: resource for gibberellins and crop abiotic stress resistance. Crit Rev Biotechnol 35:62–74

Khiralla A, Spina R, Yagi S, Mohamed I, Laurain-Mattar D (2017) Endophytic fungi: occurrence, classification, function and natural products. In: Hughes E (ed) Endophytic fungi diversity, characterization and biocontrol. Nova Publishers, New York, pp 1–38

Kim HY, Choi GJ, Lee HB, Lee SW, Kim HK, Jang KS et al (2007) Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. Lett Appl Microbiol 44(3):332–337

Kumar KK, Dara SK (2021) Fungal and bacterial endophytes as microbial control agents for plant-parasitic nematodes. Int J Environ Res Public Health 18:4269. https://doi.org/10.3390/ijerph18084269

Latch GCM, Hunt WF, Musgrave DR (1985) Endophytic fungi affect growth of perennial ryegrass. N Z J Agric Res 28:165–168

Li H, Zhang S, Lu J, Liu L, Uluko H, Pang X, Sun Y, Xue H, Zhao L, Kong F et al (2014) Antifungal activities and effect of *Lactobacillus casei* AST18 on the mycelia morphology and ultrastructure of *Penicillium chrysogenum*. Food Control 43:57–64

Likar M (2011) Dark septate endophytes and mycorrhizal fungi of trees affected by pollution. In: Pirttila AM, Frank AC (eds) Endophytes of forest trees. Springer Science+Business Media, Dordrecht, pp 189–201. https://doi.org/10.1007/978-94-007-1599-8

Liu Z, Ma L, He X, Tian C (2014) Water strategy of mycorrhizal rice at low temperature through the regulation of PIP aquaporins with the involvement of trehalose. Appl Soil Ecol 84:185–191

Liu SS, Wang WQ, Li M, Wan SB, Sui N (2017) Antioxidants and unsaturated fatty acids are involved in salt tolerance in peanut. Acta Physiol Plant 39:207. https://doi.org/10.1007/s11738-017-2501-y

Lugtenberg B, Caradus J, Johnson L (2016) Fungal endophytes for sustainable crop production. FEMS Microbiol Ecol 92(12):fiw194

Ma J, Janouskova M, Li Y, Yu X, Yan Y, Zou Z et al (2015) Impact of arbuscular mycorrhizal fungi (AMF) on cucumber growth and phosphorus uptake under cold stress. Funct Plant Biol 42:1158–1167

Malinowski D (1995) Rhizomatous ecotypes and symbiosis with endophytes as new possibilities of improvement in competitive ability of meadow fescue (*Festuca pratensis*). Ph.D., thesis. Swiss Federal Institute of Technology, Zurich

Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci 40:923–940. https://doi.org/10.2135/cropsci2000.404923x

Malinowski D, Leuchtmann A, Schmidt D, Nsberger J (1997) Growth and water status in meadow fescue (*Festuca pratensis*) is differently affected by its two natural endophytes. Agron J 89:673–678

Mane RS, Vedamurthy AB (2018) The fungal endophytes: sources and future prospects. J Med Plants Stud 6(2):121–126

Marulanda A, Barea JM, Azcon 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 28:115–124. https://doi.org/10.1007/s00344-009-9079-6

Meenatchi A, Ramesh V, Bagyalakshmi, Kuralarasi R, Shanmugaiah V, Rajendran A (2016) Diversity of endophytic fungi from the ornamental plant—*Adenium obesum*. Stud Fungi 1(1):34–42

Menjivar RD, Hagemann MH, Kranz J, Cabrera JA, Dababat AA, Sikora RA (2011) Biological control of *Meloidogyne incognita* on cucurbitaceous crops by the non-pathogenic endophytic fungus *Fusarium oxysporum* strain 162. Int J Pest Manag 57:249–253

Mirjalili MH, Farzaneh M, Bonfill M, Rezadoost H, Ghassempour A (2012) Isolation and characterization of *Stemphylium sedicola* SBU-16 as a new endophytic taxol-producing fungus from Taxus baccata grown in Iran. FEMS Microbiol Lett 328(2):122–129

Mishra M, Prasad R, Varma A (2015) Endophytic fungi: biodiversity and functions. Int J Pharm BioSci 6(1):18–46

Mochizuki T, Ohki ST (2012) Cucumber mosaic virus: viral genes as virulence determinants. Mol Plant Pathol 13:217–225

Morse LJ, Day TA, Faeth SH (2002) Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. Environ Exp Bot 48:257–268

Morsy M, Cleckler B, Armuelles-Millican H (2020) Fungal endophytes promote tomato growth and enhance drought and salt tolerance. Plants 9:877. https://doi.org/10.3390/plants9070877

Murphy BR, Doohan FM, Hodkinson TR (2013) Fungal endophytes of barley roots. J Agric Sci 152:602–615. https://doi.org/10.1017/S0021859613000348

Murphy BR, Doohan FM, Hodkinson TR (2014) Yield increase induced by the fungal root endophyte *Piriformospora indica* in barley grown at low temperature is nutrient limited. Symbiosis 62:29–39

Murphy BR, Doohan FM, Hodkinson TR (2018) From concept to commerce: developing a successful fungal endophyte inoculant for agricultural crops. J Fungi 4(1):E24

Nair DN, Padmavathy S (2014) Impact of endophytic microorganisms on plants, environment and humans. Sci World J 2014:250693

Ownley BH, Gwinn KD, Vega FE (2010) Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl 55(1):113–128

Pandey P, Irulappan V, Bagavathiannan MV, Senthil-Kumar M (2017) Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front Plant Sci 8:537. https://doi.org/10.3389/fpls.2017.00537

Paradis R, Dalpe Y, Charest C (1995) The combined effect of arbuscular mycorrhizas and short-term cold exposure on wheat. New Phytol 129:637–642

Patle PN, Navnage NP, Ramteke PR (2018) Endophytes in plant system: roles in growth promotion, mechanism and their potentiality in achieving agriculture sustainability. Int J Chem Stud 6(1): 270–274

Paul NC, Lee HB, Lee JH, Shin KS, Ryu TH, Kwon HR et al (2014) Endophytic fungi from *Lycium chinense* Mill and characterization of two new Korean records of Colletotrichum. Int J Mol Sci 15(9):15272–15286

Pedranzani H, Tavecchio N, Gutierrez M, Garbero M, Porcel R, Ruiz-Lozano JM (2015) Differential effects of cold stress on the antioxidant response of mycorrhizal and non-mycorrhizal Jatropha curcas (L.) plants. J Agric Sci 7:35–43

Pedranzani H, Rodrıguez-Rivera M, Gutierrez M, Porcel R, Hause B, Ruiz-Lozano JM (2016) Arbuscular mycorrhizal symbiosis regulates physiology and performance of Digitaria eriantha plants subjected to abiotic stresses by modulating antioxidant and jasmonate levels. Mycorrhiza 26:141–152

Pelaez F (2005) Biological activities of fungal metabolites. In: An Z (ed) Handbook of industrial mycology. Marcel Dekker, New York

Peters K, Breitsameter L, Gerowitt B (2014) Impact of climate change on weeds in agriculture: a review. Agric Sustain Dev 34:707–721. https://doi.org/10.1007/s13593-014-0245-2

Pimentel IC, Glienke-Blanco C, Gabardo J, Stuart RM, Azevedo JL (2006) Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.)Merril) under different environmental conditions. Braz Arch Biol Technol 49(5):705–711

Piyasena KGNP, Wickramarachchi WART, Kumar NS, Jayasinghe L, Fujimoto Y (2015) Two phytotoxic azaphilone derivatives from *Chaetomium globosum*, a fungal endophyte isolated from *Amaranthus viridis* leaves. Mycology 6(3–4):158–160

Porcel R, Aroca R, Cano C, Bago A, Ruiz-Lozano JM (2006) Identification of a gene from the arbuscular mycorrhizal fungus *Glomus intraradices* encoding fora 14-3-3 protein that is upregulated by drought stress during the AM symbiosis. Microb Ecol 52:575–582. https://doi.org/10.1007/s00248-006-9015-2

Porras-Sorianoa A, Soriano-Martin M, Porras-Piedraa A, Azcon R (2009) Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. J Plant Physiol 166:1350–1359

Potshangbam M, Devi SI, Sahoo D, Strobel GA (2017) Functional characterization of endophytic fungal community associated with Oryza sativa L. and Zea mays L. Front Microbiol 8:325

Powthong P, Jantrapanukorn B, Thongmee A, Suntornthiticharoen P (2013) Screening of antimicrobial activities of the endophytic fungi isolated from *Sesbania grandiflora* (L.) Pers. J Agric Sci Technol 15:1513–1522

Prasad R, Kamal S, Sharma P, Oelmuller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. J Basic Microbiol 53:1016–1024

Prasad R (2017) Fungal nanotechnology: applications in agriculture, industry, and medicine. Springer International Publishing (ISBN: 978-3-319-68423-9). https://www.springer.com/gb/book/9783319684239

Prasad R, Gupta N, Kumar M, Kumar V, Wang S, Abd-Elsalam KA (2017) Nanomaterials act as plant defense mechanism. In: Prasad R, Kumar M, Kumar V (eds) Nanotechnology. Springer, Singapore, pp 253–269

Purahong W, Hyde KD (2011) Effects of fungal endophytes on grass and non-grass litter decomposition rates. Fungal Divers 47:1–7

Rajamani T, Suryanarayanan TS, Murali TS, Thirunavukkarasu N (2018) Distribution and diversity of foliar endophytic fungi in the mangroves of Andaman Islands, India. Fungal Ecol 36:109–116

Rajamanikyam M, Vadlapudi V, Amanchy R, Upadhyayula SM (2017) Endophytic fungi as novel resources of natural therapeutics. Braz Arch Biol Technol 60:e17160542

Ravel C, Courty C, Coudret A, Charmet G (1997) Beneficial effects of *Neotyphodium lolii* on the growth and the water status in perennial ryegrass cultivated under nitrogen deficiency or drought stress. Agronomie 17:173–181

Redman RS, Dunigan DD, Rodriguez RJ (2001) Fungal symbiosis: from mutualism to parasitism, who controls the outcome, host or invader? New Phytol 151:705–716

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermo-tolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. Science 298:1581

Redman RS, Kim YO, Woodward CJ, Greer C, Espino L, Doty SL et al (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6:e14823. https://doi.org/10.1371/journal.pone.0014823

Rehman K, Fatima F, Waheed I, Akash MSH (2018) Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem 119(1):157–184

Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59:1109–1114. https://doi.org/10.1093/jxb/erm342

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Rouissi W, Ugolini L, Martini C, Lazzeri L, Mari M (2013) Control of postharvest fungal pathogens by antifungal compounds from *Penicillium expansum*. J Food Prot 76:1879–1886

Ruotsalainen AL, Kytcoviita MM (2004) Mycorrhiza does not alter low temperature impact on *Gnaphalium norvegicum*. Oecologia 140:226–233

Saikkonen K, Ion D, Gyllenberg M (2002) The persistence of vertically transmitted fungi in grass metapopulations. Proc Biol Sci 2669(1498):1397–1403

Saithong P, Panthavee W, Stonsaovapak S, Congfa L (2010) Isolation and primary identification of endophytic fungi from Cephalotaxus mannii trees. Maejo Int J Sci Technol 4(3):446453

Salas ML, Mounier J, Valence F, Coton M, Thierry A, Coton E (2017) Antifungal microbial agents for food biopreservation—A review. Microorganisms 5:37. https://doi.org/10.3390/microorganisms5030037

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A (2019) The global burden of pathogens and pests on major food crops. Nat Ecol Evol 3:430–439. https://doi.org/10.1038/s41559-018-0793-y

Sharma N, Gautam AK (2018) Pathogenicity events in plant pathogenic bacteria: a brief note. J New Biol Rep 7(3):141147

Sharma P, Kharkwal A, Abdin M, Varma A (2016) *Piriformospora indica*-mediated salinity tolerance in *Aloe vera* plantlets. Symbiosis 72(10):1007

Sharma IP, Chandra S, Kumar N, Chandra D (2017) PGPR: heart of soil and their role in soil fertility. In: Meena V, Mishra P, Bisht J, Pattanayak A (eds) Agriculturally important microbes for sustainable agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-10-5589-8_3

Shelke DB, Pandey M, Nikalje GC, Zaware BN, Suprasanna P, Nikam TD (2017) Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes. Plant Physiol Biochem 118:519–528

Shelke DB, Nikalje GC, Chambhare MR, Zaware BN, Suprasanna P, Nikam TD (2019a) Na+ and $Cl^{-}$ induce differential physiological, biochemical responses and metabolite modulations in vitro in contrasting salt-tolerant soybean genotypes. 3 Biotech 9:91

Shelke DB, Nikalje GC, Nikam TD, Maheshwari P, Punita DL, Rao KRSS, Kavi Kishor PB, Suprasanna P (2019b) Chloride (Cl−) uptake, transport, and regulation in plant salt tolerance. In: Roychoudhury A, Tripathi D (eds) Molecular plant abiotic stress: biology and biotechnology, 1st edn., pp 241–268. https://doi.org/10.1002/9781119463665.ch13

Sherameti I, Tripathi S, Varma A, Oelmuller R (2008) The root-colonizing endophyte *Piriformospora indica* confers drought tolerance in Arabidopsis by stimulating the expression of drought stress-related genes in leaves. Mol Plant Microbe Interact 21:799–807

Sibanda EP, Mabandla M, Chisango T, Nhidza AF, Mduluza T (2018) Endophytic fungi from *Vitex payos*: identification and bioactivity. Acta Mycol 53(2):1111

Singh LP, Gill SS, Tuteja N (2011) Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal Behav 6(2):175–191

Sleator RD, Hill C (2001) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol Rev 26:49–71

Sonaimuthu V, Krishnamoorthy S, Johnpaul M (2010) Taxol producing endophytic fungus *Fusarium culmorum* SVJM072 from medicinal plant of Tinospora cordifolia—a first report. J Biotechnol 150:S425

Sonawane H, Borde M, Nikalje G, Terkar A, Math S (2020) HR-LC-MS based metabolic profiling of *Fusarium solani* a fungal endophyte associated with *Avicennia officinalis*. Curr Res Environ Appl Mycol 10(1):262–273

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of pacific yew. Science 260:214–216. https://doi.org/10.1126/science.8097061

Stone J, White J, Polishook J (2004) Endophytic fungi. In: Mueller G, Foster M, Bills G (eds) Measuring and monitoring biodiversity of fungi. Inventory and monitoring methods. Elsevier Academic Press, Boston, pp 241–270

Strobel G (2003) Endophytes as sources of bioactive products. Microb Infect 5(6):535–544

Strobel GA, Miller RV, Martinez-Miller C, Condron MM, Teplow DB, Hess WM (1999) Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis* cf. quercina. Microbiology 145(8):1919–1926. https://doi.org/10.1099/13500872-145-8-1919

Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl Soil Ecol 15(2):183–190

Sun C, Johnson JM, Cai D, Sherameti I, Oelmüller R, Lou B (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017. https://doi.org/10.1016/j.jplph.2010.02.013

Suryanarayanan TS, Hawksworth DL (2005) Fungi from little explored and extreme habitats. In: Deshmukh SK, Rai MK (eds) Biodiversity of fungi: their role in human life. Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi, pp 33–48

Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R, Murali TS (2009) Fungal endophytes and bioprospecting. Fungal Biol Rev 23:9–19

Tayung K, Barik BP, Jha DK, Deka DC (2011) Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Fusarium solani* isolated from bark of Himalayan yew. Mycosphere 2(3):203–213

Terhonen E, Blumenstein K, Kovalchuk A, Asiegbu FO (2019) Forest tree microbiomes and associated fungal endophytes: functional roles and impact on forest health. Forests 10(1):42. https://doi.org/10.3390/f10010042

Thirumalai E, Dastjerdi R, Doll K, Venkatachalam A, Karlovsky P, Suryanarayanan TS (2013) Mycotoxins of endophytic *Fusarium mangiferae* and *F. pallidoroseum* from betel leaves (Piper betle L.). In: 35th Mycotoxin workshop, Ghent, Belgium

Trognitz F, Hackl E, Widhalm S, Sessitsch A (2016) The role of plant–microbiome interactions in weed establishment and control. FEMS Microbiol Ecol 92:138. https://doi.org/10.1093/femsec/fiw138

van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci 11:184–191

Vandenkoornhuyse P, Baldauf SL, Leyval C, Straczek J, Young JPW (2002) Extensive fungal diversity in plant roots. Science 295(5562):2051. https://doi.org/10.1126/science.295.5562.2051

Varma A, Prasad R, Tuteja N (2017) Mycorrhiza: nutrient uptake, biocontrol, ecorestoration. Springer International Publishing (ISBN: 978-3-319-68867-1). http://www.springer.com/us/book/9783319688664

Varma A, Swati T, Prasad R (2020) Plant microbe symbiosis. Springer International Publishing (ISBN: 978-3-030-36247-8). https://www.springer.com/gp/book/9783030362478

Vaz ABM, Fonseca PLC, Badotti F, Skaltsas D, Tome LMR, Silva AC et al (2018) A multiscale study of fungal endophyte communities of the foliar endosphere of native rubber trees in Eastern Amazon. Sci Rep 8:16151. https://doi.org/10.1038/s41598-018-34619-w

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. Biol Control 46:72–82

Verma S, Nizam S, Verma PK (2013) Biotic and abiotic stress signalling in plants. In: Stress signaling in plants: genomics and proteomics perspective, vol. 1, pp 25–49

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M 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 JL, Li T, Liu GY, Smith JM, Zhao ZW (2016) Unraveling the role of dark septate endophyte (DSE) colonizing maize (*Zea mays*) under cadmium stress: physiological, cytological and genic aspects. Sci Rep 6:22028. https://doi.org/10.1038/srep22028

Waqas M, Khan AL, Kang SM, Kim YH, Lee IJ (2014) Phytohormone-producing fungal endophytes and hardwood-derived biochar interact to ameliorate heavy metal stress in soybeans. Biol Fertil Soils 50:1155–1167

Waqas MA, Kaya C, Riaz A, Farooq M, Nawaz I, Wilkes A, Li Y (2019) Potential mechanisms of abiotic stress tolerance in crop plants induced by Thiourea. Front Plant Sci 10:1336. https://doi.org/10.3389/fpls.2019.01336

White RH, Engelke MC, Morton SJ, Johnson-Cicalese JM, Ruemmele BA (1992) *Acremonium* endophyte effects on tall fescue drought tolerance. Crop Sci 32:1392–1396

Wu QS, Zou YN (2010) Beneficial roles of arbuscular mycorrhizas in *Citrus* seedlings at temperature stress. Sci Hortic 125:289–293

Yu H, Zhang L, Li L, Zheng C, Guo L, Li W et al (2010) Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165(6):437–449

Zafar SA, Noor MA, Waqas MA, Wang X, Shaheen T, Raza M (2018) Temperature extremes in cotton production and mitigation strategies. In: In past, present and future trends in cotton breeding. IntechOpen, London. https://doi.org/10.5772/intechopen.74648

Zakaria L, Jamil MIM, Anuar ISM (2016) Molecular characterisation of endophytic fungi from roots of wild banana (*Musa acuminata*). Trop Life Sci Res 27(1):153–162

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23(5): 753771

Zhao J, Fu Y, Luo M, Zu Y, Wang W, Zhao C et al (2012) Endophytic fungi from pigeon pea [*Cajanus cajan* (L.) Millsp.] produce antioxidant cajaninstilbene acid. J Agric Food Chem 60: 4314–4319

Zhou L, Li C, Zhang X, Johnson R, Bao G, Yao X et al (2015) Effects of cold shocked Epichloe infected *Festuca sinensis* on ergot alkaloid accumulation. Fungal Ecol 14:99–104

Zhou W, Starr JL, Krumm JL, Sword GA (2016) The fungal endophyte *Chaetomium globosum* negatively affects both above-and belowground herbivores in cotton. FEMS Microbiol Ecol 92: fiw158

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273

Zhu XC, Song FB, Xu HW (2010a) Influence of arbuscular mycorrhiza on lipid peroxidation and antioxidant enzyme activity of maize plants under temperature stress. Mycorrhiza 20:325–332

Zhu XC, Song FB, Xu HW (2010b) Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil 331:129–137

Zhu XC, Song FB, Liu TD, Liu SQ (2010c) Arbuscular mycorrhizae reducing water loss in maize plants under low temperature stress. Plant Signal Behav 5:591–593

Ziska LH, Tomecek MB, Gealy DR (2010) Evaluation of competitive ability between cultivated and red weedy rice as a function of recent and projected increases in atmospheric CO2. Agron J 102:118–123. https://doi.org/10.2134/agronj2009.0205

Zlatev Z, Lidon FC (2012) An overview on drought induced changes in plant growth, water relations and photosynthesis. Emir J Food Agric 24:57–72

# Chapter 8
# Mining the Potential and Biodiversity of *Trichoderma* in the Domain of Agriculture

**Disha Changela, Ketaki Ramani, Kruti Dangar, Kishan Vachhani, Mihir Raval, and Ashish Kalasava**

**Abstract** The agriculture sector faces a challenge to look out for alternative practices that enhance crop yields eco-friendliness. Therefore, the involvement of pesticides in agriculture needs a shift. The exploration has brought into the spotlight the use of microorganisms called biocontrol agents. *Trichoderma* species are among the most admired fungi with abilities to promote plant growth and biological control potential. In this chapter, we put forward the merit of *Trichoderma* concerning biocontrol, plant growth and its application in the process of decomposition along with its biodiversity. This investigation would aid us to appreciate the advantages brought forth by *Trichoderma* to the agriculture industry and in the sustenance of environment-friendly agricultural practice.

**Keywords** *Trichoderma* · Biocontrol agent · Plant growth enhancer · Decomposer

## 8.1 Introduction

The current obstacles in the agriculture sector are decreasing the rates of plant diseases and simultaneously enhancing productivity yields (Lithourgidis et al. 2011). The pathogenic organisms have developed resistance due to the continuous

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_8

use of chemical pesticides (Ramakrishnan et al. 2019). Moreover, these are hazardous chemicals with deleterious effects on flora and fauna (Mahmood et al. 2016). Immoderate employment of pesticides and synthetic fertilizers renders the living organisms to the elevated toxicity of chemical compounds. Due to these concerns, the prospect of food production and environmental safety are under threat. Hence these issues have provoked several researchers to explore alternative methods with noteworthy eco-friendly preferences. In this regard, biological control agents (BCAs) have become potential candidates as substitutes to conventional pesticides (Bailey et al. 2010; Strauch et al. 2011). BCAs are natural rivals of plant pathogens and are often isolated from the soil (Brito et al. 2014; Pane et al. 2012). The fungus *Trichoderma* has been known for its potential to serve as BCA (Verma et al. 2007). Reports cite the role of *Trichoderma* spp. in suppressing the growth of plant pathogenic microorganisms (Abbas et al. 2017; Freeman et al. 2004). This role of *Trichoderma* spp. has been attributed to the production of myriad secondary metabolites (Contreras Cornejo et al. 2016; Reino et al. 2008). *Trichoderma* spp. have been reported in controlling plant diseases like root rot disease, damping off, wilt, fruit rot, etc. (Begum et al. 2010; Howell 2002; Li et al. 2018; Mbarga et al. 2012; Sallam et al. 2019). In addition to this, *Trichoderma* spp. have been known to have plant growth-promoting capability (Stewart and Hill 2014). Literature studies have also emphasized the role of *Trichoderma* spp. as a natural decomposition agent (Wiedow et al. 2007). Biological control strategies are the need of the hour to tackle the issues as mentioned above in the agriculture industry. Thus, this emphasizes the role of *Trichoderma* spp. as a biocontrol agent, plant growth-promoting agent and decomposition agent (Fig. 8.1).

## 8.2 Trichoderma

*Trichoderma* is a filamentous fungus belonging to the genera *Hypocrea*, family *Hypocreaceae* and order *Hypocreales* of *Ascomycota* (Kredics et al. 2014; Kubicek et al. 2019). These fungi are fast-growing saprophytes and ubiquitously distributed in almost all types of soils over extensive geographic locations and climatic zones (Naar and Kecskés 1998). They are known for their rapid growth and the capability to assimilate a diverse range of substrates (Zafra et al. 2015; Sala et al. 2021). Hence these can be seen to flourish on soil containing organic substrates and can be simply isolated from natural agricultural soil, forest soil and decaying plant organic matter (Howell 2003). Also, they found symbiotic relationships with plants (Harman et al. 2004). The potency of *Trichoderma* spp. in producing various lytic enzymes fosters their widespread distribution in varying habitats and ecological plasticity. This fungal sp., with its ability to produce volatile compounds like 6-pentyl-2pyrone, can be detected by coconut smell (Klein and Eveleigh 2002). *Trichoderma* spp. have been reported to enhance crop production. Firstly, they act as biocontrol agents. They protect against plant pathogenic microorganisms and secondly because of its plant growth-promoting potential. The outstanding trait of this genus is that they

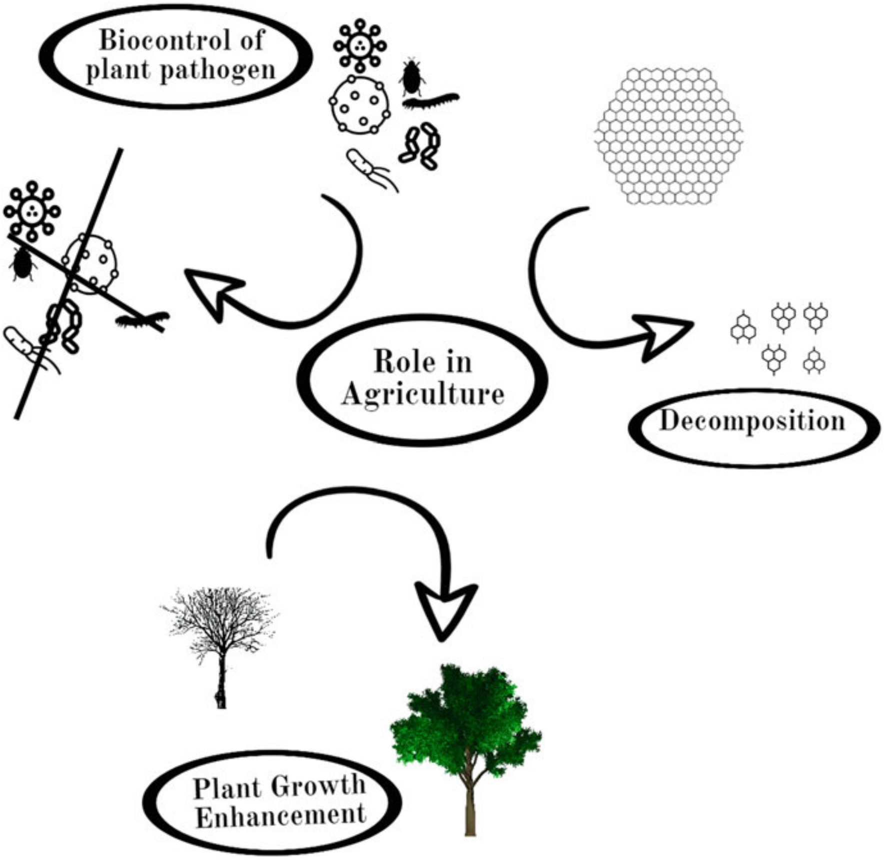

**Fig. 8.1** Role of *Trichoderma* in agriculture

possess the potential to inhibit other pathogenic fungi known to cause root rot and wilt diseases (Santoro et al. 2014; Verma et al. 2007). *Trichoderma* species have also been accounted as endophytic fungi and habitually found to be successful competitor of phytopathogens (Kim et al. 2012; Woo et al. 2006). *Trichoderma* has been classified into five sections: *Saturnisporum*, *Pachybasium*, *Longibrachiatum*, *Trichoderma* and *Hypocreanum*. Waghunde et al. (2016) have reported the existence of 10,000 species belonging to the genus *Trichoderma*.

The ecological distribution of *Trichoderma* species is affected by climatic conditions. It has been reported that *T. harzianum* grows well in warm climates. On the other hand, *T. viride* and *T. polysporum* favour cooler temperatures (Sarhy-Bagnon et al. 2000). *T. hamatum* is tolerant to high moisture compared to other species (Jiang et al. 2016).

## 8.3 Morphological Characteristics of *Trichoderma*

In laboratory conditions, *Trichoderma* has been found to grow on different nutrient sources such as Malt Agar (MA), Czapek Dox Agar (CDA) and Potato Dextrose Agar (PDA). The diagnostic feature of *Trichoderma* is that they have been found to produce conidia/spores characterized by green colour (Chaverri et al. 2003; Rey et al. 2001). Some species have thick-walled chlamydospores (Lu et al. 2004).

The primary method for identifying *Trichoderma* spp. is morphology-based identification (Zhang et al. 2005). They grow fast at the optimum temperature range of 25–30 °C (Latifian et al. 2007). Initially, *Trichoderma* appears white and cotton-like and then later develops yellowish-green to deep green compact tufts at the centre of a growing spot-on agar surface. *Trichoderma* employs various carbon and nitrogen sources as a growth medium for sporulation (Gao et al. 2007; Seyis and Aksoz 2005). The shape of conidia varies from ellipsoidal to oblong, with some species having globose to sub-globose (Bissett et al. 2003; Jaklitsch et al. 2006). The colour of conidia is primarily green, but it is grey, white and yellow (Jaklitsch et al. 2006).

## 8.4 Biodiversity of *Trichoderma*

The knowledge of the distribution of *Trichoderma* spp. is continually evolving due to current advances in resolving the taxonomy of the genus. Consequently, in future years, we can anticipate better understanding the biogeography of *Trichoderma* spp. as research is pursued in new regions and to resolve complex species aggregates (Hoyos-Carvajal and Bissett 2011). For example, Samuels (2006) determined that the species commonly cited in the literature, *Trichoderma koningii*, is a relatively uncommon species restricted to temperate Europe and North America in the strict sense. However, within the *T. koningii* aggregate, they isolated numerous new species, namely, *T. caribbaeum* var. *aequatoriale*, *T. koningiopsis* and *T. ovalisporum* as endophytes *Theobroma* species in tropical America and *T. ovalisporum* also from the woody liana *Banisteriopsis caapi* in Ecuador (Singh et al. 2020). *T. koningiopsis* (previously identified as *T. koningii*) was common in tropical America, also occurring on natural substrata in East Africa, Europe and Canada, from ascospores in eastern North America, and as an endophyte in *Theobroma*.

*T. stilbohypoxyli*, described as a parasite of *Stilbohypoxylon* species in Puerto Rico, was more common in the tropics. Samuels et al. (1998) reported the diversity of *Trichoderma* section *Longibrachiatum*, revealing diversity in Neotropical areas resulting in the description of new species in this section. Jaklitsch et al. (2006), in revising the *T. viride* species complex, reported *T. viridescens* as a species found in Peru at high elevation and *T. neokoningii* in a tropical region in Peru. *T. scalesiae* is also described as a new species isolated as an endophyte from the trunk of the daisy tree (*Scalesia pedunculata*) in the Galapagos Islands of Ecuador. *T. paucisporum* was a mycoparasite of *Moniliophthora roreri* on pods of *Theobroma cacao* in Ecuador, and *T. gamsii*, a cosmopolitan species that has been found in Italy, Rwanda, South Africa and Romania as well as Guatemala (Hoyos-Carvajal and Bissett 2011). Recent studies were undertaken to find biocontrol agents in specific crops such as cocoa that also has resulted in the determination of new species in Neotropical regions (Jaklitsch et al. 2006; Samuels 2006). Currently, 89 species are accepted within the fungal genus of *Trichoderma* (Samuels 2006).

## 8.5 Role of *Trichoderma* as Biocontrol Agent

Biocontrol is defined as living organisms to control the pest population (Kubicek et al. 2001; Regnault-Roger 2012), as shown in Fig. 8.2. It has been reported that *Trichoderma* spp. are majorly employed biocontrol agents against a wide range of pathogens (Papavizas 1985). For the successful establishment of *Trichoderma*, it is applied during seedling. The biocontrol action of *Trichoderma* spp. involves the recognition and invasion of pathogens known as mycoparasitism (Sharma et al. 2011). In the next stage, plants' resistance towards diseases is induction by alteration of root architecture during the interaction with pathogens (Nawrocka and Małolepsza 2013).

The biocontrol action of *Trichoderma* depends on varied factors like soil salinity, temperature, pH and nutrient availability (Poosapati et al. 2014). The biocontrol

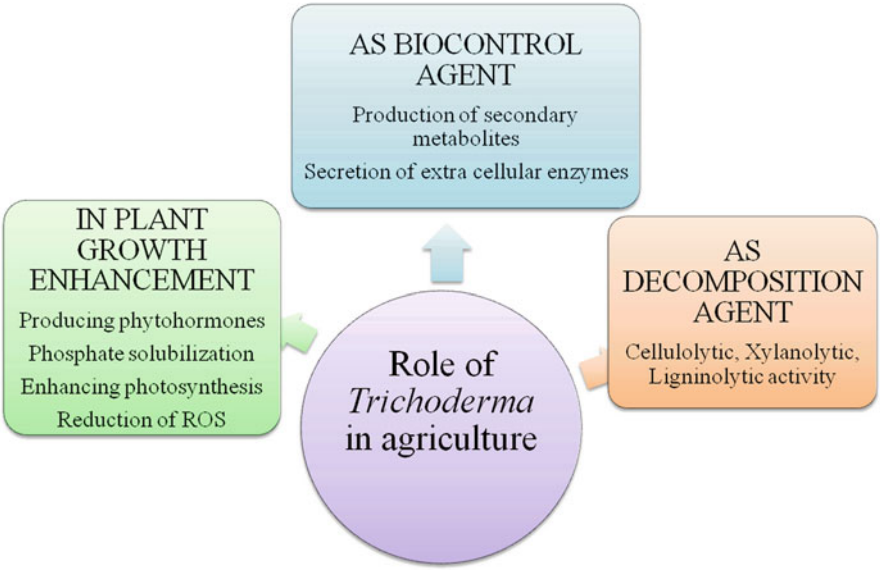

**Fig. 8.2** Multifaceted role of *Trichoderma* in the domain of agriculture

action is influenced by *Trichoderma* strain involved, the pathogenicity of the target and the crop grown (Naseby et al. 2000). Numerous reports have cited the role of *Trichoderma* spp. in suppressing the growth of plant pathogenic organisms such as *Pythium arrhenomanes*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Alternaria tenuis* and *Botrytis cinerea* (Table 8.1). Commercially available powder or granules of *Trichoderma* species as biocontrol agent includes *T. viride*, *T. virens* and *T. harzianum* (Dubey et al. 2009, 2011). The intention behind the application of *Trichoderma* strains on crops is to control the phytopathogens (Minchiotti et al. 2021). Input cost concerning crop productivity used to evaluate the efficacy of *Trichoderma* strains as a biocontrol agent. For *Trichoderma*, it has been found that the input cost for crop productivity is relatively low compared with chemical pesticides (Masso et al. 2016). Furthermore, it has been found out that *Trichoderma* spp. also enhance the productivity yield, leading to revenue augmentation. In a study by Imran et al. (2020), the application of the cocktail of *Trichoderma* with compost onto crops can be a promising alternative to expensive chemical fertilizers. Thus, the application of *Trichoderma* is a sustainable approach from the standpoint of maintenance of soil health. *Trichoderma* has been implicated to serve as a biocontrol agent through the following mechanisms.

### *8.5.1 Antibiosis*

Antibiosis is a phenomenon in which a biocontrol agent expels a compound that reduces or inhibits the growth of pathogens in their vicinity (El Komy et al. 2015). *Trichoderma* species are known to produce small-sized diffusible compounds or antibiotics that inhibit the growth of other microorganisms. The biocontrol potential of two endophytic *Trichoderma* spp. against *Fusarium solani*, the causative agent of root rot disease, was checked on plant common bean (Toghueo et al. 2016). Both the *Trichoderma* spp. could inhibit the growth of *F. solani* and spore germination. One of the *Trichoderma* spp. showed a minimum inhibitory concentration (MIC) of 0.66 mg/mL against *F. solani* germination. Wonglom et al. (2019) illustrated the capability of *Trichoderma* spp. T76-12/2 to inhibit the growth of mycelia of *Sclerotium* spp., which is the causative agent of fruit rot of snake fruit and stem rot of lettuce. The presence of antifungal compounds along with alcohol (phenylethyl alcohol) and sesquiterpene (epi-cubenol) was confirmed by gas chromatography-mass spectrometry (GC/MS) which inhibited the growth of *Sclerotium* spp. Myriad of metabolites with biocontrol effect can be discovered from *Trichoderma* spp. Saravanakumar and Wang (2020) have identified eight dominant compounds in the ethyl acetate fraction against phytopathogens by molecular modelling method in *T. aureoviride* SKCGW013. Out of these eight compounds, −2H-pyran, 3-bromo-2-butoxytetrahydro--cis showed high docking inhibitory energy against the targeted proteins.

**Table 8.1** List of *Trichoderma* spp. and the biocontrol of phytopathogens

| S. No. | Plant | Name of disease | Causative pathogen | *Trichoderma species* | References |
|---|---|---|---|---|---|
| 1. | Date palm trees | Black scorch disease | *Ceratocystis radicicola* | *Trichoderma harzianum* | Al-Naemi et al. (2016) |
| 2. | Groundnut | Collar rot | *Aspergillus niger* | *Trichoderma viride* | Gajera et al. (2016) |
| 3. | Soybean | – | Root-knot nematode (*Meloidogyne incognita*) | *Trichoderma harzianum* T22 | Izuogu and Abiri (2015) |
| 4.. | Soybean | Charcoal rot disease | *Macrophomina phaseolina* | *Trichoderma harzianum* | Khalili et al. (2016) |
| 5. | Maize | Foliar disease (*Curvularia* leaf spot) | *Curvularia lunata* | *Trichoderma harzianum* | Saravanakumar et al. (2016a) |
| 6. | Tomato | *Fusarium* wilt disease | *Fusarium oxysporum* f.sp. *lycopersici* | *Trichoderma asperellum* strain T34 | Segarra et al. (2010) |
| 7. | Jerusalem artichoke | Southern stem rot | *Sclerotium rolfsii* | *Trichoderma harzianum* | Sennoi et al. (2013) |
| 8. | Cassava plants (*Manihot esculenta*) | Cassava root rot | *Fusarium solani* | *Trichoderma hamatum* URM 6656<br>*Trichoderma aureoviride* URM 5158 | da Silva et al. (2016) |
| 9. | Tomato | *Fusarium* wilt disease | *Fusarium oxysporum f.* sp. *lycopersici* | *Trichoderma harzianum* | Sundaramoorthy and Balabaskar (2013) |
| 10. | Cucumber | *Fusarium* wilt disease | *Fusarium oxysporum* f. sp. *cucumerinum* | *Trichoderma asperellum* strain CCTCC-RW0014 | Saravanakumar et al. (2016b) |
| 11. | Brinjal and okra | Damping off and wilt diseases | *Fusarium oxysporum*<br>*Pythium aphanidermatum* | *Trichoderma harzianum* | Sain and Pandey (2018) |
| 12. | Orchard | *Armillaria* root rot | *Armillaria gallica* | *Trichoderma atroviride* SC1 | Pellegrini et al. (2014) |

(continued)

**Table 8.1** (continued)

| S. No. | Plant | Name of disease | Causative pathogen | *Trichoderma species* | References |
|---|---|---|---|---|---|
| 13. | Pearl millet | Downy mildew disease | – | *Trichoderma* sp. (UOM PGPF 37) | Murali et al. (2012) |
| 14. | Tobacco | – | Tobacco mosaic virus | *Trichoderma pseudokoningii* SMF2 | Luo et al. (2010) |
| 15. | Onion | Purple blotch | *Alternaria porri* | *Trichoderma harzianum*<br>*Trichoderma longibrachiatum* | Abo-Elyousr et al. (2014) |
| 16. | Chinese cabbage | Soft rot disease | *Pectobacterium carotovorum* | *Trichoderma pseudokoningii* SMF2 | Li et al. (2014) |
| 17. | Lettuce (*Lactuca sativa*) | Leaf spot | *Corynespora cassiicola* and *Curvularia aeria* | *Trichoderma spirale* T76–1 | Baiyee et al. (2019) |
| 18. | Soybean | White mould disease | *Sclerotinia sclerotiorum* | *Trichoderma asperelloides* | Sumida et al. (2018) |
| 19. | Wild mushrooms | Boxwood blight | *Calonectria pseudonaviculata* | *Trichoderma koningiopsis* Mb2 | Kong and Hong (2017) |
| 20. | Tomato | – | *Meloidogyne javanica* | *Trichoderma hamatum*<br>*T. viride*<br>*T. asperellum*<br>*T. atroviride*<br>*T. harzianum* | Javeed et al. (2016) |
| 21. | Tomato | – | *Phytophthora cinnamomi* | *Trichoderma atroviride* | Macías-Rodríguez et al. (2018) |
| 22. | Onion | Onion white rot | *Sclerotium cepivorum* | *Trichoderma asperellum* | Rivera-Mendez et al. (2020) |
| 23. | – | – | *Fusarium oxysporum* | *Trichoderma pseudokoningii* SMF2 | Shi et al. (2012) |
| 24. | Common beans | – | *Sclerotinia sclerotiorum* | *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma tomentosum*, *Trichoderma koningiopsis*, *Trichoderma erinaceum* | Lopes et al. (2012) |
| 25. | Ginseng (*Panax ginseng*) | Leaf spot disease and root rot disease | *Botrytis cinerea* and *Cylindrocarpon destructans* | *Trichoderma citrinoviride* | Park et al. (2019) |

| | | | | | |
|---|---|---|---|---|---|
| 26. | *Juglans mandshurica* | – | *Alternaria alternata* | *Trichoderma asperellum TaspHu1* | Yu et al. (2020) |
| 27. | Cucumber and corn | Cucumber *fusarium* wilt and corn stalk rot | *Fusarium oxysporum f. sp. cucumerinum Owen* and *Fusarium graminearum* | *Trichoderma asperellum GDFS1009* | Wu et al. (2017) |
| 28. | Wheat | Stem rust disease | *Puccinia graminis* Pers. f. sp. *tritici* | *T. harzianum* and *T. viride* | El-Sharkawy et al. (2018) |
| 29. | Onion | *Fusarium* basal rot | *Fusarium oxysporum* f. sp. *cepae* | *T. gamsii*, *T. hamatum* and *T. harzianum* | Bunbury-Blanchette and Walker (2019) |

### *8.5.2 Mycoparasitism*

In the process of mycoparasitism, there occurs a direct attack of one fungal species on another one. Mycoparasitism is the process in which the growth of *Trichoderma* occurs by recognition of the host and secretion of extracellular enzymes followed by penetration of hyphae and lysis of host (Kullnig et al. 2000; Mach et al. 1999). Zhang and Zhuang (2020) reported *Trichoderma brevicrassum* strain TC967 with a potential of diminishing cucumber disease caused by *Rhizoctonia solani*. *T. brevicrassum* showed high chitinase activity in agar medium, which inhibited *Rhizoctonia solani* (72.14%). It also showed strong mycoparasitic ability by coiling and penetrating the hyphae of the pathogen. In a study by Mazrou et al. (2020), the antagonistic activity of 12 *Trichoderma* isolates was checked against soil-borne pathogens, namely, *Rhizoctonia solani*, *Pythium ultimum* and *Alternaria solani*. The scanning electron microscopy data revealed the coiling of the hyphae of pathogens, and the *Trichoderma* isolates formed aspersorium-like structures. In addition, the *Trichoderma* isolates produced lytic enzymes and led to a breakdown of pathogens' hyphae. Sánchez et al. (2019) indicated the significance of *Trichoderma* strains in controlling *Phytophthora* collar rot of pear. *Trichoderma* isolates were evaluated for inhibiting mycelia growth (MG) and mycoparasitism against four *Phytophthora* spp., pathogens of pear. Among the isolates, *Trichoderma harzianum* 1330 and 1377 strains reduced the pear collar rot by 97% compared with diseased control. de Sousa et al. (2021) reported that *Trichoderma asperellum* inhibited the germination of conidia and the formation of appressoria in *Magnaporthe oryzae*. The infection process of *M. oryzae* was hindered by mycoparasitism and antibiosis along with the production of lytic enzymes.

### *8.5.3 Induced Resistance*

*Trichoderma* may promote a plant to produce a chemical that protects it from the pathogen, induced resistance (Cai et al. 2013; Shoresh et al. 2010; Yan et al. 2021). *Trichoderma harzianum* and methyl jasmonate were found to induce resistance against *Bipolaris sorokiniana* in bread wheat (Singh et al. 2019). The opposition was induced by enhancing phenylpropanoid activities**.** The combined application was also found to significantly enhance the activities of defence-related enzymes, namely, catalase, ascorbate peroxidase, phenylalanine ammonia lyase (*PAL)* and peroxidase, in the plants under treatment. The transcript level of *PAL* and *peroxidase* genes was checked by using semi-quantitative PCR for confirmation. The mechanisms responsible for *T. harzianum*-induced resistance in cucumber plants against *Fusarium* wilt were elucidated by Chen et al. (2017). *T. harzianum* isolate TH58 suppressed reactive oxygen species over accumulation and improved root cell viability under *F. oxysporum* infection. Inoculation of TH58 reversed the FO-induced cell division block and upregulated expression levels of cell cycle-related genes. Thus, *T. harzianum* enhanced the plant resistance against *Fusarium* wilt disease. The impact of *T. asperellum* IZR D-11 in inducing resistance in the plant *Quercus robur* was evaluated by Oszako et al. (2021). In this study, *T. asperellum* IZR D-11 reduced the progression of disease powdery mildew caused by *Erysiphe alphitoides* which infected leaves of *Quercus robur*. Volatile compounds like daucene, dauca-4(11),8-diene and isodaucene which GC-MS identified were attributed to induced resistance.

## 8.6 Role of *Trichoderma* in Plant Growth Enhancement

The role of *Trichoderma* spp. is limited to serve as a biocontrol agent and has been reported to enhance plant growth (Asghar and Kataoka 2021) (Fig. 8.2). Singh et al. (2016) studied the impact of *Trichoderma asperellum* BHUT8 on seed germination and development of six vegetable crops. The study focused on figuring out the most effective spore dose for each of six vegetable crops that effectively enhances plant growth promotion. The potential of *Trichoderma* spp. from the Amazon biome as promising biofertilizer agents was explored by Bononi et al. (2020). *Trichoderma* spp. strains showed positive responses in the promotion of soybean growth and enhanced the efficiency of P uptake. Harman et al. (2021) have shown the importance of *Trichoderma* in upregulating genes that enhances photosynthesis in plants. This is implicated by activating biochemical pathways that reduce reactive oxygen species. *Trichoderma* has also been reported to stimulate plant growth promotion by the production of phytohormones (Stewart and Hill 2014). The effect of *Trichoderma longibrachiatum* KH, *T. longibrachiatum* MA and *T. harzianum* on tomato growth under water-deficit stress was studied by Khoshmanzar et al. (2020). The results indicated that *Trichoderma longibrachiatum* KH increased the root volume and increased shoot and root potassium uptake. Establishment of vegetation on red mud sites is a considerable challenge. Anam et al. (2019) have highlighted the significance of *Trichoderma asperellum* in tolerating heavy metal(loid)s (HMs). The fungus also had desirable plant growth-promoting traits and improved the growth and chlorophyll content and simultaneously reduced the oxidative stress of sorghum-sudangrass seedlings grown in red mud leachate. Li et al. (2015) studied the role of *Trichoderma harzianum* in solubilization of phosphate and micronutrients like Fe, Mn, Cu and Zn in tomato plants which were grown in soil and hydroponic conditions. Inoculation with *T. harzianum* significantly improved the biomass and nutrient uptake of tomato seedlings. In recent research by Cai et al. (2015), the potential of *Trichoderma harzianum* strain SQR-T037 and bio-organic fertilizer in stimulating tomato plant growth under field conditions were studied. *T. harzianum* could efficiently colonize tomato roots and stimulate biomass accumulation.

## 8.7 Role of *Trichoderma* as Natural Decomposition Agent

The biological process of degradation and breaking down organic materials into smaller particles is called decomposition. Fungi act as decomposers and play a crucial role in this process. The decomposition process by fungi assists in nutrient cycling by returning nutrients from dead organic matter back into the soil. Literature studies have shown that *Trichoderma* spp. are good natural decomposers that boost the decomposition rate of organic matter (Fig. 8.2).

In a study by Siddiquee et al. (2017), two potential *Trichoderma* strains were used to compost empty fruit bunches (EFB) from the oil palm industry. The results illustrated that the bio-composting of oil palm fibres using *Trichoderma* had great potential for enhancing soil micronutrient leading to enhancement of plant growth performance and crop yield production. Błaszczyk et al. (2016) have explored the species diversity of *Trichoderma* which were obtained from samples of wood collected in the forests of the Central Europe. The study showed the positive correlation between cellulolytic and xylanolytic activity of these species and the wood decay processes. The capability of *Trichoderma* strains in modification of post-industrial lignin with ligninolytic enzymes was examined in a study by Bohacz and Korniłłowicz-Kowalska (2020). The isolated strains were found to secrete horseradish-like peroxidase (HRP-like), superoxide dismutase-like (SOD-like), xylanase and phenolic compounds and lead to biotransformation of dark post-industrial lignin. Sharma et al. (2012) demonstrated biodegradation of crop residues and press mud cake under pit conditions using *Trichoderma* species. Inoculation by *Trichoderma* increased N, P, K, S content in the resultant compost when treated with urea and dung.

## 8.8 Conclusion

The current innovations in the field of agriculture have led to an increase in crop yield. But some of them have disparaging effects on the environment. Thus, alternative approaches need to be explored that augments crop yield without adversely affecting the environment. Literature mining suggests that *Trichoderma* species are well-known biocontrol agents. In addition to this, their role as plant growth enhancer and decomposition agent is also noteworthy. Thus, in a nutshell, *Trichoderma* species aids in sustainable agriculture by diminishing different crop diseases, fuels plant growth and development and improves the composting process.

