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R. Z. Sayyed
Naveen Kumar Arora
M. S. Reddy *Editors*

Plant Growth Promoting Rhizobacteria for Sustainable Stress Management

Volume 1: Rhizobacteria in Abiotic Stress Management

Springer

Microorganisms for Sustainability

Volume 12

Series editor
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Foreword

National Academy of Agricultural Sciences

Prof. Panjab Singh
President

Achieving sustainable agricultural production while keeping the environmental quality, agroecosystem functions, and biodiversity is a real challenge in the present agricultural scenario. The traditional use of chemical inputs (fertilizers, pesticides, nutrients, etc.) poses serious threats to crop productivity, soil fertility, and the nutritional value of farm produce. Global concern over the demerits of chemicals in agriculture has diverted the attention of researchers toward sustainable agriculture by utilizing the potential of plant growth-promoting rhizobacteria (PGPR). Therefore, management of pests and diseases, agroecosystem well-being, and health issues for humans and animals has become the need of the hour. The use of PGPR as biofertilizers, plant growth promoters, biopesticides, and soil and plant health managers has gained considerable attention among researchers, agriculturists, farmers, policymakers, and consumers.

The application of PGPR as a bioinoculant can help in meeting the expected demand of global agricultural productivity to feed the world's booming population, which is projected to reach around 9 billion by 2050. However, to be a useful and effective bioinoculant, PGPR strain should possess high rhizosphere competence, usefulness to soil rhizobacteria, broad-spectrum activity and tolerance to various biotic and abiotic stresses. PGPR-mediated plant growth promotion and biocontrol is now gaining worldwide importance and acceptance as eco-friendly and effective bioinoculants for sustainable agriculture. However, the performance of PGPR is subject to various abiotic factors such as salinity, temperature (high/low), drought, metal ions, and presence of various toxic compounds. Only those PGPR that establish themselves and can manage such abiotic stress can perform better as plant growth-promoting and biocontrol agents.

This book, which has 17 chapters encompassing the influence of various abiotic factors on the performance of PGPR and written by different experts from India and abroad, is to highlight salient features on the application of PGPR in agricultural crop plants to lend a hand to scientists working in this field. ***PGPR in abiotic stress management*** is a timely effort for sustainable agriculture. I compliment the authors and hope the teachers and researchers working in this area will make use of this publication.

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Prof. Panjab Singh

Preface

The future of agriculture greatly depends on our ability to enhance crop productivity without sacrificing long-term production potential. Agriculture primarily depends on the use of natural resources such as land, soil, water, and nutrients. As demand for food increases and climate change and natural ecosystem damage imposes new constraints, sustainable agriculture has an important role to play in safeguarding natural resources, reducing greenhouse gas emissions, halting biodiversity loss, and caring for valued agricultural practices. Agricultural productivity rests on the foundation of microbial diversity in the soil. The application of microorganisms, such as the diverse bacterial species of plant growth-promoting rhizobacteria (PGPR), represents an ecologically and economically sustainable strategy for agriculture. PGPR are associated with plant roots and augment plant growth and disease management, elicit "induced systemic resistance" to salt and drought, and increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing the accumulation of nitrates in soils. Increased incidences of abiotic and biotic stresses impacting agricultural productivity in principal crops are being witnessed all over the world. Extreme events like prolonged droughts, intense rains and flooding, heat waves, and frost damages are likely to further increase in the future due to climate change. Enhancement of plant drought stress tolerance by PGPR has been increasingly documented in the literature. However, most studies to date have focused on PGPR-plant root interactions, but very little is known about PGPR's role in mediating physiochemical and hydrological changes in the rhizospheric soil that may impact plant drought stress tolerance. A reduction in fertilizer use would lessen the effects of water contamination from fertilizer runoff and lead to savings for farmers.

There is a need to develop simple and low-cost biological methods for the management of abiotic stress, which can be used on short-term basis. PGPR could play a significant role in this respect if we can exploit their unique properties of tolerance to extremities, their ubiquity and genetic diversity, and their interaction with crop plants and develop methods for their successful deployment in agriculture production.

With the advent of climate change, global agriculture faces a multitude of challenges. The most prominent among these are abiotic stresses imposed by increased incidences of drought, extremes of temperature, and unseasonal flooding. Such atmospheric threats, coupled with edaphic stresses, pose severe challenges to food production. While several agronomic and plant breeding strategies have been proposed to overcome these phenomena, the utilization of PGPR is receiving increased attention globally.

Achieving sustainable agricultural production while keeping the environmental quality, agroecosystem function, and biodiversity is a real challenge in the current agricultural practices. The traditional use of chemical fertilizers and pesticides poses serious threats to crop productivity, soil fertility, and the nutritional value of farm produce. Global concern over the demerits of chemicals in agriculture has diverted the attention of researchers toward sustainable agriculture by utilizing PGPR. Therefore, management of pests and diseases, agroecosystem well-being, and health issues for humans and animals has become the need of the hour. The use of PGPR as biofertilizers, plant growth promoters, biopesticides, and soil and plant health managers has gained considerable attention among researchers, agriculturists, farmers, policymakers, and consumers.

The application of PGPR as biostimulants can help in meeting the expected demand of global agricultural productivity to feed the world's booming population, which is predicted to reach around 9 billion by 2050. However, to be a useful and effective bioinoculant, PGPR strains should possess high rhizosphere competence, safety to the environment, plant growth promotion and biocontrol potential, compatibility with agronomic practices with broad-spectrum activity, and tolerant to various biotic and abiotic stresses. In view of this, the need for a better PGPR to complement the increasing agro-productivity as one of the crucial drivers of the economy has been highlighted.

PGPR-mediated plant growth promotion and biocontrol is now gaining worldwide importance and acceptance as eco-friendly and effective bioinoculants for sustainable agriculture. However, the performance of PGRR is subject to various abiotic factors such as salinity, temperature (high/low), drought, metal ions, and presence of various toxic compounds. Only those PPGR that establish themselves and can manage such abiotic stress can perform better as plant growth-promoting and biocontrol agents.

The prime aim and objective of this book is to highlight salient features on the application of PGPR in agricultural crop plants to lend a hand to scientists throughout the world working in this field. PGPR in abiotic stress management is a timely effort for sustainable agriculture. These also provide excellent tools for understanding the stress tolerance, adaptation, and response mechanisms that can be subsequently engineered into crop plants to cope with climate change-induced stresses.

This book is composed of 17 chapters encompassing the influence of various abiotic factors on the performance of PGPR to comprehend the information that has been generated on the abiotic stress-alleviating mechanisms of PGPR and their abiotic stress alleviation potential. Agricultural crops grown on saline soils suffer on an account of high osmotic stress, nutritional disorders and toxicities, poor soil physical conditions, and reduced crop productivity. The various chapters in this book focus on the enhancement of productivity under stressed conditions and increased resistance of plants against salinity stress by the application of PGPR.

It has been an immense pleasure to edit this book, with continued cooperation of the authors. We wish to thank Dr. Mamta Kapila, Ms. Raman Shukla, and Mr. Sivachandran Ravanan at Springer, India, for their generous cooperation in completion of this book.

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Naveen Kumar Arora, PhD in Microbiology, professor and head in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India, is a renowned researcher in the field of Environmental Microbiology and Biotechnology. His specific area of research is rhizosphere biology and plant growth-promoting rhizobacteria (PGPR). He has more than 60 research papers published in premium international journals and several articles published in magazines and dailies. He is editor of 15 books, published by Springer, member of several national and international societies and in editorial board of 4 journals and reviewer of several international journals. He is also the editor in chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has been advisor to 118 postgraduate and 9 doctoral students. He has also received awards for excellence in research by the Honorable Governor of Uttar Pradesh, Asian PGPR Society and Samagra Vikas Welfare Society. Although an academician and researcher by profession, he has a huge obsession for the wildlife and its conservation and has authored a book, *Splendid Wilds*. He is president of the Society for Conservation of Wildlife and is also secretary of the Society for Environmental Sustainability (website: www.ses-india.org).

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1 The Role of Plant Growth-Promoting Rhizobacteria to Modulate Proline Biosynthesis in Plants for Salt Stress Alleviation

Shamim Ahmed, Aritra Roy Choudhury, Poulami Chatterjee, Sandipan Samaddar, Kiyoon Kim, Sunyoung Jeon, and Tongmin Sa

Abstract

Soil salinization causes serious problem to environmental resources and human health in many countries. Around 1.5 billion hectares of cultivated lands are present in the world. It is estimated that almost 5% of the cultivated land (77 million) and 6% of total surface land is affected by salinity. Agricultural crops and their productivity are severely affected by salt stress. Many physiological mechanisms within the plants are regulated when exposed to salt stress. The salinity tolerance measurement has a great demand to asses the regulatory variations, growth, and survival parameters. Microorganisms that colonize the roots could play a significant role in this aspect. Rhizobacteria which possess properties such as salt tolerance, nutrient uptake ability, synthesis of compatible solutes, production of plant growth-promoting hormones, biocontrol potential, and their interaction with crop plants is known as plant growth-promoting rhizobacteria (PGPRs). Proline is one of the essential compatible solute for both plant and bacteria to respond against osmotic imbalance and ionic toxicity. Proline biosynthesis occurs in cytosol and mitochondria of a cell and modulates their functions in various cellular physiological pathways. It can also influence the proliferation and apoptosis of cell and regulate specific gene expression to alleviate salt stress. Rhizobacteria having plant growth promoting characteristics can be used as a suitable bioinoculant to promote growth and productivity through different mechanisms in addition to the accumulation of proline as osmoregulators.

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1.1 Introduction

The recent adversity of salinity is one of the ferine factors for crop production around the globe. In addition to global climate change, salt stress causes serious reduction of crop production, which accounts up to 20–50% of yield loss (Shrivastava and Kumar 2015). Intensive breeding of tolerant varieties, farm improvement, and quality resource management can help to overcome salinity stress. Nowadays, these technologies are quite intensive, tardy, and prolix (Grover et al. 2011) being less eco-friendly to solve that matter. The production of agrochemicals is energetically expensive and dependent on fossil fuels that are nonrenewable resources, which makes it no longer sustainable.

In agricultural point of view, it is essential to develop an easily applicable technique for the farmers. Cost-effective biological methods for salinity stress management within a short-term basis might be the appropriate alternative. To enhance the availability of essential plant nutrients and their mobilization (especially phosphorus) for crop production, biological inoculation (living organisms containing strains of specific bacteria, fungi, or algae) has high demand. The recent concern is to improve the existing bio-inoculation techniques for the development of next-generation biofertilizer.

1.2 Salt Stress

Researchers have been studying the various responses of plants to abiotic stress for developing techniques which can ramify the stress effect. Salinity stress affects the growth and survival of the plant. Due to increase in poor irrigation facilities and soil salinization, the soil becomes saline (EC >4 dSm^{-1}) or sodic (EC < 4dSm^{-1}). Sodium absorption ratio in saline soil is less than 13 (pH < 8.5) and in sodic soil is more than 13 (pH < 8.5) (Selvakumar et al. 2014). The plant's exposure to salinity results in negative impact on various physiological and biochemical pathways which affects the growth and survivability. Hence, it is important to measure the degree of salinity of a particular cultivable land (Albaladejo et al. 2017).

1.2.1 Adverse Effects of Salt Stress in Plant

Salt stress negatively affects the plants in three distinct ways, viz., osmotic imbalance, ionic toxicity, and reduction in nutrient uptake (Selvakumar et al. 2014). Osmotic imbalance due to high salinity condition makes plants harder to take up water by root from that soil. The immediate effect of salt exposure results in loss of turgidity, cell dehydration and ultimately cell death. On the other hand, adverse effects of salinity on plant growth may also result to impairment of the supply of photosynthetic assimilates or hormones to the growing tissues (Ashraf 2004). Under salt stress, ionic toxicity occurs through the replacement of K^+ by Na^+ which induces conformational changes in proteins (Maathuis and Amtmann 1999). For several enzymes, K^+ acts as a cofactor and cannot be substituted by Na^+ (Pessarakli 2016). Na^+ and Cl^-ions are mostly uptaken by the cell vacuoles and organic solutes which are compatible with metabolic activity even at high concentrations are accumulated in the cytosol (Baetz et al. 2016). These compatible solutes helps to balance the osmotic pressure of the ions in the vacuoles (Flowers and Colmer 2008). Many current studies reported that salt stress not only adversely affects the growth and development of plant but also hinders their seed germination, seedling growth, and enzyme activity (Seckin et al. 2009). High salinity has been reported to induce ROS formation and accumulation in the plant cell (Chawla et al. 2013). Overall, salinity has adverse effect on plethora of biochemical and physiological activities of plants (Tabur and Demir 2010).

1.2.2 Adverse Effects of Salt Stress in Bacteria

Microbial diversity, composition, and their abundance are also affected by soil salinity (Borneman et al. 1996). The bacterial and actinobacterial abundance was observed to be drastically reduced when salinity level increased about 5% (Omar et al. 1994). NifH expression and nitrogenase activity level were inhibited by nitrogen fixation in *Azospirillum* sp. under salt-containing rhizospheric soil (Tripathi et al. 2002). Root exudation and decomposition of organic matter by microorganisms were also affected by increasing salinity of the soil (Ondrasek et al. 2010).

1.3 Potential Use of Bio-inoculant for Salt Stress Alleviation

Microorganisms, which can colonize the roots, might play a significant role for the alleviation of salt stress. The exploitation of their unique properties for salt tolerance can be considered for development of effective bio-inoculant for plant growth promotion and salt stress alleviation. The general properties for the development of a potential bio-inoculant should include salt tolerance, production of plant growth-promoting hormones, genetic diversity, synthesis of compatible solutes, and their positive interaction with crop plants. An increasing number of farmers are choosing biofertilizers (Chatzipavlidis et al. 2013) since they are harmless for the soil and can help reduce the negative impact of global climate change. Biofertilizers can supplement nutrients to plants, particularly micronutrients, and contribute to increasing soil organic matter, in addition to being active in small numbers and able to self-multiply (Berg 2009).

1.4 Microorganisms for the Alleviation of Salt Stress

Beneficial soil microorganisms can promote growth and increase productivity through various mechanisms such as nutrient mobilization, hormone secretion, and disease suppression (Table 1.1). It is also becoming clear that their effects will be more far-reaching. Diverse halotolerant bacterial groups mostly belong to four phyla, δ-Proteobacteria, α-Proteobacteria, Bacteroidetes, and Verrucomicrobia, which are involved in alleviating salt stress. The genera *Microbulbifer* (*Alteromonadales*), *Pelagibius* (*Rhodospirillales*), *Halomonas* (*Oceanospirillales*), *Marinoscillum* (*Sphingobacteriales*), *Fulvivirga* (*Flexibacteraceae*), *Haloferula* (*Verrucomicrobiales*), *Pelagicoccus* (*Puniceicoccales*), and *Marinobacter* (*Alteromonadales*) were exclusively enriched in the rhizospheric soil, with the exception that *Marinobacter* was more abundant in the root endosphere than in the bulk or rhizosphere soil (Yuan et al. 2016) (Table 1.1).

1.5 The Role of Plant Growth-Promoting Rhizobacteria (PGPRs) for the Alleviation of Salt Stress

Plant growth-promoting rhizobacteria (PGPRs) could enhance crop yield under salinity conditions through nutrient uptake and plant growth-promoting characteristics (Fig. 1.1). PGPR as rhizo-remediators could prevent the deleterious effects of xenobiotics and act as biocontrol agents by producing antibiotics (Bouizgarne 2013). They can trigger induced local or systemic resistance for biotic and abiotic stress tolerance (Jacobsen 1997; Somers et al. 2004; Aseri et al. 2008; Glick et al. 2007; Van Loon 2007). Instead of using chemical fertilizer, their application as bio-inoculants for agricultural purposes would be a suitable alternative (Bloemberg and Lugtenberg 2001; Vessey 2003). The dominant α-Proteobacteria and γ-Proteobacteria communities in bulk soil and root endosphere tend to be phylogenetically clustered and contribute to salt stress acclimatization, nutrient solubilization, and competitive root colonization (Yuan et al. 2016). The effective existence of bacteria in the saline environment due to excessive accumulation of secondary metabolites may result in better root colonization and plant growth. Accumulation of small organic molecules also known as compatible solutes in response to salinity is reported in all living groups to a variable extent (Saharan and Nehra 2011).