## References

Abbas A, Jiang D, Fu Y (2017) *Trichoderma Spp.* as antagonist of *Rhizoctonia solani*. J Plant Pathol Microbiol 8:402. https://doi.org/10.4172/2157-7471.1000402

Abo-Elyousr KA, Abdel-Hafez SI, Abdel-Rahim IR (2014) Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. J Phytopathol 162:567–574. https://doi.org/10.1111/jph.12228

Al-Naemi FA, Ahmed TA, Nishad R, Radwan O (2016) Antagonistic effects of *Trichoderma harzianum* isolates against *Ceratocystis radicicola*: pioneering a biocontrol strategy against black scorch disease in date palm trees. J Phytopathol 164:464–475. https://doi.org/10.1111/jph.12472

Anam GB, Reddy MS, Ahn YH (2019) Characterization of *Trichoderma asperellum* RM-28 for its sodic/saline-alkali tolerance and plant growth promoting activities to alleviate toxicity of red mud. Sci Total Environ 662:462–469. https://doi.org/10.1016/j.scitotenv.2019.01.279

Asghar W, Kataoka R (2021) Effect of co-application of *Trichoderma sp.* with organic composts on plant growth enhancement, soil enzymes and fungal community in soil. Arch Microbiol 1:1. https://link.springer.com/article/10.1007/s00203-021-02413-4

Bailey KL, Boyetchko SM, Längle 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. https://doi.org/10.1016/j.biocontrol.2009.05.003

Baiyee B, Pornsuriya C, Ito SI, Sunpapao A (2019) *Trichoderma spirale* T76-1 displays biocontrol activity against leaf spot on lettuce (*Lactuca sativa L.*) caused by *Corynespora cassiicola* or *Curvularia aeria*. Biol Control 129:195–200. https://doi.org/10.1016/j.biocontrol.2018.10.018

Begum MF, Rahman MA, Alam MF (2010) Biological control of Alternaria fruit rot of chili by *Trichoderma species* under field conditions. Mycobiology 38:113–117. https://www.tandfonline.com/doi/abs/10.4489/MYCO.2010.38.2.113

Bissett J, Szakacs G, Nolan CA et al (2003) New species of *Trichoderma* from Asia. Can J Bot 81:570–586. https://doi.org/10.1139/b03-051

Błaszczyk L, Strakowska J, Chełkowski J et al (2016) *Trichoderma species* occurring on wood with decay symptoms in mountain forests in Central Europe: genetic and enzymatic characterization. J Appl Genet 57:397–407. https://doi.org/10.1007/s13353-015-0326-1

Bohacz J, Korniłłowicz-Kowalska T (2020) Modification of post-industrial lignin by fungal strains of the genus *Trichoderma* isolated from different composting stages. J Environ Manag 266:110573. https://doi.org/10.1016/j.jenvman.2020.110573

Bononi L, Chiaramonte JB, Pansa CC et al (2020) Phosphorus-solubilizing *Trichoderma spp.* from Amazon soils improve soybean plant growth. Sci Rep 10:1–13. https://doi.org/10.1038/s41598-020-59793-8

Brito JP, Ramada MH, de Magalhães MT et al (2014) Peptaibols from *Trichoderma asperellum TR356* strain isolated from Brazilian soil. Springerplus 3:1–10. https://doi.org/10.1186/2193-1801-3-600

Bunbury-Blanchette AL, Walker AK (2019) *Trichoderma species* show biocontrol potential in dual culture and greenhouse bioassays against fusarium basal rot of onion. Biol Control 130:127–135. https://doi.org/10.1016/j.biocontrol.2018.11.007

Cai F, Yu G, Wang P, Wei Z et al (2013) Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. Plant Physiol Biochem 73:106–113. https://doi.org/10.1016/j.plaphy.2013.08.011

Cai F, Chen W, Wei Z, Pang G et al (2015) Colonization of *Trichoderma harzianum strain SQR-T037* on tomato roots and its relationship to plant growth, nutrient availability and soil microflora. Plant Soil 388:337–350. https://doi.org/10.1007/s11104-014-2326-z

Chaverri P, Castlebury LA, Overton BE, Samuels GJ (2003) *Hypocrea/Trichoderma*: species with conidiophore elongations and green conidia. Mycologia 95:1100–1140. https://doi.org/10.1080/15572536.2004.11833023

Chen SC, Zhao HJ, Wang ZH et al (2017) *Trichoderma harzianum*-induced resistance against *Fusarium oxysporum* involves regulation of nuclear DNA content, cell viability and cell cycle-related genes expression in cucumber roots. Eur J Plant Pathol 147:43–53. https://link.springer.com/content/pdf/10.1007/s10658-016-0978-7.pdf

Contreras Cornejo HA, Macías Rodríguez L, DelVal E, Larsen J (2016) Ecological functions of *Trichoderma spp.* and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiol Ecol 92:fiw036. https://doi.org/10.1093/femsec/fiw036

da Silva JAT, de Medeiros EV, da Silva JM et al (2016) *Trichoderma aureoviride* URM 5158 and *Trichoderma hamatum* URM 6656 are biocontrol agents that act against cassava root rot through different mechanisms. J Phytopathol 164:1003–1011. https://doi.org/10.1111/jph.12521

de Sousa TP, Chaibub AA, Cortes MVDCB, Batista TFC, de Andrade Bezerra G, da Silva GB et al (2021) Molecular identification of *Trichoderma sp.* isolates and biochemical characterization of antagonistic interaction against rice blast. Arch Microbiol 203:3257–3268. https://doi.org/10.1007/s00203-021-02307-5

Dubey SC, Bhavani R, Singh B (2009) Development of Pusa 5SD for seed dressing and Pusa biopellet 10G for soil application formulations of *Trichoderma harzianum* and their evaluation for integrated management of dry root rot of mungbean (Vigna radiata). Biol Control 50:231–242. https://doi.org/10.1016/j.biocontrol.2009.04.008

Dubey SC, Bhavani R, Singh B (2011) Integration of soil application and seed treatment formulations of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani*. Pest Manag Sci 67:1163–1168. https://doi.org/10.1002/ps.2168

El Komy MH, Saleh AA, Eranthodi A, Molan YY (2015) Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato Fusarium wilt. Plant Pathol J 31:50. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4356605/

El-Sharkawy HH, Rashad YM, Ibrahim SA (2018) Biocontrol of stem rust disease of wheat using arbuscular *mycorrhizal fungi* and *Trichoderma spp*. Physiol Mol Plant Pathol 103:84–91. https://doi.org/10.1016/j.pmpp.2018.05.002

Freeman S, Minz D, Kolesnik I et al (2004) *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry. Eur J Plant Pathol 110:361–370. https://doi.org/10.1023/B:EJPP.0000021057.93305.d9

Gajera H, Katakpara ZA, Patel S, Golakiya B (2016) Antioxidant defense response induced by *Trichoderma viride* against *Aspergillus niger Van Tieghem* causing collar rot in groundnut (*Arachis hypogaea L.*). Microb Pathog 91:26–23. https://doi.org/10.1016/j.micpath.2015.11.010

Gao Z, Jun C, Pan Y, Wu X et al (2007) Inhibitory mechanisms of *Trichoderma harzianum* TH-1 against the pathogens of Verticillium Wilt and Fusarium Wilt of cotton. J Cotton Sci 3

Harman GE, Howell CR, Viterbo A et al (2004) *Trichoderma species* opportunistic avirulent plant symbionts. Nat Rev Microbiol 2:43–56. https://doi.org/10.1038/nrmicro797

Harman G, Doni F, Khadka RB, Uphoff N (2021) Endophytic strains of *Trichoderma* increase plants' photosynthetic capability. J Appl Microbiol 130(2):529–546

Howell C (2002) Cotton seedling preemergence damping-off incited by *Rhizopus oryzae* and *Pythium spp.* and its biological control with Trichoderma spp. Phytopathology 92:177–180. https://doi.org/10.1094/PHYTO.2002.92.2.177

Howell C (2003) Mechanisms employed by *Trichoderma species* in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis 87:4–10

Hoyos-Carvajal L, Bissett J (2011) Biodiversity of *Trichoderma* in neotropics. The dynamical processes of biodiversity-case studies of evolution and spatial distribution. Intech:303–320

Imran A, Arif M, Shah Z, Bari A (2020) Soil application of *Trichoderma* and peach (*Prunus persica L.*) residues possesses biocontrol potential for weeds and enhances growth and profitability of soybean (Glycine max). Sarhad J Agric 36:10–20. https://doi.org/10.1094/PHYTO.2002.92.2.177

Izuogu NB, Abiri T (2015) Efficacy of *Trichoderma harzianum* T22 as a biocontrol agent against root-knot nematode (*Meloidogyne incognita*) on some soybean varieties. Croat J Food Sci Technol 7:47–51. https://doi.org/10.17508/CJFST.2015.7.2.04

Jaklitsch WM, Samuels GJ, Dodd SL, Lu BS et al (2006) *Hypocrea rufa/Trichoderma viride*: a reassessment and description of five closely related species with and without warted conidia. Stud Mycol 56:135–177. https://doi.org/10.3114/sim.2006.56.04

Javeed M, Al-Hazmi A, Molan Y (2016) Antagonistic effects of some indigenous isolates of *Trichoderma spp.* against *Meloidogyne javanica*. Pak J Nematol 34:183–191. https://doi.org/10.18681/pjn.v34.i02.p183

Jiang Y, Wang JL, Chen J et al (2016) *Trichoderma* biodiversity of agricultural fields in east China reveals a gradient distribution of species. PLoS One 11:e0160613. https://doi.org/10.1371/journal.pone.0160613

Khalili E, Javed MA, Huyop F et al (2016) Evaluation of *Trichoderma* isolates as potential biological control agent against soybean charcoal rot disease caused by Macrophomina phaseolina. Biotechnol Biotechnol Equip 30:479–488. https://doi.org/10.1080/13102818.2016.1147334

Khoshmanzar E, Aliasgharzad N, Neyshabouri M et al (2020) Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. Int J Environ Sci Technol 17: 869–878. https://doi.org/10.1007/s13762-019-02405-4

Kim CS, Park MS, Kim SC, Maekawa N et al (2012) Identification of *Trichoderma* a competitor of shiitake mushroom (*Lentinula edodes*) and competition between *Lentinula edodes* and *Trichoderma species* in Korea. Plant Pathol J 28:137–148

Klein D, Eveleigh D (2002) Ecology of *Trichoderma Trichoderma* and *gliocladium* 1, 57–74

Kong P, Hong C (2017) Biocontrol of boxwood blight by *Trichoderma koningiopsis* Mb2. Crop Prot 98:124–127. https://doi.org/10.1016/j.cropro.2017.03.015

Kredics L, Hatvani L, Naeimi S (2014) Biodiversity of the genus *Hypocrea/Trichoderma* in different habitats. In: Biotechnology and biology of Trichoderma. Elsevier, pp 3–24. https://doi.org/10.1016/B978-0-444-59576-8.00001-1

Kubicek C, Mach R, Peterbauer C, Lorito M (2001) *Trichoderma*: from genes to biocontrol. J Plant Pathol 83:11–23. https://www.jstor.org/stable/41998018

Kubicek CP, Steindorff AS, Chenthamara K et al (2019) Evolution and comparative genomics of the most common *Trichoderma species*. BMC Genomics 20:1–24. https://doi.org/10.1186/s12864-019-5680-7

Kullnig C, Mach RL, Lorito M, Kubicek CP (2000) Enzyme diffusion from *Trichoderma atroviride* (T. harzianum P1) to *Rhizoctonia solani* is a prerequisite for triggering of *Trichoderma* ech42 gene expression before mycoparasitic contact. Appl Environ Microbiol 66:2232–2234. https://doi.org/10.1128/AEM.66.5.2232-2234.2000

Latifian M, Hamidi-Esfahani Z, Barzegar M (2007) Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Bioresour Technol 98:3634–3637. https://doi.org/10.1016/j.biortech.2006.11.019

Li HY, Luo Y, Zhang XS, Shi WL et al (2014) *Trichokonins* from *Trichoderma pseudokoningii* SMF2 induce resistance against gram-negative *Pectobacterium carotovorum subsp. carotovorum* in Chinese cabbage. FEMS Microbiol Lett 354:75–82. https://doi.org/10.1111/1574-6968.12427

Li RX, Cai F, Pang G, Shen QR et al (2015) Solubilization of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. PLoS One 10:e0130081. https://doi.org/10.1371/journal.pone.0130081

Li YT, Hwang SG, Huang YM, Huang CH (2018) Effects of *Trichoderma asperellum* on nutrient uptake and Fusarium wilt of tomato. Crop Prot 110:275–282. https://doi.org/10.1016/j.cropro.2017.03.021

Lithourgidis A, Dordas C, Damalas CA, Vlachostergios D (2011) Annual intercrops: an alternative pathway for sustainable agriculture. Aust J Crop Sci 5:396–410. https://search.informit.org/doi/10.3316/informit.281409060336481

Lopes FAC, Steindorff AS, Geraldine AM et al (2012) Biochemical and metabolic profiles of *Trichoderma strains* isolated from common bean crops in the Brazilian Cerrado, and potential antagonism against *Sclerotinia sclerotiorum*. Fungal Biol 116:815–824. https://doi.org/10.1016/j.funbio.2012.04.015

Lu B, Druzhinina IS, Fallah P et al (2004) *Hypocrea/Trichoderma* species with pachybasium-like conidiophores: teleomorphs for *T. minutisporum* and *T. polysporum* and their newly discovered relatives. Mycologia 96:310–342. https://doi.org/10.1080/15572536.2005.11832980

Luo Y, Zhang DD, Dong XW et al (2010) Antimicrobial peptaibols induce defense responses and systemic resistance in tobacco against tobacco mosaic virus. FEMS Microbiol Lett 313:120–126. https://doi.org/10.1111/j.1574-6968.2010.02135.x

Mach RL, Peterbauer CK, Payer K, Jaksits S et al (1999) Expression of two major chitinase genes of *Trichoderma atroviride (T. harzianum* P1) is triggered by different regulatory signals. Appl Environ Microbiol 65:1858–1863. https://doi.org/10.1128/AEM.65.5.1858-1863.1999

Macías-Rodríguez L, Guzmán-Gómez A, García-Juárez P, Contreras-Cornejo HA (2018) *Trichoderma* atroviride promotes tomato development and alters the root exudation of carbohydrates, which stimulates fungal growth and the biocontrol of the phytopathogen *Phytophthora cinnamomi* in a tripartite interaction system. FEMS Microbiol Ecol 94:fiy137. https://doi.org/10.1093/femsec/fiy137

Mahmood I, Imadi SR, Shazadi K et al (2016) Effects of pesticides on environment. In: Plant, soil and microbes. Springer, pp 253–269

Masso C, Mukhongo RW, Thuita M et al. (2016) Biological inoculants for sustainable intensification of agriculture in sub-Saharan Africa smallholder farming systems. In: Climate change and multi-dimensional sustainability in African agriculture. Springer, pp 639–658

Mazrou YS, Makhlouf AH, Elseehy MM (2020) Antagonistic activity and molecular characterization of biological control agent *Trichoderma harzianum* from Saudi Arabia. Egypt J Biol Pest Control 30:4. https://doi.org/10.1186/s41938-020-0207-8

Mbarga JB, Ten Hoopen GM, Kuaté J et al (2012) *Trichoderma asperellum:* a potential biocontrol agent for *Pythium myriotylum*, causal agent of cocoyam (*Xanthosoma sagittifolium*) root rot disease in Cameroon. Crop Prot 36:18–22. https://doi.org/10.1016/j.cropro.2012.02.004

Minchiotti MC, Vargas LI, Madoery RR (2021) Phospholipase A activity and the biocontrol potential of *Trichoderma harzianum* and *Trichoderma atroviride*. Biocontrol Sci Tech 1:17. https://doi.org/10.1080/09583157.2021.1940864

Murali M, Amruthesh K, Sudisha J et al (2012) Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. J Phytol 4:30–36. http://journal-phytology.com/.../7891

Naar Z, Kecskés M (1998) Factors influencing the competitive saprophytic ability of *Trichoderma species*. Microbiol Res 153:119–129. https://doi.org/10.1016/S0944-5013(98)80029-3

Naseby D, Pascual J, Lynch J et al (2000) Effect of biocontrol strains of *Trichoderma* on plant growth*, Pythium ultimum* populations, soil microbial communities and soil enzyme activities. J Appl Microbiol 88:161–169. https://doi.org/10.1046/j.1365-2672.2000.00939.x

Nawrocka J, Małolepsza U (2013) Diversity in plant systemic resistance induced by *Trichoderma*. Biol Control 67:149–156. https://doi.org/10.1016/j.biocontrol.2013.07.005

Oszako T, Voitka D, Stocki M et al (2021) *Trichoderma asperellum* efficiently protects Quercus robur leaves against *Erysiphe alphitoides*. Eur J Plant Pathol 159(2):295–308. https://link.springer.com/article/10.1007/s10658-020-02162-y

Pane C, Villecco D, Campanile F, Zaccardellin M (2012) Novel strains of *Bacillus*, isolated from compost and compost-amended soils, as biological control agents against soil-borne phytopathogenic fungi. Biocontrol Sci Tech 22:1373–1388. https://doi.org/10.1080/09583157.2012.729143

Papavizas G (1985) Trichoderma and Gliocladium: biology, ecology, and potential for biocontrol. Annu Rev Phytopathol 23:23–54

Park YH, Mishra RC, Yoon S et al (2019) Endophytic *Trichoderma citrinoviride* isolated from mountain-cultivated ginseng (*Panax ginseng*) has great potential as a biocontrol agent against ginseng pathogens. J Ginseng Res 43:408–420. https://doi.org/10.1016/j.jgr.2018.03.002

Pellegrini A, Prodorutti D, Pertot I (2014) Use of bark mulch pre-inoculated with *Trichoderma atroviride* to control Armillaria root rot. Crop Prot 64:104–109. https://doi.org/10.1016/j.cropro.2014.06.007

Poosapati S, Ravulapalli PD, Tippirishetty N et al (2014) Selection of high temperature and salinity tolerant *Trichoderma* isolates with antagonistic activity against *Sclerotium rolfsii*. Springerplus 3:1–11. https://doi.org/10.1186/2193-1801-3-641

Ramakrishnan B, Venkateswarlu K, Sethunathan N, Megharaj M (2019) Local applications but global implications: can pesticides drive microorganisms to develop antimicrobial resistance. Sci Total Environ 654:177–189. https://doi.org/10.1016/j.scitotenv.2018.11.041

Regnault-Roger C (2012) Trends for commercialization of biocontrol agent (biopesticide) products. In: Plant defence: biological control. Springer, pp 139–160

Reino JL, Guerrero RF, Hernández-Galán R, Collado IG (2008) Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem Rev 7:89–123. https://link.springer.com/content/pdf/10.1007/s11101-006-9032-2

Rey M, Delgado-Jarana J, Benitez T (2001) Improved antifungal activity of a mutant of *Trichoderma harzianum* CECT 2413 which produces more extracellular proteins. Appl Microbiol Biotechnol 55:604–608. https://doi.org/10.1007/s002530000551

Rivera-Mendez W, Obregón M, Moran-Diez ME et al (2020) *Trichoderma asperellum* biocontrol activity and induction of systemic defenses against *Sclerotium cepivorum* in onion plants under tropical climate conditions. Biol Control 141:104145. https://doi.org/10.1016/j.biocontrol.2019.104145

Sain SK, Pandey AK (2018) Evaluation of some *Trichoderma harzianum* isolates for the management of soilborne diseases of brinjal and okra. Proc Natl Acad Sci India Sect B Biol Sci 88:905–914. https://doi.org/10.1007/s40011-016-0824-x

Sala A, Vittone S, Barrena R, Sánchez A et al (2021) Scanning agro-industrial wastes as substrates for fungal biopesticide production: use of *Beauveria bassiana* and *Trichoderma harzianum* in solid-state fermentation. J Environ Manag 295:113113. https://doi.org/10.1016/j.jenvman.2021.113113

Sallam NM, Eraky AM, Sallam A (2019) Effect of *Trichoderma spp.* on fusarium wilt disease of tomato. Mol Biol Rep 46:4463–4470. https://doi.org/10.1007/s11033-019-04901-9

Samuels GJ (2006) *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96:195–206. https://doi.org/10.1094/PHYTO-96-0195

Samuels GJ, Petrini O, Kuhls K et al (1998) The *Hypocrea schweinitzii* complex and *Trichoderma sect. Longibrachiatum*. Stud Mycol 41:1–54

Sánchez AD, Ousset MJ, Sosa MC (2019) Biological control of Phytophthora collar rot of pear using regional *Trichoderma strains* with multiple mechanisms. Biol Control 135:124–134. https://doi.org/10.1016/j.biocontrol.2019.05.012

Santoro PH, Cavaguchi SA, Alexandre TM et al (2014) In vitro sensitivity of antagonistic *Trichoderma atroviride* to herbicides. Braz Arch Biol Technol 57:238–243

Saravanakumar K, Wang MH (2020) Isolation and molecular identification of *Trichoderma* species from wetland soil and their antagonistic activity against phytopathogens. Physiol Mol Plant Pathol 109:101458. https://doi.org/10.1016/j.pmpp.2020.101458

Saravanakumar K, Fan L, Fu K (2016a) Cellulase from *Trichoderma harzianum* interacts with roots and triggers induced systemic resistance to foliar disease in maize. Sci Rep 6:1–18. https://doi.org/10.1038/srep35543

Saravanakumar K, Yu C, Dou K (2016b) Synergistic effect of *Trichoderma* derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum f. sp. cucumerinum*. Biol Control 94:37–46. https://doi.org/10.1016/j.biocontrol.2015.12.001

Sarhy-Bagnon V, Lozano P, Saucedo-Castañeda G (2000) Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* in liquid and solid state cultures. Process Biochem 36:103–109. https://doi.org/10.1016/S0032-9592(00)00184-9

Segarra G, Casanova E, Avilés M, Trillas I (2010) *Trichoderma asperellum* strain T34 controls Fusarium wilt disease in tomato plants in soilless culture through competition for iron. Microb Ecol 59:141–149. https://doi.org/10.1007/s00248-009-9545-5

Sennoi R, Singkham N, Jogloy S (2013) Biological control of southern stem rot caused by Sclerotium rolfsii using *Trichoderma harzianum* and *arbuscular mycorrhizal* fungi on *Jerusalem artichoke (Helianthus tuberosus L.)*. Crop Prot 54:148–153. https://doi.org/10.1016/j.cropro.2013.08.011

Seyis I, Aksoz N (2005) Effect of carbon and nitrogen sources on xylanase production by *Trichoderma harzianum* 1073 D3. Int Biodeter Biodegr 55:115–119. https://doi.org/10.1016/j.ibiod.2004.09.001

Sharma P, Kumar V, Ramesh R, Saravanan K et al (2011) Biocontrol genes from *Trichoderma* species: a review. Afr J Biotechnol 10:19898–19907. https://doi.org/10.5897/AJBX11.041

Sharma BL, Singh SP, Sharma ML (2012) Bio-degradation of crop residues by *Trichoderma species* vis-a vis nutrient quality of the prepared compost. Sugar Tech 14:174–180. https://doi.org/10.1007/s12355-011-0125-x

Shi M, Chen L, Wang XW et al (2012) Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. Microbiology 158:166–175. https://doi.org/10.1099/mic.0.052670-0

Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–24. https://doi.org/10.1146/annurev-phyto-073009-1144503

Siddiquee S, Shafawati SN, Naher L (2017) Effective composting of empty fruit bunches using potential Trichoderma strains. Biotechnol Rep 13:1–7

Singh V, Upadhyay RS, Sarma BK, Singh HB (2016) *Trichoderma asperellum* spore dose depended modulation of plant growth in vegetable crops. Microbiol Res 193:74–86. https://doi.org/10.1016/j.micres.2016.09.002

Singh UB, Malviya D, Singh S et al (2019) *Trichoderma harzianum* and *methyl jasmonate* induced resistance to Bipolaris sorokiniana through enhanced phenylpropanoid activities in bread wheat (*Triticum aestivum L.*). Front Microbiol 10:1697. https://doi.org/10.3389/fmicb.2019.01697

Singh R, Tomer A, Prasad D, Viswanath H (2020) Biodiversity of *Trichoderma* species in different agro-ecological habitats. In: Trichoderma: agricultural applications and beyond. Springer, pp 21–40

Stewart A, Hill R (2014) Applications of *Trichoderma* in plant growth promotion. In Biotechnology and biology of Trichoderma. Elsevier, pp 415–428

Strauch O, Strasser H, Hauschild R, Ehlers RU (2011) Proposals for bacterial and fungal biocontrol agents. In: Regulation of biological control agents. Springer, pp 267–288

Sumida CH, Daniel JF, Araujod APC et al (2018) *Trichoderma asperelloides* a*ntagonism* to nine *Sclerotinia sclerotiorum* strains and biological control of white mold disease in soybean plants. Biocontrol Sci Tech 28:142–156. https://doi.org/10.1080/09583157.2018.1430743

Sundaramoorthy S, Balabaskar P (2013) Biocontrol efficacy of *Trichoderma spp.* against wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici*. J Appl Biol Biotechnol 1:36–40. https://doi.org/10.7324/JABB.2013.1306

Toghueo RMK, Eke P, Zabalgogeazcoa Í et al (2016) Biocontrol and growth enhancement potential of two endophytic *Trichoderma spp.* from Terminalia catappa against the causative agent of common bean root rot (Fusarium solani). Biol Control 96:8–20. https://doi.org/10.1016/j.biocontrol.2016.01.008

Verma M, Brar SK, Tyagi R, Surampalli R, Valero J (2007) Antagonistic fungi, *Trichoderma spp.* panoply of biological control. Biochem Eng J 37:1–20. https://doi.org/10.1016/j.bej.2007.05.012

Waghunde RR, Shelake RM, Sabalpara AN (2016) *Trichoderma:* a significant fungus for agriculture and environment. Afr J Agric Res 11:1952–1965

Wiedow D, Baum C, Leinweber P (2007) Inoculation with *Trichoderma saturnisporum* accelerates wheat straw decomposition on soil: (Inokulation mit *Trichoderma saturnisporum* beschleunigt den Abbau von Weizenstroh auf dem Boden). Arch Agron Soil Sci 53:1–12. https://doi.org/10.1080/03650340601054213

Wonglom P, Daengsuwan W, Si I, Sunpapao A (2019) Biological control of Sclerotium fruit rot of snake fruit and stem rot of lettuce by *Trichoderma sp.* T76-12/2 and the mechanisms involved. Physiol Mol Plant Pathol 107:1–7. https://doi.org/10.1016/j.pmpp.2019.04.007

Woo S, Scala F, Ruocco M, Lorito M (2006) The molecular biology of the interactions between *Trichoderma spp.*, phytopathogenic fungi and plants. Phytopathology 96:181–185. https://doi.org/10.1094/PHYTO-96-0181

Wu Q, Sun R, Ni M et al (2017) Identification of a novel fungus, *Trichoderma asperellum* GDFS1009 and comprehensive evaluation of its biocontrol efficacy. PLoS One 12:e0179957. https://doi.org/10.1371/journal.pone.0179957

Yan Y, Mao Q, Wang Y et al (2021) *Trichoderma harzianum* induces resistance to root-knot nematodes by increasing secondary metabolite synthesis and defense-related enzyme activity in *Solanum lycopersicum L.* Biol Control 158:104609. https://doi.org/10.1016/j.biocontrol.2021.104609

Yu Z, Wang Z, Zhang Y et al (2020) Biocontrol and growth-promoting effect of *Trichoderma asperellum* TaspHu1 isolate from Juglans mandshurica rhizosphere soil. Microbiol Res 242:126596. https://doi.org/10.1016/j.micres.2020.126596

Zafra G, Moreno-Montaño A, Absalón ÁE, Corters-Espinosa DV et al (2015) Degradation of polycyclic aromatic hydrocarbons in soil by a tolerant strain of *Trichoderma asperellum*. Environ Sci Pollut Res 22:1034–1042. https://doi.org/10.1007/s11356-014-3357-y

Zhang Y, Zhuang WY (2020) *Trichoderma brevicrassum* strain TC967 with capacities of diminishing cucumber disease caused by Rhizoctonia solani and promoting plant growth. Biol Control 142:104151. https://doi.org/10.1016/j.biocontrol.2019.104151

Zhang CL, Druzhinina IS, Kubicek CP, Xu T (2005) *Trichoderma* biodiversity in China: evidence for a north to south distribution of species in East Asia. FEMS Microbiol Lett 251:251–257. https://doi.org/10.1016/j.femsle.2005.08.034

# Chapter 9

# Current Approaches for Alleviating Abiotic Stress Tolerance in Crops: Role of Beneficial Microorganisms

**Sahana Basu, Sujoy Sarkar, Ram Prasad, and Gautam Kumar**

**Abstract** Global climate change is evolving as a potential threat to the sustainable food security. Abiotic stresses are the most important limiting factors to the agricultural crop productivity. Therefore, exploration of efficient and economical technologies for the alleviation of abiotic stresses in plants are necessary to encounter the food security. The rhizosphere and phyllosphere of plants are colonized by various microorganisms, establishing neutral, detrimental or beneficial associations with their respective host plants. The beneficial microorganisms sustain various physiological activities in plants under extreme climatic conditions. They also promote the abiotic stress tolerance of plants, thereby improving plant growth and productivity. Therefore, amelioration of abiotic stresses in crop plants by using the microorganisms is opening a promising avenue in enhancing the agricultural productivity. The chapter summarizes the role of beneficial microorganisms in enhancing the crop plants' productivity though alleviation of abiotic stresses for the agricultural sustainability.

**Keywords** Abiotic stress · Crop · Microorganism · Heavy metals · Reactive oxygen species · Plant growth-promoting bacteria

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_9

## 9.1 Introduction

The sessile nature of plants often compels them to confront environmental challenges caused by the universal climate change. Environmental stresses including salinity, drought, extreme temperatures and heavy metal toxicity have devastating impacts on the growth and yield of major crop plants throughout the world. Abiotic stress frequently affects around 90% of the agricultural lands resulting in above 70% loss of global crop productivity (Mantri et al. 2012). Current climate prediction models indicate a gradual increase in the ambient temperature with erratic rainfall to affect various ecosystems posing a considerable threat to the future food security. According to the Food and Agriculture Organization of the United Nations (FAO), the global food production requires to be increased by 70% to meet the food demand for the estimated global population of ~9.7 billion by the year 2050 (F.A.O. (Food and Agriculture Organization of the United Nations) 2017). It is, therefore, a major challenge to take necessary steps to safeguard the abiotic stress-induced loss of agricultural productivity.

Abiotic stresses severely impede the seed germination and fruiting patterns of the agricultural crop plants. Individual or combination of different abiotic stresses induces morphological, physiological, biochemical and molecular alterations in plants that adversely affect the growth, biomass and productivity (Kumar et al. 2009a). Several abiotic stresses also disturb the plant water relations, thereby reducing the water use efficiency. Abiotic stresses increase the overproduction of reactive oxygen species (ROS) including hydrogen peroxide ($H_2O_2$), hydroxide ions ($OH^{\cdot}$), singlet oxygen ($^1O_2$) and superoxide anion ($^{\cdot}O_2^{-}$) through enhanced leakage of electrons from the electron transport chain to the molecular oxygen (Basu et al. 2021a). Excessive ROS accumulation in plant cells leads to oxidative damages by oxidizing photosynthetic pigments, membrane lipids, nucleic acids and proteins, thereby inducing the tissue-specific programmed cell death (Nath et al. 2016, 2017; Kapoor et al. 2019; Kundu et al. 2020).

Abiotic stress tolerance in plants is associated with the maintenance of the cellular redox homeostasis mediated by antioxidant defence system-induced ROS scavenging (Dwivedi et al. 2019; Basu and Kumar 2021). The enzymatic antioxidants facilitating the ROS detoxification include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Basu et al. 2022). The non-enzymatic antioxidants mediating ROS scavenging include ascorbic acid, carotenoids, reduced glutathione (GSH), flavonoids and α-tocopherol. Growth enhancers like proline and soluble sugars also confer abiotic stress tolerance by interfering with the metabolic processes through osmotic adjustment.

## 9.2 Plant Growth-Promoting Microorganisms

Microbes possess the ability to enhance the plant growth by synthesizing various microbial products. Bacteria derived from the plant rhizosphere have been demonstrated to have beneficial effects on the roots of their host plants. The presence of plant growth-promoting rhizobacteria (PGPR) on the plant roots has been found to have positive direct and indirect effects on the plant growth with amelioration of various abiotic stresses (Basu and Kumar 2020a). The beneficial microbes can enhance plant growth through the induction of systemic resistance, antibiosis and competitive omission. These rhizospheric microorganisms, with their unique characteristics, diversity and relationship with plants require to be further exploited to address their potential role in abiotic stress tolerance in plants (Prasad et al. 2015). For instance, *Bacillus* species can form endospores that are extremely resilient to harsh environmental conditions and can also secrete metabolites that stimulate plant growth and health. Some *Bacillus subtilis* strains also emit various types of volatile organic compounds assisting plants to recover from stress. *Bacillus* species also secrete exopolysaccharides and siderophores that inhibit the movement of toxic ions and help in maintaining the ionic equilibrium, consequently facilitating the water uptake by plant roots. Thus, the successful application of beneficial microbes provides a model for enhancing abiotic stress tolerance and adaptation to climate change. There is a potential to improve the beneficial interactions between plants and microbes by further evaluation and identification of new microbial isolates having significant effect in the rhizosphere. Technology could be used to identify PGPR that might have a beneficial impact on abiotic stress tolerance, soil fertility, nutrient acquisition and ultimately crop productivity (Basu et al. 2020b). Further research is needed to screen and identify beneficial microbial isolates that form plant-associated microbial communities and enhance overall plant health and vigour. The use of a multidisciplinary approach that includes physiology, molecular biology and biotechnology could provide new prospects and formulations with massive potential to manage abiotic stress in crop plants (Singh and Jha 2017).

Abiotic stress tolerance in crop plants may be achieved through chemical fertilizer applications, breeding programs, tissue culture methods and genetic engineering, which are expensive and time-consuming and have adverse effects on the environment. The use of plant growth-promoting endophytic bacteria (PGPEB) is an alternative and eco-friendly approach for improving agricultural crop production by ameliorating the negative effect of abiotic stresses on economically important plant species throughout the world (Khan et al. 2020). The agricultural, coastal and geothermal plant endophytes can colonize both eudicot and monocot plants conferring abiotic stress tolerance. Abiotic stress tolerance is often correlated with the increased ROS accumulation. The ability of bacterial and fungal endophytes to confer abiotic stress tolerance in plants may provide a novel strategy for mitigating the impacts of global climate change on agricultural crop plant species (Rodriguez and Redman 2008; Godoy et al. 2021) (Fig. 9.1; Table 9.1).

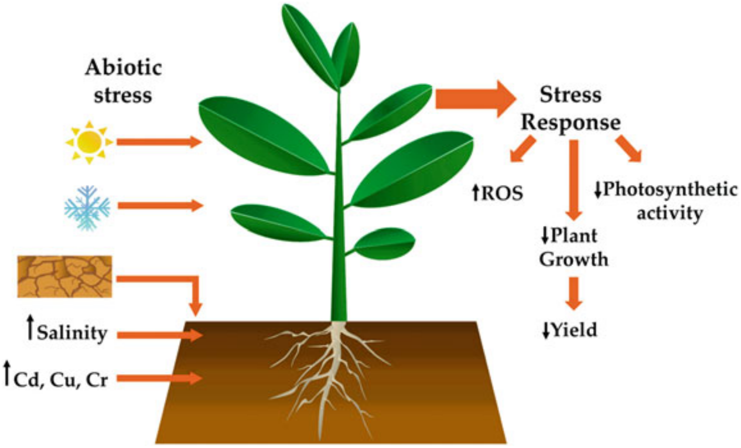

**Fig. 9.1** Abiotic stress impact on crop yield (Godoy et al. 2021)

### 9.2.1 *Salinity Stress*

Salinity is a major abiotic stress limiting productivity of important agricultural crop species (Basu et al. 2017; Mishra et al. 2021). Salinity-affected area exceeds 20% of the global agricultural land (Fouda et al. 2019). Early exposure to salinity leads to ion toxicity within the plant cells followed by disruption of osmotic balance when the stress persists for longer duration (Kumar et al. 2022a). Combined effect of the ionic and osmotic stresses alters the plant growth and development (Basu et al. 2021b). Salinity also interrupts the ion homeostasis in plant cells and impedes the internal solute balance (Kumar et al. 2009b, 2012). Continuous climate change is rapidly increasing the risk of soil salinization which has been predicted to affect 50% of the arable cropland by the year 2050 (F.A.O. (Food and Agriculture Organization of the United Nations) 2017). It is, therefore, a serious concern to take necessary steps for alleviating the deleterious effect of salinity in crops to encounter the food security.

Plant growth-promoting bacteria (PGPB) play a major role in the alleviation of salinity stress in plants (Basu and Kumar 2020a). The endophytic PGPB induce the growth of the host plants under salinity stress by facilitating the nutrient uptake. They also enhance the antioxidant activities in the host plants under salt stress, thereby maintaining the redox homeostasis. Egamberdieva et al. (2016) have revealed the dual interaction of rhizobia (*Bradyrhizobium japonicum*) and endophytic PGPB (*Stenotrophomonas rhizophila*) to alleviate the salinity stress in soybean (*Glycine max*) plants by inducing their growth and productivity. The PGPB induce salinity tolerance in the host plants by facilitating the nitrogen fixation, phytohormone production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, extracellular polymeric substance (EPS) production and biofilm formation (Basu and Kumar 2020b; Kumar et al. 2020a). The siderophore-producing

**Table 9.1** Plant growth-promoting microorganism-mediated amelioration of different abiotic stresses

| Abiotic stress | Microorganisms | | Plants | Reference |
|---|---|---|---|---|
| Salinity | *Fusarium culmorum* (FcRed1) | | *Oryza sativa*, *Lycopersicon esculentum* | Rodrigue et al. (2008) |
| | *Pseudomonas putida CW4* | | *L. esculentum* | Yan et al. (2014) |
| Drought | *Curvularia protuberata* (Cp4666D)<br>*Fusarium culmorum* (Fc18, FcRed1) | | *O. sativa*, *L. esculentum*, *Triticum aestivum*, *Citrullus lanatus* | Rodrigue et al. (2008) |
| | *Colletotrichum magna* (path-1) | | *L. esculentum*, *Capsicum annuum* | Redman (2001) |
| | *Colletotrichum magna* (L2.5) | | *L. esculentum*, *Capsicum annuum* | |
| | *Colletotrichum musae* (927) | | *L. esculentum*, *C. annuum* | |
| | *Colletotrichum orbiculare* (683) | | *L. esculentum* | |
| | *Colletotrichum gloeosporioides* (95-41A) | | *L. esculentum* | |
| Submergence | *Pseudomonas putida* UW4 | | *Brassica napus* | Farwell et al. (2007) |
| | *Achromobacter xylosoxidans*<br>*Serratia ureilytica*<br>*Herbaspirillum seropedicae*<br>*Ochrobactrum rhizosphaerae* | | *Ocimum sanctum* | Barnawal et al. (2012) |
| Heat | *Curvularia protuberata* (Cp4666D, CpMH206) | | *L. esculentum* | Rodrigue et al. (2008) |
| Chilling | *Trichoderma harzianum* (OMG16)<br>*Bacillus atrophaeus* (ABI02) | | *Zea mays*<br>*O. sativa*<br>*T. aestivum*<br>*Cicer arietinum*<br>*Solanum melongena*<br>*C. annuum* | Abdel Latef et al. (2020a) |
| Heavy metals | As | *Micrococcus luteus* | *Vitis vinifera* | Ivan et al. (2017) |
| | Cu | *Bacillus circulans*, *Paenibacillus polymyxa* | *Z. mays* | Abdel Latef et al. (2020b) |
| | Cr | *Staphylococcus arlettae* (MT4) | *Helianthus annuus* | Qadir et al. (2020) |
| | Cd | *Bacillus siamensis* | *T. aestivum* | Awan et al. (2020) |

rhizobacteria have also been reported to ameliorate salinity stress and increasing the iron (Fe) availability in saline soils (Ferreira et al. 2019). Application of plant growth-promoting rhizobacteria (PGPR) has been found to maintain the growth and productivity of French bean (*Phaseolus vulgaris*) (Kumar et al. 2020b). Bokhari et al. (2019) have reported different halophilic phosphate-solubilizing *Bacillus* strains to ameliorate salinity stress in maize (*Zea mays*). The study also determined the involvement of different osmolytes in salinity tolerance of maize plants. Among different *Bacillus* sp. strains, *B. cereus*, *B. subtilis* and *B. circulans* have been found to significantly enhance the growth and fresh weight of the inoculated plants under saline conditions. Two salinity-tolerant strains of *B. circulans* have been found to promote plant growth only in the presence of salt. Co-inoculation of maize plants with *Rhizobium* sp. and *Pseudomonas* sp. has been revealed to show enhanced salinity tolerance with decreased electrolyte leakage and maintenance of leaf water contents (Zelicourta et al. 2013). High abundances of halophilic PGPB *Pseudomonas stutzeri* and *Virgibacillus koreensis* in the rhizosphere of *Calotropis procera* have been shown to induce the plant growth under saline condition (Al-Quwaie 2020). A recent study showed co-inoculation of plant growth-promoting microorganisms *B. cereus*, *B. megaterium*, *Trichoderma longibrachiatum* and *T. simmonsii* to boost simultaneous salinity and drought tolerance in soybean plants by improving seed germination, seedling growth and $K^+$ uptake (Bakhshandeh et al. 2020). Another study Abdel Latef et al. (2020a, b) showed inoculation of maize plants with PGPB (*Azospirillum lipoferum* or *Azotobacter chroococcum*) to confer salinity tolerance by reinforcing plant growth and improving physiological activities. Co-inoculation of alfalfa (*Medicago sativa*) plants with salt-tolerant PGPB (*Hartmannibacter diazotrophicus* and *Pseudomonas* sp.) has been shown to enhance salinity tolerance with sustainable plant growth, fresh weight, nodule number, chlorophyll content, relative water content, membrane stability, $K^+/Na^+$ ratio and photosynthetic performances (Ansari et al. 2019). Inoculation of wheat (*Triticum aestivum*) plants with PGPB (*Stenotrophomonas maltophilia*) has shown to ameliorate the salinity stress with increased $K^+$ uptake, proline level and antioxidant enzyme activities (Singh and Jha 2017). The PGPB *Burkholderia phytofirmans* has been reported to enhance salinity tolerance in *Arabidopsis thaliana* (Pinedo et al. 2015).

Arbuscular mycorrhizal fungi (AMF) also significantly contribute in the salinity tolerance in plants (Basu and Kumar 2021) (Fig. 9.2). The AMF improve growth, nutrient uptake and productivity of crop plants under salinity stress (Daei et al. 2009). The study showed AMF to enhance the nitrogen and phosphorus uptake in wheat plants under salinity stress. The AMF also increased the gaseous exchange through stomata and improved the respiration and transpiration eventually affecting the water use efficiency of the host plants. The AMF have also been revealed to increase the osmolyte (carbohydrates and electrolytes) concentrations in plant roots to maintain the osmotic equilibrium under salinity stress. The AMF enhance the magnesium (Mg) uptake in host plants, thereby regulating the negative effect of Na on the leaf chlorophyll content (Miransari et al. 2009). Thus, AMF improve the host plants' photosynthetic activities under salinity stress. The AMF also enhance

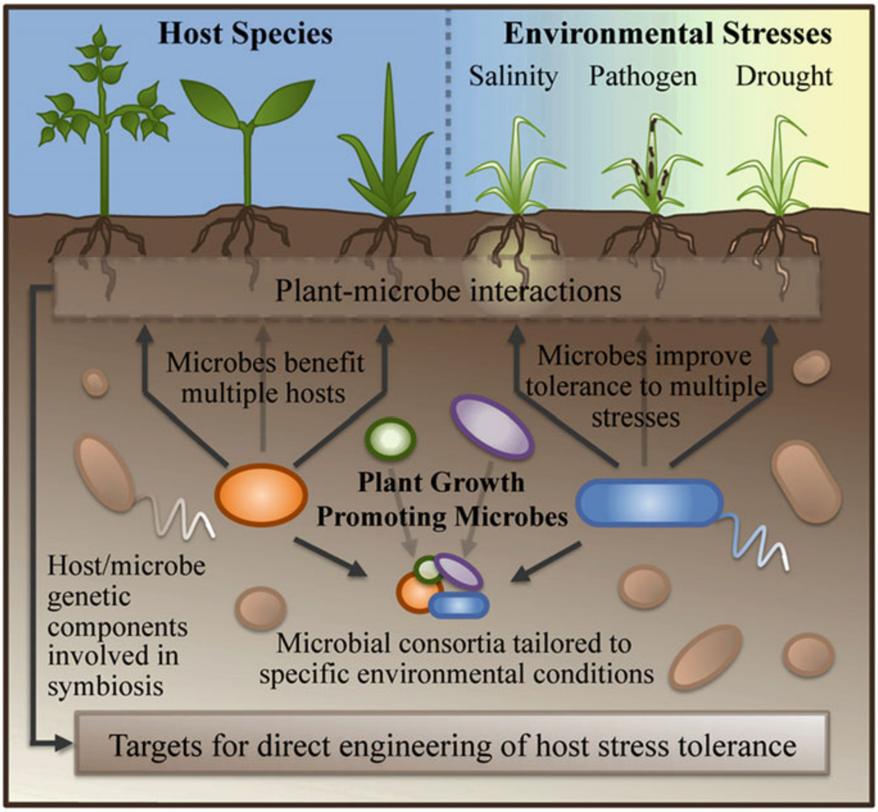

**Fig. 9.2** Advantages of plant growth-promoting microbe-mediated approaches to improving stress tolerance in crops (Coleman-Derr and Tringe 2014)

nutrient uptake in roots by increasing the hydraulic conductivity ultimately inducing the root development in host plants (Giri et al. 2003). The AMF enhance the potassium (K) uptake and sustain the $K^+/Na^+$ ratio leading to improved plant growth under saline conditions. The co-inoculation of PGPB (*Bacillus subtilis*) and AMF (*Claroideoglomus etunicatum*, *Funneliformis mosseae* and *Rhizophagus intraradices*) has been revealed to induce resistance in Talh tree (*Acacia gerrardii*) against the adverse impacts of salinity stress (Hashem et al. 2016). The plants co-inoculated with PGPR and AMF showed increased level of osmoprotectants (proline, phenol and glycine betaine contents) and improved antioxidant enzyme activities with reduced lipid peroxidation.

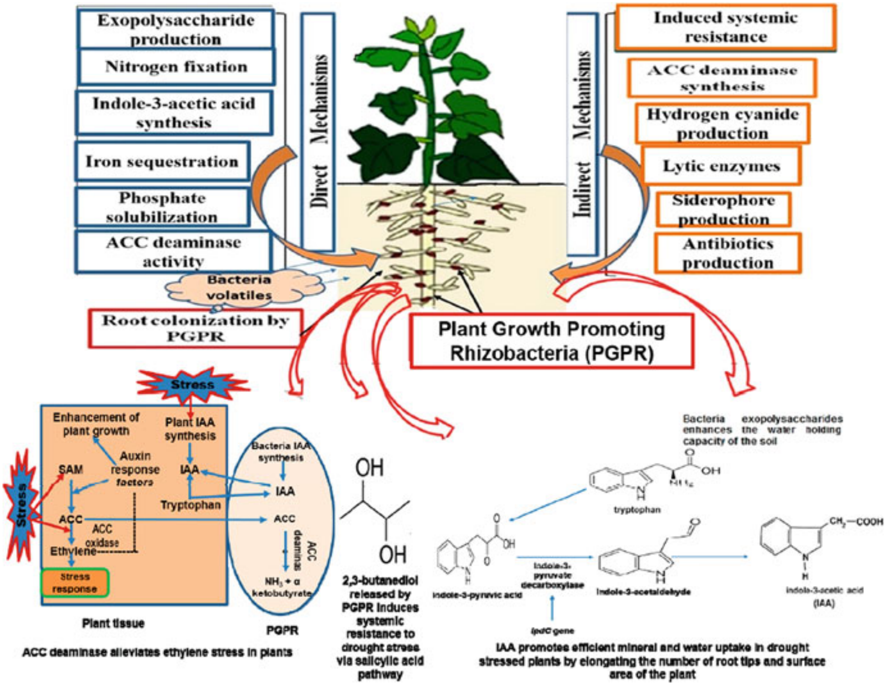

**Fig. 9.3** Mechanistic approach-mediated PGPR in alleviating drought stress and plant growth promotion (Ojuederie et al. 2019)

### 9.2.2 Drought Stress

Drought is also one of the major abiotic stresses limiting the agricultural crop production, thereby causing global food insecurity (Kumar et al. 2020c). Continuous climate change associated with increased air temperature, rainfall anomalies and shifts in the monsoon patterns has been expected to cause frequent drought events throughout the world posing serious threat to the agricultural productivity. Drought stress has been analysed to cause 21, 40 and 50% yield reductions in wheat, maize and rice, respectively (Daryanto et al. 2016). Incidence of drought stress during the pre-anthesis stage shortens the anthesis and the grain filling duration of the cereals, thereby reducing the yield. Drought stress influences the water relations leading to osmotic imbalance in plants (Basu et al. 2021c). It also impedes the plant growth, dry-matter accumulation, canopy temperature, water use efficiency and photosynthetic activities (Basu et al. 2017). Drought also severely impacts the nutrient relations of plants (Garg 2003).

The role of various microorganisms in amelioration of drought stress in plants has been extensively studied (Ojuederie et al. 2019; Fig. 9.3). The PGPR has been shown to enhance the drought stress tolerance in *Acacia abyssinica* (Getahun et al.

2020) and maize (Abdel Latef et al. 2020a, b). Various PGPB strains including *Acinetobacter calcoaceticus*, *Paenibacillus polymyxa*, *Pseudomonas putida* and *P. fluorescens* have been found to enhance drought tolerance in *A. abyssinica*. Drought-tolerant *Rhizobium* sp. has been found to alleviate drought stress in *Sesbania*. Colonization of the roots of wheat plants with *Paenibacillus* sp. and *Bacillus* sp. have been reported to enhance the drought tolerance. The EPS secretion from the microbes provides a suitable environment for chemical reactions, nutrient enrichments and protection against drought by improving the water-holding capacity and fertility of soil through aggregation as observed in *Azospirillum*. Application of *Bacillus subtilis* has been found to be potentially beneficial in enhancing drought tolerance in *Acacia gerrardii* (Hashem et al. 2016). Inoculation of *Sambucus williamsii* with PGPB *Acinetobacter calcoaceticus* has been reported to enhance drought tolerance (Liu et al. 2019). Similarly, another PGPB *Paenibacillus polymyxa* has been found to improve drought tolerance in *Arabidopsis thaliana*. The co-inoculation of two PGPB *Acinetobacter* sp. and *Pseudomonas* sp. has been revealed to enhance drought tolerance in grapevines by maintaining the shoot biomass (Getahun et al. 2020). In pennyroyal plants co-inoculation of *Azotobacter* and *Azospirillum* strains have been reported to impart drought tolerance by increasing the biosynthesis of secondary metabolites. The PGPB *Burkholderia phytofirmans* has been shown to mitigate drought in wheat and maize (Meena et al. 2017). Rhizosphere of cotton (*Gossypium hirsutum*) plants has been reported to predominantly contain *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, *Gemmatimonadetes* and *Proteobacteria* under non-stressed conditions (Ullah et al. 2019). However, Chloroflexi and Gemmatimonadetes have been mostly found in the drought-stressed rhizosphere, which could play an important role to improve drought tolerance in plants. These two phyla have been found to help cotton plants in sustaining various physiological functions under drought stress, thereby conferring drought tolerance. The cluster analyses showed *Acidothermus*, *Gemmatimonas*, *Jatrophihabitans*, *Sphingomonas*, *Sphingopyxis* and *Streptomyces* to be relatively abundant in the cotton rhizosphere under normal and drought conditions as compared to the control bulk soil. In addition to the antibiotic and antifungal role of *Streptomyces*, their roles in plant growth promotion under drought stress have also been well documented. Therefore, these bacteria may enhance growth of cotton plants under drought stress. Plant roots change the physical and chemical properties of the soil, and the secretion of root substances modulates microorganisms in the rhizosphere. On the other hand, some microorganisms failed to grow in drought-stressed soils. Some microorganisms adhere to the roots and may subsequently enhance growth and drought tolerance. The soil bacterial community might be important to plants in maintaining essential functions. The PGPB *Pseudomonas fluorescens* and *P. putida* have also been revealed to be exceptional in enhancing drought tolerance in black henbane (*Hyoscyamus niger*) plants through sustainable plant growth, chlorophyll, proline, leaf relative water content and enhanced antioxidant enzyme activities (Ghorbanpour et al. 2013). Higher membrane transport in PGPB has been predicted to enhance salinity and drought tolerance by regulating $Na^+/K^+$ ratio and $H^+$-ATPase of the plasma membrane. Higher metabolic pathways have also been found to contribute in improved drought tolerance in plants (Ullah et al. 2019).

The AMF symbioses also play an important role in enhancing drought tolerance in plants (Basu and Kumar 2020c). They bind to the soil particles with glomalin and alter the moisture retention capability of soil, thereby inducing the host plants' growth under drought (Auge 2001). Additionally, higher nutrient uptake by the AMF enhances the surface area and density of host plant roots consequently improving their drought tolerance (Subramanian et al. 2006). The AMF facilitates the plant water movement, inducing the hydration and physiological activities of the host plants under drought stress (Porcel and Ruiz-Lozano 2004). The mycorrhizal plants are also able to absorb several forms of nitrogen promoting plant growth under drought conditions. The AMF improve the host plants' biomass under drought stress through increased accumulation of organic products such as glycine betaine, proline, carbohydrates (mannitol, sucrose) and inorganic ions ($Cl^-$, $K^+$) (Ruiz-Lozano et al. 2006). Furthermore, the AMF alter the physiology and gene expression of the host plants, thereby enhancing drought tolerance. The mycorrhizal plants also exhibit higher antioxidant enzyme activities under drought stress that enhance drought tolerance through enhanced ROS scavenging and sustain plant growth.

### *9.2.3 Submergence*

Submergence is one of the principal abiotic stresses detrimentally affecting the growth and productivity of important crop species. It severely affects about 10% of the total land worldwide. The flood frequency has been predicted to affect about 42% of the global land by the end of the twenty-first century, thereby impeding the food security (IPCC 2014). Submergence has been characterized by low light intensity, impaired gaseous exchange (Basu et al. 2020a). The $O_2$ diffusion is reduced 10,000 times under submergence than the normal air leading to hypoxic or anoxic conditions that inhibit the aerobic respiration of plants. Submergence severely affects the physiological activities in plants, including chlorophyll content, membrane stability and photosynthetic gaseous exchange (Basu et al. 2021a). Incidence of complete submergence for more than a week during the early vegetative stage significantly reduces the dry-matter production resulting in dramatic yield loss.

Submergence-induced higher ethylene concentration inhibits plant growth and leads to chlorosis or cell death. Bacterial ACC deaminase has been found to play an essential role in reducing the excessive ethylene level by catabolizing its precursor (ACC) into ammonia and α-ketobutyrate, thereby alleviating the negative effects of submergence (Sasidharan et al. 2017). The ACC deaminase has been firstly isolated from the bacterium *Pseudomonas* sp. and yeast *Hansenula saturnus*. This enzyme was also found in *Pseudomonas chlororaphis* and different strains of *Pseudomonas putida*. The ACC deaminase has also been found to be produced by fungi and endophytic bacteria (Sarkar et al. 2017). The bacterium *P. putida* has been reported to produce ACC deaminase ameliorating the submergence and metal stress in

*Brassica napus* (Farwell et al. 2007). Co-inoculation of different ACC deaminase-producing PGPR strains including *Achromobacter xylosoxidans*, *Ochrobactrum rhizosphaerae*, *Serratia ureilytica* and *Herbaspirillum seropedicae* have been found to confer submergence tolerance in *Ocimum sanctum* (Barnawal et al. 2012).

### 9.2.4 Heat Stress

Global climatic changes along with constant elevation in atmospheric temperature severely influence plant growth and productivity of major agricultural crops. During 1979 to 2003, the annual mean maximum and minimum atmospheric temperature has been found to increase by 0.35 °C and 1.13 °C, respectively (Peng et al. 2004). Intergovernmental Panel on Climate Change (IPCC) has predicted the constant weather change to increase the average universal temperature with a frequency of 0.18 °C every decade (IPCC 2014). Short episodes of heat stress coinciding with sensitive developmental stages have been reported to cause a significant reduction in grain yield. Increase in the temperature from 3 to 4 °C can cause a decrease in agricultural crop productivity by approximately 15–35% in Asia and Africa and 25–35% in the Middle East. Heat stress reduces the life cycle of plants through premature ripening which causes declined crop biomass due to lesser accumulation of assimilates (Dwivedi et al. 2017). Heat stress also affects the grain filling duration and the grain filling rate of plants ultimately decreasing the grain yield (Dwivedi et al. 2019).

The role of different PGPB in alleviating heat stress has been widely examined in various crop plants. A study reported the strain of PGPB *Pseudomonas* to enhance the high-temperature (47–50 °C) tolerance of sorghum seedlings. Rhizobacterial isolates have also been found to confer high-temperature (45 °C) tolerance in plants (Getahun et al. 2020). Thermotolerant *Bacillus cereus* has been found to produce biologically active metabolites, such as indole-3-acetic acid, gibberellin and organic acids. Inoculation of *B. cereus* has been reported to confer heat stress tolerance in soybean plants with improved plant growth, biomass, chlorophyll content and reduced abscisic acid (ABA) and salicylic acid (SA) content. The inoculated plants have been found to exhibit increased antioxidant enzyme activities (ascorbic acid peroxidase, superoxide dismutase), glutathione and amino acid contents under heat stress. The PGPB inoculation also augmented the heat stress response and increased heat shock protein (GmHSP) expression in plants. Plant growth-promoting endophytic bacteria (PGPEB) have also been reported to enhance heat stress tolerance in chickpea, wheat, tomato and potato. The PGPEB can synthesize phytohormones that help in increasing heat stress tolerance by enhancing biofilm formation, reducing ABA levels and increasing HSP levels (Khan et al. 2020). Another study showed plant growth-promoting thermotolerant *Pseudomonas putida* to enhance heat tolerance in wheat by improving plant growth, dry biomass, tiller, spikelet and grain formation (Ali et al. 2011). Inoculation has also been observed to improve the cellular metabolite (proline, chlorophyll, sugars, starch, amino acids and proteins) levels, enhance the antioxidant enzyme activities (SOD, APX and CAT) and reduce the membrane injury under heat stress. The PGPB colonization on the plant root surface has been seen to mitigate the negative effects of climate change on plant growth. A recent study showed the PGPB *Bacillus cereus* to mitigate heat stress in tomato (Mukhtar et al. 2020). Bacterial inoculation has been revealed to significantly promote plant growth and biomass under heat stress. The EPS production and ACC-deaminase activity have been observed to be significantly increased in the inoculated plants. The AMF have been found to ameliorate heat stress in thermophilic plants (Bunn et al. 2009). They extend the extra radical hyphae into the soil and increase the host plants' access to water and nutrients, thereby promoting root growth under heat stress.

### 9.2.5 Low-Temperature Stress

Low-temperature or chilling stress is one of the major abiotic stresses severely affecting plant growth and hindering productivity of important agricultural crop plants (Liu et al. 2018). Most of the tropical and subtropical crop plants, including rice and maize, are extremely sensitive to chilling stress. Seed germination, physiological and biochemical performances are disrupted under low-temperature stress. Therefore, amelioration of chilling stress in crop plants has become a major challenge to encounter the food security. The PGPM play an important role in this background.

Maize seedlings inoculated with PGPR have been shown to confer chilling tolerance (Abdel Latef et al. 2020a, b). Plants under extreme cold conditions survive either through avoiding supercooling of tissue water or through freezing tolerance (Meena et al. 2017). Inoculation of *Trichoderma* or *Bacillus* is a suitable strategy to improve the chilling tolerance in plants. Although co-cultivation of *Trichoderma* and *Bacillus* strains on artificial growth media was frequently characterized by antagonisms in many plant species including *Oryza sativa*, *Triticum aestivum*, *Cicer arietinum*, *Solanum melongena* and *Capsicum annuum*, synergistic beneficial effects were reported after co-inoculation. This included stimulation of seed germination and plant growth promotion in cold and wet soils. In a recent study, Moradtalab et al. (2020) conducted a pre-selection trial with a range of fungal and bacterial PGPM strains based on *Penicillium* sp. with cold-protective properties, a cold-tolerant strain of *Bacillus atrophaeus* and a microbial consortium product (MCP), based on a combined formulation of *Trichoderma harzianum* and *Bacillus* spp. with Zn/Mn supplementation. Inoculation with *T. harzianum* has been observed to promote maize root colonization. The inoculated plants showed increased ABA/cytokinin ratio and increased concentrations of jasmonic (JA) and SA with increased enzymatic and non-enzymatic antioxidant-mediated ROS detoxification. Additional supplementation with Zn and Mn further increased plant growth, shoot IAA and total antioxidants leading to decreased oxidative damage in plants under cold stress. Another study revealed the inoculation of cold-tolerant endophytic bacteria

*Pseudomonas vancouverensis* and *P. frederiksbergensis* to confer low-temperature (10–12 °C) tolerance in tomato plants (Subramanian et al. 2015). The inoculated plants showed induced expressions of CRT repeat binding factors (LeCBF1 and LeCBF3) under chilling stress. Similarly, PGPEB *Burkholderia phytofirmans* has been reported to provide increased chilling tolerance in *Arabidopsis* (Su et al. 2015).

### 9.2.6 Heavy Metal Toxicity

Heavy metals (HMs) are the food chain contaminants affecting the growth and productivity of crop plants (Kamal et al. 2010; Thakare et al. 2021; Sonowal et al. 2022). Continuous climate change has been predicted to exaggerate the HMs (arsenic, cadmium, lead, chromium, mercury) contamination in the soil causing substantial yield loss of major crop plants (Kumar et al. 2022b). The excessive intake of HMs by the crop plants also impairs the growth, photosynthetic activities, mineral nutrition and metabolic reactions in plants (Kumar et al. 2021). It is, therefore, a serious worldwide concern to take necessary steps to counteract the problem of HMs toxicity in crop plants (Sarkar et al. 2022).

Numerous studies have shown the PGPR to confer HM tolerance in different crop species. A recent study showed Cd toxicity to decrease the abundance of *Actinobacteria* in the rhizosphere of *Brassica napus*, whereas increased in the rhizosphere of *B. juncea*. In the phyllosphere of *B. napus*, abundance of *Rhodanobacter* sp., *Rickettsia* sp. and *Massililia* sp. has been found to be increased, whereas *Acinetobacter* sp., *Achromobacter* sp. and *Buchera* sp. have been found to decrease under Cd toxicity. The *B. juncea* phyllosphere showed increase in *Gibbsiella* sp., *Lysobacter* sp. and *Stenotrophomonas* sp., while *Gaiell* sp., *Herbaspirillum* sp. and *Telluria* sp. were found to decrease under Cd toxicity (Du et al. 2021). The PGPB has shown *Bacillus anthracis* to confer Cd tolerance in *Sesbania sesban* through higher seed germination (Ali et al. 2021). Another study showed the PGPR strains *Bacillus circulans* and *Paenibacillus polymyxa* to enhance copper tolerance in maize plants (Abdel Latef et al. 2020b). The PGPR strain *Staphylococcus arlettae* has been shown to alleviate chromium toxicity in sunflower plants by restricting its uptake and strengthening the plant antioxidant defence system (Qadir et al. 2020). *Bacillus siamensis* has been shown to improve Cd tolerance in wheat plants by restricting the Cd accumulation and enhancing the antioxidant defence system (Awan et al. 2020). Inoculation of PGPB *Micrococcus luteus* has been shown to impart arsenic (arsenite) tolerance in grapevine with increased biomass and antioxidant potential (Ivan et al. 2017).

## 9.3 Conclusion

Soil microorganisms are directly and indirectly beneficiary for the agricultural crop plants. Application of the PGPM is widely studied in amelioration of various abiotic stresses like salinity, drought, heat, cold and heavy metals, which severely affect plant growth, physiological activities and agricultural productivity. However, most of the studies are restricted to the laboratory conditions. Therefore, the studies should be executed under the field conditions for sustainable agricultural productivity to keep pace with both the increasing population and continuously changing climate.

## References

Abdel Latef AAH, Alhmad MFA, Kordrostami M, Abo-Baker ABAE, Zakir A (2020a) Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions. J Plant Growth Regul 39:1293–1306

Abdel Latef AAH, Zaid A, Abo-Baker A-BA-E, Salem W, Alhmad MFA (2020b) Mitigation of copper stress in maize by inoculation with *Paenibacillus polymyxa* and *Bacillus circulans*. Plants 9(11):1513

Ali SZ, Sandhya V, Grovera M, Linga VR, Bandi V (2011) Effect of inoculation with a thermotolerant plant growth promoting *Pseudomonas putida* strain AKMP7 on growth of wheat (*Triticum* spp.) under heat stress. J Plant Interact 6(4):239–246

Ali J, Ali F, Ahmad I, Rafique M, Munis MFH, Hassan WS, Sultan T, Iftikhar M, Chaudhary JH (2021) Mechanistic elucidation of germination potential and growth of Sesbania sesban seedlings with Bacillus anthracis PM21 under heavy metals stress: an in vitro study. Ecotoxicol Environ Saf 208(111769):1–11

Al-Quwaie DA (2020) Bacterial community dynamics with rhizosphere of *Calotropis procera* and *Senna alexandrina* desert plants in Saudi Arabia. Bioinformation 16(8):567–578

Ansari M, Shekari F, Mohammadi MH, Juhos K, Vegvari G, Biro B (2019) Salt-tolerant plant growth-promoting bacteria enhanced salinity tolerance of salt-tolerant alfalfa (*Medicago sativa* L.) cultivars at high salinity. Acta Physiol Plant 41:195

Auge RM (2001) Water relations, drought and vesicular arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Awan SA, Ilyas N, Khan I, Raza MA, Rehman A, Rizwan M, Rastogi A, Tariq R, Brestic M (2020) *Bacillus siamensis* reduces cadmium accumulation and improves growth and antioxidant defense system in two wheat (*Triticum aestivum* L.) varieties. Plants 9:878

Bakhshandeh E, Gholamhosseini M, Yaghoubian Y, Pirdashti H (2020) Plant growth promoting microorganisms can improve germination, seedling growth and potassium uptake of soybean under drought and salt stress. Plant Growth Regul 90:123–136

Barnawal D, Bharti N, Maji D, Chanotiya CS, Kalra A (2012) 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene generation. Plant Physiol Biochem 58:227–235

Basu S, Kumar G (2020a) Plant microbe interaction for changing endophytic colonization to improve plant productivity. In: Verma JP, Macdonald CA, Gupta VK, Podile AP (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 137–148

Basu S, Kumar G (2020b) Nitrogen fixation in a legume-rhizobium symbiosis: the roots of a success story. In: Varma A, Tripathi S, Prasad R (eds) Plant microbe symbiosis. Springer, Singapore, pp 35–53

Basu S, Kumar G (2020c) Stress signalling in the phytomicrobiome: breadth and potential. In: Kumar M, Kumar V, Prasad R (eds) Phyto-microbiome in stress regulation. Springer, Singapore, pp 245–268

Basu S, Kumar G (2021) Exploring the significant contribution of silicon in regulation of cellular redox homeostasis for conferring stress tolerance in plants. Plant Physiol Biochem 166:393–404

Basu S, Giri RK, Benazir I, Kumar S, Rajwanshi R, Dwivedi SK, Kumar G (2017) Comprehensive physiological analyses and reactive oxygen species profiling in drought tolerant rice genotypes under salinity stress. Physiol Mol Biol Plants 23:837–850

Basu S, Kumar G, Kumari N, Kumari S, Shekhar S, Kumar S, Rajwanshi R (2020a) Reactive oxygen species and reactive nitrogen species induce lysigenous aerenchyma formation through programmed cell death in rice roots under submergence. Environ Exp Bot 177:104118

Basu S, Kumar G, Chhabra S, Prasad R (2020b) Role of soil microbes in biogeochemical cycle for enhancing soil fertility. In: Verma JP, Macdonald CA, Gupta VK, Podile AP (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 149–157

Basu S, Kumari S, Kumar P, Kumar G, Rajwanshi R (2021a) Redox imbalance impedes photosynthetic activity in rice by disrupting cellular membrane integrity and induces programmed cell death under submergence. Physiol Plant 172(3):1764–1778

Basu S, Kumar A, Benazir I, Kumar G (2021b) Reassessing the role of ion homeostasis for improving salinity tolerance in crop plants. Physiol Plant 171(4):502–519

Basu S, Kumari S, Kumar A, Shahid R, Kumar S, Kumar G (2021c) Nitro-oxidative stress induces the formation of roots' cortical aerenchyma in rice under osmotic stress. Physiol Plant 172(2): 963–975

Basu S, Prabhakar AA, Kumari S, Kumar RR, Shekhar S, Prakash K, Singh JP, Singh GP, Prasad R, Kumar G (2022) Micronutrient and redox homeostasis contribute to *Moringa oleifera*-regulated drought tolerance in wheat. Plant Growth Regul. https://doi.org/10.1007/s10725-022-00795-z

Bokhari A, Essack M, Lafi FF, Andres-Barrao C, Jalal R, Alamoudi S, Razali R, Alzubaidy H, Shah KH, Siddique S, Bajic VB, Hirt H, Saad MM (2019) Bioprospecting desert plant *Bacillus* endophytic strains for their potential to enhance plant stress tolerance. Sci Rep 9(1):18154

Bunn R, Lekberg Y, Zabinski C (2009) Arbuscular mycorrhizal fungi ameliorate temperature stress in thermophilic plants. Ecology 90(5):1378–1388

Coleman-Derr D, Tringe SG (2014) Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. Front Microbiol 5:283. https://doi.org/10.3389/fmicb.2014.00283

Daei G, Ardekani M, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:217–225

Daryanto S, Wang L, Jacinthe PA (2016) Global synthesis of drought effects on maize and wheat production. PLoS One 11:e0156362

Du Y, Zhang D, Zhou D, Liu L, Wu J, Chen H, Jin D, Yan M (2021) The growth of plants and indigenous bacterial community were significantly affected by cadmium contamination in soil–plant system. AMB Express 11(103):1–13

Dwivedi SK, Basu S, Kumar S, Kumar G, Prakash V, Kumar S, Mishra JS, Bhatt BP, Malviya N, Singh GP, Arora A (2017) Heat stress induced impairment of starch mobilisation regulates pollen viability and grain yield in wheat: study in eastern Indo-Gangetic plains. Field Crops Res. 206:106–114

Dwivedi SK, Basu S, Kumar S, Kumari S, Kumar A, Jha S, Mishra JS, Bhatt BP, Kumar G (2019) Enhanced antioxidant enzyme activities in developing anther contributes to heat stress alleviation and sustains grain yield in wheat. Funct Plant Biol 46(12):1090–1102

Egamberdieva D, Jabborova D, Berg G (2016) Synergistic interactions between *Bradyrhizobium japonicum* and the endophyte *Stenotrophomonas rhizophila* and their effects on growth and nodulation of soybean under salt stress. Plant Soil 405:35–45

F.A.O. (Food and Agriculture Organization of the United Nations) (2017) The future of food and agriculture—trends and challenges. FAO, Rome

Farwell AJ, Vesely S, Nero V, Rodriguez H, McCormack K, Shah S et al (2007) Tolerance of transgenic canola plants (*Brassica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated field site. Environ Pollut 147:540–545

Ferreira MJ, Silva H, Cunha A (2019) Siderophore-producing rhizobacteria as a promising tool for empowering plants to cope with iron limitation in saline soils: a review. Pedosphere 29:409–420

Fouda A, Hassan SED, Eid AM, El-Din Ewais E (2019) The interaction between plants and bacterial endophytes under salinity stress. In: Jha S (ed) Endophytes and secondary metabolites. Springer Nature, Switzerland, pp 591–607

Garg BK (2003) Nutrient uptake and management under drought: nutrient-moisture interaction. Curr Agric 27:1–8

Getahun A, Muleta D, Assefa F, Kiros S (2020) Plant growth-promoting Rhizobacteria isolated from degraded habitat enhance drought tolerance of Acacia (*Acacia abyssinica* Hochst. ex Benth.) seedlings. Int J Microbiol 2020:8897998

Giri B, Kapoor R, Mukerji KG (2003) Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol Fertil Soils 38:170–175

Ghorbanpour M, Hatami M, Khavazi K (2013) Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of Hyoscyamus niger under water deficit stress. Turk J Biol 37:350–360

Godoy F, Olivos-Hernández K, Stange C, Handford M (2021) Abiotic stress in crop species: improving tolerance by applying plant metabolites. Plants 10:186. https://doi.org/10.3390/plants10020186

Hashem A, Abd Allah EF, Alqarawi AA, Al-Huqail AA, Shah MA (2016) Induction of osmoregulation and modulation of salt stress in *Acacia gerrardii* Benth. by arbuscular mycorrhizal fungi and *Bacillus subtilis* (BERA 71). Biomed Res Int 2016:6294098

IPCC (2014) Climate change 2014: synthesis report. In: Core Writing Team, Pachauri RK, Meyer LA (eds) Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. IPCC, Geneva, p 151

Ivan FP, Salomon MV, Berli F, Bottini R, Piccoli P (2017) Characterization of the As(III) tolerance conferred by plant growth promoting rhizobacteria to in vitro-grown grapevine. Appl Soil Ecol 109:60–68

Kamal S, Prasad R, Varma A (2010) Soil microbial diversity in relation to heavy metals. In: Sherameti I, Varma A (eds) Soil heavy metals, vol 19. Springer-Verlag, Berlin Heidelberg, pp 31–64

Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J (2019) Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene. https://doi.org/10.1016/j.plgene.2019.100182

Khan MA, Asaf S, Khan AL, Jan R, Kang SM, Kim KM, Lee IJ (2020) Thermotolerance effect of plant growth promoting *Bacillus cereus* SA1 on soybean during heat stress. BMC Microbiol 20(1):175

Kumar G, Purty RS, Singla-Pareek SL, Pareek A (2009a) Maintenance of stress related transcripts in tolerant cultivar at a level higher than sensitive one appears to be a conserved salinity response among plants. Plant Signal Behav 4:431–434

Kumar G, Purty RS, Sharma MP, Singla-Pareek SL, Pareek A (2009b) Physiological responses among *Brassica* species under salinity stress show strong correlation with transcript abundance for SOS pathway-related genes. J Plant Physiol 166:507–520

Kumar G, Kushwaha HR, Purty RS, Kumari S, Singla-Pareek SL, Pareek A (2012) Cloning, structural and expression analysis of OsSOS2 in contrasting cultivars of rice under salinity stress. Genes Genom Genomics 6:34–41

Kumar A, Singh S, Gaurav AK, Srivastava S, Verma JP (2020a) Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front Microbiol 11:1216

Kumar V, Kumar P, Khan A (2020b) Optimization of PGPR and silicon fertilization using response surface methodology for enhanced growth, yield and biochemical parameters of French bean (*Phaseolus vulgaris* L.) under saline stress. Biocatal Agric Biotechnol 23:101463

Kumar S, Dwivedi SK, Basu S, Kumar G, Mishra JS, Koley TK, Rao KK, Choudhary AK, Mondal S, Kumar S, Bhakta N, Bhatt BP, Paul RK, Kumar A (2020c) Anatomical, agro-morphological and physiological changes in rice under cumulative and stage specific drought conditions prevailed in eastern region of India. Field Crops Res 245:107658

Kumar A, Basu S, Kumar G (2021) Evaluating the effect of seed-priming for improving arsenic tolerance in rice. J Plant Biochem Biotechnol 31(1):197–201

Kumar G, Basu S, Singla-Pareek SL, Pareek A (2022a) Unraveling the contribution of *OsSOS2* in conferring salinity and drought tolerance in a high-yielding rice. Physiol Plant 174(1):e13638

Kumar A, Basu S, Rishu AK, Kumar G (2022b) Revisiting the mechanisms of arsenic uptake, transport and detoxification in plants. Environ Exp Bot 194:104730

Kundu P, Gill R, Nehra A, Sharma KK, Hasanuzzaman M, Prasad R, Tuteja N, Singh Gil SS (2020) Reactive oxygen species (ROS) management in engineered plants for abiotic stress tolerance. In: Tuteja N, Tuteja R, Passricha N, Saifi SK (eds) Advancement in crop improvement techniques. Elsevier, pp 241–262

Liu X, Zhou Y, Xiao J, Bao F (2018) Effects of chilling on the structure, function and development of chloroplasts. Front Plant Sci 9:1715

Liu F, Ma H, Peng L, Du Z, Ma B, Liu X (2019) Effect of the inoculation of plant growth-promoting rhizobacteria on the photosynthetic characteristics of *Sambucus williamsii* Hance container seedlings under drought stress. AMB Express 9(1):1–9

Mantri N, Patade V, Penna S, Ford R, Pang E (2012) Abiotic stress responses in plants: present and future. In: Abiotic stress responses in plants. Springer, New York, pp 1–19

Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, Singh DP, Prabha R, Sahu PK, Gupta VK, Singh HB, Krishanani KK, Minhas PS (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172

Miransari M, Rejali F, Bahrami HA, Malakouti MJ (2009) Effects of arbuscular mycorrhiza, soil sterilization, and soil compaction on wheat (*Triticum aestivum* L.) nutrient uptake. Soil Tillage Res 104:48–55

Mishra M, Wungrampha S, Kumar G, Singla-Pareek SL, Pareek A (2021) How do rice seedlings of landrace Pokkali survive in saline fields after transplantation? Physiology, biochemistry, and photosynthesis. Photosynth Res 150:117–135

Moradtalab N, Ahmed A, Geistlinger J, Walker F, Hoglinger B, Ludewig U, Neumann G (2020) Synergisms of microbial consortia, N forms, and micronutrients alleviate oxidative damage and stimulate hormonal cold stress adaptations in maize. Front Plant Sci 11:396

Mukhtar T, ur Rehman S, Smith D, Sultan T, Seleiman MF, Alsadon AA, Amna, Ali S, Chaudhary HJ, Solieman THI, Ibrahim AA, Saad MAO (2020) Mitigation of heat stress in *Solanum lycopersicum* L. by ACC-deaminase and exopolysaccharide producing *Bacillus cereus*: effects on biochemical profiling. Sustainability 12:2159

Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. Front Plant Sci 7:1574. https://doi.org/10.3389/fpls.2016.01574

Nath M, Bhatt D, Prasad R, Tuteja N (2017) Reactive oxygen species (ROS) metabolism and signaling in plant-mycorrhizal association under biotic and abiotic stress conditions. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, pp 223–232

Ojuederie OB, Olanrewaju OS, Babalola OO (2019) Plant growth promoting rhizobacterial mitigation of drought stress in crop plants: implications for sustainable agriculture. Agronomy 9(11):712. https://doi.org/10.3390/agronomy9110712

Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. Proc Natl Acad Sci U S A 101:9971–9975

Pinedo I, Ledger T, Greve M, Poupin MJ (2015) *Burkholderia phytofirmans* PsJN induces long-term metabolic and transcriptional changes involved in *Arabidopsis thaliana* salt tolerance. Front Plant Sci 6:466

Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55: 1743–1750

Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant growth-promoting Rhizobacteria (PGPR) and medicinal plants. Springer International Publishing, Cham, pp 247–260

Qadir M, Hussain A, Hamayun M, Shah M, Iqbal A, Murad W (2020) Phytohormones producing rhizobacterium alleviates chromium toxicity in *Helianthus annuus* L. by reducing chromate uptake and strengthening antioxidant system. Chemosphere 258:127386

Redman RS, Dunigan DD, Rodriguez RJ (2001) Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytol 151(3):705–716

Rodrigue RJ, Henson J, Volkenburgh EV, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2(4):404–416

Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can’t make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59(5):1109–1114

Ruiz-Lozano JM, Porcel R, Aroca R (2006) Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes? New Phytol 171: 693–698

Sarkar A, Ghosh PK, Pramanik K, Mitra S, Soren T, Pandey S, Mondal MH, Maitai TK (2017) A halotolerant Enterobacter sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Res Microbiol 4:68–79

Sarkar S, Basu S, Prasad R, Kumar G (2022) Phytoremediation: Mechanistic approach for eliminating heavy metal toxicity from environment. In: Prasad R (ed) Phytoremediation for environmental sustainability. Springer, Singapore, pp 513–543

Sasidharan R, Hartman S, Liu Z, Martopawiro S, Sajeev N, van Veen H, Yeung E, Voesenek LACJ (2017) Signal dynamics and interactions during flooding stress. Plant Physiol 176:1106–1117

Singh RP, Jha PN (2017) The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants. Front Microbiol 8:1945

Sonowal S, Nava AR, Joshi SJ, Borah SN, Islam NF, Pandit S, Prasad R, Sarma H (2022) Biosurfactants assisted heavy metals phytoremediation: green technology for the united nations sustainable development goals. Pedosphere 2(1):198–210. https://doi.org/10.1016/S1002-0160(21)60067-X

Su F, Jacquard C, Villaume S, Michel J, Rabenoelina F, Clement C et al (2015) Burkholderia phytofirmans PsJN reduces impact of freezing temperatures on photosynthesis in Arabidopsis thaliana. Front Plant Sci 6:810

Subramanian K, Santhanakrishnan P, Balasubramanian P (2006) Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci Hortic 107:245–253

Subramanian P, Mageswari A, Kim K, Lee Y, Sa T (2015) Psychrotolerant endophytic Pseudomonas sp. strains OB155 and OS261 induced chilling resistance in tomato plants (*Solanum lycopersicum* Mill.) by activation of their antioxidant capacity. Mol Plant-Microbe Interact 28:1073–1081

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Curr Res Biotechnol. https://doi.org/10.1016/j.crbiot.2021.02.004

Ullah A, Akbar A, Luo Q, Khan AH, Manghwar H, Shaban M, Yang X (2019) Microbiome diversity in cotton rhizosphere under normal and drought conditions. Microb Ecol 77(2): 429–439

Yan J, Smith MD, Glick BR, Liang Y (2014) Effects of ACC deaminase containing rhizobacteria on plant growth and expression of Toc GTPases in tomato (*Solanum lycopersicum*) under salt stress. Botany 92:775–781

Zelicourta AD, Al-Yousifb M, Hirt H (2013) Rhizosphere microbes as essential partners for plant stress tolerance. Mol Plant 6(2):242–245

# Chapter 10
# Role of Engineered Microbes in Sustainable Agriculture

**Blaise Manga Enuh and Pınar Aytar Çelik**

**Abstract** For a long time, agricultural output had been solely dependent on available environmental resources, and increasing pressure on these natural resources to meet the needs of an increasing population continues to disrupt the natural systems of the planet which has led to various consequences. In the past, scientific advancements from the use of manure to breeding experiments by Mendel were used to develop methods for the improvement of agricultural production and thus saving people from mass starvation. Scientific "fixes" have nevertheless brought forth other unforeseen issues because of the introduction of new variables. Increasing concerns over the effects of these fixes on the environment, other creatures, and ultimately humans have led to the inclusion of safety considerations and the need to consider as much as possible minimal safety limits and tests on products impacted by scientific technology. As such, recently, holistic concepts such as the circular economy and sustainable agriculture are increasingly considered with approaches inline or promoting these agendas given more attention. Among the novel approaches that promote sustainability is metabolic engineering. As a field, it has evolved over the years leveraging technological improvements in genome sequencing, computational biology, and gene editing to help bring forth innovations that have contributed to mitigating the effects on nature of intensive agricultural practices while reducing global hunger. This chapter discusses the role of engineered microbes, technologies, advancements, and future perspectives in the improvement of agriculture.

**Keywords** Engineered microbes · Sustainable agriculture · Cell factories · Biocontrol · Metabolic engineering

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_10

## 10.1 Role of Microbes in Sustainable Agriculture

Over the years, to obtain food in the form of livestock and crops, man has developed and assembled various components to form suitable agricultural systems. Agricultural practices have been modified in several ways over the years to increase output in order to meet the increasing needs of the growing population. Presently, in the face of growing populations, the corresponding global demand for crops is projected to increase by a minimum of 60% by 2050 (Arif et al. 2020). Also, considering the recently pressing need to transition to more sustainable processes in industries that will require more fiber as starting material, there is going to be a continuous increase in the demand for agricultural output and pressure on natural resources (Arif et al. 2020). These and many other growing concerns are the focus of research to provide innovative approaches aimed at solving new challenges encountered in agriculture.

Many such innovative approaches leverage the important role microbes play in nutrient conversion both in the environment and plants (Singh et al. 2019). Microbes can be artificially introduced in agricultural settings to achieve a fertile environment that will in turn improve crop productivity (Hendrickson et al. 2008). Microbes are the prime nutrient cyclers in the environment and have intimate relationships with plants. As these microbial nutrient cycling processes occur in open systems, when using microbes in agriculture, considering the diversity of the environment and socioeconomic factors is necessary. Importantly, the unintended effects of approaches based on microbial interactions on other systems also have to be properly understood. Therefore, using microbes in sustainable agriculture will relate to the minimization of input investments and maximization of output gains to meet increasing demands while protecting soil health and water quality.

## 10.2 Role of Metabolic Engineering

Metabolic engineering involves modifying genetic and regulatory processes in cells to optimize a desired function in the cell. Many engineered microbes have shown important roles with potential for promoting sustainable agriculture (Table 10.1). Nevertheless, the deployment of engineered microbes in open systems such as in agriculture elicits a lot of ethical concerns especially the contamination of the natural gene pool. These ethical concerns have severely limited the use of engineered microorganisms in agriculture. This has led to the development of methods to evaluate the function of genetically modified microbes before deployment. The idea is to put bacteria communities into microscopic containers and monitor their behaviours in mimicked environmental processes. These containers are often developed with 3D printing such as root exudate collectors, microfluidic-based platforms such as RootChip, Kchip, RootArray, tracking root interaction systems, PlantChip, static droplet arrays, etc. (Ke et al. 2020). To prevent the transfer of transgenic genes to other organisms and prevent their survival and propagation in other environments,

**Table 10.1** Examples of engineered microbes and relevant roles in agriculture

| Application | Microorganism | Research goal | Reference |
|---|---|---|---|
| Biofertilizers | *Rhizobium leguminosarum* | Enhancing nitrogen-fixing efficiency by increasing catalase activity | Orikasa et al. (2010) |
| | *Rhizobium meliloti* and *Bradyrhizobium japonicum* | Improve nitrogen fixation and competitive activity | Ronson et al. (1990) |
| | *Azotobacter chroococcum* | Production of stimulants for *Orobanche crenata* seed germination and nitrogen fixation | Khalaf et al. (1991) |
| | *Rhizobia* and *Azotobacter* | Genetically engineered for temperature, drought, and salt tolerance to improve nitrogen to promote plant growth in severe environment | El-Saidi and Ali (1993) |
| | *Anabaena* sp. PCC 7120 (A. 7120) | Photosynthetic production of ammonium | Higo et al. (2018) |
| Bioremediation | *R. leguminosarum* bv. *trifolii* strain R3 | Legume-rhizobia symbionts for arsenic methylation in arsenic bioremediation | Zhang et al. (2017) |
| | *Mesorhizobium huakuii* subsp. *rengei* B3 | Legume-rhizobia symbionts for cadmium- and copper-polluted soils | Ike et al. (2007) |
| | *Pseudomonas putida* | Degradation of organophosphates, pyrethroids, and carbamates from pesticides | Liu et al. (2006), Gong et al. (2018) |
| | *P. putida* | Increase bioadsorption capacity of heavy metals | Valls et al. (2000) |
| | *Sphingobium* sp. JQL4-5 | Degradation of methyl parathion and fenpropathrin | Yuanfan et al. (2010) |
| | *E. coli* | Simultaneous degradation of organophosphorus, carbamate, and pyrethroid pesticides | Lan et al. (2006) |
| Biopesticides production | *P. putida* WCS358r | Improve antifungal activity in rhizospheres of wheat plants | Glandorf et al. (2001) |
| | *Bacillus thuringiensis* subspecies *kurstaki* | Novel insecticidal proteins and increased activity against fall armyworm | All et al. (1994) |
| | *B. thuringiensis* | Improving the larvicidal activity of cry genes | Ja et al. (1996) |
| Bioherbicide production | *Xanthomonas campestris pv. Campestris* (XCC) | Improving virulence and host range of the plant pathogen using "Bialaphos" genes | Charudattan et al. (1996) |
| | *Sclerotinia sclerotiorum* | Development of auxotrophic properties to increase efficacy against *Cirsium arvense* | Harvey et al. (1998) |
| Waste management | *E. coli* | Ability to co-utilize cellobiose and xylose for biofuel production | Vinuselvi and Lee (2012) |

(continued)

**Table 10.1** (continued)

| Application | Microorganism | Research goal | Reference |
|---|---|---|---|
| | *Saccharomyces cerevisiae* | Production of ethanol from xylose | Hahn-Hägerdal et al. (2001) |
| | *Clostridium cellulolyticum* | Synthesis of n-butanol using cellulose as substrate | Gaida et al. (2016) |
| | *Bacillus subtilis* | Production of para-aminobenzoic acid using xylose as substrate | Averesch and Rothschild (2019) |
| | *Saccharomyces cerevisiae* and *Actinotalea fermentans* | Synthesis of methyl halides from nonfood sources | Bayer et al. (2009) |
| Synthesis of high-value metabolites | *E. coli* | Production of monolignols | Chen et al. (2017) |
| | *Saccharomyces cerevisiae* | Production of tropane alkaloids | Srinivasan and Smolke (2019) |
| | *S. cerevisiae* | Production of artemisinin | Paddon et al. (2013) |
| | *E. coli* | Production of the artemisinin precursor amorpha-4,11-diene | Tsuruta et al. (2009) |
| | *S. cerevisiae* | Industrial production of isoprenoid | Meadows et al. (2016) |
| | *S. cerevisiae* and *E. coli* | Production of paclitaxel precursor | Zhou et al. (2015) |
| | *S. cerevisiae* | Production of (S)-reticuline | DeLoache et al. (2015) |
| | *E. coli* | Production of opiates | Nakagawa et al. (2016) |
| Food systems | *Lactobacillus plantarum* | Production of sorbitol | Ladero et al. (2007) |
| | *S. cerevisiae* | Production of xylitol | Kogje and Ghosalkar (2017) |

containment systems such as genetic firewalls, auxotrophies, DNA watermarks, regulation of essential genes, and expression of toxic ones have been developed (Stirling and Silver 2020; Ke et al. 2020).

Standards for levels of containment required for deployment of suitable engineered organisms and the development of new containment systems have been established (Stirling and Silver 2020), and risk assessment methods for GMM have been presented (Rycroft et al. 2019). In addition to this, programs for biosecurity and biosafety have been initiated to study the effects of genetically modified organisms across species and generations. It is expected that these safeguards and containment programs will improve safety in the deployment of

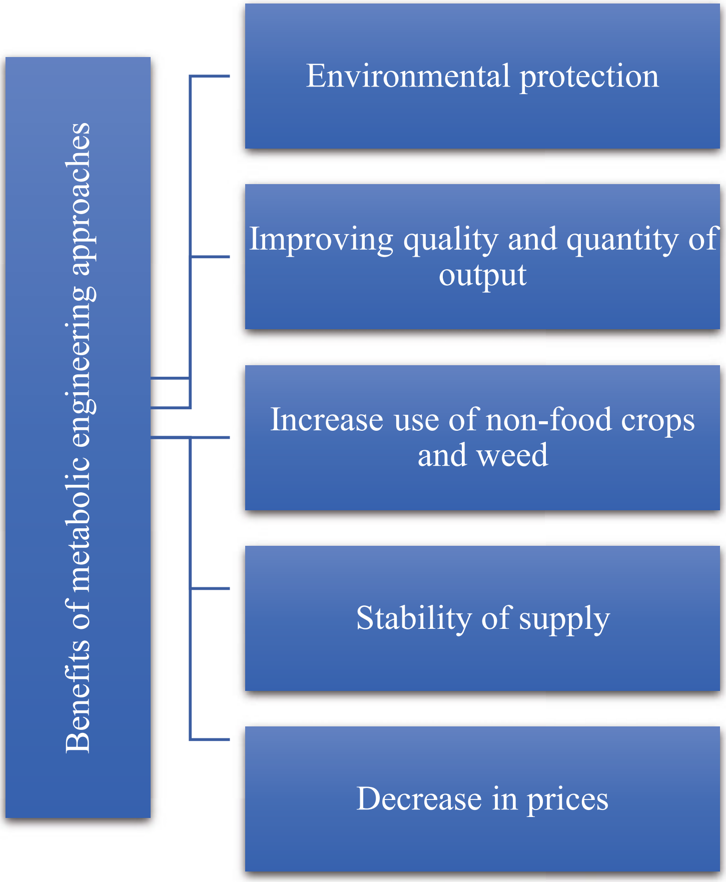

**Fig. 10.1** Areas of application of metabolic engineering approaches in agriculture

genetically modified organisms into the environment. With the rapid increase in genetic engineering technologies and their applications in agriculture, there will be a need to constantly update these regulatory frameworks to keep up. This will also mean the deployment and use of safer products that in the long-run will help gain public acceptance of products impacted by genetic engineering. Several benefits are anticipated from the use of engineered microbes in agriculture (Fig. 10.1). A summary of engineered microbes and their potential applications in agriculture is included in Table 10.1.

## 10.3 Strategies for Metabolic Engineering Applicable in Agriculture

As previously mentioned, metabolic engineering involves the introduction of genetic changes in organisms by using recombinant DNA technology tools. Approaches used in metabolic engineering depend on the goal determined after cellular functions have been carefully analyzed (Nielsen 2001). These strategies can be used alone or in combination to achieve engineering goals in agriculture. These are as follows.

### *10.3.1 Heterologous Production of the Desired Metabolite*

Plant metabolites such as artemisinin, flavonoids, and isoprenoids can be produced in mutant bacteria through the introduction of the synthesis pathway into bacteria. This process when successful has the advantage of avoiding challenges associated with posttranslational modifications in eukaryotic cells. Though the introduction of novel pathways could be toxic to microbes, there is a potential for the production of more diverse metabolites with more potent activity (Pfeifer and Khosla 2001; Tsuruta et al. 2009; Paddon et al. 2013; Mora-Pale et al. 2013; Trantas et al. 2015).

### *10.3.2 Extending the Range of Substrate to Be Used*

Agricultural by-products are lignocellulosic materials containing cellulose, hemicellulose, and lignin that are the most abundant renewable organic resource on earth. Novel pathways that can break down these compounds and enable their use as substrates for the production of high-value products by efficient industrial microbial strain can be engineered in microbes. This can enable more efficient use of agricultural by-products in a renewable way (Aristidou and Penttilä 2000).

### *10.3.3 Introducing Pathways for the Degradation of Xenobiotics*

The increasing use of xenobiotic compounds in agriculture is having negative consequences on the environment. To degrade these new synthetic compounds, completely novel pathways can be engineered in microbes to use them as substrates (McGuinness et al. 2007).

### *10.3.4 Improving the Physiology of the Cell or Optimizing Metabolism*

Cell structural characteristics can be modified to provide more surface area for the accumulation of bioproducts, increasing the secretion of metabolite (Bu et al. 2020), reducing the consumption of precursors by competing products (Hendry et al. 2017), and optimization of flux to increase productivity (Song et al. 2017). These approaches can be used alone or in combination with other approaches to increase the production titer of high-value products and make them more competitive.

## 10.4 Applications of Metabolic Engineering in Agriculture

### *10.4.1 Increasing Yield and Resilience of Plants*

Plants grow within ecosystems which often change in properties with time. For ecosystems with defined structures such as high salt concentrations in dry parts of the world, successful agriculture can be quite challenging. Microbes, however, due to their ubiquitous nature, have evolved various mechanisms to thrive in such extreme environments. For this reason, microbial genes are explored and exploited to engineer transgenic plants to impart desirable traits as tolerance to adverse conditions which in turn increases production (Gupta et al. 2013).

Another approach with biotechnology-based solutions to improve both crop yields and resilience that is gaining ground is the direct manipulation of the holobiont of plants through microbiome engineering. Microbiomes play a role in boosting plant growth, fighting against crop diseases, and mitigating abiotic stress. In addition to microbiome engineering, new practices in agriculture using this approach include microbiome breeding, transplantation, and targeted microbiome engineering, for example, by strategic soil amendments to maintain beneficial microbes or use a cocktail of microbial consortia directly on the soil as probiotic agents.

It is expected that these approaches will contribute to bring faster and more sustainable solutions to challenges in agriculture related to differences in soil type, environmental/climatic conditions, growth stage, and genotype of the plant through a more purpose-directed and effective way (Arif et al. 2020).

### *10.4.2 Rhizosphere Strengthening*

Plant growth-promoting microbes have been shown to have various beneficial effects through the improvement of plant development by triggering the secretion of growth hormones, antioxidants, and siderophores as well as improving plant nutritional capacity. Important microbe species that produce such effects include rhizobia, *Trichoderma* sp., endophytes, and arbuscular mycorrhizal fungi (AMF). Microbes in the rhizosphere can be engineered to improve plant-microbe interactions such that plants are resilient to long-term environmental perturbations including effects that could result from climate change (Ahkami et al. 2017).

### *10.4.3 Increasing the Photosynthetic Efficiency of Plants*

The ubiquitous nature of bacteria allows them to dwell in several extreme environments where they are endowed with efficient systems for obtaining nutrients and survival. In agriculture, cyanobacteria which like plants are autotrophic can serve as an important source of information to enhance the output of crops. Agriculture is greatly affected by location and the nature and availability of light in each area. Photosynthetic pigments capture light energy in plants but are often limited in their use of solar energy because of their specificity for particular wavelengths.

With the development of new gene editing tools, bacteriochlorophylls in cyanobacteria and purple bacteria with wider range of light capture wavelength can be engineered as chimeras with plant chlorophylls to increase their light-harvesting capacity (Swainsbury et al. 2019). Light-harvesting protein chimeras from bacteria and plant sources could help in the development of more efficient light harvesters which will translate into more energy synthesized and improved plant growth.

Other approaches benefiting from cyanobacteria metabolism can be used to modify processes along the photosynthesis pathways. For instance, the carboxysomes of cyanobacteria have been introduced into the chloroplasts of plants to improve their $CO_2$ fixing ability. It has been discovered that plant RuBisCO function at suboptimal levels which limits the amount of carbon fixed and hence lower nutrients acquisition in plants. These cyanobacterial carboxysomes could help improve the ability of plants to fix atmospheric carbon, improve output, and have important implications for natural resource management (Goold et al. 2018).

### *10.4.4 Biofertilizers*

Maintaining soil health is increasingly a major requirement for the development of sustainable agricultural systems. Traditional soil enrichment approaches used chemical fertilizers to enrich the soil with particular nutrients of interest. Though highly effective, in the long term, they have been the cause of gradual degradations in soil fertility, disruption of soil microbiome, and health. More sustainable biofertilizers made from exclusively living organisms are becoming the ingredients of choice to increase soil fertility while maintaining soil health. Biofertilizers are beneficial in agriculture through the acceleration of mineral uptake, increasing crop yield, stimulating plant growth, fixing nutrients and increasing availability in soil, increasing resistance against drought, and cost-effectiveness. Microbes frequently used in biofertilizer formulations include *Rhizobium*, *Azotobacter*, *Anabaena* (nitrogen fixers), *Pseudomonas putida*, and *mycorrhizal fungi* (Giri et al. 2019; Ali et al. 2020).

Considering the safety implications of applying engineered microbes in the soil, tools to engineer beneficial soil organisms such as *Anabaena* with good stability for environmental application (Chaurasia et al. 2008) including those using recent highly scalable CRISPR-Cpf1, CRISPRi technologies that produce better and markerless mutants have been developed (Higo et al. 2018; Niu et al. 2019). With improved genetic engineering tools, more environmentally friendly mutant microbes will be engineered that will improve and encourage the use of biofertilizers.

## *10.4.5 Biocontrol of Other Competing Organisms*

### 10.4.5.1 Bioinsecticides and Biofungicides

Chemical pesticides based on halogens, carbamate, and organophosphorus compounds are widely used to control pests in agricultural systems. Their use has led to secondary effects such as high toxicity to other nontargeted animals, humans, and groundwater. Biological pesticides on the other hand can be biofungicides such as those containing *Trichoderma* or bioinsecticides such as those containing *Bacillus thuringiensis*. The use of biopesticides as alternative to chemical pesticides comes with several advantages like better biodegradability, better effectiveness and selectivity, and environmental friendliness (Singh et al. 2017) which fulfill requirements for sustainability. Biopesticides are however slower to adopt due to limitations like slow kill rates, difficulties of production, costs, appropriate formulations, and previously reported poor performances (Glare et al. 2016; Bhattacharyya et al. 2016).

Various subspecies of *Bacillus thuringiensis* are used as bioinsecticides to control beetle larvae (var. *tenebrionis*), caterpillars (var. *kurstaki*, *entomocidus*, *galleriae*, and *aizawai*), and mosquito and blackfly larvae (var. *israeliensis*). Certain strains of *Bacillus subtilis*, *B. pumilus*, *Pseudomonas fluorescens*, *P. aureofaciens*, and *Streptomyces* spp. prevent plant diseases by outcompeting plant pathogens in the rhizosphere, producing antifungal compounds and promoting plant and root growth (Singh et al. 2017). Biofungicides on the other hand have been used in both the phylloplane and rhizosphere to control plant diseases caused by fungi, bacteria, or nematodes including some insect pests and weeds (Singh et al. 2017). Considering these various functions, engineering bacteria species with industrial potential could improve specificity, kill rates, strain resilience in production, and performance during application.

#### 10.4.5.2 Bioherbicides

Bioherbicides are biological agents that are used for weed control. Weed competes with crops for nutrients; therefore it has a direct effect on the quality and quantity of the output of crops. The active components of bioherbicides are living microorganisms which are applied in high inoculum rates in a plant-specific manner for weed management. They have advantages over chemical-based herbicides such as increased selectivity and reduced risks of erosion. Despite clear expected advantages of environmental friendliness over chemical herbicides, bioherbicide production and commercialization has been limited due to several environmental (aerial, soil, or aquatic), technological (mass production and formulations), and commercial constraints (market, patent issues, production costs, and regulations) (Auld and Morin 1995; Aneja et al. 2017). Just scores of bioherbicides brands are commercially available in the world markets, and they are generally fungal-based formulations (Aneja et al. 2017).

There is very little research information on the development and use of genetically engineered microbes in bioherbicide development. This is probably in part due to many failed attempts to develop effective mutants as bioherbicides (Duke et al. 2015). However, bacteria such as the pseudomonads which have good infection ability, good quorum sensing systems, and antagonistic and phytopathogenic properties (Rekadwad and Ghosh 2018) could serve as important platforms for the development of highly efficient biocontrol agents against crop weed. Possible engineering approaches include expanding the range of plant pathogens to be targeted (Charudattan et al. 1996), improving the virulence of the biocontrol agent, and developing microbial mutants producing weed-specific phytotoxins (Zidack et al. 2001; van der Does and Rep 2007).

An additional dimension could be added to protect the environment against the development of supervirulent microbes through gene transfer by including auxotrophic characteristics into engineered strains such that the strain disappears with the elimination of the weed (Miller et al. 1989; Sands and Miller 1993; Duke et al. 2015).

## 10.5 Cell Factories for the Biosynthesis of High-Value Metabolites

Plants are sources of many high-value products and metabolites such as medicines, supplements, flavors, etc. This translates into a high dependence on plants in agriculture to meet the increasing demands of these products. Developing agricultural systems to grow plants for this purpose also means dependence on arable land and use of water resources. Furthermore, the long generation time for plants and their seasonality hinder the ability to constantly produce and supply plant-derived products.

With the development of genetic engineering, some of these challenges are being overcome. However, engineering plants compared to simpler organisms such as microbes encounter more issues like long generation times, scalability, and polyploidy of their genomes. Better approaches using engineered bacteria have helped overcome many more challenges encountered because of pressure on natural resources and the use of plants in general. Instead of plants, microbes engineered with plant metabolism can produce plant metabolites more sustainably (Trivedi et al. 2017).

Yeasts are well-characterized microbes with eukaryotic machinery able to produce plant metabolites upon transfer of the pathways responsible for these metabolites from plants into yeasts. This not only provides the opportunity to address the challenges of using plants but also includes the additional advantage of using plant-derived feedstock from agricultural wastes and the relatively cheaper cost of developing engineered yeast. This is possible due to improvements in sequencing technologies and better engineering tools that enable the exploration and characterization of metabolic pathways for high-value products in plants and incorporating them in small unicellular organisms (Moses et al. 2017; Goold et al. 2018).

For agricultural systems dependent on market conditions and price fluctuations, engineered microbe platforms provide an opportunity to generate a wide variety of commodities with production unaffected by seasons. This could translate to a significant contributor to sustainable economic development through cheaper production, increase in output, stable supply, and a viable market (Paddon et al. 2013; Goold et al. 2018). Improvements in technologies especially genetic engineering continue to be of consistent relevance and support to overcome challenges related to generating high-value products from plants.

New technologies using biosensors promise to provide many innovative approaches for solving persistent challenges in bioproduct synthesis (Goold et al. 2018). For more complex products, consortia of multiple microbes can be devised to reconstitute the synthesis pathway. The advantages are the possibility to construct and optimize pathways in parallel which helps reduce the time for product formation, ability to use the properties unique to each microbe, microbial synergistic effects on increasing productivity, and fewer feedback inhibition-related problems (Zhou et al. 2015). Today, though many phytochemicals can be produced from microbial cell factories using the approaches mentioned before, there are still many pathways for phytochemicals that are still to be known, including precursor supply in microbial hosts, obstruction of product transport, and low enzyme activities.

With continuous use of high-throughput technologies and exploration of more plant pathways, innovative approaches to produce new high-value phytochemicals, increase production, and lower prices will emerge (Liu et al. 2017). Enzyme mining from native and nonnative hosts, enhancement of enzyme activities, optimization, and enhancement of reaction efficiencies of multienzyme pathways in microbial hosts are approaches where new technologies can be applied to advance our understanding and improve the production of phytochemicals from microbes (Li et al. 2018).

## 10.6 Soil Remediation

Plant-based agriculture over the years has led to the emergence of land use concerns. The increasing need for agricultural produce will not be met through cultivating the currently available land for large-scale commercial purposes. Furthermore, contamination from industrial processes render a lot more arable land unsuitable for agriculture. Some of these effects though reversible, close to half the number of farms continue to experience nutrient depletions that are very difficult to mitigate with traditional soil amendments and chemical fertilizers (Arif et al. 2020). Other methods such as biostimulation and bioaugmentation achieve remediation efficiencies of only about 60% (Wu et al. 2016).

The increasing number of novel pollutants also makes bioremediation difficult with traditional methods; therefore fields like metabolic engineering offer the possibility to develop microbial systems with specific degradation ability for new compounds (Dangi et al. 2019). With the proper characterization of the structure and activity of microbial communities as a result of increasing molecular technologies, it is increasingly possible to predict the factors required to improve the balance in microbial communities and ecosystems (Pieper and Reineke 2000). By engineering the microbiome, the composition of soil microbes can be modified to improve ecosystems and by so doing improve the growth of plants (Foo et al. 2017). Engineering techniques that can be applied to this end include optimization of enzymes structure and substrate range (Holloway et al. 1998; Chen et al. 1999; Sharma et al. 2018). Other techniques employing microbial consortia are comparatively less developed (Brune and Bayer 2012) because they require the development of more sophisticated detection and monitoring systems (Kylilis et al. 2018).