1.6 Importance of Compatible Solutes to Mitigate Salt Stress After Inoculation of PGPRs

Compatible solutes are usually nontoxic, low molecular weight organic compounds and easily soluble at high cellular concentrations (Hayat et al. 2012). At low concentrations, these solutes presumably have another role, perhaps in stabilizing the tertiary structure of proteins, and function as osmoprotectants. These solutes provide protection to plants from stress by contributing to cellular osmotic adjustment,

Table 1.1 List of bacterial endophytes with the possible mechanism of alleviating salt stress

Pathway	Bacteria	Plants	References
Cytokinin signaling and stimulation of shoot biomass	*Bacillus subtilis*	*Lactuca sativa*	Arkhipova et al. (2007)
Expression of salt stress-related *RAB18* plant gene	Root-associated plant growth-promoting rhizobacteria (PGPRs)	*Oryza sativa*	Jha et al. (2014)
Tissue-specific regulation of sodium transporter HKT1	*Bacillus subtilis* GB03	*Arabidopsis thaliana*	Zhang et al. (2008)
SA-dependent pathway	*Pseudomonas syringae* DC3000, *Bacillus* sp. strain L81, *Arthrobacter oxidans*	*Arabidopsis thaliana*	Barriuso et al. (2008)
4-Nitroguaiacol and quinoline promoter	*Pseudomonas simiae*	Soybean seed germination	Vaishnav et al. (2016)
Phytohormones as elicitor molecule	Cyanobacteria and cyanobacterial extracts	*Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Gossypium hirsutum*	Singh (2014)
Reduction in Na+ level and increase in K+ level	*Pseudomonas koreensis* strain AK-1	*Glycine max* L. Merrill	Kasotia et al. (2015)
High hydraulic conductance, increased root expression of two ZmPIP isoforms	*Bacillus megaterium*	*Zea mays*	Marulanda et al. (2010)
High osmotic root hydraulic conductance due to increased active solute transport through roots	*Glomus intraradices* BEG 123	*Phaseolus vulgaris*	Aroca et al. (2007)
Increased root but decreased shoot proline concentrations	*Glomus etunicatum*	*Glycine max*	Sharifi et al. (2007)
Reduction of proline content	*Brachybacterium saurashtrense*, *Brevibacterium casei*, *Haererohalobacter* sp.	Peanut (*Arachis hypogaea*)	Shukla et al. (2012)
Increased accumulation of proline	*Burkholderia*, *Arthrobacter*, and *Bacillus*	*Vitis vinifera*, *Capsicum annuum*	Barka et al. (2006)
Phytohormone production and proline accumulation	*Azospirillum* sp. *B. aquimaris* SU8	Wheat (*T. aestivum*)	Zarea et al. (2012), Bal et al. (2013)

(continued)

Table 1.1 (continued)

Pathway	Bacteria	Plants	References
Accumulation of carbohydrates	*Glomus fasciculatum*	*Phragmites australis*	Al-Garni (2006), Porcel and Ruiz-Lozano (2004)
	Glomus intraradices	*Glycine ma*	
High stomatal conductance and photosynthesis	*Azospirillum brasilense* and *Pantoea dispersa* (co-inoculation)	*Capsicum annuum*	del Amor and Cuadra-Crespo (2012)
Decreased root and shoot Na+ accumulation and enhanced root K+ concentrations	*Glomus intraradices* BAFC 3108	*Lotus glaber*	Sannazzaro et al. (2006), Rabie (2005), Daei et al. (2009), Kaya et al. (2009)
	Glomus clarum	*Vigna radiata*,. *Capsicum annuum*,. *Triticum aestivum*	
	Glomus etunicatum		
Decreased root transcriptional expression of a high-affinity K+ transporter (*AtHKT1*) decreasing root Na+ import	*Bacillus subtilis*	*Arabidopsis*	Zhang et al. (2008)
Exopolysaccharide production, and reduced availability of Na$^+$ for plant uptake	Exopolysaccharide-producing bacteria, i.e., *Bacillus*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Paenibacillus*	Wheat (*T. aestivum*)	Ashraf and Harris (2004), Ashraf (2004), Kohler et al. (2006), Nadeem et al. (2010), Upadhyay et al. (2011), Aroca et al. (2008)
		Mung bean	
Reduced concentration of ABA	*Glomus intraradices* BEG121	*Lactuca sativa*	Aroca et al. (2008), Yao et al. (2010)
	Pseudomonas putida Rs-198	*Gossypium hirsutum*	
Stimulation of persistent exudation of flavonoids	*Azospirillum brasilense* strain Cd	*Phaseolus vulgaris*	Dardanelli et al. (2008)
Root-to-shoot cytokinin signaling and stimulation of shoot biomass	*Bacillus subtilis*	*Lactuca sativa*	Arkhipova et al. (2007)
Enhanced antioxidant responses through ROS-scavenging enzymes	*Bacillus safensis*, *Ochrobactrum pseudogregnonense* *Enterobacter* sp. UPMR1	Wheat (*Triticum aestivum*)	Chakraborty (2013), Habib et al. (2016)
		Okra	

(continued)

Table 1.1 (continued)

Pathway	Bacteria	Plants	References
Degrading ACC produced and therefore reduced elevated ethylene level	*Pseudomonas putida*, *Enterobacter cloacae*, *Serratia ficaria*, and *P. fluorescens*	Wheat (*T. aestivum*)	Nadeem et al. (2013), Karthikeyan et al. (2012), Ali et al. (2014)
		Catharanthus roseus	
	Achromobacter xylosoxidans	Avocado (*Persea gratissima*)	
	Arthrobacter protophormiae		
	AUM54 *Pseudomonas fluorescens* YsS6	Rice (*Oryza sativa*)	
	*P. migulae*8R6		
	Bacillus sp., *Variovorax* sp.		
	Alcaligenes faecalis, *Bacillus pumilus*, *Ochrobactrum* sp.		
Ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) activity	*B. subtilis*, *Arthrobacter* sp.	Wheat (*T. aestivum*)	Upadhyay et al. (2012)
Biofilm, exopolysaccharide, and accumulated osmolytes	*Staphylococcus saprophyticus* (ST1)	*Lens esculenta* var. masoor 93	Arevalo-Ferro et al. (2005)
Nematodes carry more bacteria on their cuticle and increase colonization	*P. fuorescens*10586, *P. fluorescens* SBW25	*Triticum aestivum* var. Savannah	Knox et al. (2003)
	B. subtilis		
	P. corrugata		
Increased stomatal conductance and transpiration rate	*B. drentensis*	Mung bean	Mahmood et al. (2016), Ahmad et al. (2013)
Phytohormone production	*P. extremorientalis*, *P. chlororaphis*	Common bean (*Phaseolus vulgaris*)	Egamberdieva et al. (2011)
Production of gluconic acid, ACC deaminase, phytohormones	*P. pseudoalcaligenes*, *B. pumilus*	Rice (*O. sativa*)	Jha et al. (2013), Rojas-Tapias et al. (2012)
	Azotobacter chroococcum	Maize (*Z. mays*)	
Indolyl-3-acetic acid (IAA) and auxin increased	*Streptomyces* sp.	Wheat (*T. aestivum*)	Sadeghi et al. (2012)
Reduced production of ethylene and increased uptake of phosphorous and potassium	*Achromobacter piechaudii*	Tomato (*L. esculentum*)	Mayak et al. (2004)

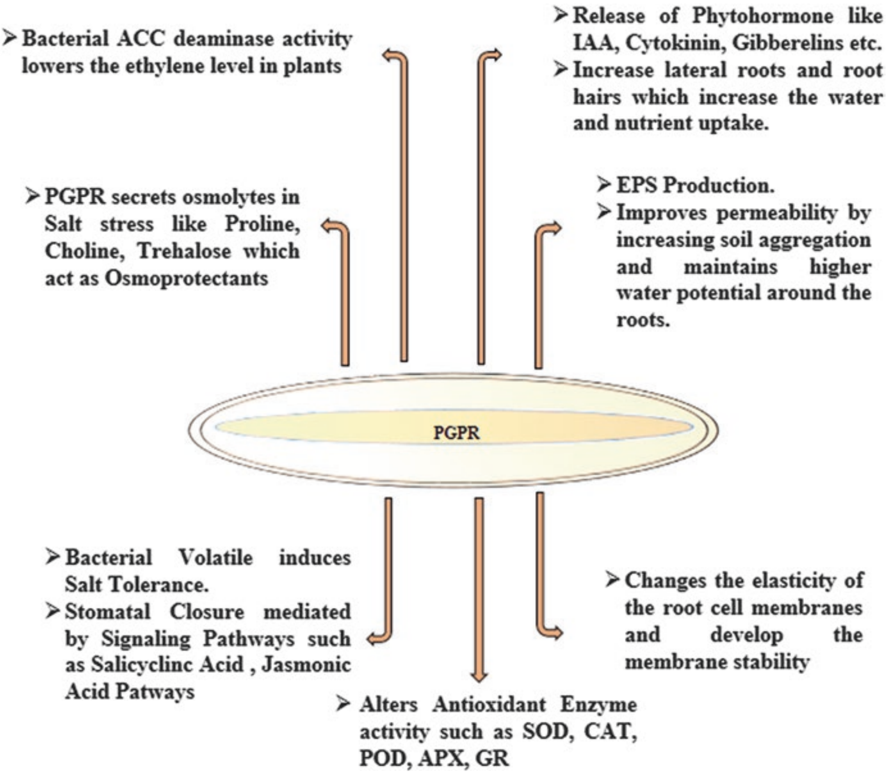

Fig. 1.1 Mechanism of plant salt tolerance induced by plant growth-promoting rhizobacteria (PGPRs)

ROS detoxification, protection of membrane integrity, and enzyme/protein stabilization (Hayat et al. 2012). Very important compatible solutes are proline, ectoine, trehalose, polyols, and sucrose and quaternary ammonium compounds (QACs) such as glycine betaine, proline, alanine, and percolate.

Accumulation of these osmolytes in bacteria and plants is an indicator of salt tolerance in response to salt stress (Bremer 2000; Gul et al. 2013). Many species of bacteria respond to increase in osmotic pressure by accumulating osmoregulatory solutes, so-called compatible solutes, up to high intracellular concentrations for coping with high external salinity. In many halophytes, proline or glycine betaine occurs at sufficiently high concentrations in leaves to compensate the osmotic stress on the cell. The concentration of compatible solutes rise up to 40 mM/tissue water when the osmotic pressure rises above 01. MPa (Flowers et al. 1977). To maintain turgor pressure in highly saline environments, considerable concentrations of solutes need to be accumulated in the cells (Imhoff 1986).

1.7 Proline as an Influential Compatible Solute for Stress Responses After PGPR Inoculation

Plants usually produce substantial amount of various compatible organic solutes under stress conditions, most commonly proline and glycine betaine (Serraj and Sinclair 2002). Proline as an osmoprotectant was discovered first in bacteria, and the relationship between proline accumulation and salt tolerance was also noticed (Csonka et al. 1988; Csonka and Hanson 1991). A wide variety of bacteria and plants respond to osmotic stress or dehydration by increasing their cellular proline levels. Proline accumulation is a sensitive physiological index for the response of plants to salt and other stresses (Liang et al. 2013) to maintain higher leaf water potential and to keep plants protected against oxidative stress (Lutts et al. 1999). On the other hand, proline also stabilizes many functional units such as ribulose bisphosphate carboxylase/oxygenase (RUBISCO) enzymes and complex II electron transport (Mäkelä et al. 2000). Proline helps the plant cell to alleviate salt stress by stabilizing subcellular structures like proteins and membranes (Huang et al. 2009). Proline also helps in scavenging free radicals and buffering cellular redox potential (Ashraf and Foolad 2007; Kohler et al. 2009). Increased total soluble sugar (TSS) content of plants under salinity stress is another vital defense strategy to cope with salinity stress. An increased amount of proline and total soluble sugar in wheat plants inoculated with PGPR significantly contributed to their osmotolerance (Upadhyay et al. 2012).

It is suggested that proline accumulation is a symptom of salt stress injury in rice and that its accumulation in salt-sensitive plants results from an increase in ornithine-δ-aminotransferase (OAT) activity and an increase in the endogenous pool of its precursor glutamate (Mansour and Ali 2017). Proline concentration in leaves, stems, and roots will increase under salt stress conditions. Proline also acts as a signaling molecule for antioxidative defense pathway and has metal chelating activity. The enzymes Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and Δ^1-pyrroline-5-carboxylate reductase (P5CR) are responsible for proline biosynthesis from its precursor, glutamate. The other pathway to synthesize proline is from ornithine, which is converted via ornithine-δ-aminotransferase (OAT) to γ-glutamate-semialdehyde (GSA) into Δ^1-pyrroline-5-carboxylate (P5C) (Liang et al. 2013). The enzymes proline dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) catabolize proline back to glutamate.

1.7.1 Proline Biosynthesis Under Stress Order

Ubiquitous pathway for proline biosynthesis is to derive glutamate via phosphorylation to γ-glutamyl phosphate by the activation of the γ-glutamyl kinase enzyme. The biosynthesis of proline from glutamate is catalyzed by three enzymatic reactions which are catayzed by γ-glutamyl kinase (GK; proB product), γ-glutamyl phosphate reductase (proA product), and Δ^1-pyrroline-5-carboxylate reductase (proI product). In general, proI on the chromosome is so distant from the operon constituted by proB and proA genes. Three proline transport systems including proline permease gene (PutP), ProP, and ProU were possessed by gram-negative bacteria *E.coli* and *S. typhimurium* (Sleator and Hill 2002). Proline was individually transported as a carbon or nitrogen source by PutP system (Sleator and Hill 2002), which plays little role in osmoadaptation. The PutP is a high affinity system which has significant homologies with PutP of *E. coli*, which is responsible for transporting proline in the cellular system. Proline was uptaken by this system, which acts independently to osmotic stimulation.

Proline accumulation in plants under stress condition usually occurred from two different precursors, glutamate and ornithine. Proline converted from glutamate is the first pathway, which involves two successive reductions: catalyzed (i) pyrroline-5-carboxylate synthase (P5CS) and (ii) pyrroline-5-carboxylate reductase (P5CR), respectively. P5CS is a bifunctional enzyme catalyzed first from the activation of glutamate by phosphorylation and second the reduction of the labile intermediate γ-glutamyl phosphate into glutamate semialdehyde (GSA), which is in equilibrium with the P5C form (Hu et al. 1992). Mitochondrial enzyme ornithine is an alternative precursor for Proline biosynthesis. It can be transaminated by ornithine-δ-amino transferase (OAT) to P5C. Glutamate pathway is the central pathway during osmotic stress. However, in young *Arabidopsis* plants, the ornithine pathway seems to contribute, and δ-OAT activity is enhanced (Roosens et al. 1998).

1.7.2 Proline Degradation

Proline degradation is a rate-determining step of its pathway similar to proline biosynthesis. Proline can be degraded by proline dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) enzymes. Proline degradation takes place in the mitochondria, while biosynthesis occurs in the cytosol and the plastids of the green tissues (e.g., chloroplasts) (Elthon and Stewart 1981; Rayapati et al. 1989; Szoke et al. 1992). Most of the plants like *Arabidopsis* have two functional Proline dehydrogenase (PDH) isoforms, (i) Proline dehydrogenase-1 (PDH1) and (ii) Proline dehydrogenase-2 (PDH2), which are localized in the mitochondria (Funck et al. 2010; Kiyosue et al. 1996). PDH1 is predominant isoform in plant and present in higher amount than PDH2. It is mainly expressed in the vasculature of leaves (Funck et al. 2010). Funck et al. (2010) suggested that proline degradation in the vasculature may provide essential energy for the plant during stress exposure.

Proline acts as a vital energy source for recovery phase under salinity stress (Szabados and Savoure 2010; Hare and Cress 1997). The salient feature of proline catabolism is to drive the oxidative phosphorylation in plants. The PDH1 mutant *Arabidopsis* showed significantly lower oxygen consumption in the root apex (Sharma et al. 2011). The recovering tissues in mitochondria get help to drive oxidative phosphorylation and ATP synthesis from proline oxidative metabolism. PDH and P5CDH expression are similarly increased during stress recovery phase (Kiyosue et al. 1996).

1.7.3 Importance of Proline to Alleviate Stress

In response to environmental stress, proline is accumulated naturally in plants similar to other microorganisms including protozoa and algae and bacteria (Csonka 1981a; Matysik et al. 2002; Szabados and Savoure 2010; Verbruggen and Hermans 2008). Plants recover from stress condition by the accumulation of proline as a signaling molecule. Proline can leverage cell proliferation or cell death and mitochondrial stress functions and regulate specific gene expression. Genetic modification of proline metabolism could escort new opportunities to boost plant tolerance from environmental stresses. The intracellular proline levels have been increased by more than 100-fold in plants during stress (Handa et al. 1983; Verbruggen and Hermans 2008). The plants tend to accumulate proline during salt stress (Yoshiba et al. 1995), drought stress (Barnett and Naylor 1966; Choudhary et al. 2005), heavy metal stress (Chen et al. 2001), UV radiation exposure (Saradhi et al. 1995), pathogen infection (Fabro et al. 2004) and oxidative stress (Yang et al. 2009). Exo- and endogenously manipulating proline levels (Hare et al. 1999) under stress conditions in plants involve reciprocal regulation of P5CS and PDH (Liang et al. 2013). Overexpression of P5CS in tobacco results in higher levels of proline, enhanced osmotolerance, flower development, and increased root biomass (Hare et al. 1999; Hong et al. 2000). Proline plays a vital role in scavenging hydroxyl radicals (Smirnoff and Cumbes 1989), chelating heavy metals (Farago and Mullen 1979), and reducing metal uptake (Wu et al. 1998) in the cytoplasm.

Proline has since been shown to accumulate high intracellular concentrations in a variety of bacteria, following exposure to osmotic stress (Measures 1975). Intracellular proline pool of many gram-positive bacteria has been shown to increase by cellular biosynthesis (Cayley et al. 1992; Whatmore and Reed 1990), whereas gram-negative bacteria achieve higher proline concentration by enhanced transport system during osmotic stress (Sleator and Hill 2002). Proline has been accounted as the most substantial part of amino acid accumulation in response to osmotic stress for gram-negative and gram-positive bacterial strains (Imhoff 1986; Hua et al. 1982). The intracellular proline level was elevated with increase in osmolarity of the medium (Perroud and Le Rudulier 1985; Imhoff 1986).

The primary response to high salinity in bacterial cell (*E. coli*) is the accumulation of K^+ and glutamate. The K^+ accumulation in the cell takes place through the action of Kdp (ion-motive P-type ATPase) and Trk (Potassium transport proteins) system (Sasaki et al. 2013). The accumulation of proline or glycine betaine in the cytosol upregulates the activity of Kef system (Potassium efflux system) which in turn depletes the glutamate pool (Sasaki et al. 2013). In various non-halophilic bacteria, the total amino acid pool increases with the increase in external osmolarity, and specifically it was noticed that proline accumulation is significant (Imhoff 1986). In general, bacterial species which accumulate proline are more salt tolerant than those which do not. Accumulation of osmolytes in bacterial strains at higher salinity might be involved for their adaptation to saline environments in the soil for improving plant growth.

1.7.4 Proline Acts as ROS Scavenger as Well as Signaling Molecule

The hydrogen peroxide and hydroxyl ions can react with free and polypeptide-bound proline (pH 7–8) to form stable free radical adducts of proline and hydroxyproline (e.g., 4-hydroxyproline and 3-hydroxyproline) (Floyd and Nagy 1984; Kaul et al. 2008; Requena et al. 2001; Rustgi et al. 1977; Trelstad et al. 1981). The reaction of hydrogen peroxide and proline has been evidenced as a very slow process, which is also reported for reaction with$O_2^{\cdot -}$. On the other hand, the facile reaction of proline with singlet oxygen ($^1O_2$) is an essential ROS-scavenging mechanism for stress alleviation.

Ali et al. (2014) reported that the production of $^1O_2$ in the thylakoids from the cotyledons of *Brassica juncea* was dramatically suppressed by proline when the plants were exposed in high illumination (Saradhi and Mohanty 1997). Due to its action as a $^1O_2$ quencher, proline may help stabilize proteins, DNA, and membranes (Matysik et al. 2002). Prolyl residues in proteins also provide protection against oxidative stress caused by $^1O_2$ (Fig. 1.2).

1.7.5 Distinct Attribute of Proline Metabolism During Stress

Proline has an advantage of being the terminal product of analogously short and high regulated pathway compared to other amino acids (Hare and Cress 1997). Proline and its immediate precursor P5C are not interconvertible, but by the action of two distinct enzymes with different mechanisms, proline catabolism can occur in a distinct subcellular compartment (Phang 1985). Therefore, since a single equilibrium reaction does not link proline and P5C, the final product of the proline biosynthetic

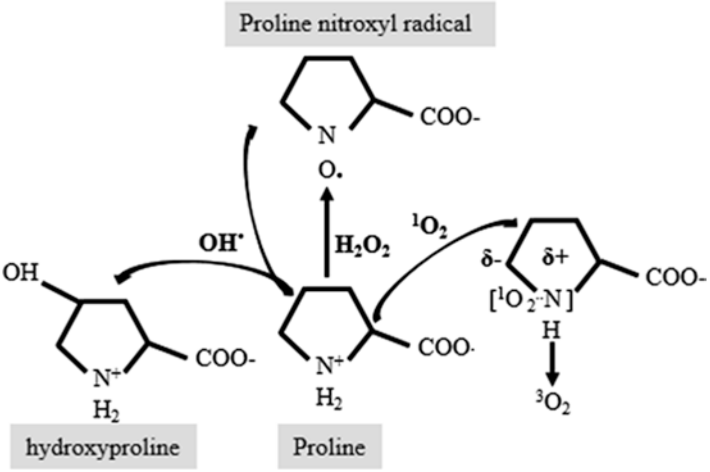

Fig. 1.2 ROS scavenging mechanisms of proline

pathway is not necessarily in equilibrium with its immediate precursor, as its α-nitrogen is a secondary amine. Proline cannot participate in the transamination or decarboxylation reactions common to other amino acids (Phang 1985). Furthermore, oxidation of proline results in the formation of 30 ATP equivalents, which is stored in the cells as energy currency (Hare and Cress 1997). These two features are likely to have contributed substantially to a role for proline in plants as a resource of value either in the acclimation to stress or in recovery upon relief from stress.

1.7.6 Characteristic Feature of Proline Under Stress Condition

The molecular mechanisms of how proline protects cells during stress are not fully understood but appear to involve its chemical properties and effects on redox systems such as the glutathione (GSH) pool. Its property often explains the function of proline in stress adaptation as an osmolyte and its ability to balance water stress (Delauney and Verma 1993). However, adverse environmental conditions often perturb to intracellular redox homeostasis and hence counteract oxidative stress. Thus, protective mechanisms of proline have also been proposed to involve the stabilization of proteins and antioxidant enzymes, direct scavenging of ROS, balance of intracellular redox homeostasis (e.g., ratio of $NADP^+$/NADPH and GSH/GSSG), and cellular signaling promoted by proline metabolism.

1.8 Proline Accumulation Thought to Be an Alternative to Mitigate Salt Stress Through PGPR Inoculation

The accumulation of proline is beneficial for survival during osmolarity imbalance in bacteria, because mutants of such bacteria confer proline overproduction, which enhanced tolerance during osmotic stress (Csonka 1981b). Increased accumulation of proline has been reported in soybean and wheat plants upon inoculation with PGPR strains which alleviated salinity stress and improved growth (Han and Lee 2005; Zarea et al. 2012). *Azospirillum* inoculation has also been reported to accumulate proline (Bashan 1999; Casanovas et al. 2003) in plants during salinity stress conditions. Maximum accumulation of proline (298 $\mu g g^{-1}$ fresh weights) was observed at 1.5 M NaCl stress for the strain *Staphylococcus haemolyticus* (ST-9), and further accumulation decreased toward increasing salt concentrations (Qurashi and Sabri 2013). Generally, there was a maximum accumulation of proline as compared to glycine betaine and choline in bacterial strains except for a few cases, i.e., 0.5 M for *Staphylococcus haemolyticus* (ST-9) and *Bacillus subtilis* (RH-4) and 1 M for *Bacillus subtilis* (RH-4) where glycine betaine accumulation was maximum (Qurashi and Sabri 2013). The previous studies have showed that the accumulation of proline during salt stress conditions tend to enhance the salt tolerance ability of plants and bacteria. Hence, proline has its noteworthy disposition to alleviate salt stress on plants as well as microorganisms.

1.9 Conclusion and Future Prospects

Overexpressing proline via transgenic approaches usually resulted in elevated concentrations and improved stress tolerance. PGPRs can alleviate plethora of abiotic stresses such as drought and salt stress through multiple mechanisms. The accumulation of osmolytes such as proline is one of those mechanisms which is believed to play an important role in amelioration of such stress conditions. Moreover, the balance between biosynthesis and degradation of Proline is also thought to be essential in the determination of the osmoprotective and developmental functions of this compatible solute. Other protective functions have been suggested for low proline levels, as it may stabilize proteins and membranes, scavenge ROS, and thus minimize cell damage. Halophytic PGPRs have their own mechanisms for osmotolerance and significant beneficiary activities to the plant under salt stress through proline accumulation.

Inoculation of PGPRs increases salt tolerance through a plethora of mechanisms. Proline accumulation is an important compatible solute which has been proposed to play an important role.

Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death, and provoke specific gene expression, which can be essential for plant recovery from stress. Understanding the interaction between a consortium of microbial inoculants and plant systems will pave a way to harness more benefits from inoculation as well as proline accumulation to improve plant growth and enhance tolerance to stress.

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Plant Growth-Promoting Rhizobacteria and Salinity Stress: A Journey into the Soil

2

Bahman Fazeli-Nasab and R. Z. Sayyed

Abstract

A large number of studies have indicated that salinity stress and saline soils are cruel environmental limiting factors that retard the growth of crop plants. Present scenario of climate change will further increase the border of the area affected by saline soils, and therefore this phenomenon will threaten the productivity of crops leading to depletion of food sources of human societies. Various strategies including soil quality management policies, improving crop resistance against salinity stress, detoxification of noxious ions, improving the quality of irrigation water, and many other effects need to be examined to decrease the detrimental consequences associated with saline soils. In this context, the use of microorganisms especially plant growth-promoting rhizobacteria (PGPR) has been proposed as a sustainable way to fortify the quality of soils to help crop plants grow under salinity stress. Recent advances in molecular soil biology studies suggested that PGPR are involved in the important physiological process associated with plant growth and development. Among the other mechanisms, improvement in water and nutrient uptake, decrease in the toxicity of hazardous ions, amelioration of photosynthesis, improvement in nitrogen fixation, regulation/modulation of physiological signaling networks are the common features exhibited by PGPR to enhance the growth of plants in saline soils. Thus, it should be noted that these miracle bacterial species are *legendary soil guards* to protect both soil texture

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and crop plants from salinity stress in the light of present and upcoming global climate changes.

Keywords
PGPR · Salt stress · Phytohormones · Osmoregulation

2.1 Introduction

The upcoming global climate changes have drastically affected the productivity of crop plants. The increased weather temperature, an imbalance in CO_2 concentration, the delayed winter rainfall pattern, the drought stress, the modified micro- and macro-ecosystems, and more importantly soil salinity are the key issues associated with global climate changes. The constant erosion of the earth's crust causes the worldwide geological changes. The main, and perhaps most important, consequent associated with earth erosion is the exchange of soil ion contents. An enormous number of chemical compounds including sodium, calcium, chloride sulfate, carbonate, manganese, and other mineral and non-mineral elements are deformed or widely spread throughout the soil texture.

The presence of these elements in the soil will change the quality of soils (especially those are currently used to cultivate crop plants), and therefore they will lose their potential to provide water and nutritional elements for plants to grow (Amozadeh and Fazeli-Nasab 2012). In addition to the earth's crust erosion, the quality of irrigation water is another important factor to change the portion of toxic ions in soils. Many studies have reported that the excessive irrigation and poor/inadequate drainage are two factors that increase the salinity of soils (Ilangumaran and Smith 2017). Though the amount of stored salts in the soil structure is directly dependent on soil type, nonetheless the quality and quantity of water for irrigation can enhance the soil salinity by changing the total amount of ions present in each layer of soils (Phogat et al. 2018).

Normally, irrigation water contains 0.1–4 kg m^{-2} salt, and this amount of water is used annually in 1–1.5 m. So, an annual amount of 1–160 tons per hectare of salt is added to agricultural land. Irrigation water evaporates and its salts remain in the soil. For saltiness, these salts should be removed from the root area of the plants by leaching and drainage techniques. There is also evidence that farmers traditionally replaced resistant plants with susceptible plants, in dealing with the salinity problem. However, use of substitute plants to deal with salinity is likely to be used as a method for a long time before the leaching technique. Substitution of saline-resistant plants is used instead of susceptible plants in saline soils in the world. Some plants, such as sugar beet, barley, cotton, sugarcane, asparagus, and dates, have a high resistance to salinity (Kafi and Mahdavi-Damghani 2005).

The increasing demand for food production (especially for cereal plants) with a significant reduction in the use of chemical agents including herbicides, fungicides, pesticides, and synthetic fertilizers is a huge global concern to affect the future of agricultural systems. A huge number of scientific studies have reported that PGPR are environmental friendly microorganisms to increase the productivity of crop plants in modern agriculture epoch. In addition to their roles in the preparation of mineral and other chemical compounds for plant root system, they also exhibit their biological activities through direct and/or indirect interaction with other soil microorganisms to provide specific environment to fortify the growth of plants (Vejan et al. 2016). PGPR can protect plants from the harmful effects of pertaining to the environmental stresses including flooding, drought, salinity, heavy metals, and phytopathogens (Mayak et al. 2004; Yildirim et al. 2006) and also manage some of these operates through specific enzymes, which stimulate physiological changes in plants at the molecular level. Among these enzymes, ACC deaminase regulates plant hormones such as ethylene (Glick 2005; Arshad and Frankenberger 2012); on the other hand, PGPR stimulate plant growth through the activity of the enzyme ACC deaminase, which causes lower plant ethylene levels resulting in longer roots (Shah et al. 1998).

2.2 Salinity

Salinity is one of the major limiting factors that cause osmotic stress and decrease plant growth and crop productivity in arid and semiarid regions. In salinity process, increase in concentration of soluble salts in the root zone is one of the major complications, and also the rhizospheric populations affect the plant productivity (Cicek and Cakirlar 2002; Tank and Saraf 2010; Fazeli-Nasab 2018).

2.3 Adverse Effects of Salinity

2.3.1 Physiological and Morphological Disturbances

Salt stress reduces many aspects of plant metabolism like growth and yield. Salinity stress increases Na^+, which eventually decreases Ca^{2+} and K^+ (Yildirim et al. 2006). Accumulation of Na^+ can cause metabolic disturbances in some processes where Na^+ (low) and K^+ or Ca^{+2} (high) are required for optimal functioning and growth (Marschner 1995; Xu et al. 1999). The ability of cells to save salts is exhausted; salts build up in the intercellular space and then kill cells and organs (Sheldon et al. 2004). At higher status of available salt, the leaf area, size, and leaf production are reduced leading to the death of the plant (Suárez and Medina 2005).

2.3.2 Disturbances in Photosynthesis

Increasing salinity in the soil decreased some plant mechanisms like photosynthesis, chlorophyll content, and stomatal conductance, and all of these mechanisms will decrease photosynthetic capacity due to the osmotic stress and partial closure of stomata (Drew et al. 1990; Han and Lee 2005; Azad et al. 2017). Accumulation of Cl^- disrupts photosynthetic cycle.

2.3.3 Effects on Plant Growth and Crop Yield

Soil salinity limits plant growth and crop production in many parts of the world, particularly in arid and semiarid areas. However plants can suffer from membrane destabilization and general nutrient imbalance (Hasegawa et al. 2000; Parida and Das 2005). Plants after exposure by salt accumulate different molecules in their organics like proline, glucose, and glycine betaine.

Salinity tolerance can be defined by maintaining plant growth in an environment containing NaCl or a mixture of salts. Bray (1997) defined salt tolerance as having a negative effect on the growth of plants that store salt in their tissues, and also Maas and Hoffman classified crops into four groups on the basis of their tolerance to salinity: (i) relatively tolerant plants, (ii) resistant plants, (iii) semi-sensitive plants, and (iv) sensitive plants (Table 2.1).

2.3.4 Mechanisms to Combat Salinity Stress

Most of the salinity problems in higher plants are due to an increase in sodium chloride, which has spread to soils in the dry and coastal areas and their water resources. The high salinity of sodium chloride causes at least three types of problems in higher plants: (1) The osmotic pressure of the external solution results in an increase in the osmotic pressure of the plant cells, which requires osmotic regulation of the plant cells in order to avoid waste. (2) Removal and transfer of nutrients such as potassium and calcium ions are interrupted by excess sodium. (3) High levels of sodium and chlorine produce direct toxic effects on membrane and enzyme

Table 2.1 Grain tolerance to salinity in some important crops (Maas and Hoffman 1977)

Crop	Salinity level threshold $^{ds}/_{m}$	Crop	Salinity level threshold $^{ds}/_{m}$
(i) Relatively tolerant plants		**(ii) Resistant plants**	
Cowpea	4.9	Sugar beet	7
Soybean	5	Cotton	7.7
Wheat	6	Barley	8
Durum wheat	5.7	Chicken	6.9
Sorghum	6.8	Wheat grass	7.5
(iii) Semi-sensitive plants		**(iv) Sensitive plants**	
Alfalfa	2	Bean	1
Corn	1.7	Carrot	1
Rice	3	Orange	1.7
Tomato	2.5	Peach	1.7
Sugarcane	1.7	Apricot	1.6
Lettuce	1.3	Plum	1.5

systems. Osmotic stress is induced in plants under drought stress conditions, and since about 100 years ago, the term salinity stress is a form of physiological drought (Rengel 1992; Ding et al. 2018).