In the future, using safe microbial chassis like *P. putida*, metabolic engineering of genetic circuits for specific degradation with the ability to resist many changing conditions will prove highly beneficial in remediating recalcitrant soils (Jaiswal and Shukla 2020).

Groundwater which is necessary for plant growth is also frequently polluted by common contaminants. For example, the frequently used 1,2,3-trichloropropane is a common contaminant that is not mineralized by any known microbe in oxic conditions. Genetically engineered microbes have nevertheless been developed that can degrade such synthetic pollutants from groundwater in combination with bioaugmentation (Janssen and Stucki 2020).

The depreciation of the quality of soils can also occur through excessive use of synthetic fertilizers or spillage of industrial wastes containing recalcitrant material. Considering the need to preserve soil health and to use remediation measures that preserve soil health in the long term, biosafety bacteria engineering could be a solution to preserve these properties. This has been demonstrated with *P. putida* strain KT2440 engineered for aerobic mineralization of 1,2,3-trichloropropane. In the study, an approach leveraging combinatorial engineering and insertion in the chromosome of the bacteria of a synthetic pathway for the degradation of 1,2,3-trichloropropane was used. The mutant bacteria were shown to utilize the compound as a sole carbon source (Gong et al. 2017). Extension of this concept to rational engineering approaches pertinent to agriculture is also being undertaken by researchers to convert microbes such as *S. cerevisiae* and *Escherichia coli* into potential bioremediation agents. These are capable of bioremediating heavy metal contamination and degrading toxic aromatic compounds (Goold et al. 2018).

### *10.6.1 Pesticide Bioremediation*

Herbicides are used in agriculture as tools to selectively grow desired crops over other plants competing for nutrients. This means higher nutrient availability to crops and increases growth. Nevertheless, there are undesirable effects that come as a result of using herbicides in the environment. They leave behind toxic metabolites resulting from partial degradation, have effects on biogeochemical cycles due to changes in microbial communities, persistent contamination, and alterations in soil fertility that affect plant nutrition (Pileggi et al. 2020). The complexity of metabolites introduced as a result of herbicide usage usually requires sophisticated approaches such as using engineered bacteria to specifically target these new agents or use bacteria acting in synergy for complete degradation.

Bacteria and fungi have been shown to degrade herbicide compounds (Erguven 2018). Knowledge of bacteria communities such as in biofilms and their structure and function is increasingly needed to develop better systems for herbicide bioremediation. A living biofouling-resistant membrane system with a beneficial bacteria strain encoding the enzyme epoxide hydrolase which degrades epichlorohydrin commonly used for the synthesis of pesticides has been demonstrated with emerging issues such as possible horizontal gene transfer addressed through bacterial chromosomal insertion of the coding sequences. Due to the importance of the risks involved in the proliferation of engineered traits in the environment, other approaches such as the introduction of programmed death after depletion of pollutants could also minimize the risks of contamination (Garbisu and Alkorta 1999; Paul et al. 2005b).

Different methodologies for the design of safer GMMs for release into the environment have also been reviewed (Paul et al. 2005a). The strain carrying the trait in the biofilm was able to control biofilm properties through a feedback circuit and producing nitric oxide to prevent the formation of biofilms by other harmful undesirable bacteria (Wood et al. 2016).

Microbial endophytes have also been shown to contribute to herbicide tolerance in plants. With metabolic engineering approaches, the range of specific tolerance traits that can be introduced into plants using endophytic bacteria is numerous. Using different beneficial endophytic bacteria that are not toxic to a plant, biotethering could be used as an accessory method for additional resistance development in crops. These are seen as cheaper alternatives to engineering plants because the cost comes as a fraction of engineering in plants (Tétard-Jones and Edwards 2016).

More responsive systems using engineered bacteria that are responsive to stress signals from plants, engineering of endophytic bacteria with phytoremediation abilities (Barac et al. 2004) and pesticide-degrading abilities (McGuinness et al. 2007) have been reported.

## 10.7 Agricultural Waste Management

Waste from agricultural systems include animal waste, food processing waste, crop waste, hazardous and toxic waste.

### *10.7.1 Crop Waste Management*

Crop wastes from agro-residues obtained after harvesting such as wheat straw, rice straw, sugarcane bagasse, rice husk often referred to as lignocellulosic substrates, and plant biomass are used by engineered microbes as substrates for high-value products like biofuels. Lignocellulosic biomass represents a cheap and the largest source of renewable carbon suitable for biotechnology production (Lin et al. 2013).

Through metabolic engineering, bacterial and yeast strains have been constructed which feature traits that are advantageous for ethanol production using lignocellulose sugars. After several rounds of modification/evaluation/modification, three main microbial platforms, *Saccharomyces cerevisiae*, *Zymomonas mobilis*, and *Escherichia coli*, have emerged, and they have performed well in pilot studies (Zaldivar et al. 2001). Thanks to genetic engineering, previous biofuel production approaches that required multiple steps in the synthesis process have been reduced to single-step processes. Also, it is now possible to use feedstock that was previously unsuitable as substrates in bioprocesses for high-value products (Majidian et al. 2018).

Biofuels are combustible organic chemicals directly or indirectly derived from biomass. Various sugars in plant biomass can be converted by microbes to biofuels (Rai et al. 2022). Currently, first-generation bioethanol derived from sugar- and starch-based feedstocks (e.g., corn, sugarcane, cereals, and sugar beets) and biodiesel derived from vegetable oil or animal fats are the most widely used biofuels. Genetically engineered microbes can be used to produce biohydrogens and biogas (Srivastava 2019). Commonly used methodologies include overexpression or deletion of enzyme systems involved in the pathway for the synthesis of the bioproduct in question and de novo biosynthesis (Lin et al. 2013). Other important chemicals such as methyl halides which are used as agricultural fumigants have also been demonstrated to be produced in high yields from engineered yeast and *Actinotalea fermentans* in a symbiotic co-culture (Bayer et al. 2009).

## 10.8 Food Systems

Industrial biotechnology is increasingly playing a big role in the food sector amidst increasing concerns to enhance global food security. Regulations, public perceptions of sustainability, and cultural differences are among important debates within this area. Cooperation between various stakeholders is required to harmonize these emerging concerns and pave a unanimous pathway forward (McCullum et al. 2003). Food packaging is part of the delivery processes of agricultural produce and adequate preservation is necessary using adequate biopolymers.

Polylactic acid plastic polymers are used in the production of homopolymers for mulching films and packaging material. Production systems with the yeast *Yarrowia lipolytica* were designed by expressing propionyl-CoA transferase and a variant of PHA synthase (Lajus et al. 2020). Other opportunities include the potential to improve the nutritional value of foods, for example, through the development of carotenoid-enriched functional crops and oilseed crops with boosted levels of omega 3 fatty acids. Metabolic rewiring could be used to greatly increase the accumulation of carotenoids with nutritional and health-promoting activity, as recently demonstrated in a proof-of-concept experiment (Goold et al. 2018).

Pigments produced from plants such as the water-soluble anthocyanins which are widely used in the food industry can be produced from bacteria as a substitute to laborious plant-based approaches. Engineered microbes make the production process easier through the elimination of complex extraction processes and offering a more sustainable approach (Zha and Koffas 2017).

There are growing concerns of food security related to the increasing highly processed foods with high-calorie contents but low nutritional value, food loss, and food waste. Metabolic engineering approaches have enabled microbes to produce nonnative chemicals by fermentation, such as human milk oligosaccharides (HMOs). Also, biological processes can be an alternative for current chemical processes, that have extreme conditions and costly purification steps.

Sugar alcohols have a wide range of sweetness and health-promoting benefits and they are being used in the food industry for this reason. Besides, some sugar alcohols produced from engineered strains such as xylitol (Kogje and Ghosalkar 2017) and sorbitol (Ladero et al. 2007) have potential applications as building blocks of various value-added chemicals.

## 10.9 Conclusions

Increasing global population and the need to ensure global food security requires the development of sustainable approaches to meet the ever-increasing needs of the population. The demands on agriculture are no longer limited to food provision but also other high-value products required to improve human lives. The transition to a circular economy as a better option toward economic and environmental sustainability requires less dependence on synthetic and chemically produced products. Agriculture provides resources for successful transition, but limitations such as increasing pressures on arable land and water resources, deforestation, seasonality, and price fluctuations negatively affect the environment and the sustainability of supply chains in bioeconomics. Increasing knowledge on plant and microbial systems thanks to recent improvements in high-throughput technologies in combination with genetic engineering presents researchers with numerous opportunities to innovate and tilt the scales once more toward sustainability. Microbial metabolic engineering is successfully addressing many challenges in agriculture though with new challenges and requirements for highly standardized regulations before implementation. The characterization of more product synthesis pathways in plants, more efficient engineering tools optimized for cell hosts, minimization of contamination of natural gene pools of other organisms, and adequate regulatory and standardization mechanisms are continuously required to improve the sustainability and acceptability of genetically engineered microbes in agriculture.

## References

Ahkami AH, Allen White R, Handakumbura PP, Jansson C (2017) Rhizosphere engineering: enhancing sustainable plant ecosystem productivity. Rhizosphere 3:233–243. https://doi.org/10.1016/j.rhisph.2017.04.012

Ali R, Zulaykha KD, Sajjad N (2020) Genetically modified microbes as biofertilizers. In: Bhat RA, Hakeem KR (eds) Bioremediation and biotechnology, Techniques for noxious substances remediation, vol 4. Springer Int Publishing, Cham, pp 275–293

All JN, Stancil JD, Johnson TB, Gouger R (1994) A genetically-modified *Bacillus thuringiensis* product effective for control of the fall Armyworm (Lepidoptera: Noctuidae) on Corn. Fla Entomol 77:437–440. https://doi.org/10.2307/3495697

Aneja KR, Khan SA, Aneja A (2017) Bioherbicides: strategies, challenges and prospects. In: Satyanarayana T, Deshmukh SK, Johri BN (eds) Developments in fungal biology and applied mycology. Springer, Singapore, pp 449–470

Arif I, Batool M, Schenk PM (2020) Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trends Biotechnol 38:1385–1396. https://doi.org/10.1016/j.tibtech.2020.04.015

Aristidou A, Penttilä M (2000) Metabolic engineering applications to renewable resource utilization. Curr Opin Biotechnol 11:187–198. https://doi.org/10.1016/S0958-1669(00)00085-9

Auld BA, Morin L (1995) Constraints in the development of bioherbicides. Weed Technol 9:638–652

Averesch NJH, Rothschild LJ (2019) Metabolic engineering of *Bacillus subtilis* for production of para-aminobenzoic acid—unexpected importance of carbon source is an advantage for space application. Microb Biotechnol 12:703–714. https://doi.org/10.1111/1751-7915.13403

Barac T, Taghavi S, Borremans B et al (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Bayer TS, Widmaier DM, Temme K et al (2009) Synthesis of methyl halides from biomass using engineered microbes. J Am Chem Soc 131:6508–6515. https://doi.org/10.1021/ja809461u

Bhattacharyya A, Duraisamy P, Govindarajan M, Buhroo AA, Prasad R (2016) Nano-biofungicides: emerging trend in insect pest control. In: Prasad R (ed) Advances and applications through fungal nanobiotechnology. Springer International Publishing, Switzerland, pp 307–319

Brune KD, Bayer T (2012) Engineering microbial consortia to enhance biomining and bioremediation. Front Microbiol 3:203. https://doi.org/10.3389/fmicb.2012.00203

Bu X, Lin J-Y, Cheng J et al (2020) Engineering endogenous ABC transporter with improving ATP supply and membrane flexibility enhances the secretion of β-carotene in *Saccharomyces cerevisiae*. Biotechnol Biofuels 13:168. https://doi.org/10.1186/s13068-020-01809-6

Charudattan R, Prange VJ, Devalerio JT (1996) Exploration of the use of the "Bialaphos Genes" for improving bioherbicide efficacy. Weed Technol 10:625–636

Chaurasia AK, Parasnis A, Apte SK (2008) An integrative expression vector for strain improvement and environmental applications of the nitrogen fixing cyanobacterium, *Anabaena sp.* strain PCC7120. J Microbiol Methods 73:133–141. https://doi.org/10.1016/j.mimet.2008.01.013

Chen W, Brühlmann F, Richins RD, Mulchandani A (1999) Engineering of improved microbes and enzymes for bioremediation. Curr Opin Biotechnol 10:137–141. https://doi.org/10.1016/S0958-1669(99)80023-8

Chen Z, Sun X, Li Y et al (2017) Metabolic engineering of *Escherichia coli* for microbial synthesis of monolignols. Metab Eng 39:102–109. https://doi.org/10.1016/j.ymben.2016.10.021

Dangi AK, Sharma B, Hill RT, Shukla P (2019) Bioremediation through microbes: systems biology and metabolic engineering approach. Crit Rev Biotechnol 39:79–98. https://doi.org/10.1080/07388551.2018.1500997

DeLoache WC, Russ ZN, Narcross L et al (2015) An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. Nat Chem Biol 11:465–471

Duke SO, Scheffler BE, Boyette CD, Dayan FE (2015) Biotechnology in weed control. In: Kirk-Othmer Encyclopedia of chemical technology. American Cancer Society, pp 1–25

El-Saidi MT, Ali AMM (1993) Growing different field crops under high salinity levels and utilization of genetically engineered Rhizobia and Azotobacter salt drought tolerant strains. In: Lieth H, Al Masoom AA (eds) Towards the rational use of high salinity tolerant plants, Agriculture and forestry under marginal soil water conditions, vol 2. Springer, Dordrecht, pp 59–65

Erguven GO (2018) Comparison of some soil fungi in bioremediation of herbicide acetochlor under agitated culture media. Bull Environ ContamToxicol 100:570–575. https://doi.org/10.1007/s00128-018-2280-1

Foo JL, Ling H, Lee YS, Chang MW (2017) Microbiome engineering: current applications and its future. Biotechnol J 12:1600099. https://doi.org/10.1002/biot.201600099

Gaida SM, Liedtke A, Jentges AHW et al (2016) Metabolic engineering of *Clostridium cellulolyticum* for the production of n-butanol from crystalline cellulose. Microb Cell Fact 15:6. https://doi.org/10.1186/s12934-015-0406-2

Garbisu C, Alkorta I (1999) Utilization of genetically engineered microorganisms (GEMs) for bioremediation. J Chem Technol Biotechnol 74:599–606. https://doi.org/10.1002/(SICI)1097-4660(199907)74:7<599::AID-JCTB82>3.0.CO;2-G

Giri B, Prasad R, Wu Q-S, Varma A (2019) Biofertilizers for sustainable agriculture and environment. Springer International Publishing. isbn:978-3-030-18932-7. https://www.springer.com/gp/book/9783030189327

Glandorf DCM, Verheggen P, Jansen T et al (2001) Effect of genetically modified *Pseudomonas putida* WCS358r on the fungal rhizosphere microflora of field-grown wheat. Appl Environ Microbiol 67:3371–3378. https://doi.org/10.1128/AEM.67.8.3371-3378.2001

Glare TR, Gwynn RL, Moran-Diez ME (2016) Development of biopesticides and future opportunities. In: Glare TR, Moran-Diez ME (eds) Microbial-based biopesticides: methods and protocols. Springer, New York, pp 211–221

Gong T, Xu X, Che Y et al (2017) Combinatorial metabolic engineering of *Pseudomonas putida* KT2440 for efficient mineralization of 1,2,3-trichloropropane. Sci Rep 7:7064. https://doi.org/10.1038/s41598-017-07435-x

Gong T, Xu X, Dang Y et al (2018) An engineered *Pseudomonas putida* can simultaneously degrade organophosphates, pyrethroids and carbamates. Sci Total Envirom 628–629:1258–1265. https://doi.org/10.1016/j.scitotenv.2018.02.143

Goold H, Wright P, Hailstones D (2018) Emerging opportunities for synthetic biology in agriculture. Genes 9:341. https://doi.org/10.3390/genes9070341

Gupta VK, Yadava LP, Donovan AO et al (2013) Microbial engineering for developing stress tolerant cultivars an innovative approach. CRC Press-Taylor & Francis Group, Boca Raton

Hahn-Hägerdal B, Wahlbom CF, Gárdonyi M et al (2001) Metabolic engineering of *Saccharomyces cerevisiae* for xylose utilization. In: Nielsen J, Eggeling L, Dynesen J et al (eds) Metabolic engineering. Springer, Berlin, pp 53–84

Harvey IC, Bourdot GW, Saville DJ, Sands DC (1998) A comparison of auxotrophic and wild strains of *Sclerotinia sclerotiorum* used as a mycoherbicide against Californian thistle (*Cirsium arvense*). Biocontrol Sci Technol 8:73–81. https://doi.org/10.1080/09583159830441

Hendrickson JR, Hanson JD, Tanaka DL, Sassenrath G (2008) Principles of integrated agricultural systems: introduction to processes and definition. Ren Agri Food Sys 23:265–271

Hendry JI, Prasannan C, Ma F et al (2017) Rerouting of carbon flux in a glycogen mutant of cyanobacteria assessed via isotopically non-stationary 13C metabolic flux analysis. Biotechnol Bioeng 114:2298–2308. https://doi.org/10.1002/bit.26350

Higo A, Isu A, Fukaya Y et al (2018) Application of CRISPR interference for metabolic engineering of the heterocyst-forming multicellular *Cyanobacterium anabaena* sp. PCC 7120. Plant Cell Physiol 59:119–127. https://doi.org/10.1093/pcp/pcx166

Holloway P, Knoke KL, Trevors JT, Lee H (1998) Alteration of the substrate range of haloalkane dehalogenase by site-directed mutagenesis. Biotechnol Bioeng 59:520–523. https://doi.org/10.1002/(SICI)1097-0290(19980820)59:4<520::AID-BIT16>3.0.CO;2-D

Ike A, Sriprang R, Ono H et al (2007) Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. Chemosphere 66:1670–1676. https://doi.org/10.1016/j.chemosphere.2006.07.058

Ja B, Kakefuda M, Gawron-Burke C (1996) Engineering *Bacillus thuringiensis* bioinsecticides with an indigenous site-specific recombination system. Appl Environ Microbiol 62:4367–4373. https://doi.org/10.1128/aem.62.12.4367-4373.1996

Jaiswal S, Shukla P (2020) Alternative strategies for microbial remediation of pollutants via synthetic biology. Front Microbiol 11:808. https://doi.org/10.3389/fmicb.2020.00808

Janssen DB, Stucki G (2020) Perspectives of genetically engineered microbes for groundwater bioremediation. Environ Sci Process Impacts 22:487–499. https://doi.org/10.1039/C9EM00601J

Ke J, Wang B, Yoshikuni Y (2020) Microbiome engineering: synthetic biology of plant-associated microbiomes in sustainable agriculture. Trends Biotechnol 39:244–261. https://doi.org/10.1016/j.tibtech.2020.07.008

Khalaf KA, Ali AM National RC (1991) Preliminary attempts to produce genetically engineered *Azotobacter chrococcum* transformants able to produce Orobanche stimulant(s) [Vicia faba]. Faba Bean Information Service

Kogje AB, Ghosalkar A (2017) Xylitol production by genetically modified industrial strain of *Saccharomyces cerevisiae* using glycerol as co-substrate. J Ind Microbiol Biotechnol 44:961–971. https://doi.org/10.1007/s10295-017-1914-3

Kylilis N, Tuza ZA, Stan G-B, Polizzi KM (2018) Tools for engineering coordinated system behaviour in synthetic microbial consortia. Nat Commun 9:2677. https://doi.org/10.1038/s41467-018-05046-2

Ladero V, Ramos A, Wiersma A et al (2007) High-level production of the low-calorie sugar sorbitol by *Lactobacillus plantarum* through metabolic engineering. Appl Environ Microbiol 73:1864–1872. https://doi.org/10.1128/AEM.02304-06

Lajus S, Dusséaux S, Verbeke J et al (2020) Engineering the yeast *Yarrowia lipolytica* for production of polylactic acid homopolymer. Front Bioeng Biotechnol 8:954. https://doi.org/10.3389/fbioe.2020.00954

Lan WS, Gu JD, Zhang JL et al (2006) Coexpression of two detoxifying pesticide-degrading enzymes in a genetically engineered bacterium. Int Biodeter Biodegr 58:70–76. https://doi.org/10.1016/j.ibiod.2006.07.008

Li S, Li Y, Smolke CD (2018) Strategies for microbial synthesis of high-value phytochemicals. Nat Chem 10:395–404. https://doi.org/10.1038/s41557-018-0013-z

Lin H, Wang Q, Shen Q et al (2013) Genetic engineering of microorganisms for biodiesel production. Bioengineered 4:292–304. https://doi.org/10.4161/bioe.23114

Liu Z, Hong Q, Xu J-H et al (2006) Construction of a genetically engineered microorganism for degrading organophosphate and carbamate pesticides. Int Biodeter Biodegr 58:65–69. https://doi.org/10.1016/j.ibiod.2006.07.009

Liu X, Ding W, Jiang H (2017) Engineering microbial cell factories for the production of plant natural products: from design principles to industrial-scale production. Microb Cell Fact 16:125. https://doi.org/10.1186/s12934-017-0732-7

Majidian P, Tabatabaei M, Zeinolabedini M et al (2018) Metabolic engineering of microorganisms for biofuel production. Renew Sust Energy Rev 82:3863–3885. https://doi.org/10.1016/j.rser.2017.10.085

McCullum C, Benbrook C, Knowles L et al (2003) Application of modern biotechnology to food and agriculture: food systems perspective. J Nutr Educ Behav 35:319–332. https://doi.org/10.1016/S1499-4046(06)60347-3

McGuinness M, Mazurkiewicz V, Brennan E, Dowling D (2007) Dechlorination of pesticides by a specific bacterial glutathione S-transferase, BphKLB400: potential for bioremediation. Eng Life Sci 7:611–615

Meadows AL, Hawkins KM, Tsegaye Y et al (2016) Rewriting yeast central carbon metabolism for industrial isoprenoid production. Nature 537:694–697. https://doi.org/10.1038/nature19769

Miller RV, Ford EJ, Zidack NJ, Sands DC (1989) A pyrimidine auxotroph of *Sclerotinia sclerotiomm* for use in biological weed control. Microbiology 135:2085–2091. https://doi.org/10.1099/00221287-135-7-2085

Mora-Pale M, Sanchez-Rodriguez SP, Linhardt RJ et al (2013) Metabolic engineering and in vitro biosynthesis of phytochemicals and non-natural analogues. Plant Sci 210:10–24. https://doi.org/10.1016/j.plantsci.2013.05.005

Moses T, Mehrshahi P, Smith AG, Goossens A (2017) Synthetic biology approaches for the production of plant metabolites in unicellular organisms. J Exp Bot 68:4057–4074. https://doi.org/10.1093/jxb/erx119

Nakagawa A, Matsumura E, Koyanagi T et al (2016) Total biosynthesis of opiates by stepwise fermentation using engineered *Escherichia coli*. Nat Commun 7:10390. https://doi.org/10.1038/ncomms10390

Nielsen J (2001) Metabolic engineering. Appl Microbiol Biotechnol 55:263–283. https://doi.org/10.1007/s002530000511

Niu T-C, Lin G-M, Xie L-R et al (2019) Expanding the potential of CRISPR-Cpf1-based genome editing technology in the *Cyanobacterium Anabaena* PCC 7120. ACS Synth Biol 8:170–180. https://doi.org/10.1021/acssynbio.8b00437

Orikasa Y, Nodasaka Y, Ohyama T et al (2010) Enhancement of the nitrogen fixation efficiency of genetically-engineered Rhizobium with high catalase activity. J Biosci Bioeng 110:397–402. https://doi.org/10.1016/j.jbiosc.2010.04.007

Paddon CJ, Westfall PJ, Pitera DJ et al (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 496:528–532. https://doi.org/10.1038/nature12051

Paul D, Pandey G, Jain RK (2005a) Suicidal genetically engineered microorganisms for bioremediation: need and perspectives. BioEssays 27:563–573. https://doi.org/10.1002/bies.20220

Paul D, Pandey G, Pandey J, Jain RK (2005b) Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol 23:135–142. https://doi.org/10.1016/j.tibtech.2005.01.001

Pfeifer BA, Khosla C (2001) Biosynthesis of polyketides in heterologous hosts. Microbiol Mol Biol Rev 65:106–118. https://doi.org/10.1128/MMBR.65.1.106-118.2001

Pieper DH, Reineke W (2000) Engineering bacteria for bioremediation. Curr Opin Biotechnol 11: 262–270. https://doi.org/10.1016/S0958-1669(00)00094-X

Pileggi M, Pileggi SAV, Sadowsky MJ (2020) Herbicide bioremediation: from strains to bacterial communities. Heliyon 6:e05767. https://doi.org/10.1016/j.heliyon.2020.e05767

Rai AK, Makishah NHA, Wen Z, Gupta G, Pandit S, Prasad R (2022) Recent developments in lignocellulosic biofuels, a renewable source of bioenergy. Fermentation 8:161. https://doi.org/10.3390/fermentation8040161

Rekadwad B, Ghosh PK (2018) Pseudomonas: a quorum sensing system for improved crop production. In: Kalia VC (ed) Quorum sensing and its biotechnological applications. Springer, Singapore, pp 181–191

Ronson CW, Bosworth A, Genova M et al (1990) Field release of genetically-engineered *Rhizobium meliloti* and *Bradyrhizobium japonicum* strains. In: Gresshoff PM, Roth LE, Stacey G, Newton WE (eds) Nitrogen fixation: achievements and objectives. Springer, Boston, pp 397–403

Rycroft T, Hamilton K, Haas CN, Linkov I (2019) A quantitative risk assessment method for synthetic biology products in the environment. Sci Total Environ 696:133940. https://doi.org/10.1016/j.scitotenv.2019.133940

Sands DC, Miller RV (1993) Evolving strategies for biological control of weeds with plant pathogens. Pest Sci 37:399–403. https://doi.org/10.1002/ps.2780370414

Sharma B, Dangi AK, Shukla P (2018) Contemporary enzyme-based technologies for bioremediation: a review. J Environ Manag 210:10–22. https://doi.org/10.1016/j.jenvman.2017.12.075

Singh R, Kumar M, Mittal A, Mehta PK (2017) Microbial metabolites in nutrition, healthcare and agriculture. 3 Biotech 7:15. https://doi.org/10.1007/s13205-016-0586-4

Singh D, Raina TK, Kumar A, Singh J, Prasad R (2019) Plant microbiome: a reservoir of novel genes and metabolites. Plant Gene. https://doi.org/10.1016/j.plgene.2019.100177

Song T-Q, Ding M-Z, Zhai F et al (2017) Engineering *Saccharomyces cerevisiae* for geranylgeraniol overproduction by combinatorial design. Sci Rep 7:14991. https://doi.org/10.1038/s41598-017-15005-4

Srinivasan P, Smolke CD (2019) Engineering a microbial biosynthesis platform for de novo production of tropane alkaloids. Nat Commun 10:3634. https://doi.org/10.1038/s41467-019-11588-w

Srivastava RK (2019) Bio-energy production by contribution of effective and suitable microbial system. Mater Sci Energy Technol 2:308–318. https://doi.org/10.1016/j.mset.2018.12.007

Stirling F, Silver PA (2020) Controlling the implementation of transgenic microbes: are we ready for what synthetic biology has to offer? Mol Cell 78:614–623. https://doi.org/10.1016/j.molcel.2020.03.034

Swainsbury DJK, Faries KM, Niedzwiedzki DM et al (2019) Engineering of B800 bacteriochlorophyll binding site specificity in the *Rhodobacter sphaeroides* LH2 antenna. Biochim Biophys Acta Bioenergy 1860:209–223. https://doi.org/10.1016/j.bbabio.2018.11.008

Tétard-Jones C, Edwards R (2016) Potential roles for microbial endophytes in herbicide tolerance in plants. Pest Manag Sci 72:203–209. https://doi.org/10.1002/ps.4147

Trantas EA, Koffas MAG, Xu P, Ververidis F (2015) When plants produce not enough or at all: metabolic engineering of flavonoids in microbial hosts. Front Plant Sci 6:7. https://doi.org/10.3389/fpls.2015.00007

Trivedi P, Schenk PM, Wallenstein MD, Singh BK (2017) Tiny microbes, big yields: enhancing food crop production with biological solutions. Microb Biotechnol 10:999–1003. https://doi.org/10.1111/1751-7915.12804

Tsuruta H, Paddon CJ, Eng D et al (2009) High-level production of amorpha-4, 11-diene, a precursor of the antimalarial agent artemisinin, in *Escherichia coli*. PLoS One 4:e4489

Valls M, de Lorenzo V, Gonzàlez-Duarte R, Atrian S (2000) Engineering outer-membrane proteins in *Pseudomonas putida* for enhanced heavy-metal bioadsorption. J Inorg Biochem 79:219–223. https://doi.org/10.1016/S0162-0134(99)00170-1

van der Does HC, Rep M (2007) Virulence genes and the evolution of host specificity in plant-pathogenic fungi. MPMI 20:1175–1182. https://doi.org/10.1094/MPMI-20-10-1175

Vinuselvi P, Lee SK (2012) Engineered *Escherichia coli* capable of co-utilization of cellobiose and xylose. Enzyme Microb Technol 50:1–4

Wood TL, Guha R, Tang L et al (2016) Living biofouling-resistant membranes as a model for the beneficial use of engineered biofilms. PNAS 113:E2802–E2811. https://doi.org/10.1073/pnas.1521731113

Wu M, Dick WA, Li W et al (2016) Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil. Int Biodeter Biodegr 107:158–164. https://doi.org/10.1016/j.ibiod.2015.11.019

Yuanfan H, Jin Z, Qing H et al (2010) Characterization of a fenpropathrin-degrading strain and construction of a genetically engineered microorganism for simultaneous degradation of methyl parathion and fenpropathrin. J Environ Manag 91:2295–2300. https://doi.org/10.1016/j.jenvman.2010.06.010

Zaldivar J, Nielsen J, Olsson L (2001) Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. Appl Microbiol Biotechnol 56:17–34. https://doi.org/10.1007/s002530100624

Zha J, Koffas MAG (2017) Production of anthocyanins in metabolically engineered microorganisms: current status and perspectives. Synth Syst Biotechnol 2:259–266. https://doi.org/10.1016/j.synbio.2017.10.005

Zhang J, Xu Y, Cao T et al (2017) Arsenic methylation by a genetically engineered Rhizobium-legume symbiont. Plant Soil 416:259–269. https://doi.org/10.1007/s11104-017-3207-z

Zhou K, Qiao K, Edgar S, Stephanopoulos G (2015) Distributing a metabolic pathway among a microbial consortium enhances production of natural products. Nat Biotechnol 33:377–383. https://doi.org/10.1038/nbt.3095

Zidack N, Tiourabaev K, Pilgeram A, et al. (2001) Valine excreting isolate of *Pseudomonas syringae* pv. tagetis exhibits enhanced virulence against Houndstongue (*Cynoglossum officinale*). Nato science series sub series I life and behavioural sciences, vol. 339, pp 279

# Chapter 11
# Extreme Microorganisms for Sustainable Agriculture

**Wei Yi, Zhang Ziyu, Huang Yuqian, Ram Prasad, and Shi-Hong Zhang**

**Abstract** Agriculture always faces many challenges, among which biotic and abiotic factors are concerned for their limiting productivity and quality of crops. To meet the human demands for the quantity and quality of agricultural products, sustainable agriculture with ecological and environment-friendly characteristics leads the direction of agricultural development. For a long time, however, people only paid more attention to agricultural productivity (yield mainly) but ignored the negative impacts of agricultural activities on the environment and ecology, resulting to the current agricultural destruction caused by such stressors as soil salinization, arid, plant disease, and pest which are getting even more serious. On the other hand, to obtain satisfied yield, more chemical fertilizers and pesticides have been applied in soils, which constitutes an uncontrollable vicious circle. Excessive use of pesticides and fertilizers not only causes environment pressure but also leads to a decline in the agricultural products, particularly a risk in the food safety. Microorganism-derived fertilizers and pesticides are alternative to chemically synthesized products. Extreme microorganisms, isolated from hyper-stressful environments, have robust vitality compared with ordinary organisms. Within the last few decades, a series of extreme microorganisms have been isolated from the unique locations such as Dead Sea, cold Antarctic, and volcano springs. A single microorganism represents a remarkable bioresource for soil bioremediation, plant growth promotion, and plant protection, but microorganism consortia play a synergistical role in obstacle environments. Thus, the application of the complete microbiomes or typical core microbiomes is going to be the key strategy for sustainable agriculture. In this chapter, we selectively introduced certain useful microorganisms living in the

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_11

different obstacle environments and highlighted their function and application in sustainable agriculture.

**Keywords** Extreme microorganism · Biofertilizer · Biopesticide · Soil remediation · Halotolerant · Halophilic

## 11.1 Introduction

Agriculture, one of the most important human activities relevant to economy, society, and environment, always faces many challenges, among which biotic and abiotic factors must be concerned for their limiting productivity and quality of crops. Sustainable agriculture is the leading direction of agricultural development. It is an agricultural development pattern by which we can make good use of agricultural natural resources, protect our ecological environment effectively, and realize good circulation of agricultural economy. It refers to an agricultural system that adopts a way of rational use and maintenance of natural resources and implements technological and institutional reforms to ensure the sustainable development of the demand for agricultural products. Sustainable agriculture continuously meets the contemporary human demand for the quantity and quality of agricultural products through the management, protection, and sustainable use of natural resources and the adjustment of farming systems and technologies, with less harming the interests of future generations. At the same time, sustainable agriculture is a technically appropriate and feasible agriculture that is economically dynamic and can be widely accepted by society. In brief, sustainable agriculture is an ecological circulation agriculture, which comprehensively considers both yield and quality of products and environmental protection.

Fertilizer is indispensable to the crops as well as to the development of agriculture, which boosted the production of crops since the populations on earth are developing in thousands of years. The earliest utilization of fertilizer to improve the crop production was the discovery of manure, which is by fermenting the human wastes and wastes in nature, such as feces of human and animals, bones, and decayed plants. The early period of human using fertilizer was pure organic and natural, it is like the discovery of a pattern that could enhance the food production, but they did not understand the principle of fertilizing. With the development of chemistry science and plant science, people start to understand the vital chemical elements that play roles in boosting the yield, such as nitrogen and phosphorus and potassium. German chemist Justus von Liebig had discovered the theory of plant mineral nutrition, which became the fundamental rule of modern chemical agriculture over the next 100 years. With the significant discovery in agriculture and organic chemistry, the growth of plant requires inorganic substances, such as carbonic acid, ammonia, magnesium oxide, phosphorus, nitric acid, and compounds of potassium. The spread of using chemical fertilizer rapidly developed and covered the world agricultural production. The crops rely on chemical fertilizer to boost yield unprecedentedly grown; on the contrary, the past traditional agriculture that relies on natural fertilizer or non-fertilizer fell into disuse rapidly.

Although in the development in agriculture, using chemical fertilizer is a great process of boosting the food production and yield, the overuse of chemical fertilizer that comes from human's greedy pursuit of agricultural production has caused severely negative impact to the soil and water and even the ecosystem, for example, the massive enhance in production and yield as well as the increase in profits brought by using chemical fertilizer. Also, driven by short-term benefits and the fact that chemical fertilizers have helped farmers increase crop yield, the producers of chemical fertilizer industry as well as the farmers who use chemical fertilizer, they all made the chemical fertilizers become the indispensable protagonist of a new age of agriculture. However, as time goes by, farmers started to discover that the needs of chemical fertilizer become larger and larger to get the same yield and crop production. And this is just the beginning of the negative impact of overuse of chemical fertilizer, when the situation of reduction of the crops becomes much more severe; farmers also notice that as the amount of chemical fertilizer increases, the yield effect begins to decline. The impact on the field yield is merely the superficial effects; the fundamental reason of the reduction of yield is that the diversity of soil microorganisms is destroyed due to the long-term overuse of monotonous chemical fertilizers, such as nitrogen fertilizer, potassium fertilizer, ammonia fertilizer, or phosphorus fertilizer; under this circumstance, the original balanced ecological chain of the microorganisms in the soil was destroyed; and the microbial population structure that could provide plant nutrients in a balanced way was simplified. This situation is perfectly explained by the law of "limiting factor" from the theory of mineral nutrition of plants from Justus von Liebig. For an organism to live and reproduce in a certain environment, it must get all the basic substances it needs, which vary according to the species and living conditions of the organism. In a steady state, in which the inflow and outflow of energy are in equilibrium, a base substance becomes a limiting factor when the availability of that base substance approaches the critical minimum required. When applying the limiting factor theory to the reduction caused by overusing chemical fertilizer, the reduction could be easily explained. To be specific, the crop yields do not depend on the need of the nutrients or the resources that can be easily absorbed in the nature, such as carbon dioxide and water; such limit is the scarcity of boron in soil, for instance, this requires us to improve crop yields and first consider the minimum factor limiting crop yield. And all these minimum factors are only regulated by soil microorganisms.

Chemical fertilizers and pesticides no doubt play important roles in ensuring stable, satisfying yields of agricultural products and promoting growth of the agricultural economy (Wang et al. 1996; Jin and Zhou 2018). However, fertilizers and pesticides used unscientifically in agriculture lead to unwanted consequences, such as degraded soil fertility, excessive pesticide residues, and agricultural source pollution. Particularly, the excessive use of pesticides and fertilizers influences the safety of the ecological environment, biodiversity, and agricultural production and further threatens human health and sustainable agricultural development (Xing and

Zhu 2000). Therefore, a safe alternative to fertilizers and pesticides is becoming increasingly urgent.

Sustainable agriculture is able to guard against the overuse of chemical fertilizers and pesticides. Specially, microorganism-based fertilizers and pesticides are alternative to chemically synthesized products. Biofertilizer, commonly defined as a substance which includes or involves the living organisms or microorganisms and is helpful in promoting the growth of the plant root system and seed germination, is the solution to stop the destruction of soil structure, water quality, and flora. The main characteristics of biofertilizer are natural and organic and on which the organic agriculture mainly depends. Biofertilizer makes nutrients that are naturally abundant in soil or atmosphere usable for plants and acts as supplements to agrochemicals; in addition, biofertilizers as essential components of organic farming play a vital role in maintaining long-term soil fertility and sustainability by fixing atmospheric dinitrogen, mobilizing fixed macro- and micronutrients, or converting insoluble phosphorus in the soil into available forms to plants, thereby increasing their efficiency and availability. The eco-friendly, easy-to-apply, nontoxic, and cost-effective nature is that biofertilizer has emerged as a highly potent alternative to chemical fertilizers (Giri et al. 2019). To sustainable agriculture, biofertilizers containing beneficial microorganisms or their metabolites are the best alternatives for replacing the conventional chemical-based fertilizers.

Like microbial fertilizers, biopesticides are defined as the directly used bioactive organisms or bioactive substances produced by organisms as pesticides, as well as synthetic pesticides with the same structure as natural compounds. Biopesticides include microbial pesticides, botanical pesticides, biochemical pesticides, genetically modified organisms, and natural enemy organisms. Beneficial microorganisms or their metabolites are the best alternatives for replacing the conventional chemical-based pesticides. Biological pesticides are generally natural or genetic modifiers, mainly including biopesticides and biochemical pesticides. Microbial pesticide is considered as the most promising alternative to chemical pesticides for its eco-friendly and economy. No matter microbial fertilizer or microbial pesticide, they are inseparable from microorganism. General microorganisms, including filamentous fungi, yeasts, bacteria, and actinomycetes, have been intensively studied and used in biofertilizer production and biological control of crop pests and diseases. However, in saline-alkaline fields, in which chemical fertilizer or pesticide overuse and other abiotic stress influenced soil, ordinary microorganism fertilizers and pesticides do not work effectively because they are unable to adapt to abiotic stress-affected environments. Under these circumstances, microorganisms originated from extreme environments are reasonable alternative.

Most halophilic organisms from different extreme environments have been isolated mainly from oceans or related places with a high concentration of salt, such as the famous Dead Sea (Moubasher et al. 1990; Xavier-Santos et al. 2004; Arakaki et al. 2014). In cold desert, Antarctic, or other specific extreme environments, many extreme organisms exist with specific resistance against the single extreme environmental factor, but also confer strong resistant ability to other extreme conditions. Till now, many studies on biodiversity and physiology have focused on the characterization of halophilic fungi present in saline and hypersaline ecosystems. Many species in ascomycetes and some in basidiomycetes have been described with the ability to grow in these environments (Butinar et al. 2005a, b).

Abiotic stresses that influence agriculture include soil salinity, drought, and extreme high or low temperatures. Second salinization or such as contaminations caused by overusing chemical fertilizers and pesticides actually belong to abiotic stress as well. Scientific utilization of beneficial extreme microorganisms is an important means to reduce harms to agriculture. Microbial application for amelioration of saline soils is gaining popularity due to its better amelioration and reduction in economic and environmental costs. Within the last few decades, a series of halophilic and alkaliphilic microorganisms have been identified. Parallelly, microorganisms isolated from drought and high-/low-temperature environments are also characterized. This chapter will focus on the extreme microorganisms and their synergistical roles in plant growth promotion, biotic stress protection, and soil remediation.

## 11.2 Halotolerant or Halophilic Microorganisms

Ancient Earth was covered in a global ocean (Burnham and Berry 2017). Studies on the microbial communities of deep subsurface sediments, saline lakes, or desert soils with variable salinity levels have indicated the presence of Bacteria and Archaea, containing a number of ubiquitous phyla including *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* (Fierer et al. 2009). A series of strains isolated from the vent of submarine volcano show not only halotolerance but also thermotolerance. In addition, *Pyrococcus abyssi*, *Sulfolobus acidocaldarius*, *Thermococcus kodakarensis*, and *Thermotoga neapolitana* make them best laboratory models for understanding the mechanisms that they have evolved to live under hostile environmental conditions. Another halotolerant *Exiguobacterium* strain was isolated from Salar de Huasco (Chilean Altiplano) and is ideal for the study on resistant mechanism and the evolution of adaptation (Remonsellez et al. 2018).

Early researches focused on prokaryotes that grow under salt stress and populate saline ecosystems such as Eubacteria, Archaea, and Algae (Oren 2002). Microbial eukaryotes also appeared in deep-sea subsurface sediments; and fungi are the most consistently detected eukaryotes in the marine sedimentary subsurface (Edgcomb et al. 2011). Most marine-derived fungi belong to halotolerant fungi which live in saline environments but do not necessarily require certain concentrations of salt; the rest of marine-derived fungi are classed as halophilic fungi because these fungi require salt concentrations of at least 0.3 M (sodium salt, e.g., NaCl) to grow optimally, and even they thrive in high-salt environments (Arakaki et al. 2014). Over the last two decades, marine fungi have been discovered accordingly in the saline environments such as in the Dead Sea, Atlantic Ocean, China Sea (Grishkan et al. 2003; Nazareth et al. 2012), and the solar salterns near to seacoast (Cantrell et al. 2006; Nayak et al. 2012). A large number of studies on biodiversity and physiology have focused on the characterization of halophilic fungi present in the sea-related saline and hypersaline ecosystems, among which *Ascomycetes* and *Basidiomycetes* have been described (Gunde-Cimermana et al. 2000; Butinar et al. 2005a, b; Evans et al. 2013; Gunde-Cimerman and Zalar 2014; Zajc et al. 2014a, b; Gonçalves et al. 2017). In general, fungal communities in hypersaline environments are dominated by *Aspergillus*, *Penicillium*, and some of their teleomorphic genera. Other genera such as *Alternaria*, *Cladosporium*, *Fusarium*, *Chaetomium*, *Wallemia*, and *Hortaea* were also reported (Gunde-Cimerman et al. 2001; Mandeel 2006; Moubasher et al. 2018). Some new species were also described from hypersaline environments including 3 *Wallemia* species (Zalar et al. 2005), 12 *Cladosporium* species (Zalar et al. 2007; Schubert et al. 2007), 2 *Emericella* species (Zalar et al. 2008), and 3 *Gymnoascus* species (Zhou et al. 2016). Similar to the communities observed in hypersaline environments (Buchalo et al. 1998, 2000; Gunde-Cimermana et al. 2000; Butinar et al. 2005a, b; Kis-Papo et al. 2003, 2014; Gunde-Cimerman and Zalar 2014), partial hypersaline fungal communities dominated by *Aspergillus* and *Penicillium* species with melanized dematiaceous forms are also observed in the soils of coastal zone or even inland lands (Moubasher et al. 1990).

Some special extreme environments are favored to isolate the halotolerant or halophilic microorganisms. A variety of filamentous fungi have been isolated from the Dead Sea, including *Gymnascella marismortui* isolated from the surface water down to a depth of 300 m (Buchalo et al. 1998). *G. marismortui* is adapted to high-salt conditions and requires high-salt concentrations (Buchalo et al. 1998, 2000). In addition, 476 fungal isolates were isolated consistently from the Dead Sea and probably form the stable core of the fungal community, including *Aspergillus terreus*, *A. sydowii*, *A. versicolor*, *Eurotium herbariorum*, *Penicillium westlingii*, *Cladosporium cladosporioides*, and *Cladosporium sphaerospermum*. However, most fungal isolates from the Dead Sea belong to the genera *Eurotium* and *Aspergillus* (Yan et al. 2005).

Lake Magadi is a hypersaline location in the East African Rift Valley, Kenya. Fifty-two fungal isolates in Lake Magadi were characterized with different pH, temperature, and salinity ranges, respectively (Orwa et al. 2020). These isolates were affiliated to 18 different genera with *Aspergillus*, *Penicillium*, *Cladosporium*, *Phoma*, and *Acremonium* being dominant. Interestingly, the different isolates could produce diverse extracellular enzymes, such as proteases, chitinases, cellulases, amylases, pectinases, and lipases. In addition, antimicrobial metabolites were noted for isolate 11M affiliated to *Penicillium chrysogenum* (99%). Cell-free extracts and crude extracts from isolate 11M had inhibitory effects on both animal and plant pathogens, indicating the promising application potential in biological protection.

China has remarkable biodiversity and many typical hypersaline environments. Research aimed at isolating and characterizing halotolerant or halophilic fungi from seas has progressed rapidly. A series of promising halophilic fungi, including *Aspergillus glaucus* CCHA, have been reported (Liu et al. 2011). Three marine-derived isolates were collected in Wenchang, Hainan Province, China, and identified as extremely halotolerant fungi: *Wallemia sebi* PXP-89 (Peng et al. 2011a), *P. chrysogenum* PXP-55 (Peng et al. 2011b), and *Cladosporium cladosporioides* PXP-49 (Xu et al. 2011). In addition, 188 marine-derived fungi were collected from the sediment in Zhoushan Sea area, the mangrove at Yunxiao Country and Jiulongjiang estuary in Fujian Province, China (Xiao et al. 2005), of which the ethyl acetate extract of strain 164 exhibited strong lethal effect on nematode *Rhabditis* sp. In another research, 31 nematode-trapping fungi recorded from mangrove habitat of Hong Kong were identified *Arthrobotrys*, *Monacrosporium*, and *Dactylella* (Swe et al. 2009). The South China Sea covers a vast area. The diversity of fungal communities in nine different deep-sea sediment samples of the South China Sea were isolated by culture-dependent methods followed by analysis of fungal internal transcribed spacer sequences (Zhang et al. 2013), in which 13 of 27 identified species were firstly reported. Moreover, three isolates might be novel phylotypes of genera *Ajellomyces*, *Podosordaria*, *Torula*, and *Xylaria*.

Many terrestrial halotolerant fungi have been characterized. Chamekh et al. (2019) identified 136 isolates from the soil of the Great Sebkha of Oran located in northwestern of Algeria. *Wallemia* sp. H15 and *Gymnoascus halophilus* H19 and H20 are obligatorily halophilic, but most isolates are halotolerant, which can still grow on PDA medium without NaCl, indicating the dominant flora of halotolerant fungi. Seventy-four percent of the strains could grow at 12.5% NaCl and five strains (*A. subramanianii* strain A1, *Aspergillus* sp. strain A4, *P. vinaceum*, and the two strains of *G. halophilus*) at 17.5%. The only strain that could grow at 20% was *Wallemia* sp. The optimum growth of most strains is 2.5% or 5% NaCl. The concentration of 10% is optimal for the growth of *G. halophilus*. The halophilic fungus *A. glaucus* CCHA from air-dried wild vegetation has been analyzed (Liu et al. 2011). This species shows extreme salt tolerance, with a salinity range of 5–32% (NaCl) required for growth. Interestingly, *A. glaucus* CCHA survives in solutions with a broad pH range of 2.0–11.5, indicating that it is a haloalkaliphilic fungus. Further investigation indicated that increasing the pH value (>8.0) can induce *A. glaucus* CCHA to produce a variety of organic acids, including citric acid, oxalic acid, and malic acid. In addition, *A. glaucus* CCHA shows resistance to aridity, heavy metal ions, and high temperature (Liu et al. 2011). The extremophilic nature of *A. glaucus* CCHA suggests that it has great promise in soil remediation applications (Fig. 11.1). Collectively, the large diversity of the fungal species, inhabiting high-salt environments, can be regarded either as halotolerant or as extremely halotolerant (Table 11.1).

## 11.3 Xerophilic, Psychrophilic, and Thermophilic Microorganisms

In the absence of water, lipids, proteins, and nucleic acids suffer structural damage of cells. The Atacama Desert located on the high northern Andean plains of Chile is one of the oldest, driest hot deserts on the Earth, while the Antarctic dry valleys are the

**Fig. 11.1** Mycoremediation of salt-affected soil using amendments supplemented with saline-tolerant fungi (*Aspergillus glaucus* CCHA and *Aspergillus terreus* (ratio = 1:1)). The area on the right received the soil amendments mixed with haloalkaliphilic fungi, but the area on the left received salt-sensitive isolates. The experiment was conducted in salt-affected soil in Dalian, Liaoning Province, China. The properties of the saline soil before organic amendments were heavy salt soil at a soil depth of 0–20 cm, NaCl = 6 g/kg, pH = 8.9, measured by Senlin Zhang and Yang Shi (2020). The photos were taken in 2020

coldest, driest places on Earth; and hot springs or volcanic vent around are extreme high-temperature environments. Research aimed at the understanding of this unique habitat and its diverse microbial ecosystems begun only a few decades ago. A milestone was a paper published in 2003, when the Atacama was shown to be a proper model of Mars. From then on, studies have been focused to examine every possible niche suitable for microbial life in this extreme environment. Habitats as different as the underside of quartz rocks, fumaroles at the Andes Mountains, and the inside of halite evaporates and caves of the Coastal Range, among others, have shown that life has found ingenious ways to adapt to extreme conditions such as low water availability (Azua-Bustos et al. 2012). All the same, diverse microorganisms still actively exist, including cyanobacteria, algae, lichens, and fungi.

Anhydrobiosis is a strategy organisms use to survive dry spells. During anhydrobiosis, cells come to contain only minimal amounts of water, and metabolic activity is rarely performed. A variety of organisms can become anhydrobiotic, including bacteria, yeast, and fungi. During the desiccation, less available water forces substances to increase in their concentration, which lead to stressful responses within a cell similar to those of a cell exposed to high salt. Microcolonial structures have been harvested from desert rock samples for cultivation and ultrastructural examination. It indicated that these microcolonial structures are fungi previously unrecognized as inhabitants of desert rocks (Staley et al. 1982).