Some of the mechanisms for avoiding salinity are presence of small leaves to reduce transpiration, fewer stomata per leaf area, the presence of thick cuticle, and an increase in root-to-crown ratio. In the atmosphere, by regulating the osmotic content of sugars, the Na^+ and Cl^- levels are limited to the limb (Reich et al. 2017).

Resistance to salinity can be elevated through five major strategies:

1. Resistance to salinity in plants by improving traditional breeding and selection.
2. In the development of plants, along with their ancestors, they may acquire the trait of salinity resistance.
3. Farming of species that have salt tolerance (halophytes) can be identified, cloned, and inoculated by modification and selection for the development of their agronomic characteristics.
4. Salinity resistance genes.
5. Salinity-resistant plant growth-promoting rhizobacteria (Carter et al. 2012).

Plant growth responds to salinity in two stages: (1) a rapid stage (osmotic phase that inhibits growth of young leaves) and (2) a slower stage (ionic stage that accelerates senescence of mature leaves) (Munns and Tester 2008). The ability of plants-against salt condition is determined by several biochemical pathways that make easy retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis (Parida and Das 2005).

2.3.5 Production of Phytohormones

Studies have shown that indoleacetic acid and cytokinin are produced from amino acids such as tryptophan and adenine which secreted from the roots. Ethylene precursor is hydrolyzed to 1- amino cyclopropane, 2-carboxylic acid (1-aminocyclopropane-1-caboxylic, ACC) by enzyme ACC deaminase (Zahir et al. 2004). Activities of PGPR cause physiological changes in the morphology of the plant, and the set of these changes have a positive effect on growth, nutrition, and plant health.

2.3.6 Plant Hormones

2.3.6.1 Ethylene

The evolution of roots approximately 400 million years ago opened up the biological colonization of the land (Jackson 2017). Ethylene is a gaseous plant growth hormone produced endogenously by almost all plants and even in soil that plays a key role in inducing several physiological functions (Saleem et al. 2007).

The hormone ethylene also known as a stress hormone is released as a physiological response to different stresses such as edaphic and adaphic. Salinity can increase biosynthesis rate of ethylene via elevated levels of ACC, which may lead to physiological changes in plant tissues. Any check on this accelerated ethylene production in plants can improve growth of plants under salt stress (Hontzeas et al. 2004).

2.3.6.2 The Effect of Ethylene in Root Growth and Development

Ethylene was known as a stress hormone that is released by the plant as a physiological response when exposed to a different kind of stresses. It has been observed that plants inoculated with PGPR having ACC deaminase activity are more resistant to the deleterious effect of stress ethylene synthesized as a consequence of stress conditions (Penrose and Glick 2003; Zahir et al. 2004). Ethylene is of great importance in plant growth and development and also in some functions like inhibition of seed germination and root growth (Nordström and Eliasson 1984). As after germination, high level of ethylene would inhibit root elongation. Inhibition of the root elongation has been prevented due to inoculation of PGPR capable of containing ACC deaminase even in the presence of high (6%) concentration of salts, NaCl (Tank and Saraf 2010). PGPR inoculation helped in seed germination followed by lowering the plant's ethylene concentration, thereby decreasing the ethylene inhibition of seedling root length, while in many plants a burst of ethylene is required to break seed dormancy (Nascimento 2003).

2.3.6.3 Stress/Wound Ethylene

The term stress is used for an external factor capable of inducing a potentially injurious strain in living organisms. Stress ethylene is one of the general phenomena observed in plant tissues subjected to various unfavorable conditions (Hyodo 2017). Several kinds of stress are related to ACC such as effects of phytopathogenic bacteria and resistance to stress from polyaromatic hydrocarbons and from heavy metals (Glick et al. 2007). Plant seed inoculated with biocontrol bacteria strongly decreases plant diseases level and may help to protect fieldworkers from exposure to pathogens (Egamberdieva et al. 2008).

Bacterial strains containing ACC deaminase can, in part, at least alleviate the stress-induced ethylene-mediated negative impact on plants (Glick et al. 1998; Glick 2005; Safronova et al. 2006). It reported that ACC deaminase bacteria conferred salt tolerance onto plants by lowering the synthesis of salt-induced stress ethylene and promoted the growth of canola in the saline environment, and also it is related that in plants Cd is the strongest heavy element inductor of ethylene biosynthesis (Cheng et al. 2007).

Ethylene production in plants is induced by various environmental factors such as wounding, physical load, disease, drought, waterlogging, chilling temperature, and exposure to various chemicals (Hyodo 2017). There is evidence that treatment with aminoethoxy vinyl glycine (AVG) prevents ethylene inhibition of root elongation (Hall et al. 1996) and also ethylene inhibitors can decrease the negative effect and the expression of stress symptoms induced by ethylene in plants (Rost et al. 1986; Elad 1990).

2.3.6.4 ACC Deaminase and Its Biochemistry

Inoculation of PGPR in pepper, bean, canola, and lettuce under salt stress has been used for mitigating the effects of salinity. Reports also showed an improvement of squash plant when applied directly or as a transplant under salinity stress (Yildirim et al. 2006).

The enzyme ACC deaminase cleaves ethylene, and also for many plants a burst of ethylene is required to break seed dormancy and germination; however, higher levels of ethylene inhibits the root elongation (Ali-Soufi et al. 2017; Soni et al. 2018). Plants that are treated with ACC deaminase-producing PGPR have been shown to exhibit more resistant to the deleterious effects of stress ethylene synthesized as a consequence of stressful conditions such as flooding (Grichko and Glick 2001), heavy metals (Grichko et al. 2000), the presence of phytopathogens (Wang et al. 2000), drought, and high salt conditions (Penrose and Glick 2003).

The activity of ACC deaminase has been widely reported in different species of Gram-negative bacteria (Wang et al. 2000; Babalola et al. 2003), Gram-positive bacteria (Belimov et al. 2001; Ghosh et al. 2003)), rhizobia (Ma et al. 2003), endophytes (Pandey et al. 2005), and fungi (Jia et al. 1999). ACC deaminase is prevalent in different kinds of bacteria, viz., *Agrobacterium* and *Azospirillum* (Blaha et al. 2006), *Alcaligenes* and *Bacillus* (Belimov et al. 2001), *Burkholderia* (Blaha et al. 2006), *Enterobacter* (Penrose and Glick 2001), *Methylobacterium* (Madhaiyan et al. 2006), *Pseudomonas* (Belimov et al. 2001), *Ralstonia solanacearum* (Arshad and Frankenberger Jr 2012), *Rhizobium* (Ma et al. 2003), *Rhodococcus* (Stiens et al. 2006), *Sinorhizobium meliloti* (Belimov et al. 2001), and *Variovorax paradoxus* (Glick 2005). Owing to ACCD activity, these bacteria are known to help plant grow under biotic and abiotic stresses condition by decreasing the level of "stress ethylene" which is inhibitory to plant growth (Singh et al. 2015). The ACC deaminase enzyme produced by several rhizobacteria catalyzes and reduces the deleterious ethylene level (Soni et al. 2018) that acts as a sink for ACC and protects stressed plants from deleterious effects of stress ethylene (Glick 2005). Reports also showed that inoculation of plant with ACC deaminase containing PGPR has also resulted in enhanced chlorophyll contents of maize and lettuce (Han and Lee 2005; Tank and Saraf 2010).

The ability of a newly isolated ACC-utilizing bacterium, *Kluyvera ascorbata* SUD165, to improve the growth of canola, tomato, and Indian mustard seedlings treated with toxic concentrations of nickel, lead, and zinc has recently been demonstrated (Shah et al. 1998; Burd et al. 2000; Safronova et al. 2006).

2.4 Mechanisms of Salt Tolerance

2.4.1 Cytoplasmic Osmotic Regulation

Halophilic bacteria accumulate more salt in the protoplasm than those that are present in the external solvent medium through active ion harvesting; therefore, the intracellular water pressure remains negative in comparison with the external solution and its enzymatic systems evolved in a manner that is carried out under conditions of high salt levels in the protoplasm. Marine algae also use their own special organic solutions for osmotic regulation to keep their sodium cytoplasm concentration low (Ashraf and Wu 1994; Cao et al. 2017). One of the problems of salt stress is the reduction of the osmotic potential of the soil solution, so that the plant can absorb water from the soil. It should reduce the osmotic potential to less than the osmotic potential of the soil.

2.4.2 The Accumulation of Substances in Vacuole

Most of the high salinity plants accumulate sodium and potassium for osmotic regulation in vacuole. However, some grasses may also use organic solvents in vacuole (Srinivas et al. 2018). In this method, sodium transfer from cytoplasm to vacuole and also the return of potassium from vacuole to cytoplasm are performed by pumps. In this way, in addition to reducing the toxicity of sodium ion in the cytoplasm, the osmotic potential of the cell also decreases (due to the accumulation of salts in the vaccine), and this way the plant will be able to absorb water and salts from the soil. The mechanism of this problem is energy, that is, the transfer of sodium from the cytoplasm to the vaccine and the transfer of potassium from vaccine to cytoplasm with energy.

2.4.3 Absorption and Replacement of Ions

The first line of defense against the addition of excess sodium into the plant is the plasma membrane of the root cell, which has low sodium permeability in all studied species. Conversely, root cells have shown a high tendency to absorb potassium, which can accumulate unlike concentration slopes (Pitman et al. 1981; Mangalassery et al. 2017). Plants that tolerate low salinity under high concentration of sodium in the root environment show a significant reduction in potassium uptake and increase sodium uptake in the shoot (Rains 1969; Makhlouf et al. 2015).

2.4.4 Movement Paths Along the Root

Water and salts can enter the root through two paths of symplast and apoplasts. Symplast is through the cytoplasmic pathway of the root cells, which extends from the epidermis to the root of the brain and is related through the connections between the adjacent cells. Apoplasts transfer material through cell walls. Entering the symplast route is the most important control point for entering salt into the plant. The water entering the apoplastic pathway is more similar to that of the intracellular solution than with the solution outside the root, although the concentration of apoplastic salt can be corrected by absorbing into the cells along the path and by exchanging the ion walls of the cell (Tester and Davenport 2003; Reddy et al. 2017).

If the only route for the transfer of salts to the xylem is apoplastic pathway, air organs should be full of salt, however, the existence of a casparian strip makes this necessary that salts and water should pass through endoderm via the entrance of the symplastic system. It has been determined that thoracic plants often have a thick layer of cork, or dendritic cellulose cells have endoderm, while mesophytes often have a thin layer of casparian strip (Esashi 2017; Zarayneh et al. 2018).

2.4.5 Recovery of Sodium from Transpiration

When the water and soluble substances reach the root from the symplast they will be transfered from xylem to the air organs. Xylem parenchyma not only prevents the entrance of salts into the transpiration system, but also can reduce salts concentration in Sap via reabsorption of salts from transmitance of root to air oragns in transpiration system. The sodium in xylem is transmited to the Phloem in the base of stem by active transport which significantly reduces the amount of sodium in the transpiration. This conclusion was achived based on the experiments that aerial parts of the stem were wounded in a circular manner so that the phloem were disconnected but xylem left in a natural state. Then, Na^{22} was applied to the environment and it was observed that the plants with injured stem transmitted higher radioactive sodium to the leaves (about 84%) compared to the uninjured stems. Probably, the readsorbed salts are returned to the tip of the root inside the phloem (Gleason et al. 2017; Keisham et al. 2018).

2.4.6 Control of Salinity Levels in Leaves

The amount of salt in the transpiration pathway is lower than that of the extra-root solution even from salt accumulation pools, for example, barley, grown in 150 mM of NaCl in its transpiration pathway, has about 5 mM of sodium chloride (Rains 1969; Makhlouf et al. 2015). Rice, wheat, and barley have two adaptation methods to tolerate salts that reach the air organs – (1) salting by growth and (2) distribution of salts to older leaves – after the accumulation of salt in older leaves, they disappear, and thus the amount of salt in the plant decreases (Munns 1993; Sarabi et al. 2017).

2.4.7 Tubers and glandular trichomes

Susceptible plants often have specific methods for managing salt in the leaves. Examples of these mechanisms are the tubers and glandular trichomes for the removal of salts to the outer surfaces of the leaves. Salt glands are known in at least 11 plant families (10 dicotyledons and 1 cotyledon family, Gramminae). Salt tubers in gramminae contain two cells, one of which is base and the other is a warhead cell. Solar cells are collected by the base cell and driven out of the warhead cell. Both cells have dense cytoplasm with a large number of mitochondria and lack central vasculature. Anatomical sacks are distinguished from salt glands in the spinach family. Particularly in *Atriplex*, where all 200 species have salt bags that contain outburst cavities that include a long and narrow leg and a cell-like cell at the top of the epidermis, different species of *Atriplex* can be identified from the shape of their salt bags. The salt solution is transmited from mesophilic cells to glandular trichomes through stem cells agianst the gradient concentration. The salt accumulates in the central vacuolic glandular trichomes, which is eventually torn and is released at the surface of the leaf. Accumulated salts in the surface of the leaf may reduce transpiration and increase light reflection; more than 80% sodium chloride entering the Atriplex leaves may be removed through glandular trichomes (Akbar et al. 1972; Hairmansis et al. 2017).

2.4.8 Broiling to Regulate Osmotic Pressure in Leaves

All salt-tolerating plants are not able to excrete salts. Many salinity-resistant and nonsaline plants tolerate temporary increase of salt in apoplasts by increasing the amount of mesophilic cell water and therefore dilutes the salts and increases their capacity to absorb the salt from the apoplast solution (Kramer 1984; Joshi et al. 2015).

2.5 Conclusion

The salinity stress and saline soils are cruel factors that adversely affect the growth of crop plants leading to decrease in agriculture productivity and hence depletion of food sources of human societies. Salinity is one of the major limiting factors that cause osmotic stress and decrease plant growth and crop productivity in arid and semiarid regions. Salt stress reduces many aspects of plant metabolism like growth and yield. Most of the salinity problems in higher plants are due to an increase in sodium chloride which has spread to soils in the dry and coastal areas and their water resources. More importantly salinity in the soil decreases plant mechanisms like photosynthesis, chlorophyll content, stomatal conductance, membrane destabilization, and general nutrient imbalance. To mitigate the salinity, many strategies including soil quality management policies, use of saline resistance varieties, detoxification of noxious ions, improvement in the quality of irrigation water etc. have been in practice. However all these strategies pose limitations and are not sustainable; in this context, the use of microorganisms especially PGPR has been proposed as a sustainable and eco-friendly way to fortify the quality of soils to help crop plants grow under salinity stress. PGPR are involved in the important physiological process associated with plant growth and development under salinity. Among the various strategies adopted to elevate salinity use of halophilic, PGPR seems to be the best alternatives. PGPR elevate salinity or exhibit tolerance to salinity through the regulation of production of stress hormone ethylene under the influence of ACC deaminase, osmotic regulation, and accumulation of salts in their cytoplasm and absorption and replacement of ions. PGPR are responsible for increasing nutrient uptake, decreasing the toxicity of hazardous ions, amelioration of photosynthesis, improvement in nitrogen fixation, regulation/modulation of physiological signaling networks, etc. These miracle bacterial species are *legendary soil guards* to protect both soil texture and crop plants from salinity stress.

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3

Dark Septate Endophytes and Their Role in Enhancing Plant Resistance to Abiotic and Biotic Stresses

Iman Hidayat

Abstract

In recent years, dark septate root endophytes (DSE) emerge as one of the potential microbial groups in enhancing plant health and resistance to environmental stresses. In this chapter, we describe the diversity of DSE, host specificity, and their role in nutrient uptake by the plant. The mechanism of plant stress tolerance to environmental stresses and possible application of DSE isolates in agricultural practices are also elucidated. Estimation of DSE diversity is difficult due to their sporulating and non-sporulating life forms of which generates problems in the DSE identification. In addition, majority members of DSE fungi showed no host specificity with their associated plant. During plant-DSE association, hyphae have shown to be important in nutrient transfer from the DSE to their host plant. Nutrients obtained by the hyphae from the soil will be translocated inter- and intracellularly into plant cells through various mechanisms. In extreme environmental condition, such as high Cd (cadmium), DSE affects plant physiology by regulating GSH (glutathione) metabolism and thiol compound contents in the host plant to alleviate Cd toxicity. Due to their potential for increasing crop productivity and resistance to abiotic and biotic stresses, the studies now focus on developing a simple and low-cost standardized formulation that is applicable to agricultural practices. It is supported by the fact that members of DSE are easily cultured and propagated in the laboratory. Current effective application of DSE involves the development of superior plant seedling through inoculation of the DSE isolates during plant propagation by tissue culture or seeds.

Keywords

Agriculture · Environmental stress · Fungal endophytes · Rhizosphere · Symbiosis

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3.1 Introduction

Maintaining crop productivity in a current environmental condition is a difficult task for farmers and agricultural practitioners worldwide. Climate change and various abiotic stresses such as drought, floods, extreme temperatures, soil salinity, deficiency of nutrients, and high soil toxic levels provide huge impacts on crop productivity, either directly or indirectly. Therefore, climate change becomes a major factor in the decline of food production, in particular in developing countries, and directly threatens world food security. For example, IPCC report in 2007 noted that production of maize and rice will decrease up to 20% and 10%, respectively, at the same time with 2 °C increase of air temperature (IPCC 2007).

Several attempts have been conducted to mitigate the effects produced by climate change to crop productivity, especially drought and heat. These include (1) developing new crop varieties that are tolerant to drought, heat, and salt via breeding, genetic modification, or other methods (Umezawa et al. 2006; Akhter et al. 2010; Bunnag and Ponthai 2013; Kumar et al. 2014; Swain et al. 2017; Zu et al. 2017); (2) planting time management (Cantelaube and Terres 2005; Cardoso et al. 2010; Bussay et al. 2015; Martins et al. 2018); (3) irrigation adjustment and optimization (Ćosić et al. 2015; Djurovic et al. 2016; Lopez et al. 2017; Winter et al. 2017); and (4) developing suitable fertilizer or symbiotic agents for particular environment (Wang et al. 2007; Boari et al. 2016; Bahrami-rad and Hajboland 2017). Among these attempts, developing suitable fertilizer or plant symbiotic agents for particular crops in particular environments is considered as one of the important factors in alleviating climate change threats to global food production and food security, especially in the current condition where soil bearing capacity to support crop productivity has been decreasing.

Plant symbiotic agents such as plant growth-promoting rhizobacteria (PGPR) (Kaushal and Wani 2016; Vurukonda et al. 2016), vesicular-arbuscular mycorrhiza (VAM) (Sullia 1991), and dark septate endophytes (DSE) (Jumpponen and Trappe 1998; Liu et al. 2017) have been known for their capability in enhancing plant health, plant productivity, and plant resistance to biotic and abiotic stresses through various mechanisms. Among them, members of DSE have relatively been unknown for their identity among other microorganisms, diversity across various hosts and habitats, biology, and their mechanism in supporting plant tolerance to environmental stresses. Therefore, this chapter will describe and elucidate various aspects of DSE such as distribution and specificity of the DSE from various hosts, their roles in affecting plant physiological and biochemical processes to resist environmental stresses, mechanism of macro- and micronutrients acquisition by the DSE from soil to plant cells, and their application in the management of good agricultural practices.

3.2 Definition, Diversity, and Specificity of DSE

The term DSE (dark septate endophyte) was firstly introduced by Read and Haselwandter (1981) who introduced "dark septate hyphae" term for sterile, dark, septate hyphae and microsclerotia of fungal isolates colonized various alpine plant roots. Many definitions of DSE have further been introduced from several authors (Grünig et al. 2011). One of the most commonly used definitions is the definition published by Jumpponen and Trappe (1998) which states that DSE is conidial or sterile fungi (Deuteromycotina, Fungi imperfecti) likely to be ascomycetous and colonizing plant roots. However, in this chapter, DSE is defined as a fungal endophyte with melanin hyphae and living in plant roots without causing any visible symptoms. DSE belong to Class 4 of endophytes and possess a capability to exit the root and spread in the soil to aid the plant in nutrients acquisition, especially P (Mandyam and Jumpponen 2005). The presence of melanin in the hyphae is a distinct character of DSE, separated from other endophytic and symbiotic fungi living in plant roots. Many fungal endophytes are characterized by having white hyphae such as *Fusarium*, *Colletotrichum*, *Phoma*, *Phomopsis*, *Pestalotiopsis*, and so on. In fact, these fungal taxa with white hyphae are typically fast growing on artificial media and, therefore, very often found from various reports of fungal endophytic research (Hyde and Soytong 2008; Rodriguez et al. 2009; Ko Ko et al. 2011). In contrast to fungal endophytes with white hyphae, melanized hyphae taxa of DSE belong to a slow-growing group of fungi. They are often overgrowth by whitish hyphae fungi during isolation processes. In addition, lack of sporulation of many DSE taxa hampers identification process, although molecular-based identification method is available. Limitation of a molecular method is DNA sequence quality and database information which contains sequences from noncultivated environmental samples that may lead to the erroneous names. Therefore, estimating species number of fungi which belongs to DSE is a difficult task for a mycologist.

Research reports that estimate DSE diversity is still rare. In general, the study of DSE is more focused on its application in improving plant immunity against plant disease and increasing plant growth in various environmental conditions. One of the information containing DSE diversity estimation data and the list of DSE taxa was reported by Grünig et al. 2011). Majority of these taxa belong to asexual fungi (Hyphomycetes and Coelomycetes) of Helotiales and Pleosporales (Table 3.1). Helotiales is a fungal order which contains about 10 families, 501 genera, and 3881 described species, while Pleosporales contains about 23 families, 332 genera, and 4764 described species (Kirk et al. 2008).

Distribution of DSE on various plant species was reported by Jumpponen and Trappe (1998) who noted that nearly 600 plant species from 320 genera and 114 families are colonized by DSE fungi. These include lower plant and higher plant, from cold temperate area, such as arctic or alpine regions (Schadt et al. 2001; Ruotsalainen et al. 2002; Newsham et al. 2009; Walker et al. 2010), to warmer temperate area like Japan (Usuki and Narisawa 2007) and also tropical regions (Rains et al. 2003; Diene et al. 2010; Takashima et al. 2014). However, DSE diversity from the warmer and tropical area is still unexplored.

Table 3.1 List of common fungal genera belonging to DSE

Genera	Host plant	References
Chloridium, Phialocephala, Phialophora	*Betula alleghaniensis, Picea rubens, Pinus resinosa*	Wilcox and Wang (1987)
Phialocephala sp.	*Betula pendula, Pinus sylvestris*	Menkis et al. (2004)
Acephala	*Pinus sylvestris, Picea abies*	Grünig and Sieber (2005)
Heteroconium	*Triticum aesticum*	Kwaśna and Bateman (2007) and Narisawa et al. (2007)
Phaeomollisia	*Picea abies, Vaccinium* spp.	Grünig et al. (2009)
Leptodonthium, Mollisia, Rhizoscyphus	*Colobanthus quitensis, Deschampsia antarctica, Colobanthus quitensis*	Upson et al. (2009)
Helminthosporium	*Sorghum bicolor*	Diene et al. (2010)
Acephala, Cadophora, Chloridium, Cryptosporiopsis, Dermea, Didymella, Didymosphaeria, Embellisia, Gaeumannomyces, Herpotrichia, Leptodontidium, Leptosphaeria, Macrophomina, Meliniomyces, Monodictys, Nectria, Neonectria, Oidiodendron, Periconia, Phialocephala, Phialophora, Phoma, Pseudocercospora, Saccharicola, Taeiniolella, Vibrissea	*Picea abies, Pinus* spp., *Cassiope mertensiana, Alnus viridis, Populus* sp., *Sorbus aucuparia, Vaccinium* spp., *Leucorchis albida, Calluna vulgaris, Aralia nudicaulis, Carex* spp., *Myricaria prostata, Rhododendron albiflorum, Tsuga heterophylla, Nothofagus procera, Quercus* spp., *Betula pubescens, Gaultheria shallon, Ledum groenladicum, Platanthera hyperborea, Pedicularis bracteosa, Abies alba, Alnus rubra, Erica tetralix, Paja brava, Linum* sp., *Poa pratensis, Oryza sativa, Salix oppositifolia, Solanum tuberosum, Elymus farctus, Tilia petiolaris, Holcus lanatus, Cyclamen persicum, Triticum aesticum, Chrysanthemum morifolium, Malus sylvestris, Saussurea involucrata, Humulus lupulus, Saccharum officinarum, Heteropappus semiprostratus*	Grünig et al. (2011)
Cadophora, Phialophora, Rhizopycnis, Periconia, Curvularia, Microdochium	*Ailanthus altissima, Ambrosia artemisiifolia, Asclepias syriaca, Ephedra dystachia, Festuca vaginata, Fumana procumbens, Helianthemum ovatum, Juniperus communis, Medicago minima, Populus alba, Stipa borysthenica*	Knapp et al. (2012)

(continued)

Table 3.1 (continued)

Genera	Host plant	References
Pseudosigmoidea	None (soil)	Diene et al. (2013)
Scolecobasidium	*Solanum lycopersicum* cv. Gohobi	Mahmoud and Narisawa (2013)
Cadophora, Cladophialophora, Cryptosporiopsis, Leohumicola, Leptodontidium, Phialocephala, Pseudoclathrosphaerina, unidentified Helotiales	*Huperzia selago, H. serrata and Lycopodium clavatum* (*Lycopodiaceae*)	Takashima et al. (2014)
Aquilomyces, Darksidea, Flavomyces, Periconia	*Stipa borysthenica*, *Festuca vaginata*, *Bromus tectorum*	Knapp et al. (2015)
Alternaria, Ascochyta, Cladosporium, Coniothyrium, Nigrospora, Microdiplodia, Hypoxylon, Curvularia, Paraphaeosphaeria, Phoma, Cladophialophora, Dokmania, Cytospora, Leptosphaerulina, Exophiala, Leohumicola, Nigrospora	*Euterpe edulis, Cecropia glaziovii, Guapira opposita, Bathysa australis, Mollinedia schottiana, Coussarea* sp., *Myrcia spectabilis*	Bonfim et al. (2016)
Cladosporium, Cyphellophora, and *Phialophora*	Various plants belong to Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Commelinaceae, Cruciferae, Cyperaceae, Juncaceae, Polygonaceae	Liu et al. (2017)

Host specificity in fungal endophytes, especially DSE, is debatable. Jumpponen and Trappe (1998) suggested that little or no host specificity in DSE-plant association due to many DSE species colonizes more than one host plants. For example, *Chloridium paucisporum* was found on *Betula alleghansis*, *Picea rubens*, and *Pinus resinosa* (Jumpponen and Trappe 1998). However, it was also reported that the same endophytic fungal species that colonizes different host plants produce specific metabolites the same or similar to their hosts (Hidayat et al. 2016). *Fusarium oxysporum* is a common endophytic fungus from many hosts, but only *F. oxysporum* from *Cinchona calisaya* was capable of producing quinine and cinchonidine (Hidayat et al. 2016). This information shows that fungal physiology is affected by the compounds produced by their host. The specific physiological characters of fungal endophytes provide a significant contribution to their taxonomical rank. In addition, host specificity in fungal endophytes is actually found on grass endophytes. Several taxa belong to clavicipitaceous fungi such as *Epichloë typhina* and *Neotyphodium coenophialum* which is specific to grass (Tsai et al. 1994; Schardl 1996). Kageyama et al. (2008) also noted the presence of specificity among several DSE taxa on particular hosts. In their study, host preference or specificity was identified when RFLP groups were limited to a single host. For example, RFLP groups of the Pezizales were exclusively obtained from *Bouteloua gracilis*, whereas the RFLP groups of the Helotiales were obtained from *Gutierrezia sarothrae* (Kageyama et al. 2008). Grünig et al.

(2011), in the review of distribution and host specificity of the *Phialocephala fortinii* s.lat-*Acephala applanata* species complex (PAC), noted that PAC species host specificity is low or lacking because most species were found from a broad range of woody plants, except *A. applanata* on Pinaceous plants. Although several species of DSE showed specificity to their hosts, we found that the majority of the DSE fungi are generalists. For example, *Leptodontidium orchidicola*, *Piriformospora indica*, *P. fortinii*, and *H. chaetospira* have been known to be associated with a wide range of hosts (Fernando and Currah 1996; Franken 2012; Narisawa et al. 2000). Weishample and Bedford (2006) also showed that some beneficial DSE isolated from monocots could be transferred to eudicots plants and still function as mutualists, indicating the non-host specificity of the DSE. It is probably related to the high adaptability of DSE to form a symbiosis with various plant species as their hosts from different environmental conditions. This evidence is supported by the presence of conspecific species, where the same species in different hosts are capable of producing different metabolite compounds according to their hosts.

3.3 Development of DSE-Root Association

It has been known that DSE (non-mycorrhizal fungi), together with mycorrhizal fungi, colonized the plant root system (Mandyam and Jumpponen 2005; Schmidt et al. 2008; Lukešova et al. 2015). They are very important for plants nutrients acquisition and survival. However, a mycorrhizal-plant association is well understood than those of DSE-plant association because the DSE does not produce structures like mycorrhizal morphology (Trappe 1998). In addition, methods in observing DSE colonization in the roots need to be developed in order to obtain more understanding about DSE colonization in the root system. DSE-pant association in root system is microscopically examined by staining roots with biological stains such as trypan blue, acid fuchsin, chlorozol black, or Sudan IV, which specifically bind to chitin in the fungal walls (Kormanik et al. 1980; Phillips and Hayman 1970; Barrow and Aaltonen 2001). Barrow and Aaltonen (2001) also noted that fungus-specific staining method is important as they found that extensive hyaline hyphae colonization in the roots was not evident by using conventional staining methods.

Several studies reported that hyphae of DSE colonize plant roots in the epidermis and the cortex and formed microsclerotia in the cells of the roots without causing diseased symptom to the plants (Hashiba and Narisawa 2005; Usuki and Narisawa 2007; Andrade-Linares et al. 2011). In the study of DSE-host symbiosis development using *Heteroconium chaetospira* and Chinese cabbage as host, Hashiba and Narisawa (2005) found that *H. chaetospira* hyphae penetrate through the outer epidermal cells which pass into the inner cortex, and the cortical cells, including the root tip region. Once the fungal hyphae get to the cortical cells, the nutrients will be absorbed from the hyphae through the symplastic pathway and/or the coupled transcellular pathway (Barberon and Geltner 2014). The development of *H. chaetospira* hyphae in the epidermis and outer cortical layer of the Chinese cabbage root were found within 3 weeks after inoculation but few in the inner cortical layer. Heavy colonization of epidermis and cortical layers were found at 8 weeks after inoculation, and microsclerotia or irregular lobes were also formed. This hyphal penetration of *H. chaetospira* does not produce pathogenic symptoms and resistance responses from the Chinese cabbage. It is related to the colonization capacity of *H. chaetospira* that does not pass through the vascular system of the host. The vascular system colonization of the host by fungal hyphae is an essential indicator in differentiating many of benefiting fungi and plant pathogenic fungi. In most of plant pathogenic fungi, for example, *Fusarium* wilt disease or other soilborne diseases, vascular system colonization by fungal diseases generally occur (Li et al. 2011). In banana, the hyphae of *Fusarium oxysporum* f.sp. *cubense* (Foc) enter the host through a similar mechanism with DSE fungi and further colonize the vascular system, in particular xylem, and block water transport from root to other plant organs. Lack of water supply causes wilting in the leaves and reduces photosynthesis activity and other physiological processes (Ghag et al. 2015).

Although many DSE fungal species colonization does not reach a vascular system of the roots, however, a few cases showed penetration of the vascular tissue of host roots, such as in *Phialocephala fortinii* (Yu et al. 2001). In this case, DSE can have detrimental effects on the host plant, depending on the environmental condition (Jumpponen and Trappe 1998). In root colonization by *P. fortinii* as summarized by Jumpponen and Trappe (1998), initial colonization is started by superficial hyphae (Currah and Van Dyk 1986; Hashiba and Narisawa 2005) that grow along the depressions between adjacent epidermal cells and pass through adjoining epidermal cell walls by narrow penetration tubes which occasionally arise from structures similar to appressorium (Currah and Tsuneda 1993; Hashiba and Narisawa 2005). The hyphae can further grow in the space between cortical cells, parallel to the main axis of the host root, and from cell to cell within the epidermis (Barrow and Altonen 2001). During this superficial colonization, "a loose hyphal network on the root surface" (Stoyke and Currah 1993) or "loose wefts of hyphae" (O'Dell et al. 1993) will develop. The hyphae further penetrate through the outer part of cortical cells (Stoyke and Currah 1993; O'Dell et al. 1993). Within cortical cells, DSE hyphae usually form rounded, chlamydospore-like structures or clusters of cells with inflated, rounded, and thick-walled structures. These structures are often dark in color due to the incorporation of melanin and called as "thick pseudoparenchymatic mass" (Robertson 1954), "groups of swollen cells" (Deacon 1973), "sclerotia" (Stoyke and Currah 1993), "microsclerotia" (Haselwandter 1987; Jumpponen et al. 1998), or "sclerotial bodies" (Wilcox and Wang 1987). However, in several hosts, these structures are often expressed in different forms, mostly resembling mycorrhizal form (Stoyke and Currah 1991; Currah et al. 1993). These reports showed that host plant control (Wilcox and Wang 1987) plays important roles in determining the structures of DSE clusters of cells within cortical cells. In addition, time is also an important factor in determining the mature state of these structures. To simplify and generalize terminology in various phases of DSE hyphae development during colonization of plant roots, Jumpponen and Trappe (1998) propose several terms such as "runner hyphae" for the individual superficial fungal hyphae between epidermal cells, "superficial net" for the superficial colonization, "appressorium" for the swollen structure preceding penetration through a host cell wall, "penetration tube" for the thin structure penetrating through the cell wall, and "microsclerotia" for the intracellular groups of rounded and thick-walled cells.