**Table 11.1** The representative halotolerant fungi

| Species/strain | Source | [Na+] range | Reference |
|---|---|---|---|
| *Wallemia* sp. | Great Sebkha of Algeria | 2.5–20% | Chamekh et al. (2019) |
| *Aspergillus subramanianii* A1 | Great Sebkha of Algeria | 0–17.5% | Chamekh et al. (2019) |
| *Aspergillus* sp. strain A4 | Great Sebkha of Algeria | 0–17.5% | Chamekh et al. (2019) |
| *Penicillium vinaceum* | Great Sebkha of Algeria | 1–17.5% | Chamekh et al. (2019) |
| *Gymnoascus halophilus* S1-2 | Great Sebkha of Algeria | 2.5–17.5% | Chamekh et al. (2019) |
| *Aspergillus penicillioides* | Dead Sea, Israel | 10–30% | Nazareth et al. (2012) |
| *Eurotium herbariorum* | Dead Sea, Israel | 2–31% | Butinar et al. (2005a, b) |
| *Gymnascella marismortui* | Dead Sea, Israel | 5–30% | Buchalo et al. (1998) |
| *Sodiomyces* sp., *Acrostalagmus luteoalbus*, *Emericellopsis alkaline*, *Thielavia* sp., *Alternaria sect. Soda* | Russia, Mongolia, Kazakhstan, Kenya, Tanzania, Armenia | NR | Grum-Grzhimaylo et al. (2016) |
| *Hortaea werneckii* | Ljubljana | 5–31% | Gunde-Cimermana et al. (2000) |
| *Myrothecium* sp. IMER1 | Wuhan, China | 0–5% | Zhang et al. (2007) |
| *Aspergillus glaucus* CCHA | Dalian, China | 5–31 | Liu et al. (2011) |
| *Cladosporium cladosporioides* PXP-49 | Hainan, China | 0–20% | Xu et al. (2011) |
| *Wallemia sebi* PXP-89 | Hainan, China | 0–20% | Peng et al. (2011a) |
| *Penicillium chrysogenum* PXP-55 | Hainan, China | 0–20% | Peng et al. (2011b) |

Note: *NR* no report

Black microcolonial fungi and black yeasts are among the most stress-resistant eukaryotic organisms known on Earth. They mainly inhabit bare rock surfaces in hot and cold deserts of all regions of the Earth. The environment of those fungi is especially characterized by extreme changes from humidity to long periods of desiccation and extreme temperature differences. A key to the understanding of microcolonial fungi ecology is the question about metabolic activity versus dormancy in the natural environments (Zakharova et al. 2013). Black microcolonial fungi and black yeasts together with lichens and cyanobacteria are among the most stress-tolerant organisms on the Earth (Badali et al. 2008). Black microcolonial fungi can be found in the hot deserts of Arizona (USA) (Palmer et al. 1987) and the cold Antarctic deserts, in which 1604 fungal or pseudofungal records belonging to 135 genera and 232 species and infraspecific taxa are reported (Onofri et al. 2004). Cryptoendolithic communities in the Antarctic desert represent the limit of microbial life in Antarctica. Many microfungi living in these communities were isolated, most of which have the appearance of meristematic black yeasts (Azua-Bustos et al. 2012). In other extreme environments, a bacterial strain GFAJ-1 of the *Halomonadaceae*, isolated from Mono Lake, California, is able to substitute arsenic for phosphorus to sustain its growth (Wolfe-Simon et al. 2011), suggesting a unique life on Earth.

Such extreme microorganisms are very useful in assisting plants to overcome abiotic stress. *Bacillus thuringiensis* AZP2 and *Paenibacillus polymyxa* B isolated from hostile environments were used to induce drought resistance to wheat crops (Timmusk et al. 2014). Meanwhile, *Bacillus licheniformis* K11 has also been used to induce drought resistance by producing auxins and ACC deaminase (Lim and Kim 2013). Two bacterial strains (*Bacillus* sp. and *Enterobacter* sp.) were characterized with a high potential to lag the effects of drought on seedlings of wheat (*Triticum aestivum*) and corn (*Zea mays*) (Jochum et al. 2019), and *A. glaucus* CCHA can be used to remediate salt-affected soil (Wei and Zhang 2018).

## 11.4 Roles of Individual Extreme Microorganisms

Hydrolysis of fungal cell wall is the main weapon to break through the first barrier, to realize parasitism, further to complete antagonism, and ultimately to achieve the goal for biological control. Thus, the secretion of hydrolytic enzymes, such as chitinases, glucanases, and proteases (Gruber and Seidl-Seiboth 2012; Kubicek et al. 2011; Mandujano-González et al. 2016), plays a critical role in cell wall degradation of fungi (Seidl et al. 2009). According to previous studies and statistics, approximately 35% of crop yields are lost to diseases in the field, and about 70–80% of plant diseases are caused by fungal pathogens. Specially, chitin, glucan, and protein comprise the cell walls of many fungi, including some yeasts, and make up the structural frameworks of nematode as well as of plant pests.

*Trichoderma* spp. as the typical mycoparasite fungi have been successfully and widely used in agriculture practice and antagonize many phytopathogenic fungi through many mechanisms including mycoparasitism, antagonism, competition, and induced systemic resistance in related plants. Particularly, mycoparasitism is the most important biocontrol mechanism adopted by *Trichoderma* or other mycoparasites against plant pathogens. It involves tropic growth of biocontrol agent toward the target organism and then sets up interactions between mycelia of both mycoparasitic fungus and host fungus. Mycoparasitic fungus hypha coils around host fungus and finally causes dissolution of target pathogen hyphal cell wall or membrane by the activity of corresponding enzymes. Therefore, chitinases are mainly studied in *Trichoderma*, *Penicillium*, *Lecanicillium*, *Neurospora*, *Mucor*, *Metarhizium*, *Beauveria*, *Lycoperdon*, *Thermomyces*, and *Aspergillus*, which have been extensively studied (Krause et al. 2000; Haki and Rakshit 2003; Kristensen et al. 2008; Sarkar et al. 2010; Trincone 2010, 2011; Hamid et al. 2013).

Cell wall-degrading enzymes of *Trichoderma* is a promising alternative for inhibiting food storage diseases. In addition to chitinases, the other enzymes such as aspartic protease P6281 secreted by *T. harzianum* have been verified to be important in mycoparasitism on phytopathogenic fungi (Deng et al. 2018). The recombinant P6281 (rP6281) expressed in *Pichia pastoris* showed the high activity, whose activity was observed at pH 2.5 and 40 °C, and the enzyme was stable in the pH range of 2.5–6.0. rP6281 significantly inhibited spore germination and growth of plant and animal pathogenic fungi such as *Botrytis cinerea*, *Mucor circinelloides*, *A. fumigatus*, *A. flavus*, *Rhizoctonia solani*, and *Candida albicans*. Transmission electron microscopy revealed that rP6281 efficiently damages the cell wall of *B. cinerea*. In addition, the protease significantly inhibited the development of *B. cinerea* that causes rotting of apple, orange, and cucumber, indicating that rP6281 may be developed as an effective anti-mold agent for fruit storage.

The biological control efficacy is determined by the activity and stability of enzymes secreted from biocontrol agents. In previous practice of biocontrol of diseases, the short validity period of biological control agents and the difficulty of field inoculation have led to poor control effect. *Piriformospora indica*, a plant-root-colonizing basidiomycete fungus, has been isolated in the Indian Thar desert and was shown to provide strong growth-promoting activity during its symbiosis with a broad spectrum of plants (Verma et al. 1998; Prasad et al. 2013). *P. indica* that is a multiple functional and useful endophyte fungus has been intensively investigated in promoting plant growth, abiotic stress resistance, and disease resistance (Gill et al. 2016; Nath et al. 2016; Narayan et al. 2017; Bajaj et al. 2018). Meanwhile, the endophytic fungus *P. indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield (Waller et al. 2005).

The halotolerant or halophilic organisms living in high saline environment at most likely contain the series of enzymes with stable and specific activities. Actually, the specific conditions, such as salinity and pH extremes in high osmosis environment, make the fungal enzymes superior to homologous enzymes from terrestrial fungi (Jones 2000; Gomes et al. 2008; Madhu et al. 2009; Pang et al. 2011; Intriago 2012; Passarini et al. 2011; Rämä et al. 2014). Therefore, the first task is to screen biocontrol fungi from marine-derived fungi, which is the important strategy for solving abovementioned defects of biocontrol agents.

The diversity of fungal communities in nine different deep-sea sediment samples of the South China Sea was investigated (Zhang et al. 2013). Recently, 28 fungal strains have been isolated from different natural marine substrates of Italy sea and plate screened for their production of chitinolytic activity (Pasqualetti et al. 2019). Two apparently best producers are *Trichoderma lixii* IG127 and *Clonostachys rosea* IG119, in which IG127 appeared to be a slight halotolerant fungus, while *C. rosea* IG119 clearly showed to be a halophilic marine fungus (Pasqualetti et al. 2019).

The beneficial effect of microbial application on saline-alkali soil has been reported by Sahin et al. (2011). Suspension mixture composed of three fungal isolates (*Aspergillus* FS 9, 11 and *Alternaria* FS 8) and two bacterial strains (*Bacillus subtilis* OSU 142 and *Bacillus megaterium* M3) were used with leaching water and applied to the soil columns in the Igdir plain of northeastern Turkey (Sahin et al. 2011). Gypsum is an economical alternative for replacing sodium with calcium in remediating saline-alkali soils (Gharaibeh et al. 2009; Oad et al. 2002). In the experimental process, gypsum was applied for the saline-alkali soil pretreatment, and the microorganisms are not halotolerant or halophilic (Aslantas et al. 2007; Turan et al. 2006). Thus, the final results should not be out of the function of microbes. Anyway, an enlightened example for mycoremediation of saline-alkali soil by using haloalkaliphilic fungi was exemplary. Actually, in our lab, we also got the similar results in mycoremediation salt-affected soil by using salt-tolerant fungal groups (Fig. 11.1). Haloalkaliphilic fungus *Aspergillus glaucus* CCHA, a fungal species with extreme tolerance to saline and alkaline conditions, has significant potential value in industrial and agricultural applications. Our group has been assessing the potential of *A. glaucus* CCHA in the mycoremediation of saline-alkaline soil in the Songnen plain of northeastern China (one of the three most famous saline and alkaline lands in the world) for 6 years (Shi and Zhu 2016). This study primarily indicates that the applied amendments mixed with haloalkaliphilic fungi significantly encourage steady growth and yield of rice in comparison to that achieved in the control plot.

## 11.5 Synergistical Role of Extreme Microbiomes

Global patterns were reported in belowground communities (Fierer et al. 2009). Microbial consortia seem to function synergistically and are able to compete for certain ecological niches. Therefore, the inoculation with microbial consortia is a more effective approach than inoculation with a single strain (Berendsen et al. 2018; Woo and Pepe 2018). Microbial consortia are promising probiotics as plant biostimulants for sustainable agriculture (Woo and Pepe 2018). The role of a simplified synthetic microbial consortium formed by seven strains of four phyla identified by culture-dependent techniques was evaluated to be very useful (Niu et al. 2017). A greatly simplified synthetic bacterial community was assembled by maize roots, which consist of seven strains (*Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Ochrobactrum pituitosum*, *Herbaspirillum frisingense*, *Pseudomonas putida*, *Curtobacterium pusillum*, and *Chryseobacterium indologenes*) representing three of the four most dominant phyla found in maize roots (Niu et al. 2017). The elimination of *E. cloacae* led to the complete loss of the community, suggesting an important role of the key species in the functioning of the total community. In another investigation, a plant-beneficial bacterial consortium was associated with disease-induced assemblage (Berendsen et al. 2018) and bioprospecting derived from plant-associated microbiomes (Müller et al. 2016).

Similarly, plant microbiome is gaining considerable interest since they play an important role in the regulation of plant metabolism (Pieterse et al. 2014; Müller et al. 2016). Microbiome selected from the tenth generation of *A. thaliana* was inoculated in A. *thaliana* and *Brassica rapa* soils, whose characteristics transferred (Panke-Buisse et al. 2015). Specially, systemic resistance was induced by beneficial microbes (Pieterse et al. 2014). And selection on soil microbiomes also reveals reproducible impacts on plant function (Panke-Buisse et al. 2015). Therefore, engineering microbiomes is expected to improve plant and animal health (Mueller and Sachs 2015).

The native plant microbiome of extreme environments could represent an unequaled source of stress-ameliorating microorganism, and the natural microbiome engineering by using extreme microbiome could represent a promising and eco-friendly alternative to ensure the global food security. Plant holobiome has been developed over the centuries to adapt to the different terrestrial biomes. Particularly, cold environments, such as Antarctic, and dry environments, such as deserts, have aroused great curiosity regarding the assembly of microbial communities and microbe-plant interactions. Microbial ecology of hot desert was edaphic systems (Makhalanyane et al. 2015). The Atacama Desert in Chile, considered the driest in the world, has a great microbial diversity that is still largely unknown taxonomically (Bull et al. 2016). Some bacterial groups and their activity can influence the growth and flowering of native plants (Araya et al. 2020; Astorga-Eló et al. 2020). Although the study of the microbiome in extreme environments is still an incipient area, some authors have begun to identify the complex interactions between the microbiome and vegetation associated with these hostile environments.

Drought-tolerant plant growth-promoting rhizobacteria were associated with foxtail millet in a semiarid and their potential in alleviating drought stress (Niu et al. 2018). Some plant-associated microbiomes have been identified in deserts habitats. For example, microbiomes of three *Agave* species were identified and distributed in central Mexico and in southern California, which are capable of conferring resistance to high temperatures and low water availability (Coleman-Derr et al. 2016). Moreover, the holobiome of succulent plants native to arid and semiarid ecosystems also represents microorganisms capable of conferring drought resistance (Fonseca-García et al. 2016). Bacterial communities present in desert soils typically contain a number of ubiquitous phyla including *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* (Fierer et al. 2009). Prokaryotic community structure and metabolisms in shallow subsurface of Atacama Desert play as alluvial fans after heavy rains to repair and prepare for next dry period (Fernández-Martínez et al. 2019). In the case of fungi, most of the studies have identified phyla that included members of *Basidiomycota* and *Ascomycota*, with high taxonomic diversity and consideration as both thermophilic and thermotolerant fungi (Fierer et al. 2012; Makhalanyane et al. 2015). Archeal taxa are relatively rare across many environments, but seem to be particularly abundant in desert soils, such as Thaumarchaeota as the most representative phylum (Fierer et al. 2012; Marusenko et al. 2013). In addition, ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands (Marusenko et al. 2013). Endophytic fungi with dematiaceous septate hyphae capable of mineralizing peptides and amino acids have been found in the rhizosphere of *D. antarctica*, indicating that they increase the availability of nitrogen for the plant (Upson et al. 2009). Similarly, cold desert such as Antarctic has also been studied. The Antarctic pristine environment is the most extreme land on the planet and represents an interesting and unique habitat for the colonization and survival of microbial life.

Several studies related with PGPM isolated from extreme environment have been widely reported, for example, the isolated native bacteria from rhizospheric arid soils, and evaluated both growth-promoting capabilities and antagonistic potential against fungi and phytopathogenic nematodes (El-Sayed et al. 2014). The bacteria exhibited capacities to fix atmospheric nitrogen; produce ammonia, indole-3-acetic acid (IAA), and siderophores; solubilize phosphate and zinc; and showed a potential antagonist against some phytopathogenic fungi and a species of nematodes (*Meloidogyne incognita*) to varying degrees. Endophytic fungi present in *C. quitensis* modulate the content of salicylic acid, jasmonate, indole-3-acetate, and ABA in shoot tissue of plants exposed to UV-B radiation, which indicate that these endophytic fungi could modulate the hormonal content of *C. quitensis* to improve its ecophysiological performance under high UV-B radiation. Moreover, bacterial strains of foxtail millet (*Setaria italica* L.) were identified from a drought-tolerant crop grown in semiarid regions in northeast China (Niu et al. 2018). Four isolated strains had the ability to generate ACC deaminase, as well as tolerance to drought. PGPM (*Bacillus cereus* and *Planomicrobium chinense*) isolated from the rhizosphere of rained area (Karak) in Pakistan were combined with salicylic acid to improve sunflower resistance (Khan et al. 2018). Moreover, endophytic symbiont yeasts (*Cryptococcus victoriae*, *Cystobasidium laryngis*, *Rhodotorula mucilaginosa*, *Sporidiobolus ruineniae*, and *Leucosporidium golubevii*) have been identified in leaves of both vascular plants that could directly or indirectly promote the fitness of host plants (Santiago et al. 2017).

The phenotype of *Triticum aestivum* subsp. *aestivum* was possibly modified using host-mediated microbiome engineering as a strategy to improve the crop resistance to drought stress (Jochum et al. 2019). This phenomenon could eventually be explained to be associated with different abiotic stress such as high radiation and freezing, among others. Moreover, the rhizobacterium *Bacillus subtilis* can promote the biofilms formation in the roots and consequently increase plant defenses (Rudrappa et al. 2008). On the other hand, plant root-secreted malic acid was found to recruit beneficial soil bacteria (Rudrappa et al. 2008). *Arabidopsis thaliana* recruit three bacterial phylum (*Proteobacteria*, *Firmicutes*, and *Bacteroidetes*) in the rhizosphere after activation of foliar defense by the downy mildew pathogen (Berendsen et al. 2018). Plant growth-promoting traits and phylogenetic affiliation of rhizobacteria were associated with wild plants grown in arid soil in vitro antagonistic activity (El-Sayed et al. 2014). In the case of emerging infectious disease, lichen-forming fungi isolated from *Everniastrum cirrhatum* lichen have important antimicrobial properties against *Fusarium moniliforme*, *F. oxysporum*, and *F. udum* (Javeria et al. 2014).

Although little knows about the microbial diversity of desert environments, new metagenomic data have shown the functional diversity and a large abundance of genes involved in biogeochemical cycles, which has much less than other terrestrial biomes and could generate functional trophic chains (Makhalanyane et al. 2015). Cross-biome metagenomic analyses of soil microbial communities can expectedly understand their functional attributes (Fierer et al. 2012).

## 11.6 Remarks and Prospects

Fertile soil is a vital complex that involves numerous species and immense biomass; soil organisms, particularly soil fungi, have significant effects on the soil ecosystem. Soil inhabitant fungi build a metabolic bridge between insoluble organic matter and soil nutrients by degrading cellulose; and soil microbiomes perform systemically multiple biological processes.

Within the last few decades, a series of extreme microorganisms have been characterized in some unique locations. Individually, such microorganism represents a remarkable bioresource for certain stress adaption, contaminated soil remediation, plant growth promotion, or disease and pest protection, but microorganisms with different roles play function synergistically in a defined extreme environment. Therefore, the identification and application of the complete microbiomes or typical core microbiomes is going to be the key strategy for sustainable agriculture. Agriculture based on beneficial extreme environmental microorganisms or complete microbiomes is sustainable agriculture, which is also organic agriculture for human health.

**Acknowledgments** The related work in our lab was partially supported by grants from the National Natural Science Foundation of China (Grant Nos. 31671972 and 31670141) and a project of the Local scientific research of Department of Education of Liaoning Province of China (Grant No. 01032920021 to S.-H.Z.) and special talent introduction of Shenyang Agricultural University of China (Grant No. 880420019 to S.-H.Z.). The authors would like to thank the Zhang Lab members (Jilin University and Shenyang Agricultural University), who contributed to fungal isolation and field trials, as well as thank Dr. Liang Yue (SYAU) for his improvement and suggestions on the whole article.

## References

Arakaki RL, Monteiro DA, Boscolo M, Dasilva R, Gomes E (2014) Halotolerance, ligninase production and herbicide degradation ability of basidiomycetes strains. Braz J Microbiol 44(4):1207–1214. https://doi.org/10.1590/S1517-83822014005000014

Araya JP, González M, Cardinale M, Schnell S, Stoll A (2020) Microbiome dynamics associated with the atacama flowering desert. Front Microbiol 10:3160. https://doi.org/10.3389/fmicb.2019.03160

Aslantas R, Cakmakci R, Sahin F (2007) Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. Sci Hortic 111:371–377

Astorga-Eló M, Zhang Q, Larama G, Stoll A, Sadowsky MJ, Jorquera MA (2020) Composition, predicted functions and co-occurrence networks of rhizobacterial communities impacting flowering desert events in the Atacama desert, Chile. Front Microbiol 11:571. https://doi.org/10.3389/fmicb.2020.00571

Azua-Bustos A, Urrejola C, Vicuña R (2012) Life at the dry edge: microorganisms of the Atacama desert. FEBS Lett 586(18):2939–2945. https://doi.org/10.1016/j.febslet.2012.07.025

Badali H, Gueidan C, Najafzadeh MJ, Bonifaz A, van den Ende AH, de Hoog GS (2008) Biodiversity of the genus Cladophialophora. Stud Mycol 61:175–191. https://doi.org/10.3114/sim.2008.61.18

Bajaj R, Huang Y, Gebrechristos S, Mikolajczyk B, Brown H, Prasad R, Varma A, Bushley K (2018) Transcriptional responses of soybean roots to colonization with the root endophytic fungus *Piriformospora indica* reveals altered phenylpropanoid and secondary metabolism. Sci Rep 8:10227. https://doi.org/10.1038/s41598-018-26809-3

Berendsen RL, Vismans G, Yu K, Song Y, de Jonge R, Burgman WP, Burmølle M, Herschend J, Bakker PAHM, Pieterse CMJ (2018) Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J 12(6):1496–1507. https://doi.org/10.1038/s41396-018-0093-1

Buchalo AS, Nevo E, Wasser SP, Oren A, Molitoris HP (1998) Fungal life in the extremely hypersaline water of the Dead Sea: first records. Proc Biol Sci 265(1404):1461–1465. https://doi.org/10.1098/rspb.1998.0458

Buchalo AS, Nevo E, Wasser SP, Oren A, Molitoris HP, Volz PA (2000) Fungi discovered in the Dead Sea. Mycol Res News 104:132–133. https://doi.org/10.1080/mmy.38.1.91.96

Bull AT, Asenjo JA, Goodfellow M, Gómez-Silva B (2016) The Atacama desert: technical resources and the growing importance of novel microbial diversity. Annu Rev Microbiol 70: 215–234. https://doi.org/10.1146/annurev-micro-102215-095236

Burnham AD, Berry AJ (2017) Formation of Hadean granites by melting of igneous crust. Nat Geosci 10(6):457–461. https://doi.org/10.1038/ngeo2942

Butinar L, Sonjak S, Zalar P, Plemenitas A, Gunde-Cimerman N (2005a) Melanized halophilic fungi are eukaryotic members of microbial communities in hypersaline waters of solar salterns. Bot Mar 48:73–79. https://doi.org/10.1515/BOT.2005.007

Butinar L, Zalar P, Frisvad JC, Gunde-Cimerman N (2005b) The genus Eurotium—members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol Ecol 51(2): 155–166. https://doi.org/10.1016/j.femsec.2004.08.002

Cantrell SA, Casillas-Martínez L, Molina M (2006) Characterization of fungi from hypersaline environments of solar salterns using morphological and molecular techniques. Mycol Res 110 (Pt 8):962–970. https://doi.org/10.1016/j.mycres.2006.06.005

Chamekh R, Deniel F, Donot C, Jany JL, Nodet P, Belabid L (2019) Isolation, identification and enzymatic activity of halotolerant and halophilic fungi from the Great Sebkha of Oran in northwestern of Algeria. Mycobiology 47(2):230–241. https://doi.org/10.1080/12298093.2019.1623979

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytol 209(2):798–811. https://doi.org/10.1111/nph.13697

Deng JJ, Huang WQ, Li ZW, Lu DL, Zhang Y, Luo XC (2018) Biocontrol activity of recombinant aspartic protease from Trichoderma harzianum against pathogenic fungi. Enzym Microb Technol 112:35–42. https://doi.org/10.1016/j.enzmictec.2018.02.002

Edgcomb VP, Beaudoin D, Gast R, Biddle JF, Teske A (2011) Marine subsurface eukaryotes: the fungal majority. Environ Microbiol 13(1):172–183. https://doi.org/10.1111/j.1462-2920.2010.02318.x

El-Sayed WS, Akhkha A, El-Naggar MY, Elbadry M (2014) In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Front Microbiol 5:651. https://doi.org/10.3389/fmicb.2014.00651

Evans S, Hansen RW, Schneegurt MA (2013) Isolation and characterization of halotolerant soil fungi from the great salt plains of Oklahoma. Cryptogam Mycol 34(4):329

Fernández-Martínez MÁ, Dos Santos SR, Moreno-Paz M, Gallardo-Carreño I, Blanco Y, Warren-Rhodes K, García-Villadangos M, Ruiz-Bermejo M, Barberán A, Wettergreen D, Cabrol N, Parro V (2019) Prokaryotic community structure and metabolisms in shallow subsurface of Atacama desert playas and alluvial fans after heavy rains: repairing and preparing for next dry period. Front Microbiol 10:1641. https://doi.org/10.3389/fmicb.2019.01641

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. Ecol Lett 12(11):1238–1249. https://doi.org/10.1111/j.1461-0248.2009.01360.x

Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL, Owens S, Gilbert JA, Wall DH, Caporaso JG (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci U S A 109(52):21390–21395. https://doi.org/10.1073/pnas.1215210110

Fonseca-García C, Coleman-Derr D, Garrido E, Visel A, Tringe SG, Partida-Martínez LP (2016) The cacti microbiome: interplay between habitat-filtering and host-specificity. Front Microbiol 7:150. https://doi.org/10.3389/fmicb.2016.00150

Gharaibeh MA, Eltaif NI, Shunnar OF (2009) Leaching and reclamation of calcareous saline–sodic soil by moderately saline and moderate-SAR water using gypsum and calcium chloride. J Plant Nutr Soil Sci 172:713–719. https://doi.org/10.1002/jpln.200700327

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:332. https://doi.org/10.3389/fmicb.2016.00332

Giri B, Prasad R, Wu Q-S, Varma A (2019) Biofertilizers for sustainable agriculture and environment. Springer International Publishing. isbn:978-3-030-18932-7. https://www.springer.com/gp/book/9783030189327

Gomes DN, Cavalcanti MA, Fernandes MJ, Lima DM, Passavante JZ (2008) Filamentous fungi isolated from sand and water of "Bairro Novo" and "Casa Caiada" beaches, Olinda, Pernambuco, Brazil. Braz J Biol 68(3):577–582. https://doi.org/10.1590/s1519-69842008000300016

Gonçalves VN, Vitoreli GA, de Menezes GCA, Mendes CRB, Secchi ER, Rosa CA, Rosa LH (2017) Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. Extremophiles 21(6):1005–1015. https://doi.org/10.1007/s00792-017-0959-6

Grishkan I, Nevo E, Wasser SP (2003) Soil micromycete diversity in the hypersaline Dead Sea coastal area, Israel. Mycol Progress 2:19–28. https://doi.org/10.1007/s11557-006-0040-9

Gruber S, Seidl-Seiboth V (2012) Self versus non-self: fungal cell wall degradation in *Trichoderma*. Microbiology (Reading) 158(Pt 1):26–34. https://doi.org/10.1099/mic.0.052613-0

Grum-Grzhimaylo AA, Georgieva ML, Bondarenko SA, Debets AJM, Bilanenko EN (2016) On the diversity of fungi from soda soils. Fungal Divers 76:27–74. https://doi.org/10.1007/s13225-015-0320-2

Gunde-Cimerman N, Cerovac S, Zalar P (2001) Biodiversity of filamentous fungi in the saltpans Secovlje. Acta Biologica Slovenica (Slovenia) 44:25–30

Gunde-Cimerman N, Zalar P (2014) Extremely halotolerant and halophilic fungi inhabit brine in solar salterns around the globe. Food Technol Biotechnol 52:170–179. https://doi.org/10.1021/jf5012819

Gunde-Cimermana N, Zalarb P, de Hoogc S, Plemenitasd A (2000) Hypersaline waters in salterns—natural ecological niches for halophilic black yeasts. FEMS Microbiol Ecol 32(3):235–240. https://doi.org/10.1111/j.1574-6941

Haki GD, Rakshit SK (2003) Developments in industrially important thermostable enzymes: a review. Bioresour Technol 89(1):17–34. https://doi.org/10.1016/s0960-8524(03)00033-6

Hamid R, Khan MA, Ahmad M, Ahmad MM, Abdin MZ, Musarrat J, Javed S (2013) Chitinases: an update. J Pharm Bioallied Sci 5(1):21–29. https://doi.org/10.4103/0975-7406.106559

Intriago P (2012) Marine microorganisms: perspectives for getting involved in cellulosic ethanol. AMB Express 2(1):46. https://doi.org/10.1186/2191-0855-2-46

Javeria S, Shahi SK, Shahi MP, Yadava YK (2014) Isolation of lichen forming fungus of Everniastrum cirrhatum and evaluate its antagonistic and antimicrobial activity. Am J Ethnomed 1:361–367

Jin S, Zhou F (2018) Zero growth of chemical fertilizer and pesticide use: China's objectives, Progress and challenges. J Resour Ecol 9(1):50–58. https://doi.org/10.5814/j.issn.1674-764x.2018.01.006

Jochum MD, McWilliams KL, Borrego EJ, Kolomiets MV, Niu G, Pierson EA, Jo YK (2019) Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. Front Microbiol 10:2106. https://doi.org/10.3389/fmicb.2019.02106

Jones EBG (2000) Marine fungi: some factors influencing biodiversity. Fungal Divers 4:53–73

Khan N, Zandi P, Ali S, Mehmood A, Shahid MA (2018) Impact of salicylic acid and PGPR on the drought tolerance and phytoremediation potential of helianthus annus. Front Microbiol 9:2507. https://doi.org/10.3389/fmicb.2018.02507

Kis-Papo T, Oren A, Wasser SP, Nevo E (2003) Survival of filamentous fungi in hypersaline Dead Sea water. Microb Ecol 45(2):183–190

Kis-Papo T, Weig AR, Riley R, Peršoh D, Salamov A, Sun H et al (2014) Genomic adaptations of the halophilic Dead Sea filamentous fungus Eurotium rubrum. Nat Commun 5(1):1–8

Krause A, Stoye J, Vingron M (2000) The SYSTERS protein sequence cluster set. Nucleic Acids Res 28(1):270–272. https://doi.org/10.1093/nar/28.1.270

Kristensen JB, Meyer RL, Laursen BS, Shipovskov S, Besenbacher F, Poulsen CH (2008) Antifouling enzymes and the biochemistry of marine settlement. Biotechnol Adv 26(5): 471–481. https://doi.org/10.1016/j.biotechadv.2008.05.005

Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M et al (2011) Genome Biol 12(4):R40. https://doi.org/10.1186/gb-2011-12-4-r40

Lim JH, Kim SD (2013) Induction of drought stress resistance by multi-functional PGPR Bacillus licheniformis K11 in pepper. Plant Pathol J 29(2):201–208. https://doi.org/10.5423/PPJ.SI.02.2013.0021

Liu XD, Liu JL, Wei Y, Tian YP, Fan FF, Pan HY, Zhang SH (2011) Isolation, identification and biologic characteristics of an extreme halotolerant Aspergillus sp. J Jilin Univ (In Chinese; abstract in English.) 9:548–552. https://doi.org/10.3724/SP.J.1011.2011.00462

Madhu KM, Beena PS, Chandrasekaran M (2009) Extracellular beta-glucosidase production by a marine *Aspergillus sydowii* BTMFS 55 under solid state fermentation using statistical experimental design. Biotechnol Bioprocess Eng 14(4):457–466. https://doi.org/10.1007/s12257-008-0116-2

Makhalanyane TP, Valverde A, Gunnigle E, Frossard A, Ramond JB, Cowan DA (2015) Microbial ecology of hot desert edaphic systems. FEMS Microbiol Rev 39(2):203–221. https://doi.org/10.1093/femsre/fuu011

Mandeel QA (2006) Biodiversity of the genus Fusarium in saline soil habitats. J Basic Microbiol 46(6):480–494. https://doi.org/10.1002/jobm.200510128

Mandujano-González V, Villa-Tanaca L, Anducho-Reyes MA, Mercado-Flores Y (2016) Secreted fungal aspartic proteases: a review. Rev Iberoam Micol 33(2):76–82. https://doi.org/10.1016/j.riam.2015.10.003

Marusenko Y, Bates ST, Anderson I, Johnson SL, Soule T, Garcia-Pichel F (2013) Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. Ecol Process 2:1–10. https://doi.org/10.1186/2192-1709-2-9

Moubasher AH, Abdel-Hafez SII, Bagy MMK, Abdel-Satar MA (1990) Halophilic and halotolerant fungi in cultivated desert and salt marsh soils from Egypt. Acta Mycol 26:65–81. https://doi.org/10.5586/am.1990.013

Moubasher AAH, Abdel-Sater MA, Soliman ZSM (2018) Diversity of yeasts and filamentous fungi in mud from hypersaline and freshwater bodies in Egypt. Czech Mycol 70:1–32

Mueller UG, Sachs JL (2015) Engineering microbiomes to improve plant and animal health. Trends Microbiol 23(10):606–617. https://doi.org/10.1016/j.tim.2015.07.009

Müller CA, Obermeier MM, Berg G (2016) Bioprospecting plant-associated microbiomes. J Biotechnol 235:171–180. https://doi.org/10.1016/j.jbiotec.2016.03.033

Narayan OP, Verma N, Singh AK, Oelmüller R, Kumar M, Prasad D, Kapoor R, Dua M, Johri AK (2017) Antioxidant enzymes in chickpea colonized by *Piriformospora indica* participate in defense against the pathogen *Botrytis cinerea*. Sci Rep 7(1):13553. https://doi.org/10.1038/s41598-017-12944-w

Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. Front Plant Sci 7:1574. https://doi.org/10.3389/fpls.2016.01574

Nayak SS, Gonsalves V, Nazareth SW (2012) Isolation and salt tolerance of halophilic fungi from mangroves and solar salterns in Goa–India. Indian J Mar Sci 41(2):164–172. https://doi.org/10.1029/2011JC007201

Nazareth S, Gonsalves V, Nayak S (2012) A first record of obligate halophilic aspergilli from the dead sea. Indian J Microbiol 52(1):22–27. https://doi.org/10.1007/s12088-011-0225-z

Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. Proc Natl Acad Sci U S A 114(12):E2450–E2459. https://doi.org/10.1073/pnas.1616148114

Niu X, Song L, Xiao Y, Ge W (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol 8:2580. https://doi.org/10.3389/fmicb.2017.02580

Oad FC, Samo MA, Soomro A, Oad DL, Oad NL, Siyal AG (2002) Amelioration of salt affected soils. Pak J Appl Sci 2:1–9. https://doi.org/10.3923/jas.2002.1.9

Onofri S, Selbmann L, Zucconi L, Tosi S, de Hoog GS (2004) The mycota of continental Antarctica. Terra Antarct Rep 1:32–42

Oren A (2002) Halophilic microorganisms and their environments. In: Seckbach J (ed) Cellular origin & life in extreme habitats & astrobiology. Kluwer Academic Publishers, Dordrecht. https://doi.org/10.1007/0-306-48053-0

Orwa P, Mugambi G, Wekesa V, Mwirichia R (2020) Isolation of haloalkaliphilic fungi from Lake Magadi in Kenya. Heliyon 6(1):e02823. https://doi.org/10.1016/j.heliyon.2019.e02823

Palmer FE, Emery DR, Stemmler J, Staley JT (1987) Survival and growth of microcolonial rock fungi as affected by temperature and humidity. New Phytol 107:155–162. https://doi.org/10.2307/2434887

Pang KL, Chow RKK, Chan C, Vrijmoed LLP (2011) Diversity and physiology of marine lignicolous fungi in arctic waters: a preliminary account. Polar Res 30:1–5. https://doi.org/10.3402/polar.v30i0.5859

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(4):980–989. https://doi.org/10.1038/ismej.2014.196

Pasqualetti M, Barghini P, Giovannini V, Fenice M (2019) High production of chitinolytic activity in halophilic conditions by a new marine strain of Clonostachys rosea. Molecules 24(10):1880. https://doi.org/10.3390/molecules24101880

Passarini MR, Rodrigues MV, da Silva M, Sette LD (2011) Marine-derived filamentous fungi and their potential application for polycyclic aromatic hydrocarbon bioremediation. Mar Pollut Bull 62(2):364–370. https://doi.org/10.1016/j.marpolbul.2010.10.003

Peng X, Wang Y, Sun K, Liu P, Yin X, Zhu W (2011a) Cerebrosides and 2-pyridone alkaloids from the halotolerant fungus *Penicillium chrysogenum* grown in a hypersaline medium. J Nat Prod 74(5):1298–1302. https://doi.org/10.1021/np1008976

Peng XP, Wang Y, Liu PP, Hong K, Chen H, Yin X, Zhu WM (2011b) Aromatic compounds from the halotolerant fungal strain of Wallemia sebi PXP-89 in a hypersaline medium. Arch Pharm Res 34(6):907–912. https://doi.org/10.1007/s12272-011-0607-0

Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Prasad R, Kamal S, Sharma PK, Oelmueller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. J Basic Microbiol 53(12):1016–1102

Rämä T, Norden J, Davey ML, Mathiassen GH, Spatafora JW, Kauserud H (2014) Fungi ahoy! Diversity on marine wooden substrata in the high North. Fungal Ecol 8:46–58. https://doi.org/10.1016/j.funeco.2013.12.002

Remonsellez F, Castro-Severyn J, Pardo-Esté C, Aguilar P, Fortt J, Salinas C, Barahona S, León J, Fuentes B, Areche C, Hernández KL, Aguayo D, Saavedra CP (2018) Characterization and salt response in recurrent halotolerant Exiguobacterium sp. SH31 isolated from sediments of Salar de Huasco, Chilean Altiplano. Front Microbiol 9:2228. https://doi.org/10.3389/fmicb.2018.02228

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148(3):1547–1556. https://doi.org/10.1104/pp.108.127613

Sahin U, Eroğlum S, Sahin F (2011) Microbial application with gypsum increases the saturated hydraulic conductivity of saline–sodic soils. Appl Soil Ecol 48:247–250. https://doi.org/10.1016/j.apsoil.2011.04.001

Santiago IF, Rosa CA, Rosa LH (2017) Endophytic symbiont yeasts associated with the Antarctic angiosperms Deschampsia antarctica and Colobanthus quitensis. Polar Biol 40(1):177–183

Sarkar S, Pramanik A, Mitra A, Mukherjee J (2010) Bioprocessing data for the production of marine enzymes. Mar Drugs 8(4):1323–1372. https://doi.org/10.3390/md8041323

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, de Hoog GS, Crous PW (2007) Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardization of methods for Cladosporium taxonomy and diagnostics. Stud Mycol 58:105–156. https://doi.org/10.3114/sim.2007.58.05

Seidl V, Song L, Lindquist E, Gruber S, Koptchinskiy A, Zeilinger S, Schmoll M, Martínez P, Sun J, Grigoriev I, Herrera-Estrella A, Baker SE, Kubicek CP (2009) Transcriptomic response of the mycoparasitic fungus *Trichoderma atroviride* to the presence of a fungal prey. BMC Genomics 10:567. https://doi.org/10.1186/1471-2164-10-567

Shi Y, Zhu J (2016) Saline-alkali soil improvement fertilizer and preparation method and use method thereof. Chinese Patent, CN 105237293 A (in Chinese). http://patentool.wanfangdata.com.cn/Patent/Details?id=CN201510597529.0

Staley JT, Palmer F, Adams JB (1982) Microcolonial fungi: common inhabitants on desert rocks? Science 215(4536):1093–10955. https://doi.org/10.1126/science.215.4536.1093

Swe A, Jeewon R, Pointing SB, Hyde KD (2009) Diversity and abundance of nematode-trapping fungi from decaying litter in terrestrial, freshwater and mangrove habitats. Biodivers Conserv 18(6):1695–1714. https://doi.org/10.1007/s10531-008-9553-7

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kännaste A, Behers L, Nevo E, Seisenbaeva G, Stenström E, Niinemets Ü (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9(5):e96086. https://doi.org/10.1371/journal.pone.0096086

Trincone A (2010) Potential biocatalysts originating from sea environments. J Mol Catal B Enzym 66(3–4):241–256. https://doi.org/10.1016/j.molcatb.2010.06.004

Trincone A (2011) Marine biocatalysts: enzymatic features and applications. Mar Drugs 9(4):478–499. https://doi.org/10.3390/md9040478

Turan M, Ataoglu N, Sahin F (2006) Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. J Sustain Agric 28:99–108

Upson R, Read DJ, Newsham KK (2009) Nitrogen form influences the response of *Deschampsia antarctica* to dark septate root endophytes. Mycorrhiza 20(1):1–11. https://doi.org/10.1007/s00572-009-0260-3

Verma S, Varma A, Rexer KH, Hassel A, Kost G, Sarabhoy A, Bisen P, Bütenhorn B, Franken P (1998) 90:896–903. https://doi.org/10.1080/00275514.1998.12026983

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, von Wettstein D, Franken P, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A 102(38):13386–13391. https://doi.org/10.1073/pnas.0504423102

Wang QB, Halbrendt C, Johnson SR (1996) Grain production and environmental management in China's fertilizer economy. J Environ Manag 47(3):283–296. https://doi.org/10.1006/jema.1996.0053

Wei Y, Zhang SH (2018) Abiostress resistance and cellulose degradation abilities of haloalkaliphilic fungi: applications for Saline-alkaline remediation. Extremophiles 22:155–164. https://doi.org/10.1007/s00792-017-0986-3

Wolfe-Simon F, Switzer Blum J, Kulp TR, Gordon GW, Hoeft SE, Pett-Ridge J, Stolz JF, Webb SM, Weber PK, Davies PC, Anbar AD, Oremland RS (2011) A bacterium that can grow by using arsenic instead of phosphorus. Science 332(6034):1163–1166. https://doi.org/10.1126/science.1197258

Woo SL, Pepe O (2018) Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. Front Plant Sci 9:1801. https://doi.org/10.3389/fpls.2018.01801

Xavier-Santos S, Carvalho CC, Bonfá M, Silva R, Capelari M, Gomes E (2004) Screening for pectinolytic activity of wood-rotting basidiomycetes and characterization of the enzymes. Folia Microbiol (Praha) 49(1):46–52. https://doi.org/10.1007/BF02931645

Xiao Y, Zheng Z, Huang Y, Xu Q, Su W (2005) Nematicidal and brine shrimp lethality of secondary metabolites from marine-derived fungi Xiamen da xue xue bao. Zi ran ke xue ban. J Xiamen Uni Nat Sci 44(6):847–850. http://europepmc.org/article/CBA/604823

Xing GX, Zhu ZX (2000) An assessment of N loss from agricultural fields to the environment in China. Nutr Cycl Agroecosyst 57(1):67–73. https://doi.org/10.1023/A:1009717603427

Xu ZH, Peng XP, Wang Y, Zhu WM (2011) (22E, 24R)-3ß,5α,9α-Trihydroxyergosta-7,22-dien-6-one monohydrate. Acta Cryst E67:o1141–o1142. https://doi.org/10.1107/s160053681101347x

Yan J, Song WN, Nevo E (2005) A MAPK gene from Dead Sea fungus confers stress tolerance to lithium salt and freezing-thawing: prospects for saline agriculture. Proc Natl Acad Sci U S A 102(52):18992–18997

Zajc J, Džeroski S, Kocev D, Oren A, Sonjak S, Tkavc R, Gunde-Cimerman N (2014a) Chaophilic or chaotolerant fungi: a new category of extremophiles? Front Microbiol 5:708-1–708-5. https://doi.org/10.3389/fmicb.2014.00708

Zajc J, Kogej T, Galinski EA, Ramos J, Gunde-Cimerman N (2014b) Osmoadaptation strategy of the most halophilic fungus, Wallemia ichthyophaga, growing optimally at salinities above 15% NaCl. Appl Environ Microbiol 80(1):247–256. https://doi.org/10.1128/AEM.02702-13

Zalar P, Frisvad JC, Gunde-Cimerman N, Varga J, Samson RA (2008) Four new species of emericella from the mediterranean region of Europe. Mycologia 100(5):779–795

Zakharova K, Tesei D, Marzban G, Dijksterhuis J, Wyatt T, Sterflinger K (2013) Microcolonial fungi on rocks: a life in constant drought? Mycopathologia 175(5–6):537–547. https://doi.org/10.1007/s11046-012-9592-1

Zalar P, Sybren de Hoog G, Schroers HJ, Frank JM, Gunde-Cimerman N (2005) Taxonomy and phylogeny of the xerophilic genus Wallemia (Wallemiomycetes and Wallemiales, cl. et ord. nov.). Antonie Van Leeuwenhoek 87(4):311–328. https://doi.org/10.1007/s10482-004-6783-x

Zalar P, de Hoog GS, Schroers HJ, Crous PW, Groenewald JZ, Gunde-Cimerman N (2007) Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Stud Mycol 58:157–183. https://doi.org/10.3114/sim.2007.58.06

Zhang X, Liu Y, Yan K, Wu H (2007) Decolorization of anthraquinone-type dye by bilirubin oxidase-producing nonligninolytic fungus Myrothecium sp. IMER1. J Biosci Bioeng 104(2): 104–110

Zhou N, Zhang Y, Liu F, Cai L (2016) Halophilic and thermotolerant Gymnoascus species from several special environments, China. Mycologia 108(1):179–191

Zhang XY, Zhang Y, Xu XY, Qi SH (2013) Diverse deep-sea fungi from the South China Sea and their antimicrobial activity. Curr Microbiol 67(5):525–530. https://doi.org/10.1007/s00284-013-0394-6

# Chapter 12
# Molecular Basis of Stress-Tolerant Genes in Extreme Microorganisms

**Wei Yi, Zhang Ziyu, Li Feng-Lan, and Shi-Hong Zhang**

**Abstract** Diversity of abiotic and biotic stress responses is a great threat to agricultural and industrial development. Genetic modifications are one of the most promising methods for the improvement of economically important plants or microbes. However, most genes harboring in ordinary organism cells play little role in stress-resistant biotechnology. Some species thrive in extreme environments, such as the hypersaline marine, the cold Antarctic continent, dry desert, or hot springs, where general eukaryotes such as plants or yeasts can hardly survive, revealing that extreme environmental organisms are promising genetic resources for biotechnology. Extremophilic fungi are excellent models to provide understanding in resistant mechanisms that allow higher organisms to overcome stress; these fungi present valuable genetic resources for isolation of resistance genes to be applied in genetic engineering and biotechnology. As a model fungus, the unicellular yeast *Saccharomyces cerevisiae* exhibits characteristics responses to a variety of stressors; it has led to the discovery of two significant osmotic-resistance pathways: the high-osmolarity glycerol response (HOG1) pathway and calcineurin-dependent pathway. With the increasing number of fungal species being characterized and sequenced, extremophilic fungi are found to be better systems for the isolation of abiotic stress resistance and related genes. So far, a series of environmental stress-related genes have been investigated in diverse fungi, and no doubt these specific resistance genes could be valuable for the improvement of crop tolerance. Interestingly, several ribosomal proteins recently isolated from the extremophilic fungi have been reported to possess moonlighting functions. Collectively, a tremendous number of tolerant genes cloned from extremophilic fungi appeared to be more resistant to

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_12

abiotic stress than their homologs or orthologs cloned from ordinary fungi, though these proteins are highly conserved and exist in a wide variety of organisms. Taking into account the special characteristics/mechanisms of genes from extremophilic fungi in stress responses, the application of these types of genes might be more valuable and reliable for biotechnology.

**Keywords** Extremophilic fungi · Abiotic stress · Tolerant genes · Tolerant mechanism · Biotechnology · Calcineurin pathway

## 12.1 Introduction

From the biological and environmental evolution perspective, fungi are one of the earliest eukaryotes to colonize the ancient earth (Gray and Shear 1992; Horodyski and Knauth 1994). Considering the harsh physical environments on ancient earth, to ensure the chances of survival, fungi might be more tolerant or resistant to adverse environmental factors than the latter appeared plants or other organisms. The level of response to environmental factors differs significantly from organism to organism; however, maintenance of metabolic flux and cellular mechanisms relies upon the organisms' ability to keep their functional states stable when they are under extreme stress. This diversifies the microbial survival at specific niche (Ranawat and Rawat 2017). Within the last few decades, a number of fungal species (halophile, xerophiles, or thermophile) that can live in a variety of extreme environments have been isolated. For example, *Eurotium herbariorum* that can survive in 340 g/L total dissolved salts was isolated from the Dead Sea (Kis-Papo et al. 2001; Yan et al. 2005); *Sarcinomyces petricola* strain A95 (a representative strain of rock-inhabiting fungi) was isolated from a marble rock surface near the Philopappos Monument on Musaios Hill of Athens (Gorbushina 2007); a thermophilic fungus *Thermomyces lanuginosus* isolated is able to survive at 62 °C, the highest growth temperature recorded so far (Singh et al. 2003); and in our laboratory, the fungal strain *T. lanuginosus* MY21 isolated was observed to be capable of growing at 65 °C (Meng and Wei 2019, unpublished). The stress-tolerant molecular mechanism and corresponding stress-tolerant genes in extreme microorganisms are also gradually undeviled.

In plants, great achievements have been made in recent years in understanding the abiotic stress responses and molecular mechanisms (Bhatnagar-Mathur et al. 2008; Gupta and Huang 2014; Zhan et al. 2015), but crop-breeding practice of higher resistant varieties remains unsatisfactory due to the lack of abiotic stress resistance germplasms. Wild germplasms are of interests, as wild crops may have retained genetic information before the domestication and artificial selection of modern plants (Lam et al. 2010). Wild germplasms screening, however, is a daunting and time-consuming task; and most likely we will never find certain wild germplasms again because of their extinction in the modern agricultural practices and environmental changes. Considering the survival abilities in the extreme conditions, extremophilic fungi may provide special or different resistance mechanism compared to plant. The genetic basis of abiotic stress resistance in extremophilic fungi makes it a unique genetic resource to improve abiotic stress resistance of crops. Here we highlight the abiotic stress resistance mechanisms and resistant genes in extremophilic fungi. In addition, application strategies for genetic engineering are also discussed.

## 12.2 The Stress-Tolerant Mechanism in Extremophilic Fungi

Fungi like other eukaryotic organisms such as plants depend on signaling-receiving and transmitting systems to respond to, survive, and thrive under the imposed adverse conditions. In eukaryote microorganisms, the yeast *S. cerevisiae* is known to have moderate levels of tolerance. It presents a rather poor performance in the presence of salt, drought, extreme temperature, and other stressors (Prista et al. 2002, 2005; Serrano and Gaxiola 1994). Thus, a wild-type *S. cerevisiae* is not the best model organism, neither for salt tolerance nor for sensitivity to salt. However, *S. cerevisiae* was an excellent tool for genetic manipulation (fast growth rate and easy transformation) and has been extensively applied in field of resistance research. The high-osmolarity glycerol (HOG1) pathway which is an essential stress-signaling module has been broadly studied in fungi: from the yeast *S. cerevisiae* to the filamentous fungus *Trichoderma harzianum* (Brewster et al. 1993; Delgado-Jarana et al. 2006). HOG1 is also found in extremophilic fungi. Nevo group testified that *Eurotium herbariorum* HOG1 is highly similar to the homologs from non-extreme fungi such as *Aspergillus nidulans*, *S. cerevisiae*, and *Schizosaccharomyces pombe* (Yan et al. 2005). However, it appears that HOG1 is found exclusively in fungi and no homolog gene has been detected in plants.

The HOG1 regulation system in yeast involves two pathways, the low-osmolarity SHO1 pathway and high-osmolarity SLN1 pathway. The difference between the two pathways lies at the SHO1 and SLN1 sensors, but both pathways ultimately lead to glycerol biosynthesis and the glycerol concentration for osmotic balance (Brewster et al. 1993). Besides salt, HOG1 also responds to a variety of other stressors (Delgado-Jarana et al. 2006), suggesting cross-talking feature in HOG pathways.

Another stress-responsive system required for salt stress tolerance in yeast is calcineurin; this protein phosphatase complex is dependent on calcium ion and calmodulin (Mendoza et al. 1994; Nakamura et al. 1993). Calcineurin is required for the genes' transcription of sodium and calcium ion ATPases and a cell wall β-1,3-glucan synthase through regulating CRZ1/TCN1, the downstream zinc-finger transcription factor (Matheos et al. 1997; Stathopoulos-Gerontides et al. 1999). The salt-responsive calcineurin-CRZ1 pathway is also involved in yeast stress responses (Juvvadi et al. 2014). When CRZ1 was overexpressed in the industrial baker's yeast HS13 strain, tolerance to both salt and freeze was increased (Panadero et al. 2007).

The calcineurin pathway, unlike HOG1, is highly conserved in eukaryotes from yeast to animals, which can be searched in the public nucleic acid sequence repository (http://www.ncbi.nlm.nih.gov/genbank). In plants, the physiology functions of calcineurin have been clarified (Luan et al. 1993; Allen and Sanders 1995), and the osmotic stress resistance is associated with the increased expression of calcineurin pathway genes. In fungi, the calcineurin homologs PsCNA1/PsCNB1 from the wheat rust disease fungus *Puccinia striiformis* have been studied. Results indicated that the calcineurin signaling pathway participates in stripe rust morphogenetic differentiation, especially the formation of haustoria during the early stage of infection and during the production of urediniospores (Zhang et al. 2012). Interestingly, calcineurin may be a multifunctional enzyme, because it was required for not only drug tolerance but also hyphal growth and virulence in *Candida tropicalis* (Chen et al. 2014). In contrast, relatively little is known about the calcineurin pathway in extremophilic fungi.