Different morphological structures formed in a different stage of root colonization by DSE showed that the DSE is a polymorphic fungal group. The colonization of DSE in the root system is often visible as hyaline hyphae penetration in the cortex cells at the early stage, followed by melanized hyphopodia or microsclerotia (Jumpponen and Trappe 1998) formation in the cortex and on the root surface (Barrow and Aaltonen 2001) such as the colonization of *Vulpia ciliata* ssp. *ambigua* by *Philophora graminicola* (Newsham 1999). Different to arbuscular mycorrhizae colonization in the roots which form thin-walled arbuscules in the cortex, DSE fungi interface the cortex and further grow into sieve elements (primary plant tissue for carbon transport) as thin-walled hyphae with a very small diameter. Hyaline hyphae and melanized hyphae are, in fact, continuous (Newsham 1999; Barrow and Aaltonen 2001). In the study of DSE colonization of *Atriplex canescens*, Barrow and Aaltonen (2001) found that internal DSE fungal tissue comprises four distinct types, namely, (1) microsclerotia, (2) melanized hyphae, (3) hyaline hyphae minus lipids, and (4) hyaline hyphae plus lipids of which related to their physiological activities. The microsclerotia which contain thick melanized walls are the structure that are frequently found in dormant plants and, therefore, are considered as vegetative propagules that protect DSE during environmental stress until conditions are favorable for germination. External melanized hyphae allow DSE to function in dry soil. Hyaline hyphae are considered as the most active form of DSE and usually characterized by thin-walled and lack of chitin and melanin which allow them to penetrate among root cells and more permeable with increased potential for resource exchange with the host. Lipid accumulation in vacuoles of hyaline hyphae is strongly related to DSE physiological activity, and this suggests as a potential site for carbon exchange between the host and the DSE fungi. When the host is physiologically active, lipid-containing vacuoles in DSE hyaline or melanized hyphae become visible. Not only in the cortex, but this form is also often found in the vascular cylinder when the plant roots are physiologically active. The decrease in vacuole size and lipid content is correlated to decreases in physiological activities (Barrow and Aaltonen 2001).

3.4 Effect of DSE on Plant Acquisition of Nutrients from Soil

It has been known that members of DSE fungi possess symbiotic mutualism interaction with their hosts. For example, *Heteroconium chaetospira* was able to promote Chinese cabbage growth and enhance their resistance to pathogen (Usuki and Narisawa 2007), *Piriformospora indica* in promoting the growth of various crops such as barley and rice (Varma et al. 2012; Jogawat et al. 2013), *Cladorrhinum forcundissimum* on cotton (Gasoni and De Gurfinkel 1997), *Helminthosporium velutinum* strain 41-1 that is capable in promoting sweet sorghum (Diene et al. 2010), etc. Plant growth-promoting activity through symbiosis mutualism between DSE and their hosts is apparently related to the mechanism of nutrient transfer from soil to plant cells via DSE activity. The mechanism includes macro- and micronutrients exchange.

Nutrients acquisition from soil to plant cells through DSE activity involving the works of hyaline hyphae, dark and pigmented (melanized) hyphae, and their microsclerotia inside the plant roots. The DSE is hyphae generally capable of colonizing root tissues intro- and intercellularly without causing pathologies (Andrade-Linares et al. 2011). DSE fungi are also capable of accessing macronutrients such as carbon (C), nitrogen (N), phosphorus (P), and organic compounds such as amino acids from the soil and transferring the readily absorbed nutrients to their host plant (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005; Reeve et al. 2008). The readily absorbed nutrients provided by DSE are available due to the DSE fungi capability in degrading various organic compounds such as cellulose, starch, proteins, lipids, amino acids, and other organic compounds (Caldwell et al. 2000; Mandyam et al. 2010; Surono and Narisawa 2017). Barrow and Aaltonen (2001) showed that the most active thin-walled hyphae take an essential role in this nutrient transfer by actively growing into cortex and sieve elements of which the latter part is important in carbon exchange between DSE and host plant.

DSE produce various degrading enzymes that are capable in degrading organic matter in the soil such as cellulases, laccases, amylases, lipases, pectinases, xylanases, proteolytic enzymes, tyrosinases, and polyphenol oxidases and transfer the products into plant roots (Mandyam and Jumpponen 2005). For example, insoluble P solubilization by *Aspergillus ustus* through phosphatase activity was able to increase the P content and biomass of its host, *Arctostaphylos canescens* grown on the rock (Mandyam and Jumpponen 2005). In addition, *Phialocephala fortinii* inoculation resulted in increased levels of leaf P, N uptake from the soil, and higher plant biomass in *Pinus contorta* (Jumpponen et al. 1998). Inoculation of *Phialophora graminicola* onto the grass *Vulpia ciliate* also increases plant biomass and elevates the levels of N and P (Newsham 1999).

Macronutrients such as N are one of the limiting factors for plant growth and development because the plant needs a larger quantity of N (over 50% N in the leaf) than P during photosynthesis; therefore, N is crucial for carbon fixation (Field and Mooney 1986; Yoneyama et al. 2007). Atmospheric N exist in the forms of N_2, NO, and NO_3^-. Although some of these forms are readily deposited into a terrestrial ecosystem, however, N requires fixation by soil and/or plant symbiotic microbes, called diazotrophic microbes (Steppe 1996). After N is fixed in the terrestrial ecosystems, the N is further transformed into organic matter and must be mineralized into inorganic N to be readily absorbed by plants. In the soil, N is more abundant in the organic form than inorganic form (Aerts and Chapin 2000).

N mineralization in the soil is largely mediated by soil fungi and endophytic fungi including DSE. In AM and DSE fungi, both fungal groups excrete exo-enzyme such as proteases to break down organic matter and provide N in the soil to the plant (Schimel and Bennett 2004). Most of the fungi are usually capable of absorbing organic matter such as peptides, proteins, and amino acids through enzymatic activity. In addition, inorganic N in the soil is absorbed by fungal extraradical hyphae as nitrates (NO_3^-) or ammonium (NH), converted into arginine, and transported into the intraradical hyphae (Ngwene et al. 2010, 2013). The arginine is further converted into ammonium through the urease cycle before transferring into the plant (Cruz et al. 2007). This hypothetical pathway is supported by the increase of arginine in plant roots associated with symbiotic fungi (Tian et al. 2010). These nitrates and ammonium are considered as main pathways in N transfer during plant-fungal symbioses (Behie and Bidochka 2014).

Similar to N acquisition in the soil, P can be a limiting factor in some ecosystems. However, the P cycle differs from the N cycle because it is not influenced by the atmospheric level of P (Ruttenberg 2003). P is generally attached to rocks or minerals and released to the soil in P ions forming through geological processes such as weathering. Less than 1% of soluble P is available in the soil, and organic P is mainly available in the form of inositol phosphates, phospholipids, and nucleic acids. Therefore, conversion of organic P by extracellular enzymes from microbes such as DSE is very important for the plant (Smith and Read 1997). Although AM fungi roles in P conversion are more commonly known than DSE, however, in extreme environmental condition, DSE may replace AM in improving P supply to the host plant (Mandyam and Jumpponen 2005).

Plant generally absorbed P from the soil, directly or through association with AM and endophytes, in form PO_4^{3-} (orthophosphate). In a condition where P is lacking in the soil, plants adjust root and shoot accordingly through a phosphate starvation response (PSR) system (Poirier and Bucher 2002). In DSE-plant symbiosis, P transfer from soil involves the conversion of insoluble P or polyphosphate back into inorganic P through the enzymatic process by fungal hyphae (phosphomonoesterases and phosphatases) and then transported into host plant cortex (Smith and Smith 2011).

Transport of P from AM and fungal endophytes to host plant is usually via two transporters, viz., the first to enable P uptake and transport from the fungus and the second to mediate P uptake by the plant. In AM fungi such as *Glomus intraradices* and its host (*Medicago truncatula*), coordination of P transport is specifically via GmosPT and GvPT (specific to AM fungi) in the fungus and MtPt4 in the plant (Harrison and Van Buuren 1995; Benedetto et al. 2005). In non-mycorrhizal fungal endophytes, this process usually occurs via PiPT. In the PiPT system, P uptake from the soil is started from external hyphae and then induced host P transporters such as in the case of *Piriformospora indica* and maize (*Zea mays*) (Yadav et al. 2010). However, host specificity of *P. indica* and PiPT gene is unresolved because in several cases such as in potato, *P. indica* is not involved in the transfer of P to the host plant (Yadav et al. 2010). In *Colletotrichum tofieldiae*, an endemic endophyte of *Arabidopsis thaliana*, the endophytic fungus transfers P to shoots and promotes plant growth only under P-deficient condition (Hiruma et al. 2016). The phosphate starvation response (PSR) system of *A. thaliana* controls *C. tofieldiae* growth and colonization in the roots to facilitate a beneficial form of *C. tofeildiae* (Hiruma et al. 2016).

Micronutrients are needed by plants in small quantity, but relatively abundant in soils of most ecosystems, and available in the soils in the way P transfer to the soils. Mechanism of micronutrients acquisition from the soil through DSE involves chelating agents or enzymes works released by the DSE hyphae (Vergara et al. 2017). For example, acquisition of iron (Fe) by the plant roots associated with DSE. The Fe is one of the micronutrients that is not easily accessible by plants in the environment and very important for the mitochondria and chloroplast metabolism of the plant. This micronutrient exists as the insoluble ferric oxides form that is not ready for assimilation by plants, especially in higher soil pH. However, at lower pH, the Fe becomes more available for uptake by plant roots due to being freed from the oxide. In general, plants utilize two strategies in managing Fe deficiency at higher soil pH (Marschner and Römheld 1994). The first strategy involves the induction of a plasma membrane-bound reductase by enhanced net excretion of protons. This mechanism usually occurs in all plant species except grasses. In the second strategy, which commonly occurred on grasses, the plants increase biosynthesis and secretion of phytosiderophores (PS) which form chelates with Fe (III), and the Fe-PS complexes are then transported back into the roots (Bienfait 1989). The second strategy, in fact, has many similarities with the microbial siderophore system (the third strategy) (Bienfait 1989). In the Fe uptake by plant roots via *Phialocephala fortinii*, especially in the Fe-deficient soil, the hyphae of the *P. fortinii* synthesized high-affinity Fe-chelating siderophore hydroxamate (ferrichrome-type siderophore) to bind Fe from the soil. This led to the increase of Fe bioaccumulation in *P. fortinii* hyphae. Many fungal species, in fact, produce other types of siderophores and facilitators, such as fusarinine, coprogen, rhodotorulic acid, and rhizoferrin (Leong and Winkelmann 1998). The Fe oxidation further occurred in the hyphae to reduce iron cytotoxicity and to maintain intracellular homeostasis. The processes in fungal hyphae involve three features: (1) relies on redox cycling, (2) includes high- and low-affinity pathways; and (3) all processes are auto-regulating to maintain intracellular iron homeostasis (Kosman 2003). In the redox chemistry of Fe, first, the ferric iron system starts by reducing Fe^{3+} to Fe^{2+} through the iron reductase action (Kosman 2003). The Fe^{3+} insoluble in water at neutral pH is kinetically "labilize" and becomes soluble in the form of Fe^{2+}. In *Saccharomyces cerevisiae* model, the Fe_2^+ is reoxidized into Fe_3^+ (ferroxidation) by a multicopper oxidase enzyme or ferroxidases (Frieden and Osaki 1974).

There are limiting information regarding other micronutrient transfer from soil to host plant via DSE-plant symbiosis; however, chelating agents or enzymatic process probably takes an important role in the other micronutrient transport by DSE to host plant. The fungal ability to solubilize and accumulate metal minerals could be related to metal tolerance of the fungal strains which include DSE. In the solid metal solubilization by fungi, Burgstaller and Schinner (1993) noted that this process generally involves four mechanisms: (1) acidolysis, (2) complexolysis, (3) redoxolysis, and (4) the mycelium functioning as a "sink." The acidolysis and complex lysis are also called "heterotrophic leaching" that occurs as a result of several processes such as the discharge of protons from hyphae, the production of siderophores (for Fe), and the production of organic acids (Gadd 2000). The production of organic acids generates protons for solubilization and a metal-chelating anion that bind the metal cation (Gadd 1999). Fomina et al. (2004) reported that proton-promoted dissolution, complexolysis or ligand-promoted dissolution, and metal accumulation by the biomass play important roles in zinc phosphate solubilization by the plant-symbiotic fungi. The organic acids such as carboxylic acids are important in a metal complexing process by fungi in the soil depending on the number and dissociation properties of their carboxylic groups. Di- and tricarboxylic acids are examples of strong chelators that can transform metals such as Al, Fe, and K from insoluble compounds in the soil (Gadd 1999; Ahonen-Jonnarth et al. 2000).

3.5 Mechanism of DSE in Increasing Plant Tolerance to Environmental Stress

3.5.1 Drought and High Temperatures

Drought and extreme environmental temperatures are two related major environmental stresses causing plant stress such as osmotic and oxidative stress causing physiological and biochemical metabolism changes in plants and finally resulting in plant deaths or significant crop productivity loss. Drought can cause a shortage of groundwater, resulting in dehydration of the plant cells. This condition obstructs the process of plants cell division and development. In addition, extremely high temperature resulting in plants shriveled, chlorotic or die. In the drought or water deficits condition, plants usually respond by osmotic adjustments, production of antioxidants, and altered transcriptional and translational regulation and modify stomatal activity (Griffiths and Parry 2002).

DSE symbiosis benefits to plants are not only in nutrient uptake and diseases protection but also in increasing tolerance to drought stresses through water uptake and relations mechanisms in the root. Several studies on increasing plant resistance to drought and high temperature via symbiosis with DSE usually involve habitat-specific fungi (Rodriguez et al. 2004; Márquez et al. 2007). For example, *Curvularia protuberata*, an endophytic fungus from *Dichanthelium lanuginosum* that can confer heat tolerance (up to 65 °C) to its host, is isolated from geothermal soil of Yellowstone National Park (Redman et al. 2002). Nurdebyandaru et al. (2013) also reported that *Helmithosporium velutinum* 41-1 and *Veronaeopsis simplex* Y34 could promote chili at relatively high-temperature conditions (up to 35 °C) (Fig. 3.1). Rodriguez et al. (2008) noted that the ability to confer heat resistance plant is specific to DSE fungi isolated from geothermal plants or similar habitats and possibly a habitat-adapted phenomenon (Rodriguez and Redman 2008). The mechanism of heat tolerance in the plant through symbiosis with DSE is not fully understood. However, Márquez et al. (2007) reported one of the mechanisms where the fungus induces plant's heat tolerance via fungal RNA virus, namely, CThTV (*Curvularia* thermal tolerance virus). It was hypothesized that the symbiotic virus is providing biochemical functionality to the fungus, not the virus that directly confers heat tolerance (Márquez et al. 2007).

The mechanism of DSE or other symbionts conferred drought tolerance to the host plants is also not fully understand. Kaushal and Wani (2016) indicated that modulating hormonal balance of the plants can enhance plant growth under a

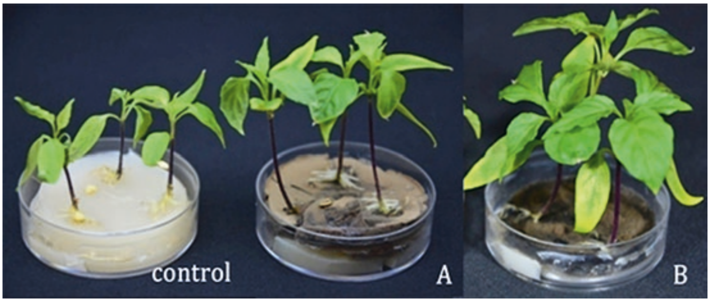

Fig. 3.1 A growth of chili treated with DSE fungi at 35 °C for 3 weeks. (From Nurdebyandaru et al. 2013). (**a**) Inoculated with *V. simplex* Y34. (**b**) Inoculated with *H. velutinum* 41-1

stressed condition. For example, in the drought tolerance assay of *Trifolium repens* induced by PGPR, Marulanda et al. (2009) found that production of indole-3-acetic acid (IAA) in inoculated plants increased shoot and root biomass and increased water uptake resulting in plant growth and survival during drought condition. A similar mechanism is also found in AM fungi (Marulanda et al. 2009) and probably also occurs in plant drought tolerance mechanism via DSE. The reason behind the influence of hormones in plant drought tolerance via hormonal balance is that well-developed root system will have the greatest ability to absorb water from soils (Marulanda et al. 2003, 2007). In the DSE-plant symbiosis, extraradical hyphae of DSE possibly grow and exploit into the soil matrix and absorb water from the soils and transport into the root cells (Bryla and Duniway 1997). Melanized hyphae of DSE are also important during this process because melanins might protect DSE hyphae from unfavorable soil conditions (Zhan et al. 2011). This mechanism is similar to AM fungi mechanism in transporting water and nutrients to their hosts. Plants will be benefiting by their increase in photosynthetic and other physiological activities for their growth, and in exchange, fungi will obtain C (carbon) from soluble sugars translocated from photosynthetic organs into host roots, thus increasing fungal growth and their activity in the roots. In addition, enhanced IAA synthesis due to the indole-3-pyruvate decarboxylase regulation gene in symbiotic microbes yields the coleoptile xylem architecture change (wider xylem vessels) in inoculated wheat, and this leads to enhanced water conductance in coleoptiles that enabled the plant to cope with osmotic stress (Pereyra et al. 2012). In another hormonal mechanism, maintaining the stability of the abscisic acid (ABA) level causes the balance in the stomatal closure to minimize transpirational loss of water via stomata and induces root branching to increase water uptake, as shown in the experiment of *Azospirillum lipoferum* inoculation to maize (Cohen et al. 2009, 2015). Another hormone, jasmonic acid (JA) and salicylic acid (SA), protects the plants from oxidative stress damages (Iqbal and Ashraf 2010).

Another plant drought tolerance mechanism through symbiosis with fungi involves induction of gene that maintains or facilitates transport of water between cells, called aquaporins. In AM fungi-plant symbiosis, drought-sensitive plants obtained physiological benefit through downregulation of eight aquaporin genes

(ZmPIP1;1, ZmPIP1;3, ZmPIP1;4, ZmPIP1;6, ZmPIP2;2, ZmPIP2;4, ZmTIP1;1, and ZmTIP2;3) by the AM symbiosis, and in three aquaporin genes (ZmPIP1;6, ZmPIP2;2, and ZmTIP4;1) were regulated by the AM fungi in the induced maize cultivars (Quiroga et al. 2017). This result showed that fungal symbionts of plants are capable in regulating water loss of their hosts through downregulation of aquaporins (Quiroga et al. 2017). In DSE-plant symbiosis, *P. indica* colonization of Chinese cabbage under drought condition promoted root and shoot growth and lateral root formation through upregulation of peroxidases (POX), catalases (CAT), and superoxide dismutases (SOD) in the leaves within 24 h (Sun et al. 2010). The drought resistance genes such as DREB2A, CBL1, ANAC072, and RD29A were upregulated in the DSE-colonized plants. The CAS mRNA level and the CAS protein level were also increased. Sun et al. (2010) also conclude that antioxidant enzyme activities, drought-related genes, and CAS (calcium-sensing receptor) are crucial targets of *P. indica* in Chinese cabbage leaves during the drought tolerance establishment.

In high-temperature condition, heat shock proteins (HSPs) that are widely distributed in fungi, animals, and plants possibly play important roles in heat stress response. Heat induces the production of reactive oxygen species (ROS) that can chemically alter or inactivate proteins, lipid membranes, and DNA (Zhang et al. 2017). In fungi, pyruvate molecules play important roles that scavenge heat-induced reactive oxygen species (ROS) (Zhang et al. 2017). By using *Metarhizium robertsii* as a model, Zhang et al. (2017) showed that pyruvate accumulation is the fastest mechanism of several *M. robertsii* scavenge ROS that efficiently reduce protein carbonylation, stabilize mitochondrial membrane potential, and promote fungal growth. Mastouri et al. (2010) reported that *Trichoderma harzianum* strain T22 was capable of reducing oxidative damages from the accumulation of ROS in stressed plants. Plant seedling inoculated with *T. harzianum* strain T22 grows well under multiple abiotic stresses (osmotic, salt, or suboptimal temperatures), biotic stress (*Pythium ultimum* disease treatment), and physiological stress (poor seed quality induced by seed aging) (Mastouri et al. 2010). It is clear that eliminate accumulation of ROS in host plant might be a possible way of inducing heat tolerance in plants by symbiotic fungi.

3.5.2 Heavy Metals in Soil

Heavy metal (HM) pollution in soils by anthropogenic activities causes severe threats to plant survival worldwide. Several plant species can survive in this environmental condition through complex physiological traits, adapting metabolic pathways, and perform symbiosis with beneficial microbes (Xu et al. 2015). AM and DSE symbiotic fungi are considered important for plant growth and survival in heavy metals contaminated soils due to their ability in accumulating HMs and/or converting them into readily absorbed minerals by the plant (Ban et al. 2012; Babu et al. 2014). Melanin in DSE hyphae is hypothesized as one of the important components in the fungal cell wall to reduce HMs toxicity due to the fungal melanin capacity in binding HM ions (Larsson and Tjälve 1978).

In AM fungi-plant symbiosis system, AM fungi are capable of translocating and distributing metal inner parenchyma cells of roots (Kaldorf et al. 1999), via the following mechanism: (1) extracellular biosorption/precipitation of metals, (2) active efflux pumping of metals out of the cell via transporter system, (3) sequestration of metals in intracellular compartments (mainly cell vacuole), (4) exclusion of metal chelates into the extracellular space, and (5) enzymatic redox reaction through conversion of metal ion into a non-toxic or less toxic state (Umar 2017). Similar to AM fungi, DSE fungi involve several ways in adapting to high HMs invested soils. The first step is similar to the acquisition of Fe from soils where DSE produce extracellular chelating compounds or binding of HMs to cell wall constituents to prevent entrance of HMs into cells. This step is followed by a series of intracellular processes, such as complexation and peptide binding, transportation, compartmentalization, ROS scavenging, etc. Zhang et al. (2008) and Diao et al. (2013) reported that superoxide dismutase (SOD) and catalase (CAT) (antioxidases) were significantly upregulated under Cd or Zn stress and showed the role of fungal melanin in cell wall to combat HMs stress (Zhan et al. 2011). Zhao et al. (2015) elucidated the process of Cd detoxification by DSE fungus, *Exophiala pisciphila*, that involves (1) extracellular function such as extracellular metal ion binding and cell wall integrity maintenance and (2) intracellular processes, such as metal ion binding and transportation, organic acid metabolic processes, organic acid transportation, ROS scavenging, redox balance, transcription factor production, sulfate assimilation, and DNA repair.

Zhao et al. (2015) also detected 104 metal binding and 32 metal transport genes associated with HMs acquisition and homeostasis among 575 differentially expressed genes (DEGs). These include Zn^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}, and Ca^{2+} binding genes. Three metallothionein (MT) genes, a family of cysteine-rich polypeptides involved in intracellular Cd detoxification, were also detected in *E. pisciphila*. Wu et al. (2012) reported that Cd^{2+} entered cells through Fe^{2+}, Ca^{2+}, or Zn^{2+} transporters; therefore, eliminating the transporters may become one of the most effective mechanisms to keep toxic metals outside of cells (Pócsi 2011). In *E. pisciphila* mechanism, Zn^{2+}, Fe^{2+}, and Ca^{2+} transporters were downregulated, indicating a strategy for the inhibition of Cd passage through the membrane cell of *E. pisciphila*. In the same study, Zhao et al. (2015) also noted that amino and organic acids are important molecules due to their role in preventing the metal's entry into cells or by facilitating intracellular HMs detoxification through the chelation of various ligands (Lin and Aarts 2012). The DSE fungus *E. pisciphila* also synthesizes and upregulates various antioxidants, such as SOD and CAT in response to oxidative stresses (Zhang et al. 2008; Ban et al. 2012).

All these studies have shown that DSE fungi apparently influence their host plant response to the high HMs environment and enhance their host plant tolerance to heavy metal stress. However, whether DSE-plant tolerance to heavy metals can be attributed to the DSE fungi or the result of symbiosis is still unknown. Therefore, understanding the mechanisms by which DSE fungi help their hosts adaptation to the HMs environment is more important in future studies.

3.5.3 Plant Defense and Immunity to Phytopathogens

Members of DSE are capable of colonizing the plant roots and proliferating endophytically in the root cells without causing disease symptoms to their host. They can enhance hosts growth performance and immunity to plant pathogen attacks. Many studies have reported improvement of crops due to the application of DSE that enhance plant performance such as nutrient uptake and adaptation to various environmental stresses. These include adaptation to abiotic factors such as drought, high temperature, poor soil quality, heavy metal's infestation in the soils (Rodriguez et al. 2004, 2008; Zhang et al. 2008; Márquez et al. 2007; Mastouri et al. 2010; Sun et al. 2010; Zhan et al. 2011; Ban et al. 2012; Wu et al. 2012; Zhao et al. 2015; Zhang et al. 2017) and protection from biotic factor like phytopathogen attacks (Narisawa et al. 1998, 2002, 2004; Andrade-Linares et al. 2011; Khastini et al. 2012, 2014). *Cadophora* sp. (Khastini et al. 2014), *H. chaetospira*, *P. fortinii* (Narisawa et al. 1998, 2002, 2004), *Leptodontidium orchidicola* (Andrade-Linares et al. 2011), *Meliniomyces variabilis* LtVB3 (Ohtaka and Narisawa 2008), *Piriformospora indica* (Kumar et al. 2009), and *Veronaeopsis simplex* Y34 (Khastini et al. 2012) are among DSE species that is capable to increase resistance of several crops such as tomato, eggplant, Chinese cabbage against phytopathogens such as *Fusarium* wilt, *Verticillium* yellow, Clubrot, *Pseudomonas syringae* pv. *macricola* (bacterial leaf spot), and *Alternaria brassicae* (*Alternaria* leaf spot), etc.

Mechanisms of DSE protecting their hosts against plant pathogen attack involve antimicrobial metabolites production (Gunatilaka 2006), fungal competition for nutrients (Serra-Witling et al. 1996), competition for infection sites and root colonization (Mandeel 2007), and plant systemic resistance induction (Kogel et al. 2006). Mandyam and Jumpponen (2005) also noted three possible mechanisms of which DSE inhibit plant pathogen attack or minimize pathogens impact on plant growth: (1) the first mechanism is competition for site colonization of plant photosynthates, (2) production of antimicrobial compounds, and (3) plant defense induction to subsequent pathogen infection.

Among these mechanisms, induction of plant defense through DSE symbiosis and siderophore production is the most common mechanisms found during greenhouse and field trial. Induced systemic resistance (ISR) can be defined as defensive capacity developed by plants when appropriately stimulated through activation or induction by diverse agents, including fungal symbiont (Van Loon et al. 1998). In the study of *Meliniomyces variabilis* LtVB3 application to suppress *Verticillium* yellow attacks on Chinese cabbage, after inoculated with *M. variabilis* LtVB3, the host developed wall appositions and thickenings in the epidermal and cortical layers (Narisawa et al. 2004). The cell wall modification and changes are possibly a signal that is followed by producing antimicrobial substances. Benhamou and Garand (2001) reported that cell wall modification is related to the production of secondary metabolite and accumulation of pathogenesis-related proteins. Production of phenylalanine ammonia-lyase and H_2O_2 were also signal of plant defense system activation (War et al. 2011). A similar mechanism was found on *V. simplex* Y34 application to Chinese cabbage in mitigating *Fusarium* disease attacks, by which *V. simplex* restricted *F. oxysporum* penetration into Chinese cabbage roots by activating the defense system (Khastini et al. 2012). Kumar et al. (2009) also found that DSE fungus, *Piriformospora indica*, protects its host (maize) through the oxidative defense, but not antibiotic production. In the host plant protection that involves siderophore production by DSE symbionts, the DSE fungi excrete the siderophores to bind Fe with high affinity from the soil, thus limiting the growth of other microorganisms in the rhizosphere. In the application of *V. simplex* Y34 to the *Chinese* cabbage root, Khastini et al. (2012) reported that *V. simplex* Y34 chelated Fe and supplied Fe to its host, at the same time, making Fe unavailable to *F. oxysporum*. This mechanism was also found in *P. fortinii* (Bartholdy et al. 2001; Narisawa et al., 2002).

3.6 DSE Application and Commercialization

Although reports on successful DSE application on various hosts were reported at the laboratory and greenhouse scales by which nutrition and environmental condition are under control, it does not negate the possibility of application in a large-scale application in the field or natural ecosystems. Successful DSE inoculants or other biocontrol agent inoculants have to be able to colonize the hosts (rhizosphere, phyllosphere, or plant organs), compete with other microorganisms, and persist in various local abiotic and biotic conditions in various settings to meet commercial inoculants requirements. It is not guaranteed that successful DSE colonizing their host will persist over time.

3.7 Factors Affecting the Scale-Up of Fungal Endophytes

Several limiting factors to scale up fungal endophytes, especially DSE, into practical use or commercial scales are needed to overcome. These include:

3.7.1 Host and Pathogen Specificity

Host and pathogen specificity is a very important concern in developing biological control at commercial scale because it affects the effectiveness of biological control agents to mitigate various pathogens on various hosts and the risks of affecting non-target organisms in the field. Therefore, several authors noted narrow host specificity as a limiting factor for the commercialization of biological control agents. Commercial products of biocontrol formula generally required a broad spectrum of target pathogens and crops. Since most of DSE members are generalists to host plants, host specificity of the DSE for a large-scale application is not a bottleneck. For example, *P. indica* is one of common DSE that is successfully applied and enhance various plants such as *Spilanthes calva*, *Withania somnifera*, *Arabidopsis thaliana*, *Adhatoda vasica*, *Nicotiana attenuata*, *N. tabacum*, *Hordeum vulgare*, *Triticum aestivum*, *Solanum lycopersicum*, *Chlorophytum* sp., *Cicer arietinum*, *C. arietinum*, *Phaseolus* *aureus*, *Brassica campestris*, *Piper nigrum*, *Glycine max*, *Foeniculum vulgare*, *Thymus vulgaris*, *Vigna mungo Fragaria x ananassa*, and *Centella asiatica* (Franken 2012). Broad-spectrum biocontrol activity of *P. indica* has been reported against *Blumeria graminis* f. sp. *tritici*, *Pseudocercosporella herpotrichoides*, and *Fusarium culmorum* (Serfling et al. 2007). In addition, *P. indica* also reported capable of reducing egg density of the soybean cyst nematode (SCN), *Heterodera glycines* (Bajaj et al. 2015). Hashiba and Narisawa (2005) also reported successful inoculation and symbiosis between *H. chaetospira* and 19 host plant species. The DSE fungus *H. chaetospira* also successfully suppresses *Pseudomonas syringae* pv. *macricola* and *Alternaria brassicae* attack on Chinese cabbage leaves (Hashiba and Narisawa 2005), indicating the broad-spectrum nature of this fungus.

Since a majority of DSE possesses a capacity to establish a new host and new environment, it will possibly create a risk to non-target species, directly or indirectly (Brodeur 2012). Although environmental risks due to biological control are difficult to estimate, however, the risks can be reduced by conducting the selection of specific biological control agents and gathering information regarding host and pathogens range of the biological control agents. Brodeur (2012) also noted that the level of risk that is acceptable depending on the importance of the diseases problem and the presence of ecologically and economically important non-target species (e.g., endangered species, crop plants, and pollinators) in the environment where the biological control agent is to be released.

3.7.2 Inoculation Technique and Symbiosis Sustainability

Finding the best microbes to enhance plant growth and increase their immunity to plant pathogens, including DSE, is hard, and optimizing their potential in the field scale is harder to carry out. The DSE cannot be applied in the field as spore suspension, but need a precise delivery system such as powdered or liquid formulation through seed treatment, root dip, etc. Therefore, determining the correct method and time to inoculate DSE into host plants is crucial to the success of DSE applications. Microbe coating of seeds with promising microbes (Ab Rahman et al. 2018), including inoculation for fungal endophytes, is one of the best options for optimizing plant-microbe interactions at the field scale. The benefit of this method for a large-scale and field application is that the inoculants can be stored/cryopreserved for a long period and cultured at any time before being applied to the seeds. The important phase to inoculate the DSE isolate is during seed germination or during embryogenesis in tissue culture. This report provides an indication of potential *P. indica* for large-scale application. Bhagobaty and Joshi (2009) reported that fungal endophyte *Penicillium verruculosum* RS7PF was able to induce germination of *Vigna radiata* and *Cicer arietinum*. The endophytic fungus may provide carbon for the seed germination through degradation of cellulose in the cuticle. In the orchid's germination, seedlings of orchids, in fact, nutritionally depend on fungal endophytes that colonize their cells (Bidartondo and Read 2008). It is called "myco-heterotrophic" (Leake 1994).