## 12.3 The Molecular Basis of Osmoregulation in Extremophilic Fungi

To remain viable, fungi under extreme conditions must regulate and keep essential cellular processes. The fluidity and components of the plasma membrane play important roles in maintaining the cell membrane physiological functions and the adaption to extreme conditions (Turk et al. 2007; Zhang et al. 2015). Plasma-membrane fluidity has been regarded as a typical indicator of fitness for survival in extreme environments (Turk et al. 2007). Unsaturated fatty acids are key compounds in the plasma membrane, and cellular unsaturated fatty acids constitutions are directly controlled by fatty acid desaturases. In *Pichia pastoris* GS115, cellular fatty acids compositions were changed with the increased or decreased expression of desaturases; in addition, deletions of fatty acid desaturases give rise to increased resistance to adverse environmental stress (Zhang et al. 2015). In Turk's study, all tested fungi showed increased plasma-membrane fluidity in response to increased salt concentrations. However, when salinity exceeded their optimal range, the extremophilic fungi (*Hortaea werneckii*, *Cryptococcus liquefaciens*) showed decreased plasma-membrane fluidity, reflecting the limitation of cell membrane remodeling.

Microorganisms have developed two main strategies for osmotic adjustment: influx of $K^+$ and accumulation of low molecular weight solutes of some organisms, such as hyper-/thermophiles, which utilize a combination of both strategies by accumulating negatively charged compatible solutes and potassium. This mechanism of osmosis adjustment occurs in extremophilic archaeal families *Halobacteriaceae* and *Haloferacaceae* (Gupta et al. 2015, 2016). But this has not been documented in majority of thermophilic microorganisms.

Another strategy relies on accumulation of compatible solutes or osmolytes, which are small organic compounds used for osmotic adjustment that do not interfere with cell function (Brown 1976; Ventosa et al. 1998). Thermophilic and hyperthermophilic organisms generally accumulate very unusual compatible solutes, namely, di*myo*-inositol phosphate, di-mannosyldi*myo*-inositol phosphate, diglycerol phosphate, mannosylglycerate, and mannosylglyceramide, which have not been identified in bacteria or archaea that grow at low and moderate temperatures (Santos and da Costa 2002).

A large variety of microorganisms, from bacteria to filamentous fungi, rely exclusively on the accumulation of compatible solutes for osmoadaptation. Compatible solutes, such as trehalose, glycine betaine, and α-glutamate, are widespread in microorganisms, while others are restricted to a few organisms. Chloride is accumulated in molar concentrations in the cytoplasm of *Halobacillus halophilus* to cope with salt stress (Saum et al. 2013). Polyols are also widespread compatible solute among fungi and algae but are very rare in bacteria and unknown in archaea. Ectoine and hydroxyectoine are examples of compatible solutes found only in bacteria (Santos and da Costa 2002).

The role of compatible solutes, however, goes beyond osmotic adjustment alone, to the protection of cells and cell components from freezing, desiccation, high temperature, and even UV radiations (Welsh 2000; Santos and da Costa 2002; Beblo-Vranesevic et al. 2016). The protective role of trehalose against several stress conditions has been amply demonstrated (Simola et al. 2000), and the accumulation of glycerol, the canonical osmolyte of yeast, has also been correlated with the acquisition of thermotolerance (Siderius et al. 2000).

Many polyols have been reported to contribute fungi to survive at high-salt concentrations or drought conditions. And among these compatible solutes, glycerol and trehalose have been extensively studied. Glycerol is the major product when extremophilic fungi, such as *Aspergillus glaucus*, grow on glucose-contained medium with high concentrations of NaCl (Liu et al. 2015). In the process of glycerol biosynthesis, key enzymes determine the production of intracellular glycerol and therefore impact on osmotic stress tolerance. Glycerol-3-phosphate dehydrogenase encoded by the gene of GPD1 in *S. cerevisiae* is important for yeast survival under osmotic stress (Albertyn et al. 1994). The yeast glycerol-3-phosphatases gpp1p and gpp2p are also essential for glycerol biosynthesis, but their roles in the cellular responses to osmotic, anaerobic, and oxidative stress are different (Pahlman et al. 2001).

To conquer high osmotic stress by biosynthesis of glycerol is inefficient and uneconomical. The active retention and uptake of glycerol are necessary when fungi are at high osmotic environments. Aquaglyceroporins (AQGPs; GlpFs in yeast) transport glycerol along with water, and other uncharged solutes are involved in osmoregulation in myriad species. The two genes encoding AQGPs in the yeast genome, Fps1 (Tamás et al. 1999, 2009; Oliveira et al. 2003) and Yfl054 (Oliveira et al. 2003; Hohmann et al. 2000), are functional glycerol facilitators. Fps1plays a key role in yeast osmoregulation by regulating intracellular glycerol levels during changes in external osmolarity (Luyten et al. 1995; Hohmann et al. 2007;

Ahmadpour et al. 2014), whereas the cellular function of Yfl054 remains uncertain (Oliveira et al. 2003). Recently, the AQGPs of arbuscular mycorrhizal fungus have received a lot of attention. The aquaglyceroporin GintAQPF2 from *Glomus intraradices*, a member of the γ subgroup (Xu et al., 2013), showed high activity when exposed to polyethylene glycol and high capacity to transport water, which is crucial for transformed yeast cells to survive osmotic stress (Li et al. 2013). In the halophilic fungus *Aspergillus glaucus*, the aquaglyceroporin gene *AgGlpF* has been demonstrated to be a water/glycerol channel (Liu et al. 2015). Interestingly, *AgGlpF* functions not only in *S. cerevisiae* and *Neurospora crassa* but also in model plants. When *AgGlpF* was expressed in *Arabidopsis thaliana*, the transgenic lines survived under high osmotic pressure and particularly under drought stress.

Another metabolite associated with osmoregulation is trehalose, the highly stable disaccharide commonly found in nature. Trehalose has multiple functions (Elbein et al. 2003), and is well-known for osmoprotection where correlations between accumulation of trehalose and high resistance to various stresses have been observed (Crowe et al. 1992). However, an unbiased study carried out by Petitjean et al. (2015) casted doubt on this long time-believed that trehalose is an osmoprotectant. By combining the use of mutant strains expressing catalytically inactive variants of Tps1, $MAL^+$ yeast strains were able to accumulate trehalose from an exogenous supply, and the authors found that the stress-protecting role of trehalose in the yeast was largely overestimated: trehalose actually was unable to protect yeast cells from dying; on the contrary, it is the Tps1 protein, the key enzyme for synthesis of trehalose, that played essential roles for yeast survival in response to temperature, oxidative, and desiccation stress (Petitjean et al. 2015).

In thermophiles, thermophilic lipids should be considered as lipids that normally function at high temperature and are termed as "thermophilic lipids" (Koga 2012). The other important factors that help bacterial membrane to withstand high temperature stress are hopanoids. In *Methylobacterium extorquens*, hopanoids interact with glycolipids in bacterial outer membranes to form a highly ordered bilayer in a manner analogous to the interaction of sterols with sphingolipids in eukaryotic plasma membranes; additionally, multidrug transport is impaired in a hopanoid-deficient mutant of the Gram-negative, which introduces a link between membrane order and an energy-dependent, membrane-associated function in prokaryotes (Sáenz et al. 2015). In *Bacillus acidocaldarius*, the production of hopanoids was increased sharply with temperature increase from 60 to 65 °C, therefore suggesting hopanoids play a role in counteracting the increased fluidity of cell membrane (Schaechter 2016). Though we do not know the concrete molecular mechanism of thermophilic lipid metabolic regulation, the research advances may indicate osmoregulation is not limited to polyols only. To uncover osmoregulation mechanisms, more polyols and moonlighting proteins must be further investigated.

## 12.4 Stress-Responsive Genes in Extremophilic Fungi

Hyperthermophilic microorganisms thrive in volcano rent water or hot springs. Chaperones play crucial roles in hyperthermophilic life. The chaperones accumulated in these hyperthermophilic microorganisms illustrate that chaperones are important for organism survival and organism must spend a substantial proportion of its metabolic energy for the folding and refolding of its proteins. In hyperthermophiles, *Thermococcus kodakarensis*, *Thermus thermophilus*, and *Sulfolobus* sp. are typical species. The thermotolerance and induction of heat-shock proteins make hyperthermophiles to adapt to hostile environmental conditions (Trent et al. 1997; Cava et al. 2009).

A predominant 55 kDa protein (TF55) was rapidly expressed, while *S. shibatae* B12 cells exposed to 88 °C environment (Trent et al. 1990). TF 55 belongs to the chaperones containing tailless-complex polypeptide family of chaperonins. The HSP60 molecular chaperonins in response to heat shocks, termed as CpkA and CpkB, have been intensively studied in *T. kodakarensis* (Fujiwara et al. 2008). The two genes were expressed in different patterns at different temperatures; but all the results supported the essential role of CpkA and CpkBin cell growth at low and high temperatures. HSP60 proteins have also been identified in methanogens also (Ambily Nath and Loka Bhararthi 2011). Transcriptional analysis of the hyperthermophilic archaea on *Sulfolobus solfataricus* revealed 26 vapBC of family TA (toxin-antitoxin) loci in its genome on raising the growth temperature from 80 to 90 °C (Cooper et al. 2009).

Cold-shock responses have been documented in almost all unicellular organisms from thermophiles such as *Thermus thermophilus* (Mega et al. 2010) and *Thermus* sp. GH5 (Yousefi-Nejad et al. 2011) to mesophiles such as *Caulobacter crescentus* (Balhesteros et al. 2010) to psychrophiles such as *Pseudoalteromonas haloplanktis* (Piette et al. 2012) and *Psychromonas sarctica* (Jung et al. 2010).

The aerobic thermophilic bacterium, *Thermus* sp. GH5, usually lives at temperatures between 70 and 75 °C. Under different cold-shock conditions, several proteins, involved in the degradation of carbon and synthesis of amino acids and nucleotides, were upregulated (Yousefi-Nejad et al. 2011), and accumulation of transaldolase and ribose 5-phosphate isomerase in late cold shock (Minic 2015).

The cold-shock protein of *Thermoanaerobacter tengcongensis* MB4, TteCspC, is involved in the survival of the organism at 50–80 °C. It is a common phenomenon that a single *csp* gene presents among most thermophilic anaerobes. In *T. tengcongensis*, there are more other cold-shock response genes, including DNA replication, recombination, and repair; transcriptional and translational regulation of genes plays effective roles in diverse processes. A number of novel low-temperature specific genes were also identified. Interestingly, the cold-shock response proteins, including TteCspC, function as molecular chaperones (Liu et al. 2014a).

In *Bacillus stearothermophilus* TLS33, eight cold-shock-induced proteins were characterized, but all these proteins have markedly different expression patterns. Interestingly, six of these cold-shock-induced proteins were correlated with the sigma B protein which plays an important role in signal transduction pathway of bacterial sporulation, suggesting the functional adaptation of this bacterium to environmental cold-shock stress (Topanurak et al. 2005).

As a salt-loving fungus, *Debaryomyces hansenii* has been extensively investigated in recent years. It is able to accumulate high concentrations of sodium without being any damages and also grow well under additional stress factors such as high temperature and extreme pH in the presence of 0.25 M NaCl (Almagro et al. 2000). Through screening *S. cerevisiae* transformants that contain the genomic library prepared from *D. hansenii* (Prista et al. 2002), a series of genes associated with salt tolerance were characterized. The *DhGZF3* gene, which encodes a GATA transcription factor homolog to Dal80 and Gzf3 in *S. cerevisiae*, has been functionally analyzed in *D. hansenii*. In *S. cerevisiae* expression system, the *DhGZF3* gene plays a role in a negative transcription factor (García-Salcedo et al. 2006). Using the cDNA library from the stress-tolerant *Rhodotorula mucilaginosa*, more than 100 *S. cerevisiae* transformants that are tolerant to concentrations of various osmolytes have been screened by Gostinčar and Turk (2012). Among the sequenced clones, 12 genes mediated increased stress tolerance that were upregulated in the *R. mucilaginosa*. Recently, from the *D. hansenii* genome database, Pereira et al. (2014) analyzed nine candidates of polyol/H(+) symporters by heterologous expression in *S. cerevisiae*. Five distinct polyol/H(+) symporters were confirmed, among which two symporters were tested to be specific for uncommon substrates as galactitol and D-(+)-chiro-inositol.

The abiotic stress resistance genes isolated from extremophilic filamentous fungi appear to be more resistant than homologs from ordinary fungi; however, in extremophilic filamentous fungi, the stress tolerance genes are relatively few. *EhHOG*, as mentioned above, is the *E. herbariorum* MAPK kinase gene similar to HOG1 homologs from *A. nidulans*, *S. cerevisiae*, *Schizosaccharomyces pombe*, and most other fungi; but hog1 mutant complemented with EhHOG outperformed the wild type under high salt and freezing-thawing conditions (Yan et al. 2005), indicating the higher genetic fitness of EhHOG in comparison with the corresponding HOG from *S. cerevisiae*. Some genes isolated from the halophilic fungus *A. glaucus* were also found to be more resistant to osmotic stress than the common fungi such as *S. cerevisiae* and *Magnaporthe oryzae*. A yeast expression library containing full-length cDNAs of *A. glaucus* was constructed and used to screen salt resistance transformants in our lab. The ribosomal protein L44 (RPL44), one of the proteins of the large ribosomal subunit 60S, was obtained according to its association with salt resistance. In comparison with the homologous sequence from *M. oryzae*, MoRPL44 in a yeast expression system, the results indicated that yeast cells with overexpressed *AgRPL44* were more resistant to salt, drought, and heavy metals than yeast cells expressing *MoRPL44* at a similar level of stress. In addition, when *AgRPL44* was introduced into *M. oryzae*, the transformants also displayed significantly enhanced tolerance to salt and drought, indicating the unique osmosis resistance ability from the halophilic fungus. Similar results were also obtained in the studies of another ribosomal protein subunit of AgRPS3aE (Liang et al. 2015), the AgglpF (Liu et al. 2015), a 60S protease subunit, and 14 other unknown or predicted genes including the cell wall-degrading enzymes such as chitinase, cellulase, and glucanase. The common features of all these genes are highly conserved, at least not specific to extremophilic fungi, but they obviously support transgenic cells or organisms surviving under stress conditions, suggesting special mechanisms to be uncovered in future and potential values for genetic engineering.

## 12.5 Transgenic Application and Concluding Remarks

Plants can benefit from foreign genes, such as CtHSR1 from the halophytic yeast *Candida tropicalis*, transferring the ability to adapt to adverse environments (Martínez et al. 2015). Many genes from diverse fungi have been successfully transferred into plants. *T. harzianum* is commonly used as a mycoparasite fungus for agriculture biological control. On the other hand, *T. harzianum* has moderate levels of tolerance to stressors; it is regarded as an active agent with abilities to induce resistance to abiotic stress in plants and to promote plant growth (Dana et al. 2006; Shoresh et al. 2010). *T. harzianum* provides an excellent genetic pool for cloning multiresistance genes. For example, ThHog1 (Delgado-Jarana et al. 2006), HSP70 (Montero-Barrientos et al. 2008, 2010), and Thkel1 (Hermosa et al. 2011) were successively characterized to be the genes responsible for resistance to salt or other stressors. Interestingly, some genes generally associated with cell wall degradation were shown to be associated with stress tolerance, when they were transferred into plants (Nicolás et al. 2014). This is not dissimilar to the ribosomal protein subunits RPL44 and RPS3aE described above. Considering the moderate levels of tolerance in *T. harzianum*, the homologous genes from extremophilic fungi could be even more resistant. Therefore, it is important and necessary to identify and characterize more genes related to stress resistance regardless of their origin and novelty.

Crops are often exposed to multiple stresses. One gene with multiple actions such as *Trichoderma* hsp70 is no doubt efficient and economic. Transgenic *Arabidopsis* containing hsp70 showed an enhanced tolerance to oxidative, osmotic, and salt stresses (Montero-Barrientos et al. 2010). The highly conserved ribosomal protein subunits like RPL44 and RPS3aE are also promising candidates for creating tolerance-enhanced crops without consideration of their biosafety (Liu et al. 2014b; Liang et al. 2015). These genes are generally in the downstream of resistant pathway and likely to have direct contribution to stress tolerance. Therefore, other physiological traits in transgenic plants may not be seriously affected, even if all these genes are overexpressed.

To a transformed gene, low levels of expression may have no anticipated function; however, high levels of expression probably affect the bioassay or productivity of the plant. Spatiotemporal expression of specific and controllable genes is in need. Inducible promoters are available in stress resistance genetic engineering. In plants, there exist many stress-responsive genes, particularly in sensitive plants (Dey et al. 2015). The resistant gene or genes from an extremophilic fungus driven by a plant-inducible promoter constitute the so-called two-component sensor systems (de Wit 1992). This strategy will solve the problem of excess cellular materials and energy (ATP) consumption.

**Acknowledgments** The related work in our lab was partially supported by grants from the National Natural Science Foundation of China (Grant Nos. 31671972 and 31670141) and a project of the Local scientific research of Department of Education of Liaoning Province of China (Grant No. 01032920021 to S.-H.Z.); Special talent introduction of Shenyang Agricultural University of China (Grant No. 880420019 to S.-H.Z.). The authors would like to thank the Zhang Lab members (Jilin University and Shenyang Agricultural University), who contributed to fungal isolation and gene cloning.

## References

Ahmadpour D, Geijer C, Tamás MJ, Lindkvist-Petersson K, Hohmann S (2014) Yeast reveals unexpected roles and regulatory features of aquaporins and aquaglyceroporins. Biochim Biophys Acta 1840:1482–1491

Albertyn J, Hohmann S, Thevelein JM, Prior BA (1994) GPD1, which encodes glycerol 3-phosphate dehydrogenase, is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high osmolarity glycerol response pathway. Mol Cell Biol 14:4135–4144

Allen GJ, Sanders D (1995) Calcineurin, a type 2B protein phosphatase, modulates the calcium-permeable slow vacuolar ion channel of stomatal guard cells. Plant Cell 7:1473–1483

Almagro A, Prista C, Castro S, Quintas C, Madeira-Lopes A, Ramos J, Loureiro-Dias MC (2000) Effects of salts on *Debaryomyces hansenii* and *Saccharomyces cerevisiae* under stress conditions. Int J Food Microbiol 56:191–197

Ambily Nath IV, Loka Bhararthi PA (2011) Diversity in transcripts and translational pattern of stress proteins in marine extremophiles. Extremophiles 15(2):129–153

Balhesteros H, Mazzon RR, da Silva CAPT, Lang EAS, Marques MV (2010) CspC and CspD are essential for *Caulobacter crescentus* stationary phase survival. Arch Microbiol 192:747–758

Beblo-Vranesevic K, Koschnitzki D, Rettberg P (2016) Ignicoccus hospitalis: a polyextremophilic microorganism with unusual high radiation tolerance. DGDR 2016

Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27(3):411–424

Brewster JL, de Valoir T, Dwyer ND, Winter E, Gustin MC (1993) An osmosensing signal transduction pathway in yeast. Science 259:1760–1763

Brown AD (1976) Microbial water stress. Bacteriol Rev 40:803–846

Cava F, Hidalgo A, Berenguer J (2009) *Thermus thermophilus* as biological model. Extremophiles 13:213–231

Chen YL, Yu SJ, Huang HY, Chang YL, Lehman VN, Silao FG, Bigol UG, Bungay AA, Averette A, Heitman J (2014) Calcineurin controls hyphal growth, virulence, and drug tolerance of Candida tropicalis. Eukaryot Cell 13(7):844–854. https://doi.org/10.1128/EC.00302-13

Cooper CR, Daugherty AJ, Tachdjian S, Blum PH, Kelly RM (2009) Role of vapBC toxin antitoxin loci in the thermal stress response of *Sulfolobus solfataricus*. Biochem Soc Trans 37:123–126

Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Annu Rev Physiol 54:579–599

Dana MM, Pintor-Toro JA, Cubero B (2006) Transgenic tobacco plants overexpressing chitinases of fungal origin show enhanced resistance to biotic and abiotic stress agents. Plant Physiol 142: 722–730

de Wit PJ (1992) Molecular characterization of gene-for-gene systems in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. Annu Rev Phytopathol 30: 391–418

Delgado-Jarana J, Sousa S, González F, Rey M, Llobell A (2006) ThHog1 controls the hyperosmotic stress response in Trichoderma harzianum. Microbiology 152(Pt 6):1687–1700

Dey N, Sarkar S, Acharya S, Maiti IB (2015) Synthetic promoters in planta. Planta 242(5): 1077–1094. https://doi.org/10.1007/s00425-015-2377-2

Elbein AD, Pan YT, Pastuszak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. Glycobiology 13:17R–27R

Fujiwara S, Aki R, Yoshida M, Higashibata H, Imanaka T, Fukuda W (2008) Expression profiles and physiological roles of two types of molecular chaperonins from the hyperthermophilic archaeon *Thermococcus kodakarensis*. Appl Environ Microbiol 74(23):7306–7312

García-Salcedo R, Casamayor A, Ruiz A, González A, Prista C, Loureiro-Dias MC, Ramos J, Ariño J (2006) Heterologous expression implicates a GATA factor in regulation of nitrogen metabolic genes and ion homeostasis in the halotolerant yeast *Debaryomyces hansenii*. Eukaryot Cell 5(8): 1388–1398

Gorbushina AA (2007) Life on the rocks. Environ Microbiol 9(7):1613–1631

Gostinčar C, Turk M (2012) Extremotolerant fungi as genetic resources for biotechnology. Bioengineered 3(5):293–297. https://doi.org/10.4161/bioe.20713

Gray J, Shear W (1992) Early life on land. Am Sci 80:444–456

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genomics 2014:701596. https://doi.org/10.1155/2014/701596

Gupta RS, Naushad S, Baker S (2015) Phylogenomic analyses and molecular signatures for the class Halobacteria and its two major clades: a proposal for division of the class Halobacteria into an emended order Halobacteriales and two new orders, Haloferacales ord. nov. and Natrialbales ord. nov., containing the novel families Haloferacaceae fam. nov. and Natrialbaceae fam. nov. Int J Syst Evol Microbiol 65(Pt 3):1050–1069. https://doi.org/10.1099/ijs.0.070136-0

Gupta RS, Naushad S, Fabros R, Adeolu M (2016) A phylogenomic reappraisal of family-level divisions within the class Halobacteria: proposal to divide the order Halobacteriales into the families Halobacteriaceae, Haloarculaceae fam. nov., and Halococcaceae fam. nov., and the order Haloferacales into the families, Haloferacaceae and Halorubraceae fam nov. Antonie Van Leeuwenhoek 109(4):565–587. https://doi.org/10.1007/s10482-016-0660-2

Hermosa R, Botella L, Keck E, Jiménez JA, Montero-Barrientos M, Arbona V, Gómez-Cadenas A, Monte E, Nicolás 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

Hohmann I, Bill RM, Kayingo I, Prior BA (2000) Microbial MIP channels. Trends Microbiol 8:33–38. https://doi.org/10.1016/S0966-842X(99)01645-5

Hohmann S, Krantz M, Nordlander B (2007) Yeast osmoregulation. Methods Enzymol 428:29–45. https://doi.org/10.1016/S0076-6879(07)28002-4

Horodyski RJ, Knauth LP (1994) Life on land in the precambrian. Science 263(5146):494–498

Jung YH, Yi JY, Jung HJ, Lee YK, Lee HK, Naicker MC, Uh JH, Jo IS, Jung EJ, Im H (2010) Overexpression of cold shock protein A of *Psychromonas arctica* KOPRI 22215 confers cold-resistance. Protein J 10(29):136–142

Juvvadi PR, Lamoth F, Steinbach WJ (2014) Calcineurin as a multifunctional regulator: unraveling novel functions in fungal stress responses, hyphal growth, drug resistance, and pathogenesis. Fungal Biol Rev 28(2–3):56–69

Kis-Papo T, Grishkan I, Oren A, Wasser SP, Nevo E (2001) Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea. Mycol Res 105:749–756

Koga Y (2012) Thermal adaptation of the archaeal and bacterial lipid membranes. Archaea 2012: 789652. https://doi.org/10.1155/2012/789652

Lam HM, Xu X, Liu X, Chen W, Yang G, Wong FL, Li MW, He W, Qin N, Wang B, Li J, Jian M, Wang J, Shao G, Wang J, Sun SS, Zhang G (2010) Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nat Genet 42(12): 1053–1059. https://doi.org/10.1038/ng.715

Li T, Hu YJ, Hao ZP, Li H, Chen BD (2013) Aquaporin genes GintAQPF1and GintAQPF2 from *Glomus intraradices* contribute to plant drought tolerance. Plant Signal Behav 8:e24030. https://doi.org/10.4161/psb.24030

Liang X, Liu Y, Xie L, Liu X, Wei Y, Zhou X, Zhang S (2015) A ribosomal protein AgRPS3aE from halophilic *Aspergillus glaucus* confers salt tolerance in heterologous organisms. Int J Mol Sci 16(2):3058–3070. https://doi.org/10.3390/ijms16023058

Liu B, Zhang Y, Zhang W (2014a) RNA-seq-based analysis of cold shock response in *Thermoanaerobacter tengcongensis,* a bacterium harbouring a single cold shock protein encoding gene. PLoS One 9(3):e93289. https://doi.org/10.1371/journal.pone.0093289

Liu XD, Xie L, Wei Y, Zhou XY, Jia B, Liu J, Zhang SH (2014b) Abiotic stress resistance, a novel moonlighting function of ribosomal protein RPL44 in the halophilic fungus *Aspergillus glaucus*. Appl Environ Microbiol 80(14):4294–4300. https://doi.org/10.1128/AEM.00292-14

Liu XD, Wei Y, Zhou XY, Pei X, Zhang SH (2015) *Aspergillus glaucus* aquaglyceroporin gene *glpF* confers high osmosis tolerance in heterologous organisms. Appl Environ Microbiol 81(19):6926–6937. https://doi.org/10.1128/AEM.02127-15

Luan S, Li W, Rusnak F, Assmann SM, Schreiber SL (1993) Immunosuppressants implicate protein phosphatase regulation of K+ channels in guard cells. Proc Natl Acad Sci U S A 90:2202–2206

Luyten K, Albertyn J, Skibbe WF, Prior BA, Ramos J, Thevelein JM, Hohmann S (1995) Fps1, a yeast member of the MIP family of channel proteins, is a facilitator for glycerol uptake and efflux and is inactive under osmotic stress. EMBO J 14:1360–1371

Martínez F, Arif A, Nebauer SG, Bueso E, Ali R, Montesinos C, Brunaud V, Muñoz-Bertomeu J, Serrano R (2015) A fungal transcription factor gene is expressed in plants from its own promoter and improves drought tolerance. Planta 242(1):39–52. https://doi.org/10.1007/s00425-015-2285-5

Matheos DP, Kingsbury TJ, Ahsan US, Cunningham KW (1997) Tcn1p/Crz1p, a calcineurin-dependent transcription factor that differentially regulates gene expression in *Saccharomyces cerevisiae*. Genes Dev 11:3445–3458

Mega R, Manzoku M, Shinkai A, Nakagawa N, Kuramitsu S, Masui R (2010) Very rapid induction of a cold shock protein by temperature downshift in *Thermus thermophilus*. Biochem Biophys Res Commun 399:336–340

Mendoza I, Rubio F, Rodriguez-Navarro A, Pardo JM (1994) The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. J Biol Chem 269:8792–8796

Minic Z (2015) Proteomic studies of the effects of different stress conditions on central carbon metabolism in microorganisms. J Proteomics Bioinform 8:080–090. https://doi.org/10.4172/jpb.1000355

Montero-Barrientos M, Hermosa R, Nicolás C, Cardoza RE, Gutiérrez S, Monte E (2008) Overexpression of a *Trichoderma*HSP70 gene increases fungal resistance to heat and other abiotic stresses. Fungal Genet Biol 45:1506–1513

Montero-Barrientos M, Hermosa MR, Cardoza RE, Gutiérrez S, Nicolás C, Monte E (2010) Transgenic expression of the *Trichoderma harzianum* hsp70 gene increases *Arabidopsis* resistance to heat and other abiotic stresses. J Plant Physiol 167:659–665

Nakamura TY, Liu Y, Hirata D, Namba H, Harada S, Hirokawa T, Miyakawa T (1993) Protein phosphatase type 2B (calcineurin)-mediated, FK506-sensitive regulation of intracellular ions in yeast is an important determinant for adaptation to high salt stress conditions. EMBO J 12: 4063–4071

Nicolás C, Hermosa R, Rubio B, Mukherjee PK, Monte E (2014) *Trichoderma* genes in plants for stress tolerance—status and prospects. Plant Sci 228:71–78

Oliveira R, Lages F, Silva-Graça M, Lucas C (2003) Fps1p channel is the mediator of the major part of glycerol passive diffusion in *Saccharomyces cerevisiae*: artefacts and re-definitions. Biochim Biophys Acta 1613:57–71. https://doi.org/10.1016/S0005-2736(03)00138-X

Pahlman AK, Granath K, Ansell R, Hohmann S, Adler L (2001) The yeast glycerol 3-phosphatases gpp1p and gpp2p are required for glycerol biosynthesis and differentially involved in the cellular responses to osmotic, anaerobic, and oxidative stress. J Biol Chem 276:3555–3563

Panadero J, Hernández-López MJ, Prieto JA, Randez-Gil F (2007) Overexpression of the calcineurin target CRZ1 provides freeze tolerance and enhances the fermentative capacity of baker's yeast. Appl Environ Microbiol 73(15):4824–4831

Pereira I, Madeira A, Prista C, Loureiro-Dias MC, Leandro MJ (2014) Characterization of new polyol/H+ symporters in *Debaryomyces hansenii*. PLoS One 9(2):e88180. https://doi.org/10.1371/journal.pone.0088180

Petitjean M, Teste MA, François JM, Parrou J, L. (2015) Yeast tolerance to various stresses relies on the Trehalose-6P synthase (Tps1) protein, not on Trehalose. J Biol Chem 290(26): 16177–16190. https://doi.org/10.1074/jbc.M115.653899

Piette F, Leprince P, Feller G (2012) Is there a cold shock response in the Antarctic psychrophile *Pseudoalteromonas haloplanktis*? Extremophiles 16:681–683

Prista C, Soeiro A, Vesely P, Almagro A, Ramos J, Loureiro-Dias MC (2002) Genes from *Debaryomyces hansenii* increase salt tolerance in *Saccharomyces cerevisiae* W303. FEMS Yeast Res 2:151–157

Prista C, Loureiro-Dias MC, Montiel V, García R, Ramos J (2005) Mechanisms underlying the halotolerant way of *Debaryomyces hansenii*. FEMS Yeast Res 5(8):693–701

Ranawat P, Rawat S (2017) Stress response physiology of thermophiles. Arch Microbiol 199(3): 391–414. https://doi.org/10.1007/s00203-016-1331-4

Sáenz JP, Grosser D, Bradley AS, Lagny TJ, Lavrynenko O, Broda M, Simons K (2015) Hopanoids as functional analogues of cholesterol in bacterial membranes. Proc Natl Acad Sci U S A 112(38):11971–11976. https://doi.org/10.1073/pnas.1515607112

Santos H, da Costa MS (2002) Compatible solutes of organisms that live in hot saline environments. Environ Microbiol 4:501–509

Saum SH, Pfeiffer F, Palm P, Rampp M, Schuster SC, Müller, V., Oesterheldt, D. (2013) Chloride and organic osmolytes: a hybrid strategy to cope with elevated salinities by the moderately halophilic, chloride dependent bacterium *Halobacillus halophilus*. Environ Microbiol 15(5): 1619–1633

Schaechter E (2016) Bacterial hopanoids: the lipids that last forever. In: Schaechter E (ed) In the company of microbes: ten years of small things considered, pp 146–149. https://doi.org/10.1128/9781555819606.ch37

Serrano R, Gaxiola R (1994) Microbial models and salt stress tolerance in plants. Crit Rev Plant Sci 13:121–138

Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43

Siderius M, Van Wuytswinkel O, Reijenga KA, Kelders M, Mager WH (2000) The control of intracellular glycerol in *Saccharomyces cerevisiae* influences osmotic stress response and resistance to increased temperature. Mol Microbiol 36:1381–1390

Simola M, Hanninen AL, Stranius SM, Makarow M (2000) Trehalose is required for conformational repair of heat-denatured proteins in yeast endoplasmic reticulum but not for maintenance of membrane traffic function after severe heat stress. Mol Microbiol 37:42–53

Singh S, Madlala AM, Prior BA (2003) *Thermomyces lanuginosus*: properties of strains and their hemicellulases. FEMS Microbiol Rev 27(1):3–16

Stathopoulos-Gerontides A, Guo JJ, Cyert MS (1999) Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. Genes Dev 13:798–803

Tamás MJ, Luyten FC, Sutherland A, Hernandez J, Albertyn J, Valadi H, Li H, Prior BA, Kilian SG, Ramos J, Gustafsson L, Thevelein JM, Hohmann S (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. Mol Microbiol 31:1087–1104. https://doi.org/10.1046/j.1365-2958.1999.01248.x

Tamás MJ, Thorsen M, Jacobson T, Vooijs R, Schat H (2009) Extracellular glutathione protects yeast cells from arsenite toxicity. Microbiology 140(Pt 8):1855

Topanurak S, Sinchaikul S, Sookkheo B, Phutrakul S, Chen ST (2005) Functional proteomics and correlated signaling pathway of the thermophilic bacterium *Bacillus stearothermophilus* TLS33 under cold- shock stress. Proteomics 5:4456–4471

Trent JD, Osipiuk J, Pinkau T (1990) Acquired thermotolerance and heat shock in the extremely thermophilic archaebacterium *Sulfolobus* sp. strain B12. J Bacteriol 172(3):1478–1484

Trent JD, Kagawa HK, Yaoi T, Olle E, Zaluzec NJ (1997) Chaperonin filaments: the archaeal cytoskeleton? Proc Natl Acad Sci U S A 94:5383–5388

Turk M, Abramović Z, Plemenitas A, Gunde-Cimerman N (2007) Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. FEMS Yeast Res 7(4):550–557

Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544

Welsh DT (2000) Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. FEMS Microbiol Rev 24:263–290

Yan J, Song WN, Nevo E (2005) A MAPK gene from Dead Sea fungus confers stress tolerance to lithium salt and freezing-thawing: prospects for saline agriculture. Proc Natl Acad Sci U S A 102(52):18992–18997

Yousefi-Nejad M, Naderi-Manesh H, Khajeh K (2011) Proteomics of early and late cold shock stress on thermophilic bacterium, *Thermus* sp GH5. J Proteome 74:2100–2111

Zhan X, Zhu JK, Lang Z (2015) Increasing freezing tolerance: kinase regulation of ICE1. Dev Cell 32(3):257–258. https://doi.org/10.1016/j.devcel.2015.01.004

Zhang H, Guo J, Voegele RT, Zhang J, Duan Y, Luo H, Kang Z (2012) Functional characterization of calcineurin homologs PsCNA1/PsCNB1 in *Puccinia striiformis* f. sp. *tritici* using a host-induced RNAi system. PLoS One 7(11):e49262. https://doi.org/10.1371/journal.pone.0049262

Zhang M, Liu Z, Yu Q, Mao J, Zhang B, Xing L, Li M (2015) Deletion of genes encoding fatty acid desaturases leads to alterations in stress sensitivity in *Pichia pastoris*. FEMS Yeast Res 15(4): fov020. https://doi.org/10.1093/femsyr/fov020

# Chapter 13
# Cellulose Degradation Microorganisms and Environmental-Friendly Solution to the Agricultural Waste Management

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**Abstract** Agricultural waste is a general term for all agricultural residues. Comprehensively, agricultural waste refers to the organic residues in agricultural activity, which includes multiple crop straws, animal and human excreta, kitchen wastes, and industrial or agricultural processing residues. For a long time, agricultural waste, namely, inedible parts of plants, has been mainly used to be raw materials for the preparation of feed, fuel, and compost. Due to the low nutrition, low calorific value, and refractory residues, agricultural wastes currently have become the abandoned agricultural wastes. However, with the progress of modern biotechnology, the so-called wastes are starting to be shown the practice value, particularly in the fields of organic agriculture and bioenergy. Cellulose, an insoluble polymer composed of long chains of β-1,4-linked glucose residues associated in microfibrils, is the major component of plant biomass and the most abundant organic compound in the biosphere. Plant cellulose can be degraded to soluble sugars by synergistic mixtures of microbial cellulases and other cell wall-degrading enzymes and is becoming an important renewable source for biofertilizer and bioenergy production. In recent years, progress has been made in isolating and identifying multifunctional cellulose degradation microorganisms and degrading enzymes. Due to the complexity of straw

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R. Prasad, S.-H. Zhang (eds.), *Beneficial Microorganisms in Agriculture*,
Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_13

cellulose structure and fermentation process, the highly efficient degradation of cellulose requires synergistic interactions of varieties of microorganisms. In addition, during microbial fermentation, the condition in fermentation system is extreme. Microorganisms must have the ability to adapt to the extreme environments in order to maintain the continuous fermentation. Therefore, extreme environmental microorganisms have become the urgent chosen for fermentation. In this chapter, we will focus on the four aspects: (1) isolation and identification of cellulose-degrading microorganisms, (2) the degradation mechanism of cellulose by microorganisms and cellulase system, (3) synergistic mechanism of varieties of microorganisms, and (4) biofertilizer and bioenergy application.

**Keywords** Cellulose degradation · Extreme microorganism · Cellulase · Biofertilizer · Bioenergy

## 13.1 Introduction

Agricultural waste is the most widely distributed and most abundant biomass in nature, which involves multiple crop straws, animal dungs, and human excreta kitchen wastes and other industrial and agricultural processing residues. For a long time, agricultural wastes have been mainly used as raw materials for the preparation of feed, fuel, and compost. Due to their low commercial value, low nutrition, low calorific value, and robust nature to be degraded, the agricultural waste currently has become the real useless wastes discarded everywhere.

However, the so-called agricultural waste contains great amount of cellulose, and cellulose is very useful biomaterials for organic agriculture. Annual production of cellulose is estimated at $4.0 \times 10^7$ tons (Bakare et al. 2005); and large quantities of industrial and agricultural cellulose waste have increasingly accumulated due to unscientific and inefficient use (Kim et al. 2003; Lee et al. 2008). There are a large number of nutrition elements such as nitrogen, phosphorus, potassium, sulfur, and magnesium in crop straws and other agricultural wastes, but these elements mostly exist in a combined state within plant cells. Basically, they chemically combine with cellulose, lignin, and hemicellulose structure and cannot be easily absorbed by plants. Cellulose degradation microorganism-dependent fermentation, that is, the composing of agricultural waste, is able to release the elements for plant absorption and utilization. Additionally, the degradation products and microbial metabolites of agricultural wastes are as well conducive to the growth and development of plants. The agricultural waste with value-added bio-product of the composting process contributes to the improvement of the soil properties and plant growth in an environment-friendly way. However, the conventional process employed for composting cellulose is time-consuming and even becomes an impediment or hazard for farmers. Therefore, an economical, efficient, and environment-friendly utilization measure for composing agricultural waste is urgent.

Cellulosic biomasses do not compete with our food resources, so they are more sustainable than starch-based biofuels. Actually, the major sources of second-generation biofuels should be agricultural wastes such as rice straw and corn stover that contain high amounts of cellulose (Saini et al. 2015). However, lignocellulose needs to be converted into sugars that can be further fermented into biofuels. As cellulose is the most abundant renewable natural product in the biosphere (Feng et al. 2007), cellulolytic microorganisms are fundamental for the transformation of cellulose into sugars that are essential nutrients for various organisms and for biofuels (Arifoglu and Ögel 2000; Bhat and Bhat 1997). It was reported that lignocellulose could be decomposed by thermochemical and biochemical processes; and the thermochemical routes, such as pyrolysis and gasification, decompose agricultural wastes into a mixture of gaseous and liquid forms of hydrocarbons (Bridgwater 2012; Tanger et al. 2013). But the processing by thermochemical route needs high temperatures and other more auxiliary suppliers and thus depends on high energy input. On the contrary, the biochemical route utilizes lignocellulolytic enzymes to degrade agricultural wastes into fermentable sugars, which requires lower temperature and produces relatively simple products (Robak and Balcerek 2018).

Lignocellulose mainly consists of cellulose (30–45%), hemicellulose (15–30%), and lignin (12–25%), although the ratios may vary among feedstocks (Isikgor and Becer 2015). Cellulose consists of β-1,4-linked glucosyl residues and is the most abundant component of lignocellulosic residues. The degradation of cellulose requires three types of cellulases for complete hydrolysis to glucose. Endoglucanases and exoglucanases cooperatively cleave the cellulose polymers into trisaccharide and disaccharide and then hydrolyzed to glucose with β-glucosidases, which is readily usable for biofuel production (Kostylev and Wilson 2012). Since the complexity of straw cellulose structure and fermentation process, industrial enzymatic degradation of lignocellulose highly depends on the synergism between xylanases, cellulases, and lignin modification enzymes. In addition, different strain has a different cellulolytic enzyme system; thus, the highly efficient degradation of cellulose requires synergistic interactions of varieties of microorganisms.

During microbial fermentation, the condition in fermentation system is extremely harsh. For example, the high temperature, low oxygen, and osmotic stresses can be formed during agricultural waste composting. Microorganisms in action must have the ability to adapt to the extreme environment in order to maintain the continuous fermentation. Therefore, extreme environmental microorganisms have become the urgent chosen for fermentation. In this chapter we focused on the following aspects: (1) isolation and identification of cellulose-degrading microorganisms, (2) the degradation mechanism of cellulose by microorganisms and cellulase system, (3) synergistic mechanism of varieties of microorganisms, and (4) biofertilizer and bioenergy application.

## 13.2 Cellulose Degradation Microorganisms Isolated from Extreme Environments

Cellulases are the key enzymes in the breakdown of lignocellulosic polysaccharides into simple sugars (Nagraj et al. 2014). The fungi are the most efficient degraders of lignocellulosic cellulose (Gutiérrez-Rojas et al. 2013; Isroi et al. 2011; Tsegaye et al. 2018; Venkatramanan et al. 2021). The long-term management of agricultural waste requires plenty of efficiency and stability of cellulases. Most commercial cellulases come from species of *Trichoderma* and *Aspergillus* and *Thermophilic* fungi. To make full use of large-scale agricultural waste, plenty of microorganisms, which produce efficient and stable cellulases, are desiderated (López et al. 2021; Sarsaiya et al. 2019; Pandit et al. 2021).

The most-studied species from the *Trichoderma* and *Aspergillus* genus have been widely used to produce industrial-scale enzymes. Different strains of *T. harzianum* have different cellulolytic activity, and the potential of these enzymes has been explored for applications in biomass degradation to the production of biofuels (Horta et al. 2014; de Castro et al. 2010). Some strains are specific for the biofertilizer through composing of plant biomass, and some are specific for the biofuel technology (Li et al. 2018a, b; Horta et al. 2014). These organisms are the sources of most enzymes comprising enzymatic cocktails that are currently available on the market (Bischof et al. 2016). *T. reesei* is still the widely studied fungus that has been widely used in metabolic engineering (Li et al. 2018a, b); and the *T. harzianum* IOC3844 is also going to be in synthetic biology (Santos et al. 2019).

In addition to the *Trichoderma* and *Aspergillus* genus, which produce and secrete cellulases to the medium (Gupta et al. 2016; Zeng and Zhuang 2019), there are many plant endophytic fungi with great ability of producing extracellular cellulases of industrial interest (Corrêa et al. 2014).

Fungal endophytes isolated from many plants mostly belong to *Ascomycota*; and they are going to be recognized as a rich source of bioactive metabolites. These endophytes play a role in plant defense, by degrading the cell wall using various hydrolytic enzymes (Goldbeck et al. 2013). They live in symbiotic association in internal plant tissues, through the major life cycle of the host (Uzma et al. 2016). A variety of cellulase, amylase, xylanase, and other enzymes have been produced by fungal endophytes isolated from several plants (Fouda and Hassan 2015). These endophytes are a potential source of enzymes of industrial interest.

More than 100 Brazilian endophytic fungi have been isolated from *Eucalyptus benthamii*, *Platanus orientalis*, *Glycine max*, *Solanum tuberosum*, and *Saccharum officinarum*, of which *Penicillium* sp. and *Phoma* sp. have a high capacity to produce extracellular enzymes (Pandey et al. 2016; Robl et al. 2013). Similarly, the endophyte *Fusarium oxysporum* also produces a high amount of cellulases (Sideney et al. 2013). *T. reesei*, *A. niger*, *A. nidulans*, and *Neurospora crassa* are the most-studied models for cellulase production (Gutiérrez-Rojas et al. 2013). All these endophytes together with various diversity of fungi relevant to species isolated from extreme environments construct a huge prospecting resource pool of cellulose degradation system (Aime and Brearley 2012; Le Calvez et al. 2009).

The Nevados National Natural Park mountain ecosystems are regarded as an extreme natural region in Colombia due to the high levels of solar radiation, low atmospheric pressure, and extreme daily changes in temperature. The unique ecosystem that contributes associated functions in hydric regulation and maintenance of endemic biodiversity makes it a preferred area for screening efficient cellulose degradation microorganisms (Avellaneda-Torres et al. 2015a, b). Totally, 108 microorganisms (74 bacterial and 34 fungal isolates) with the capacity to degrade cellulose in the CMC form were isolated from the soils. According to the cellulolytic activity, 46 microorganisms, which showed high cellulose hydrolytic activity, were characterized. The identified bacterial isolates among the organisms with cellulolytic activity belonged to the genera *Pseudomonas*, *Streptomyces*, *Rhodococcus*, *Stenotrophomonas*, *Variovorax*, *Serratia*, and *Janthinobacterium*. The identified fungal isolates belonged to the genera *Penicillium*, *Mortierella*, *Tolypocladium*, *Paecilomyces*, *Acremonium*, *Fusarium*, *Volutella*, *Hypocrea*, *Neonectria*, *Mucor*, *Aureobasidium*, and *Arthrinium*. Importantly, these microorganisms showed a synergistic mechanism during degrading cellulose, suggesting that a combination of microorganisms with various enzymatic activities be used to obtain high total cellulolytic activity.

Marine habitats have usually been regarded as a source of microorganisms that possess robust proteins that help enable them to survive in such harsh conditions. These areas likely contain a community of extremophilic microorganisms that have evolved unique characteristics to adapt to the uncommon conditions that can be found in these ecosystems. Marine fungi appear to dominate eukaryotic life in the buried marine subsurface of marine environments (Edgcomb et al. 2011). Fungi, with high species diversity (Pachiadaki et al. 2016), known to play key roles in decomposition of organic matter, represent a significant portion of the biomass in marine environment and play important roles in biogeochemical cycles and food webs (Bass et al. 2007; Gadd 2007). Thus, marine-derived fungi and their cell wall-degrading system play important roles in the recycling of agricultural wastes such as crop straws.

About 20 strains were isolated from sea mud samples collected in the East China Sea and then screened for their capacity to produce lignin-degrading enzymes (Chen et al. 2011). Out of the 20 strains, a moderately halotolerant endophytic fungus, *Pestalotiopsis* sp., had a great potential to secrete a considerable amount of laccase. The production of both cellulase and laccase by *Pestalotiopsis* sp. J63 was investigated under submerged fermentation and solid-state fermentation with various lignocellulosic by-products as substrates. The result indicated that J63 produced 0.11 U/mL cellulase when alkaline-pretreated sugarcane bagasse was used as growth substrate under submerged fermentation.

Following screening of 14 fungi isolated from the deep-sea sponge *Stelletta normani* sampled at a depth of 751 m, Batista-García et al. (2017) identified 3 halotolerant strains (*Cadophora* sp. TS2, *Emericellopsis* sp. TS 11, and *Pseudogymnoascus* sp. TS12) which displayed high CMCase and xylanase activities. The three fungi displayed psychrotolerance and halotolerant growth on CMC and xylan as sole carbon sources. These microorganisms produced series of cellulases, which displayed optimal temperature and pH values of between 50 and 70 °C and pH 5–8, respectively, together with good thermostability and halotolerance. In solid-state fermentations, TS2, TS11, and TS12 produced CMCases, xylanases, and peroxidase/phenol oxidases when grown on corn stover and wheat straw, suggesting that they can be used in future biomass conversion (Table 13.1).

Nowadays, composting has been a successful alternative for the management of agricultural organic waste, in which the biotransformation process is performed by a complex microbial community (López et al. 2021; Moreno et al. 2021). During waste processing, heat is produced because of the energy generated by exergonic aerobic reactions derived from microbial metabolism. This leads the composting to evolve through different stages driven mainly by the temperature reached in the materials being transformed. The thermal phases that define a composting process are mesophilic, thermophilic, cooling, and maturation (López et al. 2021). Based on previous research, the upper temperature limit for fungal organism growth is 62 °C (Tansey and Brock 1972), so ordinary fungi appear to dislike high-temperature environments. In composing system actually, the high temperature inside compost pile will maintain over a period of time. In order to produce high-quality compost, thermophilic or tolerant microorganisms are essential.

Composting involves the selection of a microbiota capable of resisting the high temperatures generated during the process and degrading the lignocellulose. López et al. (2021) investigated the lignocellulose-degrading thermophilic microbial community at all the stages of waste composting. The whole fungal thermophilic population exhibited lignocellulose-degrading activity, whereas roughly 8–10% of thermophilic bacteria functioned exclusively for hemicellulose degradation. Bacteria play a key role in the breakdown of hemicellulose during the entire process, whereas the degradation of cellulose and lignin is restricted to the activity of a few thermophilic fungi that persists at the end of the process. The most prevalent species were *Bacillus licheniformis* and *Aeribacillus pallidu*s. However, thermophilic fungi comprised only four species, namely, *Thermomyces lanuginosus*, *Talaromyces thermophilus*, *Aspergillus fumigatus*, and *Gibellulopsis nigrescens*, of which *A. fumigatus* and *T. lanuginosus* dominated. *Bacillus thermoamylovorans*, *Geobacillus thermodenitrificans*, *T. lanuginosus*, and *A. fumigatus* with considerable enzymatic activities were selected as potential candidates for the production of thermozymes. This study lays a foundation to further investigate the mechanisms of adaptation and acquisition of new traits among thermophilic lignocellulolytic microorganisms during composting as well as their potential utility in biotechnological processing.

**Table 13.1** Cellulose degradation fungi isolated from plants and extreme environments

| Species/strain | Biological characteristics | Cellulolytic enzymes | References |
|---|---|---|---|
| *Cladosporium cladosporioides* | Plant endophytic | Amylase, cellulase | Amirita et al. (2012) |
| *Curvularia vermiformis* | Plant endophytic | Cellulase, lipase, protease | Amirita et al. (2012) |
| *Acrimoniumterícola* | Plant endophytic | Cellulase, protease, xylanase | Bezerra et al. (2012) |
| *Fusarium lateritium* | Plant endophytic | Cellulase, protease, xylanase | Bezerra et al. (2012) |
| *Nigrospora sphaerica* | Plant endophytic | Cellulase, protease, xylanase | Bezerra et al. (2012) |
| *Penicillium aurantiogriseum* | Plant endophytic | Cellulase, protease, xylanase | Bezerra et al. (2012) |
| *Pestalotiopsis guepinii* | Plant endophytic | Cellulase, protease, xylanase | Bezerra et al. (2012) |
| *Wallemia* sp. | Halophilic | Cellulase | Chamekh et al. (2019) |
| *Aspergillus subramanianii* A1 | Halotolerant | Cellulase | Chamekh et al. (2019) |
| *Aspergillus* sp. *strain* A4 | Halotolerant | Cellulase | Chamekh et al. (2019) |
| *Penicillium vinaceum* | Halophilic | Cellulase | Chamekh et al. (2019) |
| *Gymnoascus halophilus* S1-2 | Halophilic | Cellulase | Chamekh et al. (2019) |
| *Trichoderma lixii* IG127 | Halotolerant | Cellulase | Pasqualetti et al. (2019) |
| *Clonostachys rosea* IG119 | Halotolerant | Cellulase | Pasqualetti et al. (2019) |
| *Aspergillus salisburgensis*, *Aspergillus atacamensis* | Halophilic | Cellulase | Martinelli et al. (2017) |
| *Aspergillus penicillioides* | Halophilic | Cellulase | Nazareth and Gonsalves (2014) |
| *Aspergillus nidulans* KK-99 | Halotolerant | Cellulase | Taneja et al. (2002) |
| *Eurotium herbariorum* | Halophilic | Cellulase | Butinar et al. (2005) |
| *Gymnascella marismortui* | Halophilic | Cellulase | Buchalo et al. (1998) |
| *Acrostalagmus luteoalbus*, *Emericellopsis alkaline* | Halotolerant | Cellulase | Grum-Grzhimaylo et al. (2016) |
| *Hortaea werneckii* | Halophilic | Cellulase | Gunde-Cimerman et al. (2009) |
| *Penicillium* sp., *Fusarium* sp., *Hypocrea* sp., *Mucor* sp. | Solar radiation tolerant | Cellulase | Avellaneda-Torres et al. (2015a, b) |
| *Aspergillus glaucus* CCHA | Halophilic | Cellulase | Li et al. (2018a, b) |

## 13.3 Cellulolytic System and Cellulases for Agricultural Waste Treatment

As stated above, plant biomass contains a large number of cell wall components. The cell wall is composed of cellulose, hemicellulose, and lignin. Despite great differences in composition and in the anatomical structure of cell walls across plant taxa, a high cellulose content—typically in the range of approximately 35–50% of plant dry weight—is a unifying feature (Lynd et al. 1999).

During biomass fermentation under natural conditions, aerobic degradation process is in the primary position. After the fermentation process of aerobic microorganisms in the first phase, a high temperature and anoxic environment is created accordingly, and then the following thermophilic, hypoxia-tolerant, or even anaerobic microorganisms further degrade and utilize the cellulose primarily degraded. The reason why cellulose-degrading microorganisms are capable of hydrolyzing and metabolizing this water-insoluble, solid-state, and refractory cellulose as the only carbon is that they have the ability to secrete cellulose-degrading enzymes and degrade cellulose into small molecular sugars. In cellulose system, the induced extracellular cellulases are either free or cell associated. The biochemical analysis of cellulase systems from aerobic and anaerobic bacteria and fungi has been comprehensively studied in the past decades.

Cellulose consists of β-1,4-linked glucosyl residues. Cellulases can specifically hydrolyze β-1,4-glucosidic bonds between glucosyl residues, which is distinguished from other glycoside hydrolases. The enzymatic breakage of the β-1,4-glucosidic bonds in cellulose proceeds through an acid hydrolysis mechanism, using a proton donor and nucleophile or base. The hydrolysis products can result in the double replacement mechanism either the inversion or retention of the anomeric configuration of carbon-1 at the reducing end (Withers 2001; Birsan et al. 1998). Therefore, cellulase system has specific structural characteristics and unique catalytic action (Henrissat et al. 1998). Based on the cellulase structural characteristics and enzymatic activities, cellulolytic system mainly involves three types of cellulases, which function independently and interactively. Endoglucanases (EC 3.2.1.4), namely, the 1,4-β-d-glucan-4-glucanohydrolases, cut at random at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends. Exoglucanases, which include 1,4-β-d-glucan glucanohydrolases (cellodextrinases, EC 3.2.1.74) and 1,4-β-d-glucancellobiohydrolases (cellobiohydrolases, EC 3.2.1.91), act on both the reducing or nonreducing ends of cellulose polysaccharide chains in a processive manner and then generating major products either glucose by glucanohydrolase activity or cellobiose by cellobiohydrolase activity; as to the microcrystalline cellulose, exoglucanases mainly act on the microcrystalline cellulose in the way of untwisting cellulose chains from the substrate structure (Teeri 1997). β-Glucosidases (-β-glucoside glucohydrolases, EC 3.2.1.21) act on the products of cellulose degraded by endo-/exo-glucanases, the soluble cellodextrins and cellobiose, and finally produce glucose (Fig. 13.1).

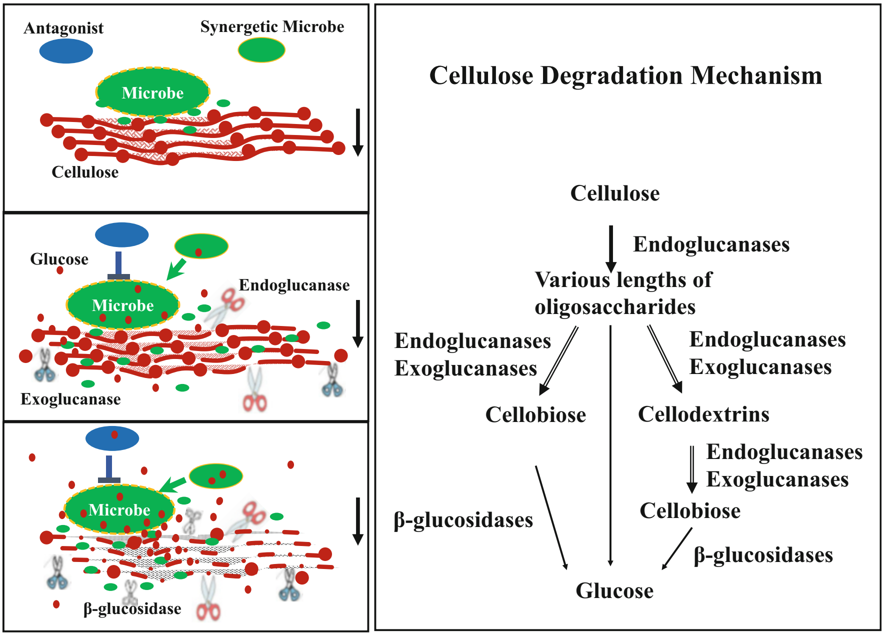

**Fig. 13.1** Cellulose degradation model. When cellulose degradation microorganisms live in cellulose environment, they produce a series of cellulose hydrolases, which gradually induced by cellulose, and finally produce glucose. In the process of cellulose degradation, the natural microbial community is in the balance of antagonism and synergy. When the synergistic effect is dominant, the cellulose degradation effect is obvious

In addition to cellulase, hydrolytic enzymes also involve hemicellulase, ligninase, and pectinase, which can degrade plant organic matter consisting with cellulose, hemicellulose, lignin, and pectin (e.g., maize, wheat, or rice straw) (Castillo and Demoulin 1997; Santos et al. 2004; Batista-García et al. 2014; Li et al. 2018a, b). The enzymatic hydrolysis of cellulose, particularly hydrogen-bonded and ordered crystalline regions, is very complex. Xylanases and β-xylosidases are the enzymes that attack the backbone of hemicellulose resulting in the production of xylose monomers. Lignin acts as physical barrier limiting the accessibility of enzymes to cellulose and hemicellulose substrates. The enzymatic hydrolysis process of cellulose is very slow. During biomass degrading, interactions of different celluloses and interactions between cellulases and other biomass hydrolases such as xylanases are important.

Much information is available on the production of cellulase enzymes using different substrates. The widely used substrate for determination of endoglucanase production is the highly soluble cellulose carboxymethylcellulose (generally termed as CMC). Actually, many microorganisms that can hydrolyze CMC via mixed β-glucan enzymes cannot degrade cellulose (Fields et al. 1998), thus the use of CMC as an enzymatic substrate is inaccurate, and many other subtracts to measure cellulase activity and accurate method should be considered. Recently, Lin et al. (2021) reported a simple, specific, and accurate method for discovering unique cellulolytic enzymes so that fungal species producing a diverse group of cellulolytic enzymes can be identified. The approach should be of value for identifying active cellulolytic enzymes that can facilitate management of agricultural wastes to fermentable sugars.

A variety of optimized cellulases have been identified from terrestrial fungi such as *Phanerochaete chrysosporium*, *Trichoderma reesei*, *Aspergillus niger*, *Penicillium oxalicum*, and *Gracilibacillus* species (Szabo et al. 1996; Sukumaran et al. 2009; Yu and Li 2015; Huang et al. 2015). Cellulases from ordinary fungi and extreme bacteria have been reported a lot; however, much less information is available on extreme fungi associated with cell wall degradation.

*Trichoderma reesei* and *Penicillium janthinellum* are known to be excellent cellulase producers, but their cellulases are not stable under alkali conditions (Mernitz et al. 1996; Wang et al. 2005; Qin et al. 2008). *Aspergillus niger*, one of the most efficient identified cellulose-degrading microorganisms, secretes large amounts of different cellulases during fermentation (Schuster et al. 2002). Endoglucanase B (EGLB), encoded by the endoglucanase gene (GenBank GQ292753) of *Aspergillus niger* BCRC31494, has been used in the fermentation industry because of its alkaline and thermal tolerance (Li et al. 2012). EGLB is a member of glycosyl hydrolase family 5 of the cellulase superfamily. When the recombinant EGLB cDNA was expressed in *Pichia pastoris*, a purified protein of 51 kDa in size was obtained. The enzyme was specific for substrates with β-1,3 and β-1,4 linkages, and it exhibited optimal activity at 70 °C and pH 4 (Li et al. 2012). Interestingly, the relative activity of recombinant EGLB at pH 9 was significantly better than that of wild-type EGLB. The advantages of endoglucanase *EGLB*, particularly its tolerance to a broad range of pH values, indicate that this enzyme has significant promise as a means of genetically improving fungi for haloalkaline soil remediation.

The soft rot ascomycetes fungus *T. reesei* is utilized for industrial production of secreted enzymes, especially lignocellulose-degrading enzymes. *T. reesei* uses several different enzymes for the degradation of plant cell wall-derived material, including 9 characterized cellulases, 15 characterized hemicellulases, and at least 42 genes predicted to encode cellulolytic or hemicellulolytic activities (Häkkinen et al. 2014). The family 7 cellobiohydrolase (Cel7A) of *T. reesei* is comprised of a 36-amino acid CBM, a linker domain with O-glycan, and a large catalytic domain with N-linked glycan and a 50-Å tunnel for processing cellulose chains. The possibility of controlled hydrolysis of microcrystalline cellulose by *T. reesei* has been analyzed. The penetration of fungus into the ordered regions of microcrystalline cellulose during incubation resulted in reduced crystallinity of nanocellulose prepared by microbial hydrolysis compared to that of acid hydrolysis (Satyamurthy et al. 2011). However, in comparison to the fungal hydrolysis system, the anaerobic bacteria consortium is much more efficient in hydrolyzing microcrystalline cellulose to produce nanocellulose in a span of 7 days with a maximum yield of 12.3%; and nanocellulose prepared by this process has a bimodal particle size distribution

(43 ± 13 and 119 ± 9 nm) (Satyamurthy and Vigneshwaran 2013). Thus, more efficient fungal hydrolyzing system must be strengthened.

Based on an analysis of the genomic sequence of haloalkaliphilic fungus *A. glaucus* CCHA, we found that *A. glaucus* CCHA expresses only one gene belonging to the GH5 family, AgCel5A. The open-reading frame of *Agcel5A* consists of 1509 base pairs that encode a polypeptide of 502 amino acids. AgCel5A has four potential N-glycosylation sites and three O-glycosylation sites, which indicates high similarity to the characterized GH5 β-glucosidases from *Aspergillus niger* (65%) and *Trichoderma reesei* (31%). AgCel5A was cloned and heterologously expressed in *Pichia pastoris* GS115. Recombinant AgCel5A exhibited maximal activity at pH 5.0. AgCel5A is much more stable than PdCel5Cfrom *Penicillium decumbens* (Liu et al. 2013); it retains more than 70% of its maximum activity at pH 8.0–10.0. In addition, AgCel5A exhibited stable degradation activity under high-salt (NaCl) conditions. In the presence of 4 M NaCl, AgCel5A retained 90% activity even after 4 h of preincubation. Interestingly, the activity of AgCel5A increased as the NaCl concentration was increased. The high resistance of AgCel5A to saline and alkaline conditions suggests that the *AgCel5A* gene is an ideal candidate for genetic improvement of soil fungi and industrial applications (Zhang et al. 2016; Li et al. 2018a, b).

The crystalline nature of cellulose microfibrils is one of the key factors influencing biomass recalcitrance. To degrade crystalline cellulose microfibrils, some bacteria are superior to fungi for two reasons. The first reason is that cellulases of some bacteria have multi-catalytic domain but fungal cellulases have single catalytic domain. However, the thermophilic fungus *Caldicellulosiruptor bescii* is capable of producing CelA cellulase to degrade highly crystalline cellulose as well as low-crystallinity substrates making it the only known cellulase to function well on highly crystalline cellulose (Brunecky et al. 2017). The *C. bescii* CelA is extremely thermostable and highly active at elevated temperatures. *C. bescii* CelA will have commercial development potential because the current commercial fungal cellulases are unstable and inefficient. Another reason is that certain bacteria can use both cell surface and periplasmic endoglucanases for the growth on cellulose. The soil bacterium *Cytophaga hutchinsonii* can actively digest crystalline cellulose, but be short of cellobiohydrolases, which are usually involved in the utilization of crystalline cellulose. Through identifying the mutants absent of eight of the endoglucanases encoded by cel5A, cel5B, cel5C, cel9A, cel9B, cel9C, cel9E, and cel9F, Zhu et al. (2016) revealed that both cell surface and periplasmic endoglucanases are required for the growth of *C. hutchinsonii* on cellulose and that novel cell surface proteins may solubilize and transport cellodextrins across the outer membrane.

In a defined biomass degradation system, the efficiency of a cellulolytic system depends on the activity and stability of cellulases. However, the enzymatic hydrolysis rate of plant biomass is often affected by various inhibition factors. Mostly, enzyme deactivation, a drop in substrate reactivity, or nonproductive binding of enzyme to lignin could be responsible for this loss of effectiveness; and xylose, xylan, and xylooligomers dramatically decrease conversion rates and yields. In a study, when xylan and various xylooligomers were added to Avicel hydrolysis at low enzyme loadings, the inhibition effect on hydrolysis system was greater than adding equal amounts of xylose derived from these materials or when added separately; and xylooligomers were more inhibitory than xylan or xylose (Qing et al. 2010). Mixed DP xylooligomers showed strong inhibition on cellulase enzymes but not on beta-glucosidase enzymes. By tracking the profile change of xylooligomers, a large portion of the xylooligomers was found to be hydrolyzed by Spezyme CP enzyme preparations, indicating competitive inhibition by mixed xylooligomers. A comparison among glucose sugars and xylose sugars also showed that xylooligomers were more powerful inhibitors than well-established glucose and cellobiose (Qing et al. 2010). This research clearly indicated that the interaction between different hydrolases in cellulose fermentation system can be realized through their products; also, it enlightens us that the complete degradation of cellulose is capable of realizing through designing microorganism consortia. Therefore, it is important to study the synergistic degradation mechanism between microorganisms.

## 13.4 Synergistic Actions of Varieties of Microorganisms

The *Daldinia caldariorum* strain D263 fungus secretes a wide range of cellulolytic and hemicellulolytic enzymes, including endoglucanases, exoglucanases, and xylanases (Lin et al. 2021). The D263 encodes at least 100 potential cellulolytic enzymes, and the proportion among different types of CAZymes in D263 is distinct from that in *T. reesei*, which have been used for commercial cellulase production. Therefore, the *D. caldariorum* D263 is multiple functional for cellulose degradation with potential application in commercial cellulase production. Nevertheless, D263 has not been applied in practice. Till now, commercial cellulase preparations for lignocellulose bioconversion are still produced by both fungi *T. reesei* and *A. niger*. There are many reasons to cause this result, but we believe that it most likely resulted from the interactions and final effect of multiple cellulolytic enzymes in D263. Just as above noted, cellulases-based biodegradation for biomass depends on the synergistic interactions of microorganism consortia; actually, it also depends on the relationship between cellulolytic enzymes and substrates even in a single microorganism.

There are antagonistic and reciprocal relationships between microorganisms in a defined environment. During cellulose degradation, the antagonistic and reciprocal relationships are quite common. The maximum cellulose conversion of *T. reesei* cellulase mixture was 15–20% higher than that of *Penicillium oxalicum* in the hydrolysis of corncob residue and Avicel. Nevertheless, both preparations hydrolyzed more than 92% of cellulose in Avicel. When added to Avicel hydrolysis residue that was less reactive to *P. oxalicum* cellulases, cellobiohydrolase I (CBH I) from *T. reesei* resulted in a higher cellulose conversion than its homologous proteins from *P. oxalicum* and *A. niger* at the same protein loadings (Du et al. 2018). The synergistic degradation action of *T. reesei* and *P. oxalicum* cellulase mixture implies that the two fungi can be applied together in agricultural waste management.

To obtain the synergistic bacteria of *Trichoderma*, co-culture of *T. asperellum* and *Bacillus amyloliquefaciens* has been carried out to induce the production of polysaccharide-hydrolyzing enzymes under both induction and repression conditions (Karuppiah et al. 2021). The enzymatic activity and its gene expression were induced by the co-culture compared to the monoculture. They found that the co-culture upregulated the transcription regulatory genes and downregulated the repressor genes under both repressor and inducer conditions, respectively. In addition, the crude enzyme produced by the co-culture and monocultures using the optimized medium containing molasses, cornmeal, and rice bran could be further used to hydrolyze the pretreated corn stover, rice straw, and wheat straw (Karuppiah et al. 2022). This research shows a promising and inexpensive method to advance the innovation on the continuous production of cellulase and xylanase under different circumstances for the bioconversion of lignocellulosic biomass into glucose for the biofuels.

In order to improve the methane yield of bioreactors fed with agricultural wastes, hydrolytic microorganisms-based consolidated bioprocessing has been extensively studied, which shows it is effective and has broad prospects. The thermophilic anaerobic bacterium *Clostridium thermocellum* is a multifunctional ethanol producer, capable of both saccharification and fermentation, that is central to the consolidated bioprocessing approach of converting lignocellulosic biomass to ethanol without external enzyme supplementation. So far, *C. thermocellum* has been one of the most promising host organisms for application of consolidated bioprocessing. The efficiency of *C. thermocellum* and *Melioribacter roseus* to degrade lignocellulosic matter was analyzed by Tsapekos et al. (2017). They found that *C. thermocellum* enhanced the methane yield by 34%, but *M. roseus* did not promote markedly the anaerobic biodegradability. Importantly, the applied strategies did not alter significantly the microbial communities (Tsapekos et al. 2017), confirming the *C. thermocellum* is a potential consolidated bioprocessing without impact on the indigenous microorganisms in biomethane production system.

Indeed, consolidated bioprocessing strategy with *C. thermocellum* could increase the methane yield up to a higher percentage; however, the indigenous microbial community was modified by the consolidated bioprocessing of *C. thermocellum* (EcemÖner et al. 2018). The impact of consolidated bioprocessing with *C. thermocellum* on the performance of anaerobic digesters depended on the inoculation ratios of *C. thermocellum*. During the process of consolidated bioprocessing, in the digester that was inoculated at the ratio of 20% (v:v), an increase in the abundance of *Ruminococcaceae* family led to a decrease in the *Bacteroidaceae* and *Synergistaceae* families. Additionally, the metabolic products of the bioaugmented strains greatly influenced the diversity of the archaeal community, and an increase in the abundance of Methanomicrobiales was observed (EcemÖner et al. 2018). In the process of application, in addition to consider the inoculation ratios of *C. thermocellum*, the biomass substrate composition in biomethane production system should also be considered (Kothari et al. 2018). Anyway, all the above studies firmly indicate that to design a synergistic microorganism consortium is important for consolidated bioprocessing.

## 13.5 Biofertilizer and Bioenergy Application

Compost, one of the most important biofertilizers, is the final product of aerobic microbial decomposition of agricultural wastes. Compost is a good alternative to chemical fertilizers, which have many negative impacts on the environment and the quality of agricultural products (López-González et al. 2015; Zhao et al. 2017). Compost is a natural, safe, and environment-friendly option for the recovery of organic fertility in the degraded soil and for supplying substances, such as nutrients, phytohormones, antioxidants, and enzymes that promote plant growth and production. The use of cellulose-degrading microorganism consortia containing the nitrogen-fixing microorganisms for composting has emerged as a promising method for enhancing the residue composting and quality of the compost (Harindintwali et al. 2020).

Air pollution has a negative impact on the biodiversity and ecosystems in the long term (Lovett et al. 2009). Recently, it is gradually aggravated with current farming activities, such as the widely concerned straw burning and overuse of the chemical fertilizers and pesticides, which causes the release of a large amount of harmful chemicals into the air (Gutiérrez-Miceli et al. 2007; Li et al. 2007; Behera et al. 2013; Doan et al. 2015). Unlike chemical fertilizers, composts have the ability to hold the nutrients and release them to the plants slowly. Composting has also been highlighted as a proven technology to reduce greenhouse gas emissions. The process of composting takes organic wastes and stabilizes them under controlled conditions. The nutrients are stabilized during composting and aggregated in the compost's organic matter, which enhances their efficient use by plants once they are applied to the soil. Thus, compost applications should be considered for reduction of air pollution and potential leaching of pesticides (Harindintwali et al. 2020).

Agricultural waste composts bioprocessed by halotolerant fungi have been beneficially used to bioremediate salt-affected soils (Sahin et al. 2011). In the study, suspensions of three fungal isolates (*Aspergillus* spp. FS 9, 11, and *Alternaria* spp. FS 8) and two bacterial strains (*Bacillus subtilis* OSU 142 and *Bacillus megaterium* M3) at $10^4$ spore/mL and $10^9$ CFU/mL, respectively, were mixed with leaching water and applied to the soil columns in the Igdir plain of northeastern Turkey; and the tested saline soils were remediated (Sahin et al. 2011). In our lab, we got a similar result in mycoremediation salt-affected soil by using *A. glaucus* CCHA with salt-tolerant fungal species. Fungi like *A. glaucus* CCHA have the ability to secrete considerable amounts of organic acids. Furthermore, with the increase of salinity and pH value, the organic acids secreted from fungi gradually increase (Chen et al. 2020). Several organic acids, such as gallic acid, gluconic acid, citric acid, itaconic acid, kojic acid, and malic acid, have been detected in the fermentation filtrate of *A. glaucus* CCHA while treated with 5% $NaHCO_3$ (Chen et al. 2020). In addition to the benefit for air conditions and soil remediation, as a biofertilizer, the compost can also promote plant to absorb multiple mineral nutrition essential for plant growth and development and provide organic carbon for the health of soil and plant roots (Tian et al. 2013). Importantly, both yield and quality of plants have been greatly improved after compost application (Lazcano et al. (2013). All the microorganisms including the inoculated fungi, their metabolites, and cellulolytic products in compost play a synergistic role in soil improvement.

In the field of bioenergy, clean energy production through using agricultural waste is an economic, environmentally friendly, and sustainable approach. However, the stability and efficiency of the natural fermentation system like biogas are very low, so it is very necessary to develop extreme environmental microorganisms and their cellulolytic enzymes. Rastogi et al. identified eight isolates capable of degrading cellulose, carboxymethyl cellulose, or ponderosa pine sawdust, which belong to the genera *Geobacillus*, *Thermobacillus*, *Cohnella*, and *Thermus*. A compost isolate WSUCF1 (*Geobacillus* sp.) was characterized to have higher growth rate and cellulase activity. WSUCF1 strain and a previously isolated thermophilic cellulose-degrading deep gold mine strain DUSELR13 (*Bacillus* sp.) were examined for their enzyme properties and kinetics. After incubation at 70 °C for 1 day, WSUCF1 and DUSELR13 retained 89% and 78% of the initial cellulase activities, respectively, suggesting that the thermostable enzymes will facilitate development of more efficient and cost-effective forms of the simultaneous saccharification and fermentation process to convert lignocellulosic biomass into biofuels (Rastogi et al. 2010).

Other solid waste like livestock manure and kitchen waste will be produced continuously. Similar to the plant biomass, their management predominantly involves unsustainable practices like landfilling and incineration. The organic fraction of these wastes typically comprises plenty of lignocellulose-rich material, but their fermentation conditions are even harsh. To identify robust species capable of fermenting this complex feedstock will also be one of the best strategies. From the eight biotechnologically useful microorganisms (*Clostridium saccharoperbutylacetonicum*, *Escherichia coli*, *Geobacillus thermoglucosidasius*, *Pseudomonas putida*, *Rhodococcus opacus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Zymomonas mobilis*), the three strains (*Z. mobilis*, *S. cerevisiae*, and *R. opacus*) were examined to be robust in fermentation system. To be exited, the three strains produced product to 69%, 70%, and 72% of the maximum theoretical fermentation yield and could theoretically produce 136 kg and 139 kg of ethanol and 91 kg of triacylglycerol per ton of wastes (Dornau et al. 2020). These species will be promising candidates for agricultural waste management.

## 13.6 Remarks

Agricultural waste composting involves the selection of a microbiota capable of resisting the high temperatures generated during the process and degrading the lignocellulose. A deep understanding of the thermophilic microbial community involved in such biotransformation is valuable to improve composting efficiency and to provide thermostable biomass-degrading enzymes for biorefinery. The value-added bio-product of the composting process contributes to the improvement of the soil properties and plant growth in an environment-friendly way. However, the conventional process employed for composting agricultural organic waste is slow and becomes an impediment for farmers who plant two or three crops a year. Therefore, in the future, we should continue to vigorously carry out isolation and identification researches on fermentation microorganisms with high efficiency, stability, and multiresistance. As for agricultural wastes for bioenergy, the process of bioenergy production is extremely complex, and there are many conditions that need to be adjusted; thus, at present, it is necessary to focus on metabolic regulation and interaction between fermentation microorganisms. At the same time, the development of commercial efficient hydrolases is also the key to solve the current problems.

**Acknowledgments** The related work in our lab was partially supported by grants from the National Natural Science Foundation of China (Grant Nos. 31671972 and 31670141) and a project of the Local scientific research of Department of Education of Liaoning Province of China (Grant No. 01032920021 to S.-H.Z.); Special talent introduction of Shenyang Agricultural University of China (Grant No. 880420019 to S.-H.Z.). The authors would like to thank the Zhang Lab members (Jilin University and Shenyang Agricultural University), who contributed to fungal isolation and field trials.

## References

Aime MC, Brearley FQ (2012) Tropical fungal diversity: closing the gap between species estimates and species discovery. Biodivers Conserv 21:2177–2180. https://doi.org/10.1007/s10531-012-0338-7

Amirita A, Sindhu P, Swetha J, Vasanthi NS, Kannan KP (2012) Enumeration of endophytic fungi from medicinal plants and screening of extracellular enzymes. World J Sci Technol 2:13–19

Arifoglu N, Ögel ZB (2000) Avicel-adsorbable endoglucanase production by the thermophilic fungus Scytalidium thermophilum type culture Torula thermophila. Enzyme Microb Technol 27:560–569

Avellaneda-Torres LM, Pulido CP, Rojas ET (2015a) Assessment of cellulolytic microorganisms in soils of Nevados Park, Colombia. Braz J Microbiol 45(4):1211–1220. https://doi.org/10.1590/s1517-83822014000400011

Avellaneda-Torres LM, Pulido CP, Rojas ET (2015b) Assessment of cellulolytic microorganisms in soils of Nevados Park, Colombia. Braz J Microbiol. 45(4):1211–1220. https://doi.org/10.1590/s1517-83822014000400011

Bakare M, Adewale I, Ajayi A, Shonukan O (2005) Purification and characterization of cellulase from the wild-type and two improved mutants of Pseudomonas fluorescens. Afr J Biotechnol 4: 898–904

Bass D, Howe A, Brown N, Barton H, Demidova M, Michelle H, Li L, Sanders H, Watkinson SC, Willcock S, Richards TA (2007) Yeast forms dominate fungal diversity in the deep oceans. Proc Biol Sci 274(1629):3069–3077. https://doi.org/10.1098/rspb.2007.1067

Batista-García RA, Balcázar-López E, Miranda-Miranda E, Sánchez-Reyes A, Cuervo-Soto L, Aceves-Zamudio D et al (2014) Characterization of lignocellulolytic activities from a moderate halophile strain of Aspergillus caesiellus isolated from a sugarcane bagasse fermentation. PLoS One 9(8):e105893

Batista-García RA, Sutton T, Jackson SA, Tovar-Herrera OE, Balcázar-López E, Sánchez-Carbente MDR et al (2017) Characterization of lignocellulolytic activities from fungi isolated from the deep-sea sponge Stelletta normani. PLoS One 12(3):e0173750

Behera SN, Sharma M, Aneja VP, Balasubramanian R (2013) Ammonia in the atmosphere: a review on emission sources, atmospheric chemistry and deposition on terrestrial bodies. Environ Sci Pollut Res Int 20(11):8092–8131. https://doi.org/10.1007/s11356-013-2051-9

Bezerra JD, Santos MG, Svedese VM, Lima DM, Fernandes MJ, Paiva LM, Souza-Motta CM (2012) Richness of endophytic fungi isolated from *Opuntia ficus-indica* Mill. (Cactaceae) and preliminary screening for enzyme production. World J Microbiol Biotechnol 28:1989–1995

Bhat MK, Bhat S (1997) Cellulose degrading enzymes and their potential industrial applications. Biotechnol Adv 15:583–620

Birsan C, Johnson P, Joshi M, MacLeod A, McIntosh L, Monem V, Nitz M, Rose DR, Tull D, Wakarchuck WW, Wang Q, Warren RA, White A, Withers SG (1998) Mechanisms of cellulases and xylanases. Biochem Soc Trans 26(2):156–160. https://doi.org/10.1042/bst0260156. PMID: 9649739

Bischof RH, Ramoni J, Seiboth B (2016) Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. Microb Cell Fact 15(1):106

Bridgwater AV (2012) Review of fast pyrolysis of biomass and product upgrading. Biomass Bioenergy 38:68–94

Brunecky R, Donohoe BS, Yarbrough JM, Mittal A, Scott BR, Ding H, Taylor LE II, Russell JF, Chung D, Westpheling J, Teter SA, Himmel ME, Bomble YJ (2017) The multi domain *Caldicellulosiruptor bescii* CelA Cellulase excels at the hydrolysis of crystalline cellulose. Sci Rep 7(1):9622. https://doi.org/10.1038/s41598-017-08985-w

Buchalo AS, Nevo E, Wasser SP, Oren A, Molitoris HP (1998) Fungal life in the extremely hypersaline water of the Dead Sea: first records. Proc Biol Sci 265(1404):1461–1465. https://doi.org/10.1098/rspb.1998.0458

Butinar L, Zalar P, Frisvad JC, Gunde-Cimerman N (2005) The genus Eurotium—members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol Ecol 51(2): 155–166. https://doi.org/10.1016/j.femsec.2004.08.002

Castillo G, Demoulin V (1997) NaCl salinity and temperature effects on growth of three wood-rotting basidiomycetes from a Papua New Guinea coastal forest. Mycol Res 101(3):341–344

Chamekh R, Deniel F, Donot C, Jany JL, Nodet P, Belabid L (2019) Isolation, identification and enzymatic activity of halotolerant and halophilic fungi from the great Sebkha of Oran in northwestern of Algeria. Mycobiology 47(2):230–241. https://doi.org/10.1080/12298093.2019.1623979

Chen HY, Xue DS, Feng XY, Yao SJ (2011) Screening and production of ligninolytic enzyme by a marine-derived fungal Pestalotiopsis sp. J63. Appl Biochem Biotechnol 165(7–8):1754–1769. https://doi.org/10.1007/s12010-011-9392-y

Chen L, Wei Y, Shi M, Li Z, Zhang SH (2020) Statistical optimization of a cellulase from Aspergillus glaucus CCHA for hydrolyzing corn and rice straw by RSM to enhance yield of reducing sugar. Biotechnol Lett 42(4):583–595. https://doi.org/10.1007/s10529-020-02804-5

Corrêa RC, Rhoden SA, Mota TR, Azevedo JL, Pamphile JA, de Souza CG, de Moraes Polizeli MLT, Bracht A, Peralta RM (2014) Endophytic fungi: expanding the arsenal of industrial enzyme producers. J Ind Microbiol Biotechnol 41(10):1467–1478. https://doi.org/10.1007/s10295-014-1496-2 de Castro AM, Pedro KCNR, da Cruz JC, Ferreira MC, Leite SGF, Pereira N (2010) Trichoderma harzianum IOC-4038: a promising strain for the production of a cellulolytic complex with significant β-glucosidase activity from sugarcane bagasse cellulignin. Appl Biochem Biotechnol 162(7):2111–2122

Doan TT, Henry-des-Tureaux T, Rumpel C, Janeau JL, Jouquet P (2015) Impact of compost, vermicompost and biochar on soil fertility, maize yield and soil erosion in northern Vietnam: a three year mesocosm experiment. Sci Total Environ 514:147–154. https://doi.org/10.1016/j.scitotenv.2015.02.005

Dornau A, Robson JF, Thomas GH, McQueen-Mason SJ (2020) Robust microorganisms for biofuel and chemical production from municipal solid waste. Microb Cell Fact 19(1):68. https://doi.org/10.1186/s12934-020-01325-0

Du J, Zhang X, Li X, Zhao J, Liu G, Gao B, Qu Y (2018) The cellulose binding region in *Trichoderma reesei* cellobiohydrolase I has a higher capacity in improving crystalline cellulose degradation than that of *Penicillium oxalicum*. Bioresour Technol 266:19–25. https://doi.org/10.1016/j.biortech.2018.06.050

EcemÖner B, Akyol Ç, Bozan M, Ince O, Aydin S, Ince B (2018) Bioaugmentation with Clostridium thermocellum to enhance the anaerobic biodegradation of lignocellulosic agricultural residues. Bioresour Technol 249:620–625. https://doi.org/10.1016/j.biortech.2017.10.040

Edgcomb VP, Beaudoin D, Gast R, Biddle JF, Teske A (2011) Marine subsurface eukaryotes: the fungal majority. Environ Microbiol 1:172–183. https://doi.org/10.1111/j.1462-2920.2010.02318.x

Feng Y, Duan C-J, Pang H, Mo X-C, Wu C-F, Yu Y, Hu Y-L, Wei J, Tang J-L, Feng J-X (2007) Cloning and identification of novel cellulase genes from uncultured microorganisms in rabbit cecum and characterization of the expressed cellulases. Appl Microbiol Biotechnol 75:319–328

Fields MW, Russell JB, Wilson DB (1998) The role of ruminal carboxymethylcellulases in the degradation of β-glucans from cereal grain. FEMS Microbiol Ecol 27:261–268

Fouda AH, Hassan SED (2015) Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.). Ann Agric Sci 60(1):95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111(Pt 1):3–49. https://doi.org/10.1016/j.mycres.2006.12.001

Goldbeck R, Ramos MM, Pereira GA, Maugeri-Filho F (2013) Cellulase production from a new strain Acremonium strictum isolated from the Brazilian biome using different substrates. Bioresour Technol 128:797–803. https://doi.org/10.1016/j.biortech.2012.10.034

Grum-Grzhimaylo AA, Georgieva ML, Bondarenko SA, Debets AJM, Bilanenko EN (2016) On the diversity of fungi from soda soils. Fungal Divers 76:27–74

Gunde-Cimerman N, Ramos J, Plemenitas A (2009) Halotolerant and halophilic fungi. Mycol Res 113(Pt 11):1231–1241. https://doi.org/10.1016/j.mycres.2009.09.002

Gupta VK, Kubicek CP, Berrin JG, Wilson DW, Couturier M, Berlin A, Filho EXF, Ezeji T (2016) Fungal enzymes for bio-products from sustainable and waste biomass. Trends Biochem Sci 41: 633–645. https://doi.org/10.1016/j.tibs.2016.04.006

Gutiérrez-Miceli FA, Santiago-Borraz J, Montes Molina JA, Nafate CC, Abud-Archila M, Oliva Llaven MA, Rincón-Rosales R, Dendooven L (2007) Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (Lycopersicum esculentum). Bioresour Technol 98(15):2781–2786. https://doi.org/10.1016/j.biortech.2006.02.032

Gutiérrez-Rojas I, Moreno-Sarmiento N, Montoya D (2013) Mechanisms and regulation of enzymatic cellulose hydrolysis in filamentous fungi: classical cases and new models. Rev Iberoam Micol 32:1–12. https://doi.org/10.1016/j.riam.2013.10.009

Häkkinen M, Valkonen MJ, Westerholm-Parvinen A, Aro N, Arvas M, Vitikainen M, Penttilä M, Saloheimo M, Pakula TM (2014) Screening of candidate regulators for cellulase and hemicellulase production in Trichoderma reesei and identification of a factor essential for cellulase production. Biotechnol Biofuels 7(1):14. https://doi.org/10.1186/1754-6834-7-14

Harindintwali JD, Zhou J, Yu X (2020) Lignocellulosic crop residue composting by cellulolytic nitrogen-fixing bacteria: a novel tool for environmental sustainability. Sci Total Environ 715: 136912. https://doi.org/10.1016/j.scitotenv.2020.136912

Henrissat B, Teeri TT, Warren RA (1998) A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. FEBS Lett 425(2):352–354. https://doi.org/10.1016/s0014-5793(98)00265-8. PMID: 9559678

Horta MAC, Vicentini R, da Silva DP, Laborda P, Crucello A, Freitas S, Kuroshu RM, Polikarpov I, da Cruz Pradella JG, Souza AP (2014) Transcriptome profile of Trichoderma harzianum IOC-3844 induced by sugarcane bagasse. PLoS One 9(2):e88689

Huang Y, Qin X, Luo XM, Nong Q, Yang Q, Zhang Z et al (2015) Efficient enzymatic hydrolysis and simultaneous saccharification and fermentation of sugarcane bagasse pulp for ethanol production by cellulase from Penicillium oxalicum EU2106 and thermotolerant Saccharomyces cerevisiae ZM1-5. Biomass Bioenergy 77:53–63. https://doi.org/10.1016/j.biombioe.2015.03.020

Isikgor FH, Becer CR (2015) Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym Chem 6:4497–4559

Isroi MR, Syamsiah S, Niklasson C, Cahyanto MN, Lundquist K, Taherzadeh MJ (2011) Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. BioResources 6:5224–5259. https://doi.org/10.15376/biores.6.4

Karuppiah V, Zhixiang L, Liu H, Murugappan V, Kumaran S, PerianaikaAnahas AM, Chen J (2022) Co-cultivation of T. asperellum GDFS1009 and B. amyloliquefaciens 1841: strategy to regulate the production of ligno-cellulolytic enzymes for the lignocellulose biomass degradation. J Environ Manag 301:113833. https://doi.org/10.1016/j.jenvman.2021.113833

Kim KC, Yoo SS, Oh YA, Kim SJ (2003) Isolation and characteristics of Trichoderma harzianum FJ1 producing cellulases and xylanase. J Microbiol Biotechnol 13:1–8

Kostylev M, Wilson D (2012) Synergistic interactions in cellulose hydrolysis. Biofuels 3:61–70

Kothari N, Holwerda EK, Cai CM, Kumar R, Wyman CE (2018) Biomass augmentation through thermochemical pretreatments greatly enhances digestion of switchgrass by *Clostridium thermocellum*. Biotechnol Biofuels 11:219. https://doi.org/10.1186/s13068-018-1216-7

Lazcano C, Arnold J, Tato A, Zaller JG, Domínguez J (2013) Compost and vermicompost as nursery pot components: effects on tomato plant growth and morphology. Spanish J Agric Res. https://doi.org/10.5424/sjar/2009074-1107

Le Calvez T, Burgaud G, Mahé S, Barbier G, Vandenkoornhuyse P (2009) Fungal diversity in Deep-Sea hydrothermal ecosystems. Appl Environ Microbiol 75:6415–6421. https://doi.org/10.1128/AEM.00653-09

Lee Y-J, Kim B-K, Lee B-H, Jo K-I, Lee N-K, Chung C-H, Lee Y-C, Lee J-W (2008) Purification and characterization of cellulase produced by Bacillus amyoliquefaciens DL-3 utilizing rice hull. Bioresour Technol 99:378–386

Li X, Wang S, Duan L, Hao J, Li C, Chen Y, Yang L (2007) Particulate and trace gas emissions from open burning of wheat straw and corn stover in China. Environ Sci Technol 41(17): 6052–60588. https://doi.org/10.1021/es0705137

Li CH, Wang HR, Yan TR (2012) Cloning, purification, and characterization of a heat- and alkaline-stable endoglucanase B from Aspergillus niger BCRC31494. Molecules 17(8): 9774–9789. https://doi.org/10.3390/molecules17089774

Li Z, Pei X, Zhang Z, Wei Y, Song Y, Chen L, Liu S, Zhang SH (2018a) The unique GH5 cellulase member in the extreme halotolerant fungus Aspergillus glaucus CCHA is an endoglucanase with multiple tolerance to salt, alkali and heat: prospects for straw degradation applications. Extremophiles 22(4):675–685. https://doi.org/10.1007/s00792-018-1028-5

Li Y-H, Zhang X-Y, Zhang F, Peng L-C, Zhang D-B, Kondo A, Bai F-W, Zhao X-Q (2018b) Optimization of cellulolytic enzyme components through engineering Trichoderma reesei and on-site fermentation using the soluble inducer for cellulosic ethanol production from corn Stover. Biotechnol Biofuels 11(1):49

Lin MC, Kuo HW, Kao MR, Lin WD, Li CW, Hung KS, Yang SC, Yu SM, Ho TD (2021) From simple and specific zymographic detections to the annotation of a fungus Daldinia caldariorum D263 that encodes a wide range of highly bioactive cellulolytic enzymes. Biotechnol Biofuels 14(1):120. https://doi.org/10.1186/s13068-021-01959-1

Liu G, Qin Y, Hu Y, Gao M, Peng S, Qu Y (2013) An endo-1,4-β-glucanase PdCel5C from cellulolytic fungus Penicillium decumbens with distinctive domain composition and hydrolysis product profile. Enzyme Microb Technol 52(3):190–195. https://doi.org/10.1016/j.enzmictec.2012.12.009

López MJ, Jurado MM, López-González JA, Estrella-González MJ, Martínez-Gallardo MR, Toribio A, Suárez-Estrella F (2021) Characterization of thermophilic lignocellulolytic microorganisms in composting. Front Microbiol 12:697480. https://doi.org/10.3389/fmicb.2021.697480

López-González JA, Suárez-Estrella F, Vargas-García MC, López MJ, Jurado MM, Moreno J (2015) Dynamics of bacterial microbiota during lignocellulosic waste composting: studies upon its structure, functionality and biodiversity. Bioresour Technol 175:406–416. https://doi.org/10.1016/j.biortech.2014.10.123

Lovett GM, Tear TH, Evers DC, Findlay SE, Cosby BJ, Dunscomb JK, Driscoll CT, Weathers KC (2009) Effects of air pollution on ecosystems and biological diversity in the eastern United States. Ann N Y Acad Sci 1162:99–135. https://doi.org/10.1111/j.1749-6632.2009.04153.x

Lynd LR, Wyman CE, Gerngross TU (1999) Biocommodity engineering. Biotechnol Prog 15(5):777–793. https://doi.org/10.1021/bp990109e

Martinelli L, Zalar P, Gunde-Cimerman N, Azua-Bustos A, Sterflinger K, Piñar G (2017) Aspergillus atacamensis and A. salisburgensis: two new halophilic species from hypersaline/arid habitats with a phialosimplex-like morphology. Extremophiles 21(4):755–773. https://doi.org/10.1007/s00792-017-0941-3

Mernitz G, Koch A, Henrissat B, Schulz G (1996) Endoglucanase II (EGII) of Penicillium janthinellum: cDNA sequence, heterologous expression and promotor analysis. Curr Genet 29(5):490–495. https://doi.org/10.1007/BF02221519. PMID: 8625430

Moreno J, López-González JA, Arcos-Nievas MA, Suárez-Estrella F, Jurado MM, Estrella-González MJ, López MJ (2021) Revisiting the succession of microbial populations throughout composting: a matter of thermotolerance. Sci Total Environ 773:145587. https://doi.org/10.1016/j.scitotenv.2021.145587

Nagraj A, Singhvi MS, Kumar R, Gokhale D (2014) Optimization studies for enhancing cellulase production by Penicillium janthinellum mutant EU2D-21 using response surface methodology. BioResource 9(2):1914–1923. https://doi.org/10.15376/biores.9.2.1914-1923

Nazareth S, Gonsalves V (2014) Aspergillus penicillioides—a true halophile existing in hypersaline and polyhaline econiches. Ann Microbiol 64:397–402

Pachiadaki MG, Rédou V, Beaudoin DJ, Burgaud G, Edgcomb VP (2016) Fungal and prokaryotic activities in the marine subsurface biosphere at Peru margin and Canterbury Basin inferred from RNA-based analyses and microscopy. Front Microbiol 7:846. https://doi.org/10.3389/fmicb.2016.00846

Pandey SS, Singh S, Babu CS, Shanker K, Srivastava NK, Kalra A (2016) Endophytes of opium poppy differentially modulate host plant productivity and genes for the biosynthetic pathway of benzylisoquinoline alkaloids. Planta 243:1097–1104. https://doi.org/10.1007/s00425-016-2467-9

Pandit S, Savla N, Sonawane JM, Sani AM, Gupta PK, Mathuriya AS, Rai AK, Jadhav DA, Jung SP, Prasad R (2021) Agricultural waste and wastewater as feedstock for bioelectricity generation using microbial fuel cells: recent advances. Fermentation 7:169. https://doi.org/10.3390/fermentation7030169

Pasqualetti M, Barghini P, Giovannini V, Fenice M (2019) High production of chitinolytic activity in halophilic conditions by a new marine strain of *Clonostachys rosea*. Molecules 24(10):1880. https://doi.org/10.3390/molecules24101880

Qin Y, Wei X, Song X, Qu Y (2008) Engineering endoglucanase II from Trichoderma reesei to improve the catalytic efficiency at a higher pH optimum. J Biotechnol 135(2):190–195. https://doi.org/10.1016/j.jbiotec.2008.03.016

Qing Q, Yang B, Wyman CE (2010) Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresour Technol 101(24):9624–9630. https://doi.org/10.1016/j.biortech.2010.06.137

Rastogi G, Bhalla A, Adhikari A, Bischoff KM, Hughes SR, Christopher LP, Sani RK (2010) Characterization of thermostable cellulases produced by Bacillus and Geobacillus strains. Bioresour Technol 101(22):8798–8806. https://doi.org/10.1016/j.biortech.2010.06.001

Robak K, Balcerek M (2018) Review of second generation bioethanol production from residual biomass. Food Technol Biotechnol 56(2):174–187. https://doi.org/10.17113/ftb.56.02.18.5428

Robl D, Delabona P, Mergel CM, Rojas JD, Costa P, Pimentel IC, Vicente VA, Pradella JG, Padilla G (2013) The capability of endophytic fungi for production of hemicellulases and related enzymes. BMC Biotechnol 13:94. https://doi.org/10.1186/1472-6750-13-94

Sahin U, Eroğlum S, Sahin F (2011) Microbial application with gypsum increases the saturated hydraulic conductivity of saline–sodic soils. Appl Soil Ecol 48:247–250

Saini JK, Saini R, Tewari L (2015) Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech 5(4): 337–353. https://doi.org/10.1007/s13205-014-0246-5

Santos IS, Da Cunha M, Machado OL, Gomes VM (2004) A chitinase from Adenanthera pavonina L. seeds: purification, characterisation and immunolocalisation. Plant Sci 167(6):1203–1210

Santos CA, Morais MA, Terrett OM, Lyczakowski JJ, Zanphorlin LM, Ferreira-Filho JA, Tonoli CC, Murakami MT, Dupree P, Souza AP (2019) An engineered GH1 β-glucosidase displays enhanced glucose tolerance and increased sugar release from lignocellulosic materials. Sci Rep 9(1):1–10

Sarsaiya S, Jain A, Kumar Awasthi S, Duan Y, Kumar Awasthi M, Shi J (2019) Microbial dynamics for lignocellulosic waste bioconversion and its importance with modern circular economy, challenges and future perspectives. Bioresour Technol 291:121905. https://doi.org/10.1016/j.biortech.2019.121905

Satyamurthy P, Vigneshwaran N (2013) A novel process for synthesis of spherical nanocellulose by controlled hydrolysis of microcrystalline cellulose using anaerobic microbial consortium. Enzyme Microb Technol 52(1):20–25. https://doi.org/10.1016/j.enzmictec.2012.09.002

Satyamurthy P, Jain P, Balasubramanya RH, Vigneshwaran N (2011) Preparation and characterization of cellulose nanowhiskers from cotton fibres by controlled microbial hydrolysis. Carbohydr Polym 83(1):122–129

Schuster E, Dunn-Coleman N, Frisvad JC, Van Dijck PW (2002) On the safety of Aspergillus niger—a review. Appl Microbiol Biotechnol 59(4–5):426–435. https://doi.org/10.1007/s00253-002-1032-6

Sideney BO, Shaiana PM, da Silva GC, Deizi G, Ivair M (2013) Production of cellulases by the endophytic fungus *Fusarium oxysporum*. J Microbial Res 3:131–134. https://doi.org/10.5923/j.microbiology.20130304.01

Sukumaran RK, Singhania RR, Mathew GM, Panday A (2009) Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. Renew Energy 34:421–424. https://doi.org/10.1016/j.renene.2008.05.008

Szabo I, Johansson G, Pettersson G (1996) Optimized cellulase production by Phanerochaete chrysosporium: control of catabolite repression by fed-batch cultivation. J Biotechnol 48:221–230. https://doi.org/10.1016/0168-1656(96)01512-X

Taneja K, Gupta S, Kuhad RC (2002) Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus Aspergillus nidulans KK-99. Bioresour Technol 85(1): 39–42. https://doi.org/10.1016/s0960-8524(02)00064-0

Tanger P, Field JL, Jahn CE, Defoort MW, Leach JE (2013) Biomass for thermochemical conversion: targets and challenges. Front Plant Sci 4:218. https://doi.org/10.3389/fpls.2013.00218

Tansey MR, Brock TD (1972) The upper temperature limit for eukaryotic organisms. Proc Natl Acad Sci U S A 69(9):2426–2428. https://doi.org/10.1073/pnas.69.9.2426

Teeri TT (1997) Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. Trends Biotechnol 15:160–167

Tian H, Gao J, Hao J, Lu L, Zhu C, Qiu P (2013) Atmospheric pollution problems and control proposals associated with solid waste management in China: a review. J Hazard Mater 252–253: 142–154. https://doi.org/10.1016/j.jhazmat.2013.02.013

Tsapekos P, Kougias PG, Vasileiou SA, Treu L, Campanaro S, Lyberatos G, Angelidaki I (2017) Bioaugmentation with hydrolytic microbes to improve the anaerobic biodegradability of lignocellulosic agricultural residues. Bioresour Technol 234:350–359. https://doi.org/10.1016/j.biortech.2017.03.043

Tsegaye B, Balomajumder C, Roy P (2018) Isolation and characterization of novel lignolytic, cellulolytic, and Hemicellulolytic bacteria from wood-feeding termite Cryptotermes brevis. Int Microbiol 22:29–39. https://doi.org/10.1007/s10123-018-0024-z

Uzma F, Konappa NM, Chowdappa S (2016) Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka. Egypt J Basic Appl Sci 3(4):335–342

Venkatramanan V, Shah S, Prasad R, Shah M (2021) Life cycle assessment of lignocellulosic waste biorefinery. In: Shah S, Venkatramanan V, Prasad R (eds) Biovalorization of waste: trends and perspectives. Springer Nature Singapore, pp 327–347

Wang T, Liu X, Yu Q, Zhang X, Qu Y, Gao P, Wang T (2005) Directed evolution for engineering pH profile of endoglucanase III from Trichoderma reesei. Biomol Eng 22(1–3):89–94. https://doi.org/10.1016/j.bioeng.2004.10.003

Withers SG (2001) Mechanisms of glycosyl transferases and hydrolyses. Carbohydr Polym 44: 325–337

Yu HY, Li X (2015) Alkali-stable cellulose from a halophilic isolate, Gracilibacillus sp. SK1 and its application in lignocellulosic saccharification for ethanol. Biomass Bioenergy 81:19–25. https://doi.org/10.1016/j.biombioe.2015.05.020

Zeng ZQ, Zhuang WY (2019) Two new species and a new Chinese record of Hypocreaceae as evidenced by morphological and molecular data. Mycobiology 47(3):280–291

Zhang YG, Lu XH, Ding YB, Zhou XK, Li L, Guo JW et al (2016) Phytoactinopolyspora alkaliphila sp. nov., an alkaliphilic actinomycete isolated from a saline-alkaline soil. Int J Syst Evol Microbiol 66(5):2058–2063

Zhao Y, Zhao Y, Zhang Z, Wei Y, Wang H, Lu Q, Li Y, Wei Z (2017) Effect of thermo-tolerant actinomycetes inoculation on cellulose degradation and the formation of humic substances during composting. Waste Manag 68:64–73. https://doi.org/10.1016/j.wasman.2017.06.022

Zhu Y, Han L, Hefferon KL, Silvaggi NR, Wilson DB, McBride MJ (2016) Periplasmic Cytophaga hutchinsonii endoglucanases are required for use of crystalline cellulose as the sole source of carbon and energy. Appl Environ Microbiol 82(15):4835–4845. https://doi.org/10.1128/AEM.01298-16

# Chapter 14
# Effects of Microbial Signaling in Plant Growth and Development

**Sahana Basu, Preeti Priyadarshini, Ram Prasad, and Gautam Kumar**

**Abstract** Agricultural sustainability is predominantly regulated by the functional equilibrium between soil processes and productivity of plants. Growth and productivity of plants frequently depend on their intimate association with the microbial organisms present in the rhizosphere. The present chapter summarizes an updated knowledge on the role of microbial signaling in regulating plant growth and also recommends future research prospects in this area. Plant roots release various organic compounds in its surrounding soil, known as root exudates leading to the induction of the beneficial rhizospheric microbes. Microorganisms also modify the plants' behavior by producing inter-organismal signaling molecules. Plant growth-promoting rhizobacteria (PGPR) play an important role in the signaling, metabolism, and hormonal homeostasis in plants. They also produce antibiotic compounds that inhibit the growth of poisonous rhizospheric microbes, thereby promoting plant growth. The PGPR also increase the availability and uptake of nutrients and provide resistance to abiotic and biotic stresses leading to agricultural sustainability. Free-living beneficial fungi in the soil microbiome efficiently spread over the rhizosphere and eliminate pathogenic fungal strains by competitive inhibition. They also contribute in the antibiotic production and elicitation of defense responses in plants. Signaling process is executed by other classes of molecules, including N-acyl-l-homoserine lactones (AHLs) and microbial volatile organic compounds (MVOCs). The AHLs and MVOCs play a crucial role in the bacterial downstream signaling, by which the bacterial genes may express. These molecules are also recognized by the plants further contributing in the improvement of plant development and defense mechanisms by upregulating different genes.

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Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-19-0733-3_14

**Keywords** Microbial signal · Plant growth-promoting rhizobacteria · Root exudate · Sustainable agriculture

## 14.1 Introduction

Plants play a pivotal role in maintaining the ecosystem. They have been established to have many evolutionary benefits, which actually help them in acclimatizing with the stressful environments. Additionally, several studies have revealed the plants to possess a complicated nutritional organization (Marschner 1995). Plant-microbe interactions comprise of rhizomicrobiome (microbes associated with root), phyllomicrobiome (microbes associated with aboveground tissues), and endosphere (microbial colonization of internal tissues) (Basu and Kumar 2020a). However, rhizomicrobiome has been extensively studied than the other components.

Root system in plants is the most important morphologically and physiologically plastic structure forming a vigorous assembly after entering inside the soil. In addition to providing anchorage, root has other major contributions directly or indirectly associated with the plant growth and development (Kumar et al. 2022a). Root system also directly perceives various environmental stresses (Kumar et al. 2022b; Mishra et al. 2021). Therefore, development of plants eventually depends upon the environment (Kumar et al. 2021). Root in the soil continuously interacts with the microbial community, supporting in the plant development and immunity. Roots also secrete various chemical components, which play a significant role in regulating the phytomicrobiome.

Soil ecosystem plays a crucial role in the assimilation of the organic compounds in soil, deterioration of living particles, eradication of pathogenic components, and detoxification (Behera and Prasad 2020). In the soil ecosystem, the microorganisms survive with the support of soil carbon, which actually comes from the plants' rhizosphere. It is the zone of soil surrounding the plant root, which harbors a variety of microbes (Shrivastava et al. 2014). Numerous studies have shown the occurrence of root-microbes and intra-inter-microbial interaction through the soil ecosystem, facilitating the soil fertility, plant growth, root and shoot formation, and development of leaf primordia and flowers (Basu et al. 2020a). However, interactions between the rhizosphere and microbes are still not completely understood.

Higher plants interact with their associated microbes in diverse ways affecting each other (Berendsen et al. 2012). Microorganisms modify the plants' behavior by producing inter-organismal signaling molecules, and plant-synthesized signaling compounds regulate the microbial populations. Thus, plants and microbes alter each other's behavior for their individual benefit leading to symbiotic association. The signaling in the phytomicrobiome stimulating plant growth has been illustrated in Fig. 14.1.

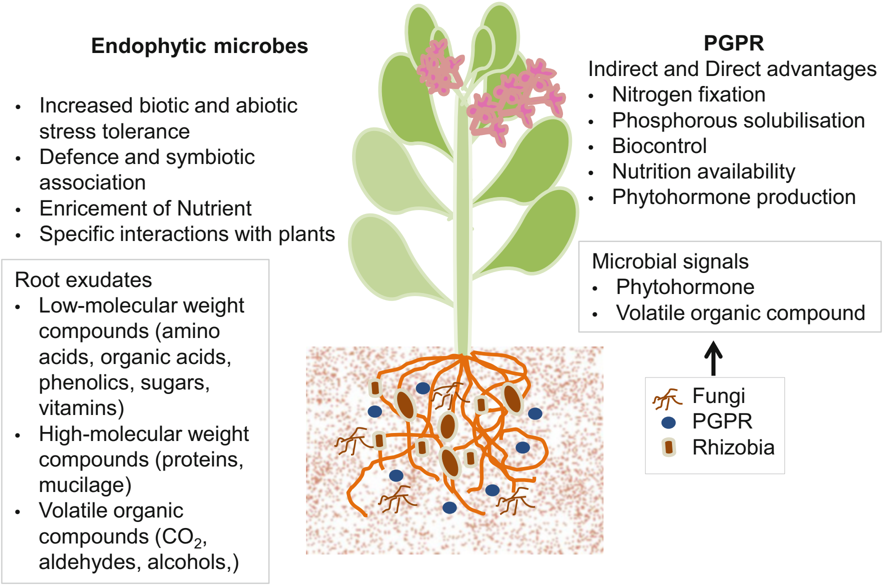

**Fig. 14.1** Cross talking of plants and microbes through signaling pathways for promotion of plant growth

## 14.2 Plant Signal: Root Exudates

During the various stages of plant growth, seed germination to flowering the plant interacts with the surrounding soil which has a wide variety of microorganisms. Plant roots release various organic compounds in its surrounding soil, known as root exudates that lead to the improvement of the soil environment (Sarkar et al. 2022). The physical, chemical, and biological nature of the soil changes during the plant growth because of root exudates and rhizodeposition (el Zahar Haichar et al. 2014). This phenomenon of change in nature of surrounding soil is known as the rhizosphere effect. Root exudates are mainly composed of amino acids, organic acids, water-soluble sugars, hormones, vitamins, amino compounds, phenolics, and sugar phosphate esters. It is estimated that around 20–40% of the carbon fixed during photosynthesis is released as exudates from the plant roots (Badri and Vivanco 2009). The nature of the exudates depends on the growth stage of the plant and the environment. Modern ecological theories correlate root exudation for the plants' benefit for stimulating the beneficial microorganisms, thereby facilitating nutrient acquisition eventually inducing plant growth (Ortiz-Castro et al. 2009).

Based on the chemical nature of the compound, root exudates are categorized into three main classes.

### *14.2.1 Low-Molecular-Weight Compounds*

They contribute to a major portion of root exudates, comprising amino acids, organic acids, phenolics, sugars, vitamins, and several secondary metabolites. These are usually transported through passive transport along the concentration gradient between the root cells and the external (soil) solution.

### *14.2.2 High-Molecular-Weight Compounds*

They include proteins and mucilage.

### *14.2.3 Volatile Organic Compounds (VOCs)*

They include carbon dioxide ($CO_2$), aldehydes, alcohols, and secondary metabolites.

The composition of the root exudates usually varies with plant species, age, and growth stages of plants' life cycle. Several abiotic factors, including soil type, temperature, light, micro-, and macronutrients also impact the chemical nature and time of exudation (Chai and Schachtman 2021). These factors often increase the rate of the exudation, thereby affecting the membrane integrity. For instance, under nitrogen-deficient conditions, root exudates assist the associations of plants with nitrogen-fixing microbes. Under this circumstance root exudates also inhibit the nitrifying and denitrifying bacteria, thereby reducing soil nitrogen losses. Ramesh et al. (2015) have revealed the anions and γ-aminobutyric acid (GABA) at the root apex to regulate the malate efflux anions channel (ALMT) under abiotic stress. Dong et al. (2004) have found the exudation of organic acids (such as oxalic acid, malic acid, and citric acid) to be predominantly increased in soybean plants under phosphate-deficient and aluminum-contaminated soil conditions. The chemicals present in the root exudates either attract microbes or produce carbon providing nutrition to the microbes.

Ecosystem also influences the nature and signaling cascade of the plant exudates. For instance, microbial infection in the plant root system assists the production of defense-oriented root exudates. The molecular pattern and the concentration of root exudates in soil are complex phenomena, which have not been completely understood so far. These exudates attract soil microbes, thereby establishing the rhizosphere effect. The composition of exudates also affects the microbial populations and activities.

## 14.3 Rhizosphere

The term “rhizosphere” was coined by a German plant physiologist Lorenz Hiltner in 1904 (Hiltner 1904). Rhizosphere (Greek “rhiza,” root and “sphere,” surrounding area) is defined as the plant root-soil interface or the seed-soil interface (Harley and Russell 1979). It has been described as the area surrounding the plant root (2–80 mm distance from root system), inhabited by distinctive microorganisms inducing the root exudation. It is a region of increased nutrient concentration and biotic activity. Enhanced interactions between the plants and soil microbes can be perceived in this region and, therefore, described as the microbe storehouse. These interactions can be symbiotic (beneficial), pathogenic (harmful), or neutral. The present chapter has discussed the beneficial aspects of the soil microbial interactions and their impact on plant growth and development.

### *14.3.1 Zones of the Rhizosphere*

The rhizosphere is categorized into three zones based on the relative distance from the root (Lynch and Whipps 1991). As the proximity decreases, the influence of the root and its exudates decreases.

#### 14.3.1.1 Endorhizosphere

It is the innermost zone, which includes the cortex and endodermis (internal) where the microbes and cations can occupy the apoplastic space (free space between cells).

#### 14.3.1.2 Rhizoplane

It is the medial zone, which includes the root epidermis and mucilage. It is the root surface directly adjacent to the soil.

#### 14.3.1.3 Exorhizosphere

It is the outermost zone contiguous to the epidermis extending from the rhizoplane out into the bulk soil.

The rhizosphere cannot be defined with a definite area or size due to the diversity and complexity of the plant root system. It is a gradient of physical, chemical, and biological properties changing along the root both radially and longitudinally. The rhizosphere isolated from the bulk soil is termed as edaphosphere (non-rhizosphere), where the rhizosphere effect is negligible or absent. It has 10- to 100-fold less microbial density than the rhizosphere. The rhizosphere is enriched in organic matter and generally more acidic than the edaphosphere. The soil in the rhizosphere is subjected to chemical changes caused by the presence of root exudates and metabolites of microbial degradation.

## *14.3.2 Rhizosphere Effect and R/E Ratio*

The rhizosphere effect indicates enhanced microbial activity in the rhizosphere. It can be quantitatively expressed with R/E ratio, where "R" denotes the amount of root exudates in rhizosphere soil and "E" is the edaphosphere soil content. This ratio is determined from the number of microbes present in the rhizosphere to that of the edaphosphere. The value of R/E ranges within 5–20.

## *14.3.3 Microbiome in Rhizosphere*

Rhizosphere harbors a variety of microbes like bacteria, fungi, actinomycetes, and algae (Campbell and Greaves 1990). Based on the nucleic acid analyses techniques of genomic molecules from soil, the samples have been revealed to exhibit a huge diversity in the rhizospheric microbial population (Basu and Kumar 2021a). Around 98% of the microbes in the soil cannot be cultured, and hence their identification, characterization, and their effect are still not known. Some microbes are in a close association with roots like mycorrhizal fungi. There are a variety of free-living microbes which include filamentous fungi of the genus *Trichoderma* and a variety of plant growth-promoting rhizobacteria (PGPR) (Prasad et al. 2015). Rhizospheric organisms that have been well studied for their beneficial effects on plant growth and health include the nitrogen-fixing bacteria, mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), biocontrol microorganisms, mycoparasitic fungi, and protozoa (Basu and Kumar 2020a, b).

### 14.3.3.1 Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are various bacterial members of several taxonomic groups colonizing the rhizosphere. Natural PGPR commonly include various species of *Pseudomonas* sp. and *Bacillus* sp. colonizing different plants such as *Arabidopsis*, barley, and rice. Numerous studies have revealed the PGPR to promote growth and productivity of different agricultural crop species plant (Backer et al. 2018; Tsukanova et al. 2017). They have also been found to confer induced systemic resistance (ISR) and systemic acquired resistance (SAR) consequently reducing phytotoxic microbial populations (Bukhat et al. 2020). PGPR play an important role in the signaling, metabolism, and hormonal homeostasis in plants

(Tsukanova et al. 2017). They also produce antibiotic compounds as well as hydrogen cyanide that inhibit the growth of poisonous rhizospheric microbes, thereby promoting plant growth. The PGPR also increase the availability and uptake of nutrients and provide resistance to abiotic and biotic stresses leading to agricultural sustainability.

#### 14.3.3.2 Beneficial Fungi

In the soil microbiome, fungi also play a crucial role in promotion of plant growth. Mycotic populations efficiently spread over the soil and rhizosphere and eliminate the pathogenic fungal strains by competitive inhibition. They also contribute in the antibiotic production and elicitation of defense responses. The beneficial plant fungal populations stabilize the pathogenic microbes by parasitizing their spore (mycoparasitism) and sclerotia, ultimately leading to biocontrol. They also produce chemical compounds and enzymes (e.g., chitinase, glucanase, and protease), which degrade the harmful mycotic populations.

The free-living fungi are beneficial inhabitants of the rhizosphere. *Trichoderma* sp. belongs to this class and possesses the mycoparasitic capabilities, which is a more predominant attribute of the free-living fungi (Harman et al. 2004). They promote the plant development without showing any detrimental effects on the plants. The root colonization of *Trichoderma* is associated with the induction of both the local and systemic resistance that is directly influenced by the production of a fungal protein elicitor molecule, designated as *small protein1* (*Sm1*), which lacks toxic activity in plants and microbes (Djonovic et al. 2006). The *Sm1* promotes the immunity-related gene expressions in plants. Several studies have shown *T. atroviride* and *T. virens* to induce plant growth by producing indole-3-acetic acid (Contreras-Cornejo et al. 2009).

## 14.4 Microbial Signaling Involved in Plant Growth

Development of plant is driven by multiple factors, including root microbial population as a major contributor. Previous workers have revealed the signaling process to be executed by certain classes of molecules, including phytohormones, N-acyl-l-homoserine lactones (AHLs), and microbial volatile organic compounds (MVOCs) (Mhlongo et al. 2018).

The MVOCs and the AHLs play a crucial role in the bacterial downstream signaling, by which the bacterial genes may express (Ortiz-Castro et al. 2009). The MVOCs are recognized by the plants further contributing in the improvement of plant development and defense mechanisms by upregulating different genes.

In addition, there are many soil bacterial populations that belong to the class of *Proteobacteria*, which help plants in the uptake of mineral nutrients and nitrogen fixation (Basu and Kumar 2020b). Endophytic microbes help in manipulation of biotic and abiotic stresses in plants (Basu and Kumar 2021a). These evidences establish the existence of a feedback loop system (Mandal et al. 2010).

## *14.4.1 Phytohormones*

Former studies have shown the rhizosphere to potentially contribute the chemical components, which are essential for maintaining the developmental cues in plants (Ortiz-Castro et al. 2009). Phytohormones have been shown to play a crucial role in the plant development, indirectly regulating the nutrient uptake and distribution (Basu et al. 2022). They are produced in root and shoot, but their effectiveness is spatial. Therefore, the shoot and root growth is completely distinct from each other in accordance to the auxin and cytokinin gradient. Fate of root and shoot development has been found to be decided by the auxin and cytokinin produced by the soil microbiota (Su et al. 2011).

Root bacterial and fungal populations produce different phytohormones such as cytokinin, auxin, and ethylene (Table 14.1). Phytohormones auxin and cytokinin possess antagonistic role in development of plants. Conversely, production of these phytohormones occasionally leads to diseased conditions in plants (e.g., infection caused by *Agrobacterium tumefaciens* or *Ustilago maydis*). Therefore, the equilibrium of auxin-cytokinin and site of hormone accumulation can determine the beneficial or detrimental role of the microbial interaction.

### 14.4.1.1 Cytokinin

Cytokinin positively regulates the growth of the whole plant, especially the shoot (Beck 1996). Several studies have revealed the involvement of different gene expressions to stimulate cytokinin production in plants. Signal perception of cytokinins involves three sensor histidine kinases—*CRE1*/*AHK4*/*WOL*, *AHK2*, and *AHK3*, which upregulate multiple response regulator expression depending on the concentration of cytokinin (Kakimoto 2003). The cytokinin receptors are also necessary for the viability and normal growth of plants. Furthermore, the cytokinin signaling upregulates the *CYCD3* gene that encodes a D-type cyclin leading to the cell cycle progression and cell division in the shoot (Riou-Khamlichi et al. 1999). Additionally, cytokinin gradient has been reported to drive the plant, microbe, and insect interactions, thereby contributing in the plant defense system (Giron et al. 2013).

Several studies have shown the PGPR to produce cytokinin, consequently promoting plant growth and biomass production (Bukhat et al. 2020). Liu et al. (2013) have shown the inoculation of *Platycladus orientalis* plants with the PGPR *Bacillus subtilis* AE016877 to stimulate the cytokinin production in shoots. Further, Tahir et al. (2017) have shown the inoculation of tomato plants with PGPR *B. subtilis* SYST2 to enhance the expression of gene for cytokinin synthesis (*SlCKX1*) with

**Table 14.1** Microorganisms stimulate plant growth by producing phytohormones

| Microorganisms | Plant growth stimulated | Reference |
|---|---|---|
| **Cytokinin** | | |
| **PGPR** | | |
| *Bacillus subtilis* SYST2 | Tomato (*Solanum lycopersicum*) | Tahir et al. (2017) |
| *Pseudomonas fluorescens* G20-18 | *Arabidopsis thaliana* | Großkinsky et al. (2016) |
| *B. subtilis* AE016877 | Oriental thuja (*Platycladus orientalis*) | Liu et al. (2013) |
| *B. megaterium* UMCV1 | *A. thaliana* | Ortiz-Castro et al. (2008a, b) |
| | | Lopez-Bucio et al. (2007) |
| *B. subtilis* IB 22 | Lettuce (*Lactuca sativa*) | Arkhipova et al. (2005) |
| **Auxin** | | |
| **PGPR** | | |
| *Azospirillum brasilense* Sp245 | *A. thaliana* | Spaepen et al. (2014) |
| *Aeromonas punctata* PNS-1 | *A. thaliana* | Iqbal and Hasnain (2013) |
| *Burkholderia cepacia* RRE25 | Rice (*Oryza sativa*) | Singh et al. (2013) |
| *Serratia marcescens* 90-166 | *A. thaliana* | Shi et al. (2010) |
| *Enterobacter cloacae* UW4 | Canola (*Brassica napus*) | Li et al. (2000) |
| **Fungi** | | |
| *Trichoderma atroviride* | Tomato | Gravel et al. (2007) |
| *T. virens* | *A. thaliana* | Contreras-Cornejo et al. (2009) |
| **Ethylene** | | |
| **PGPR** | | |
| *Achromobacter xylosoxidans* Cm4, *Pseudomonas oryzihabitans* Ep4, and *Variovorax paradoxus* 5C-2 | Potato (*Solanum tuberosum*) | Belimov et al. (2015) |
| *Variovorax paradoxus* 5C-2 | *A. thaliana* | Chen et al. (2013) |
| *Azotobacter chroococcum* AZO2 | Sesame (*Sesamum indicum*) | Dubey et al. (2012) |
| *Corynebacterium* sp. Sb26, *Rhizobium* sp. Sb16 | Rice (*Oryza sativa*) | Naher et al. (2008) |
| *Pseudomonas brassicacearum* 520-1, *P. brassicacearum* Am3 | Tomato | Belimov et al. (2007) |
| *Pseudomonas brassicacearum* Am3, *Pseudomonas marginalis* Dp | Indian mustard (*Brassica juncea*), rape (*B. napus*), and pea (*Pisum sativum*) | Safronova et al. (2006) |
| *Achromobacter piechaudii* ARV8 | Pepper (*Capsicum annuum*), tomato | Mayak et al. (2004) |
| *Pseudomonas putida* GR12-2 | Canola | Penrose et al. (2001) |
| | Barley (*Hordeum vulgare*), oat (*Avena sativa*), wheat (*Triticum aestivum*), canola, lettuce, tomato | Hall et al. (1996) |

increased cytokinin level. Another PGPR strain *B. megaterium* UMCV1 has also been found to promote the growth and biomass of *Arabidopsis* plants (Lopez-Bucio et al. 2007).

#### 14.4.1.2 Auxin

Auxin concentration along with environmental factors plays an important role in determining the root architecture in plants. The root and shoot meristem size, lateral organ primordial position, and floral morphogenesis are regulated by auxin. It also drives the cell cycle, thereby releasing the bud dormancy in plants (Tsukanova et al. 2017).

Microbial interaction with plant root induces the synthesis of auxin, including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), phenylacetic acid, 4-chlorindole-3-acetic acid, or their precursors (Spaepen et al. 2007). The IAA, major naturally occurring auxin, reciprocates the signaling during the plant-microbiota interaction. The IAA has been found to be a positive regulator of plant growth. Therefore, IAA overproduction has been suggested to induce the formation of long hypocotyls with increased numbers of lateral roots and root hairs. The amount, localization, and direction of IAA movement in plants have been proved to be affected by the PGPR (Ahmed and Hasnain 2014). Spaepen et al. (2014) have reported the PGPR strain *Azospirillum brasilense* Sp245 to produce higher concentration of IAA, thereby promoting root growth in *A. thaliana*. The PGPR strains *Aeromonas punctata* PNS-1 (Iqbal and Hasnain 2013) and *Serratia marcescens* 90-166 (Shi et al. 2010) have also been revealed to induce growth of *Arabidopsis* plants. Singh et al. (2013) have reported the IAA overproducing PGPR strain of *Burkholderia cepacia* RRE25 to stimulate growth of rice plants.

Several studies have revealed the rhizospheric fungal population to produce IAA leading to enhanced plant growth. This is an interacting system that may have a role in the fungi and plant symbiosis, where the fungi can use IAA and related compounds to interact with plants as part of their colonization strategy, consequently stimulating plant growth. Gravel et al. (2007) have shown the inoculation of tomato plants with *T. atroviride* to stimulate plant growth and yield by inducing IAA production. Contreras-Cornejo et al. (2009) have reported the plant-beneficial fungi *Trichoderma virens* to promote lateral root growth with enhanced biomass production in *Arabidopsis* through the IAA-dependent mechanism. The study has also revealed the mutations in IAA transport or signaling genes (*AUX1*, *AXR1*, *BIG*, and *EIR1*) to suppress the root growth by diminishing the effects of *Trichoderma*.

#### 14.4.1.3 Ethylene

Ethylene plays an active role in seed germination, leaf maturation, root elongation, nodulation, root branching, floral initiation, and fruit ripening at low concentrations. However, high concentrations of ethylene have been found to cause phytotoxicity leading to inhibition of root growth, defoliation, and early senescence. Several studies have shown plants to produce 1-aminocyclopropane-1-carboxylate (ACC), the precursor of ethylene under different abiotic and biotic stresses including drought (Basu et al. 2021a), submergence (Basu et al. 2020b; Basu et al. 2021b), and pathogenic infections (Basu and Kumar 2021b).

The PGPR also synthesizes ACC deaminase that helps plants by converting it to α-ketobutyrate and ammonia, thereby regulating the concentration of ACC. Therefore, the PGPR also regulates plant growth under different stress conditions by limiting the toxic level of ethylene. Hall et al. (1996) showed *Pseudomonas putida* GR12-2 to induce growth in barley, oat, wheat, canola, lettuce, and tomato seedlings. Later Penrose et al. (2001) also showed *P. putida* GR12-2 to promote plant growth in canola. Mayak et al. (2004) revealed *Achromobacter piechaudii* ARV8 to stimulate growth of pepper and tomato plants. Different strains of *Pseudomonas* spp. *P. brassicacearum* Am3 and *P. marginalis* Dp have been revealed to promote plant growth in Indian mustard, rape, peas (Safronova et al. 2006), and tomato (Belimov et al. 2007). Naher et al. (2008) showed *Corynebacterium* sp. Sb26 and *Rhizobium* sp. Sb16 to induce growth of rice plants. Strain of *Azotobacter chroococcum* AZO2 has been found to promote growth of sesame plants (Dubey et al. 2012). Belimov et al. (2015) showed strains of *Achromobacter xylosoxidans* Cm4, *Pseudomonas oryzihabitans* Ep4, and *Variovorax paradoxus* 5C-2 to stimulate growth of potato plants.

### *14.4.2 N-Acyl-L-Homoserine Lactone (AHL)*

Bacterial adaptation, proficiency, cellular communication, and reproduction are regulated by an essential strategy, known as quorum sensing (QS) (Basu and Kumar 2021a). N-Acyl homoserine lactones (AHLs) belong to a class of bacterial QS signals from gram-negative bacteria (Hartmann et al. 2021). These compounds enable bacterial cells to regulate gene expression depending on the population density. Accumulation of AHLs confers resistance against phytopathogens (Table 14.2). The AHL-producing bacterial strain *Serratia liquefaciens* MG1 has been reported to promote plant growth by inducing SAR against phytopathogenic fungus *Alternaria alternata* in tomato (Schuhegger et al. 2006). Newman et al. (2008) reported the bacteria *Bacillus* sp. and *Pseudomonas* sp. to promote SAR in plants against *Xylella fastidiosa* and *Xanthomonas* sp. by degrading the diffusible signal factor. Kusari et al. (2014) have shown endophytic bacteria associated with *Cannabis sativa* plant to regulate phytopathogenic *Chromobacterium violaceum* by inhibiting QS.

**Table 14.2** Microbial resistance against phytopathogens mediated by accumulation of N-acyl homoserine lactones (AHLs)

| Microbes | Phytopathogens | Plants | References |
|---|---|---|---|
| Endophytic bacteria | *Chromobacterium violaceum* | *Cannabis sativa* | Kusari et al. (2014) |
| *Ensifer meliloti* (*Sinorhizobium meliloti*) | *Pseudomonas syringae* | *Arabidopsis thaliana* | Zarkani et al. (2013) |
| *Bacillus* sp. and *Pseudomonas* sp. | *Xylella fastidiosa* and *Xanthomonas* sp. | | Newman et al. (2008) |
| *Serratia liquefaciens* MG1 | *P. syringae* | *Arabidopsis thaliana* | von Rad et al. (2008) |
| | *Alternaria alternata* | Tomato | Schuhegger et al. (2006) |

### *14.4.3 Microbial Volatile Organic Compound (MVOC)*

Microbial volatile organic compounds (MVOCs) are variable compounds formed through the bacterial and fungal metabolism (Korpi et al. 2009). The compounds usually have low molecular weight (e.g., hydrocarbon, alcohol, aldehyde, and ketone), distinct odour, low boiling point and high vapor pressure. MVOCs establish communication with plants by functioning as attractants, repellents, or cautioning signals. Lemfack et al. (2014) have reported approximately 350 bacterial and 69 fungal species to produce about 846 diverse MVOCs. Different MVOCs are represented in Fig. 14.2.

The roles of PGPR in stimulating plant growth through the production of diverse MVOCs are well documented (Park et al. 2015). Different species and strains of PGPR, including *Bacillus*, *Pseudomonas*, and *Serratia*, have been revealed to stimulate growth of different plant species. The PGPR or PGPR-derived products frequently require the physical contact with the plants for inducing plant growth. However, different studies have reported about the distant regulation of plant growth without direct interaction with plants, which suggests the possibility of the emission of MVOCs by the PGPR. Different MVOCs stimulating growth of various plant species have been listed in Table 14.3. Ryu et al. (2003) first revealed two MVOCs, 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol produced by PGPR strains *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a to induce growth in *A. thaliana*. Again Zou et al. (2010) showed the PGPR strain *B. megaterium* XTBG34 to promote the growth of *Arabidopsis* plants by producing 2-pentylfuran. Park et al. have reported MVOCs (2-methyl-n-1-tridecene, 2-butanone, and 13-tetradecadien-1-ol) produced by the PGPR strain *Pseudomonas fluorescens* SS101 to stimulate growth of tobacco plants. Xie et al. (2014) have shown the spermidine-producing PGPR strain *Bacillus subtilis* OKB105 to stimulate growth in tobacco plants. Further, Tahir et al. (2017) have shown another strain of *B. subtilis* SYST2 to promote growth of tomato plants by producing MVOCs (albuterol and 1,3-propanediole). Kai and Piechulla (2014) showed another PGPR *Serratia odorifera* to promote the growth of moss *Physcomitrella patens* by producing $CO_2$.

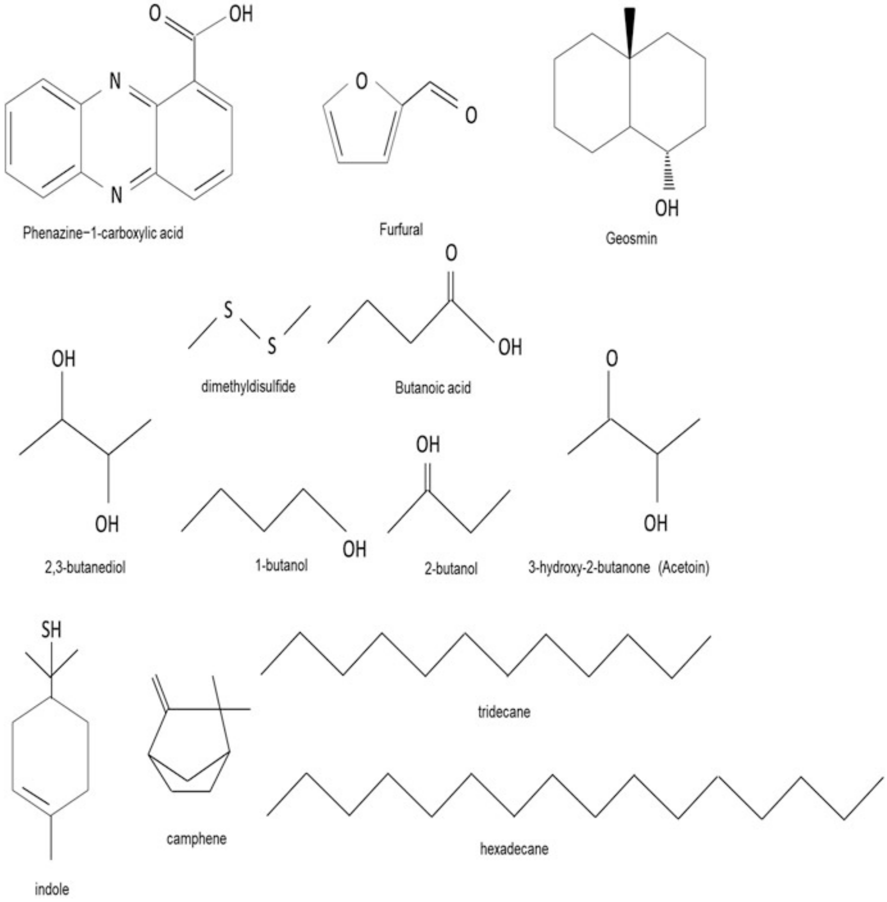

**Fig. 14.2** Different microbial volatile organic compounds (MVOCs) responsible for establishment of communication with plants

Fungi being an important associate of the phytomicrobiome release abundant MVOCs playing a key role in plant-microbe interactions. A recent study showed beneficial fungal strain of *Trichoderma hamatum* FB10 to increase the growth and biomass of cowpea, small millet, maize, green gram, and black gram seedlings by synthesizing bioactive MVOCs (butanoic acid, ethanoic acid, hexadecanoic acid, butyrolactone, and hexadecane) (Baazeem et al. 2021). Another recent study showed the fungal strain of *Cladosporium halotolerans* NGPF1 to promote growth of tobacco (*Nicotiana benthamiana*) plants through the production of two MVOCs 2-methyl-butanal and 3-methyl-butanal (Jiang et al. 2021). Further, Paul and Park (2013) showed another strain of *Cladosporium cladosporioides* CL-1 to stimulate growth and fresh weight of tobacco seedlings by synthesizing MVOCs including dehydroaromadendrene, (−)-trans-caryophyllene, tetrahydro-2,2,5,5-

**Table 14.3** Microbial volatile organic compounds (MVOCs) stimulating growth of different plant species

| Microorganisms | MVOCs produced | Plant species with induced growth | Reference |
|---|---|---|---|
| **Rhizobacteria** | | | |
| *B. subtilis* SYST2 | Albuterol and 1,3-propanediole | Tomato | Tahir et al. (2017) |
| *Pseudomonas fluorescens* SS101 | 2-Methyl-n-1-tridecene, 2-butanone, and 13-Tetradecadien-1-ol | Tobacco | Park et al. (2015) |
| *Serratia odorifera* | $CO_2$ | *Physcomitrella patens* | Kai and Piechulla (2014) |
| *Bacillus subtilis* OKB105 | Spermidine | Tobacco | Xie et al. (2014) |
| *B. megaterium* XTBG34 | 2-Pentylfuran | *Arabidopsis* | Zou et al. (2010) |
| *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a | 3-Hydroxy-2-butanone (acetoin) and 2,3-butanediol | *A. thaliana* | Ryu et al. (2003) |
| **Fungi** | | | |
| *Trichoderma hamatum* FB10 | Butanoic acid, ethanolic acid, hexadecanoic acid, butyrolactone, and hexadecane | Cowpea, small millet, maize, green gram, and black gram | Baazeem et al. (2021) |
| *Cladosporium halotolerans* NGPF1 | 2-Methyl-butanal and 3-methyl-butanal | Tobacco (*Nicotiana benthamiana*) | Jiang et al. (2021) |
| *Cladosporium cladosporioides* CL-1 | Dehydroaromadendrene, (−)-trans-caryophyllene, tetrahydro-2,2,5,5-tetramethylfuran, α-pinene, and (+)-sativene | Tobacco | Paul and Park (2013) |
| *Phoma* sp. GS8-3 | 2-Methyl-propanol and 3-methyl-butanol | Tobacco | Naznin et al. (2013) |

tetramethylfuran, α-pinene, and (+)-sativene. Naznin et al. (2013) have shown the plant growth-promoting fungal strain *Phoma* sp. GS8-3 to induce the growth of tobacco plants by producing the MVOCs, 2-methyl-propanol and 3-methyl-butanol.

## 14.5 Future Prospects

The plant rhizosphere harbors a diverse reservoir of culturable microorganisms that can be exploited to benefit mankind. Interactions of plants and rhizospheric organisms influence plants' root functions, eventually altering their growth and productivity. Therefore, understanding the soil-root and soil-seed interface is essential to manage the microorganisms for sustainable agriculture. Microbial activities and population numbers are often affected by the soil composition, which in turn has an impact on the nematodes and microarthropods that share this environment. Many rhizospheric microbes benefit crop production, reducing the dependence on chemical fertilizers to achieve high productivity. Some microbes also protect plants from the ravages of the severe disease-causing pathogens.

Exploration of rhizosphere may contribute in the enhanced application of plant growth-promoting organisms for sustainable plant growth and usage of the biocontrol agents for suppressing plant diseases and weeds. Rhizospheric organisms can also be used to enhance the formation of stable soil aggregates and as bioremediation agents of contaminated soils. Utilization of the beneficial microorganisms is fully consistent with sustainable agriculture, where the goal of paramount importance is to utilize the natural processes that promote the crops' output without irreparably damaging the natural resources. Progressive understanding of the ecology and biota in the rhizosphere may help in manipulating this zone of increased nutrients, biotic activity, and interactions to improve plant productivity and environmental quality.

Among the many recent discoveries in rhizosphere research, the ominous is finding that certain potential human pathogenic microorganisms are also successful inhabitants of this nutrient-enriched plant soil environment, and this ecology poses potential public health hazards for both producers and consumers who encounter them. An interesting thought for future exploration is the rhizosphere supports the populations of human health-promoting rhizobacteria (HHPR).

Rhizosphere bioremediation refers to the biodegradation of pollutants by microorganisms in the plant root zone. Plants play an important role to increase both microbial numbers and metabolism in soil, resulting in increased biodegradation activity. Several mechanisms elucidate the enhanced biodegradation in the rhizosphere: the root turnover increases soil organic carbon stimulating microbial activities, thereby metabolizing toxic pollutants; root exudates contain small organic acids, alcohols, and phenolic compounds that favor solubilization and bioavailability of hydrophobic pollutants; root tissues and microorganisms also secrete catabolic enzymes, such as peroxidases and laccases, involved in biodegradation mechanisms; specific compounds released by roots induce microbial enzymes and stimulate biodegradation; roots introduce oxygen in the rhizosphere, which is necessary for oxidative biodegradation by oxygenases.

In the near future, it could be expected that more studies will be conducted on this field, by which plant-microbe interaction will be more understandable. It will further open up new junctions to use microbial strains with a capability to produce the phytohormones for plant improvement under field conditions to sustain the agricultural production.

## 14.6 Conclusion

Microbes and plants have cohabited and coevolved for millions of years. The complicated interactions of plants with the rhizospheric microbial populations have not been completely understood so far. The microbial populations play a significant role in the nutrient uptake and assimilation. Additionally, they contribute in the plant development and immunity system. The cross talking of both the organisms through vibrant chemical signaling pathways indicates their symbiotic association. The present chapter has explored the potential role of the major signaling molecules in the plant-microbe interactions, which may improve the efficiency of the ecosystem. Therefore, the comprehensive analyses of microbial signaling may be effectively used to pave the way for the agricultural sustainability.

## References

Ahmed A, Hasnain S (2014) Auxins as one of the factors of plant growth improvement by plant growth promoting rhizobacteria. Pol J Microbiol 63:261–266

Arkhipova TN, Veselov SU, Melentiev AI, Martynenko EV, Kudoyarova GR (2005) Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 272:201–209

Baazeem A, Almanea A, Manikandan P, Alorabi M, Vijayaraghavan P, Abdel-Hadi A (2021) In vitro antibacterial, antifungal, nematocidal and growth promoting activities of *Trichoderma hamatum* FB10 and its secondary metabolites. J Fungi (Basel) 7(5):331

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32: 666–681

Basu S, Kumar G (2020a) Stress signalling in the phytomicrobiome: breadth and potential. In: Kumar M, Kumar V, Prasad R (eds) Phyto-microbiome in stress regulation. Environmental and microbial biotechnology, Springer, Singapore, pp 245–268

Basu S, Kumar G (2020b) Nitrogen fixation in a legume-rhizobium symbiosis: the roots of a success story. In: Varma A, Tripathi S, Prasad R (eds) Plant microbe symbiosis. Springer, Singapore, pp 35–53

Basu S, Kumar G (2021a) Plant microbe interaction for changing endophytic colonization to improve plant productivity. In: Verma JP, Macdonald CA, Gupta VK, Podile AR (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 137–147

Basu S, Kumar G (2021b) Exploring the significant contribution of silicon in regulation of cellular redox homeostasis for conferring stress tolerance in plants. Plant Physiol Biochem 166:393–404

Basu S, Kumar G, Chhabra S, Prasad R (2020a) Role of soil microbes in biogeochemical cycle for enhancing soil fertility. In: Verma JP, Macdonald CA, Gupta VK, Podile AP (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 149–157

Basu S, Kumar G, Kumari N, Kumari S, Shekhar S, Kumar S, Rajwanshi R (2020b) Reactive oxygen species and reactive nitrogen species induce lysigenous aerenchyma formation through programmed cell death in rice roots under submergence. Environ Exp Bot 177:104118

Basu S, Kumari S, Kumar A, Shahid R, Kumar S, Kumar G (2021a) Nitro-oxidative stress induces the formation of roots' cortical aerenchyma in rice under osmotic stress. Physiol Plant 172(2): 963–975

Basu S, Kumari S, Kumar P, Kumar G, Rajwanshi R (2021b) Redox imbalance impedes photosynthetic activity in rice by disrupting cellular membrane integrity and induces programmed cell death under submergence. Physiol Plant 172(3):1764–1778

Basu S, Prabhakar AA, Kumari S, Kumar RR, Shekhar S, Prakash K, Singh JP, Singh GP, Prasad R, Kumar G (2022) Micronutrient and redox homeostasis contribute to *Moringa oleifera*-regulated drought tolerance in wheat. Plant Growth Regul. https://doi.org/10.1007/s10725-022-00795-z

Beck EH (1996) Regulation of shoot/root ratio by cytokinins from roots in *Urtica dioica*: opinion. Plant Soil 185:1–12

Behera BK, Prasad R (2020) Strategies for soil management. In: Behera BK, Prasad R (eds) Environmental technology and sustainability. Elsevier, pp 143–167

Belimov AA, Dodd IC, Safronova VI, Hontzeas N, Davies WJ (2007) *Pseudomonas brassicacearum* strain Am3 containing 1-aminocyclopropane-1-carboxylate deaminase can show both pathogenic and growth-promoting properties in its interaction with tomato. J Exp Bot 58(6):1485–1495

Belimov AA, Dodd IC, Safronova VI, Shaposhnikov AI, Azarova TS, Makarova NM, Davies WJ, Tikhonovich IA (2015) Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-atered and water-limited potato (*Solanum tuberosum*). Ann Appl Biol 167:11–25

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 8:478–486

Bukhat S, Imran A, Javaid S, Shahid M, Majeed A, Naqqash T (2020) Communication of plants with microbial world: exploring the regulatory networks for PGPR mediated defense signaling. Microbiol Res 238:126486

Campbell R, Greaves MP (1990) Anatomy and community structure of the rhizosphere. In: Lynch JM (ed) The rhizosphere. John Wiley and Sons Ltd, Chichester, pp 11–34

Chai YN, Schachtman DP (2022) Root exudates impact plant performance under abiotic stress. Trends Plant Sci 27(1):80–91. https://doi.org/10.1016/j.tplants.2021.08.003

Chen L, Dodd IC, Theobald JC, Davies WJ, Belimov AA (2013) The rhizobacterium *Variovorax paradoxus* 5C-2, containing ACC deaminase, promotes growth and development of *Arabidopsis thaliana* via an ethylene-dependent pathway. J Exp Bot 64:1565–1573

Contreras-Cornejo HA, Macías-Rodriguez LI, Cortes-Penagos C, Lopez-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: 1579–1592

Djonovic S, Pozo MJ, Dangott LJ, Howell CR, Kenerley CM (2006) Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. Mol Plant Microbe Interact 19:838–853

Dong D, Peng X, Yan X (2004) Organic acid exudation induced by phosphorus deficiency and/or aluminium toxicity in two contrasting soybean genotypes. Physiol Plant 122:190–199

Dubey RC, Maheshwari DK, Kumar V, Pandey RR (2012) Growth enhancement of *Sesamum indicum* L. by rhizosphere-competent *Azotobacter chroococcum* AZO2 and its antagonistic activity against *Macrophomina phaseolina*. Arch Phytopathol Plant Protect 45:437–454

el Zahar Haichar F, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground. Soil Biol Biochem 77:69–80

Giron D, Frago E, Glevarec G, Pieterse CMJ, Dicke M (2013) Cytokinins as key regulators in plant-microbe-insect interactions: connecting plant growth and defence. Funct Ecol 27(3):599–609

Gravel V, Antoun H, Russell J, Weddell T (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39:1968–1977

Großkinsky DK, Tafner R, Moreno MV, Stenglein SA, García de Salamone IE, Nelson LM et al (2016) Cytokinin production by *Pseudomonas fluorescens* G20-18 determines biocontrol activity against Pseudomonas syringae in *Arabidopsis*. Sci Rep 6:23310

Hall JA, Peirson D, Ghosh S, Glick BR (1996) Root elongation in various agronomic crops by the plant growth promoting rhizobacterium Pseudomonas putida GR12-2. Isr J Plant Sci 44:37–42

Harley JL, Russell RS (1979) The soil-root interface. Academic, London

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56

Hartmann A, Klink S, Rothballer M (2021) Importance of N-acyl-homoserine lactone-based quorum sensing and quorum quenching in pathogen control and plant growth promotion. Pathogens 10:1561

Hiltner L (1904) Uber nevere Erfahrungen und Probleme auf dem Gebiet der Boden Bakteriologie und unterbesonderer Beurchsichtigung der Grundungung und Broche. Arbeit Deut Landw Ges Berlin 98:59–78

Iqbal A, Hasnain S (2013) *Aeromonas punctata* PNS-1: a promising candidate to change the root morphogenesis of A. thaliana in MS and sand system. Acta Physiol Plant 35:657–665

Jiang L, Lee MH, Kim CY, Kim SW, Kim PI, Min SR, Lee J (2021) Plant growth promotion by two volatile organic compounds emitted from the fungus *Cladosporium halotolerans* NGPF1. Front Plant Sci 12:794349

Kai M, Piechulla B (2014) Impact of volatiles of the rhizobacteria *Serratia odorifera* on the moss *Physcomitrella patens*. Plant Signal Behav 5:444–446

Kakimoto T (2003) Perception and signal transduction of cytokinins. Annu Rev Plant Biol 54:605–627

Korpi A, Jarnberg J, Pasanen AL (2009) Microbial volatile organic compounds. Crit Rev Toxicol 39(2):139–193

Kumar A, Basu S, Kumar G (2021) Evaluating the effect of seed-priming for improving arsenic tolerance in rice. J Plant Biochem Biotechnol 31(1):197–201

Kumar A, Basu S, Rishu AK, Kumar G (2022a) Revisiting the mechanisms of arsenic uptake, transport and detoxification in plants. Environ Exp Bot 194:104730

Kumar G, Basu S, Singla-Pareek SL, Pareek A (2022b) Unraveling the contribution of *OsSOS2* in conferring salinity and drought tolerance in a high-yielding rice. Physiol Plant 174(1):e13638

Kusari P, Kusari S, Lamshöft M, Sezgin S, Spiteller M, Kayser O (2014) Quorum quenching is an antivirulence strategy employed by endophytic bacteria. Appl Microbiol Biotechnol 98:7173–7183

Lemfack MC, Nickel J, Dunkel M, Preissner R, Piechulla B (2014) mVOC: a database of microbial volatiles. Nucleic Acids Res 42(D1):D744–D748

Li J, Ovakim DH, Charles TC, Glick BR (2000) An ACC deaminase minus mutant of Enterobacter cloacae UW4 no longer promotes root elongation. Curr Microbiol 41:101–105

Liu FC, Xing SJ, Ma HL, Du ZY, Ma BY (2013) Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in *Platycladus orientalis* container seedlings. Appl Microb Biotech 97:9155–9164

Lopez-Bucio J, Campos-Cuevas JC, Hernandez-Calderon E, Velasquez-Becerra C, Farias-Rodriguez R, Macias-Rodriguez LI, Valencia-Cantero E (2007) *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. Mol Plant Microbe Interact 20:207–217

Lynch J, Whipps J (1991) Substrate flow in the rhizosphere. In: The rhizosphere and plant growth. Springer, Dordrecht, pp 15–24

Mandal SM, Chakraborty D, Dey S (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5:359–368

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London

Mayak S, Tirosh T, Glick BR (2004) Plant growth promoting bacteria that confer resistance to water stress in tomato and pepper. Plant Sci 166:525–530

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (2018) The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112

Mishra M, Wungrampha S, Kumar G, Singla-Pareek SL, Pareek A (2021) How do rice seedlings of landrace Pokkali survive in saline fields after transplantation? Physiology, biochemistry, and photosynthesis. Photosynth Res 150:117–135

Naher UA, Radziah O, Halimi MS, Shamsuddin ZH, Mohd RI (2008) Effect of inoculation on root exudates carbon sugar and amino acids production of different rice varieties. Res J Microbiol 3: 580–587

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 28:42–49

Newman KL, Chatterjee S, Ho KA, Lindow SE (2008) Virulence of plant pathogenic bacteria attenuated by degradation of fatty acid cell-to-cell signaling factors. Mol Plant Microbe Interact 21:326–334

Ortiz-Castro R, Martinez-Trujillo M, Lopez-Bucio J (2008a) N-acyl-L homoserine lactones: a class of bacterial quorum-sensing signals alters post-embryonic root development in *Arabidopsis thaliana*. Plant Cell Environ 31:1497–1509

Ortiz-Castro R, Valencia-Cantero E, Lopez-Bucio J (2008b) Plant growth promotion by Bacillus megaterium involves cytokinin signaling. Plant Signal Behav 3:263–265

Ortiz-Castro R, Contreras-Cornejo HA, Macias-Rodriguez L, Lopez-Bucio J (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4(8):701–712

Park YS, Dutta S, Ann M, Raaijmakers JM, Park K (2015) Promotion of plant growth by Pseudomonas fluorescens strain SS101 via novel volatile organic compounds. Biochem Biophys Res Commun 461(2):361–365

Paul D, Park KS (2013) Identification of volatiles produced by Cladosporium cladosporioides CL-1, a fungal biocontrol agent that promotes plant growth. Sensors (Basel) 13:13969–13977

Penrose DM, Moffatt BA, Glick BR (2001) Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. Can J Microbiol 47:77–80

Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant growth-promoting rhizobacteria (PGPR) and medicinal plants. Springer International Publishing, Switzerland, pp 247–260

Ramesh SA, Tyerman SD, Xu B, Bose J, Kaur S, Conn V, Domingos P, Ullah S, Wege S, Shabala S, Feijo JA, Ryan PR, Gilliham M (2015) GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. Nat Commun 6:7879

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JAH (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Science 283:1541–1544

Ryu C-M, Farag MA, Hu C-H, Reddy MS, Wei H-X, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci U S A 100:4927–4932

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 42:267–272

Sarkar S, Basu S, Prasad R, Kumar G (2022) Phytoremediation: mechanistic approach for eliminating heavy metal toxicity from environment. In: Prasad R (ed) Phytoremediation for environmental sustainability. Springer, Singapore, pp 513–543

Schuhegger R, Ihring A, Gantner S, Bahnweg G, Knappe C, Vogg G et al (2006) Induction of systemic resistance in tomato plants by N-acyl-homoserine lactone-producing rhizosphere bacteria. Plant Cell Environ 29:909–918

Shi CL, Park HB, Lee JS, Ryu S, Ryu CM (2010) Inhibition of primary roots and stimulation of lateral root development in A. thaliana by the rhizobacterium *Serratia marcescens* 90-166 is through both auxin-dependent and -independent signaling pathways. Mol Cells 29:251–258

Shrivastava S, Prasad R, Varma A (2014) Anatomy of root from eyes of a microbiologist. In: Morte A, Varma A (eds) Root Engineering, vol 40. Springer-Verlag, Berlin Heidelberg, pp 3–22

Singh RK, Malik N, Singh S (2013) Improved nutrient use efficiency increases plant growth of rice with the use of IAA-overproducing strains of endophytic *Burkholderia cepacia* strain RRE25. Microb Ecol 66:375–384

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

Spaepen S, Bossuyt S, Engelen K, Marchal K, Vanderleyden J (2014) Phenotypical and molecular responses of *A. thaliana* roots as a result of inoculation with the auxin-producing bacterium *Azospirillum brasilense*. New Phytol 201:850–861

Su HJ, Thseng FM, Chen JS, Ko WH (2011) Production of volatile substances by rhizomorphs of *Marasmius crinisequi* and its significance in nature. Fungal Divers 49:199–202

Tahir HA, Gu Q, Wu H, Raza W, Hanif A, Wu L, Colman MV, Gao X (2017) Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. Front Microbiol 8:171

Tsukanova KA, Chebotar VK, Meyer JJM, Bibikova TN (2017) Effect of plant growth-promoting Rhizobacteria on plant hormone homeostasis. S Afr J Bot 113:91–102

von Rad U, Klein I, Dobrev PI, Kottova J, Zazimalova E, Fekete A et al (2008) Response of *Arabidopsis thaliana* to N-hexanoyl-DL-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229:73–85

Xie S, Wu H, Zang H, Wu L, Zhu Q, Gao X (2014) Plant growth promotion by spermidine-producing *Bacillus subtilis* OKB105. Mol Plant Microbe Interact 27:655–663

Zarkani AA, Stein E, Rohrich CR, Schikora M, Evguenieva-Hackenberg E, Degenkolb T et al (2013) Homoserine lactones influence the reaction of plants to rhizobia. Int J Mol Sci 14:17122–17146

Zou C, Li Z, Yu D (2010) *Bacillus megaterium* strain XTBG34 promotes plant growth by producing 2-pentylfuran. J Microbiol 48:460–466