Several methods to effectively inoculate DSE fungus into host plants have been reported at various scales (Varma et al. 1999; Usuki et al. 2002). In the application of *P. indica* fungus into *Spilanthes calva* and *Withania somnifera*, the mycelium of *P. indica* was homogenized in sterile water and was further applied at 1% of seed (Rai et al. 2001). The results showed that not only increase in all plant growth parameters was found but also drought tolerance adaptation of *S. calva* and *W. somnifera* during the hot March–June summer season (day temperature above 40 °C). Tefera and Vidal (2009) in the study of *Beavueria bassiana* inoculation into sorghum noted that colonization by *B. bassiana* endophytically depended upon the inoculation method, specificity of fungal isolate, and plant species. In their study, seed coating with conidial suspension followed by planting in vermiculite and sterile soil could improve the colonization of *B. bassiana* on sorghum. In the study of fungal biocontrol inoculation into tissue culture of banana, Paparu et al. (2004) noted that potentially PGP fungal endophytes must be present in plant tissue at the time the plants are attacked by pathogens. Successful colonization of plant roots by fungal endophytes and continual symbiosis from the time of inoculation onward (until field planting) is crucial in the application of the fungal endophytes. In the micro-propagated plants, DSE can be inoculated during somatic embryogenesis (Niemi et al. 2004; Jie et al. 2009). The in vitro colonization of tissue culture plants can increase adaptation to ex vitro conditions and to acclimatization.

3.8 Conclusion and Future Direction

The potential of DSE in enhancing plant overall performance has been discussed and elucidated, from nutrient uptake, adaptation to the extreme environment, and protection from plant pathogen threats. However, further difficult tasks for scientists to face are moving forward this huge potential into large-scale application or commercial application to meet world demand for foods. A rapid increase in human population will have to meet the current and future environmental condition such as less land, less water, and high temperature. Therefore, every opportunity and potential to increase food production must be explored. Since the DSE fungal application for commercial scale has several limitations, the future research should be focused on (1) examination of host specificity, pathogen specificity, and symbiosis sustainability of the potential DSE with various hosts; (2) optimization of DSE application methods at various environmental conditions, such as tropic, sub-tropic, temperate, etc.; and (3) development of DSE co-inoculation method on a commercial scale.

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4

Rhizobacteria and Abiotic Stress Management

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Abstract

With ongoing climate change, the severity, frequency and duration of different abiotic stresses have threatened the agricultural productivity around the globe. Major abiotic stresses like drought and salinity have reduced agricultural land both in the arid and semiarid regions of the world. Further decline in yield is inculcated by plant pathogens. Excessive use of chemical fertilizers induced heavy metal as secondary stress. Therefore, use of environmentally friendly approach based on plant growth-promoting rhizobacteria is a promising one to alleviate the adverse effect of stresses and improve growth of plants under such conditions. These are the natural inhabitants of diverse environment an integral part of natural ecosystem and exhibit enormous plant growth promotional capabilities. They colonize plant roots, can modulate phytohormone levels and induce local and systemic mechanism in plants that offer resistance against biotic and abiotic stress factors. When applied as biofertilizers, counteract osmotic stress, oxidative stress, provide bioprotection against heavy metals thus enhance tolerance against salinity, metal toxicity and drought stress. They change heavy metal bioavailability in soil through major processes of immobilization, transformation, acidification, precipitation, chelation, complexation, redox reactions and distribution. These bacteria also affect the physiochemical properties of saline soil by increasing organic matter content, NO_3-N, available P and K of the soil, and decrease in the ECe, CEC and SAR of rhizosphere soil renders the saline soil productive. This chapter provides a brief overview of PGPR-mediated stress-tolerance responses in plants and the molecular and the cellular mechanisms responsible to alleviate drought, salt and heavy metal stresses.

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PGPR · Drought tolerance · Salinity stress

4.1 Introduction

4.1.1 PGPR Effect on Growth and Development of Plants

PGPR escalate plant nourishment through modification of root architecture and their effectiveness and reciprocate to abiotic stresses. Besides growth enhancement, they produce phytohormones and nutrient uptake, solubilize mineral deposits and synthesize siderophores, an iron chelator to make iron available to plant roots (Glick 1995). Mineralization, decomposition, storage and/or discharge of nutrients mediated by complex PGPR community inculcates a crucial role in soil richness (Bunemann et al. 2004; Khan et al. 2018a). They work as a sink for phosphorous in the presence of liable carbon, as it rapidly immobilizes phosphorous in soil having low content of phosphorous and surge its availability to plant roots (Kang et al. 2002; Khan and Bano 2016a). They also improve plant growth by boosting the efficacy of biological N_2-fixation and augment the approachability of trace elements (Ponmurugan and Gopi 2006) (Figs. 4.1 and 4.2).

Rhizobium is a well-reported PGPR that resides inside the roots of non-leguminous plant and induces phytohormone production, solubilizes insoluble phosphate and to some degree acts as nitrogen fixer (Afzal and Bano 2008; Matiru and Dakora 2004). Inoculation of such bacterium in field enriched soil fertility and

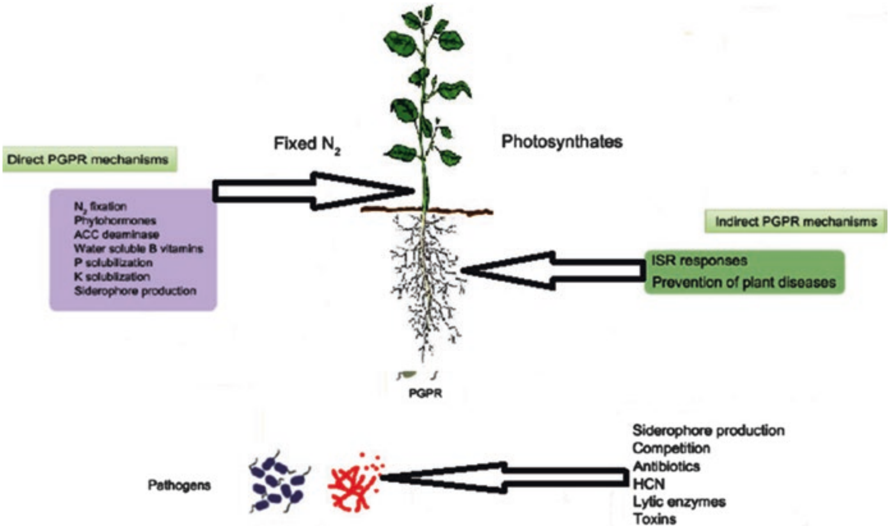

Fig. 4.1 Mechanisms of Plant growth promoting rhizobacteria (García-Fraile et al. 2015)

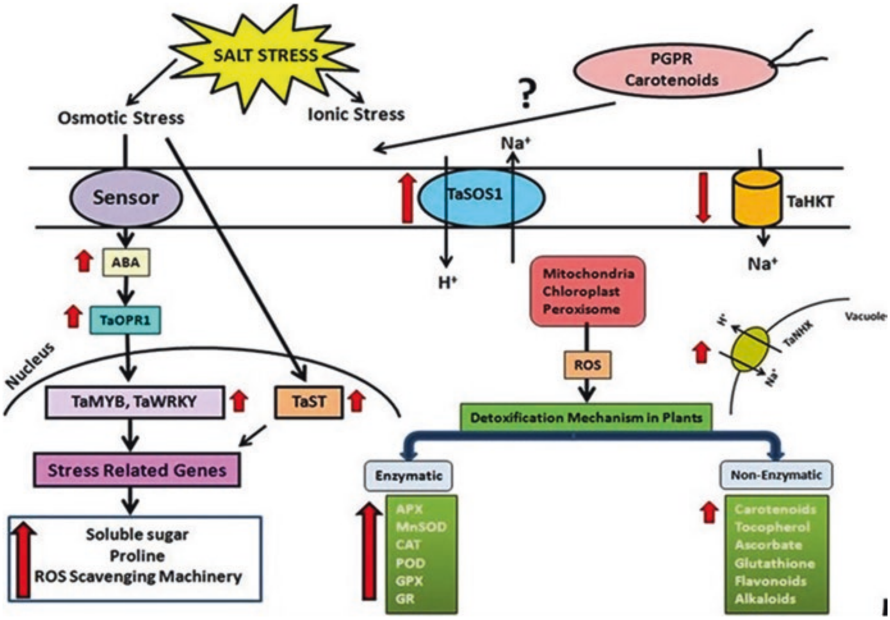

Fig. 4.2 PGPR mediated abiotic stress tolerance in plants (Bharti et al. 2016)

decreased production cost of nitrogen fertilizers for next crop (Ahmad et al. 2006). Examples include genera, i.e. Achromobacter, *Aeromonas*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Pseudomonas* and *Variovorax* (Pishchik et al. 2002; Sorty et al. 2016). Current modern agriculture technology relies on use of PGPR especially in stress-affected regions of the world. A summary of action mechanism adopted by PGPR is shown in Table 4.1.

4.1.2 Drought, Salinity and Heavy Metal Stresses

Abiotic stresses created by adverse climatic conditions are amongst the primary restrictive that decline crop productivity in different areas of the world. Report presented by FAO clearly illustrates that 3.5% of the total land area has been left unpretentious by major environmental constraint such as drought that has affected 64% of global land area, 6% salinity affected area, flood (13%), mineral deficiency 9%, acidic soils 15% and cold 57%. Riadh et al. reported that out of the 5.2 billion ha of agriculture land, 3.6 billion ha is affected by the erosion, soil degradation and salinity.

Shahbaz et al. reported that salinity causes major reductions in cultivated land area, crop productivity and quality.

Globally, more than 831 Mha of land is affected, either by salinity (397 Mha) or by sodicity (434 Mha) (Martinez-Beltran and Manzur 2005). Salinity and sodicity

Table 4.1 PGPR interaction effects in crop plants under salinity stress

PGPR species	Crop plant	Effects	References
Pseudomonas pseudoalcaligenes, *Bacillus pumilus*	*Rice (Oryza sativa)*	Augment the concentration of glycine betaine	
Raoultella planticola Rs-2, *Streptomyces* sp. strain PGPA39	*Cotton 'Micro tom' tomato*	ACC deaminase activity and production of IAA	Wu et al. (2012)
Acinetobacter sp. and *Pseudomonas* sp	*Barley and oats*	Production of ACC deaminase and indole-3-acetic acid production	
Pseudomonas pseudoalcaligenes	Salt-sensitive rice GJ-17	Reduce lipid peroxidation and SOD activity	Jha and Subramanian (2014)
Bacillus pumilus			
Brachybacterium saurashtrense (JG-06), *Brevibacterium casei* (JG-08) and *Haererohalobacter* (JG-11)	*Groundnut (Arachis hypogaea L.)*	Increase K+/Na+ and Ca^{2+}, ration and the accumulation of P and N	Shukla et al. (2012)
Rhizobium phaseoli and PGPR *(Pseudomonas syringae, Mk1; Pseudomonas fluorescens, Mk20;* and *Pseudomonas fluorescens Biotype G,* Mk25)	*Mung bean (Vignaradiata L.)*	ACC deaminase activity and increased WUE	
Rhizobium and *Pseudomonas*	*Mung bean (Vignaradiata L.)*	Responsible for the synthesis of IAA	
Pseudomonas putida, *Enterobacter cloacae*, *Serratiaficaria* and *Pseudomonas fluorescence*	*Wheat*	Enhance % germination and improve the nutrient status in wheat plant	
Pseudomonas simiae AU	*Glycine max*	Decrease in root NaCl accumulation and increase in proline and chlorophyll content	
Pseudomonas putida UW4 (ACC deaminase)	*Solanum lycopersicum*	Increased shoot growth and expression of Toc GTPase	
Dietzianatro nolimnaea	*Triticum aestivum*	Responsible for the modulation of ABA signalling cascade	Bharti et al. (2016)
Enterobacter sp. UPMR18 (ACC deaminase)	*Abelmoschus esculentus*	Increase antioxidant enzyme activities and upregulation of ROS pathway genes	

(continued)

Table 4.1 (continued)

PGPR species	Crop plant	Effects	References
Bacillus thuriengenesis NEB17	*Glycine max*	Regulate the activity of PEP carboxylase, pyruvate kinase and antioxidant glutathione-S-transferases	Subramanian et al. (2016)
Bacillus amyloliquefaciens SQR9	*Zea mays*	Upregulation of RBCS, RBCL and NHX1	Chen et al. (2016)
Bacillus amyloliquefaciens SN13	*Oryza sativa*	Upregulation of SOS1, EREBP, SERK1, NADP-Me2	

increase heavy metal toxicity as secondary stress. Metals and metalloids having specific weight greater than >5 g cm^3 are generally referred as heavy metals. In biology heavy metals include those elements which cause toxicity to human beings and environment (Tchounwou et al. 2012). They are classified into three categories, including toxic metals (e.g. Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (e.g. Pb, Pt, Ag, Au, Ru, etc.) and radionucleids (e.g. U, Th, Ra, Am, etc.) (Wang and Chen 2006; Thateyus and Ramya 2016). Heavy metals that reach hazardous levels comprised of Pb, Cr, Hg, U, Se, Zn, As, Cd, Ag, Ni, Au and Cu (Ahalya et al. 2003; Vieira and Volesky 2000). Rhizoremediation of such metals has gained an attracted attention and considerable research.

Weathering of enriched metal rocks naturally releases heavy metals in the soil (Smith 2009). Addition of anthropogenic activities, i.e. extensive use of metal-based pesticides, biosolids and manures, exploitation of mining and industrial wastes, municipal and industrial waste water, military training and weapons, etc. can result into heavy metal toxicity in soil.

Plants adaptation to salinized land polluted with heavy metals is an increasingly important problem of the world (Kholodova et al. 2010; Khan and Bano 2016b). In alkaline salt-affected soil, the damaging effect on plants is more severe (Heshmatpur and Rad 2012). Metal toxicity effect the soil physio-chemical properties like pH, soil texture and the accumulation of macronutrients as well as micronutrients hence effects the overall plant growth. It also has an inhibitory effect on plant growth, root development, photosynthetic activity and accumulation of mineral nutrient (Sen et al. 2013; Garg and Bhandari 2011).

Salinity-induced osmotic stress leads to precipitation and renders the unavailability of essential elements such as K, Ca, Fe and Zn, causing nutrient deficiency to plants. Wang et al. (2013) reported that maize shoot and root is highly reduced in the presence of Cd. Toxicity of Pb causes reduction in germination, suppressed growth, reduces length and dry mass of root and shoot, disturbs mineral nutrition and decreases protein content in maize (Ghani et al. 2010; Hussain et al. 2013).

4.1.3 Drought Tolerance

PGPR induce drought tolerance in plants by many mechanisms (Farooq et al. 2009; Khan and Bano 2016b). Parenthetically, compounds that exudate by rhizosphere bacteria also contain such osmolytes. Production of glycine-betaine by rhizobacteria acts in coordination with glycine-betaine produced by the roots of plant against environmental stresses and thus enhances tolerance in plants to varied stresses. In connection with this, the advantageous effects of osmolyte producing rhizobacteria on the seedling of rice were more momentous under severe stress condition that positively affected shoot and root dry weights. These bacteria were also tested for the production of IAA, thus helping in root proliferation and apparently improving water uptake.

Bacteria that synthesize ACC-deaminase can enhance the tolerance of root and leaf to water scarcity, ostensibly by the induction of ethylene signalling (Stenglein and Harris 2006). The ACC deaminase action of *Achromobacter piechaudi* was revealed to enhance the drought tolerance of tomato and pepper, instigating significant surges in their fresh and dry weights. PGPR inoculation reduces the production of ethylene in PGPR-treated plants as compared to control and improves the retrieval from water deficiency, though bacterial treatment did not affect relative water content (Mayak et al. 2004).

By 2030, the available freshwater will not keep up with the demand, and without more active managing practices for water resources, the complications will increase and food production will decrease in many countries of the world. Water supply directly affects the agricultural GDP. Major reservoirs (i.e. 70%) of the global freshwater are used for agriculture, thus creating chances and potentials for agro-technologist to propose solutions in order to professionally use the available freshwater sources (Ahmadi 2009; Khan et al. 2019a).

4.1.4 Mechanisms of Bacteria-Mediated Stress Tolerance

Plant growth-promoting rhizobacteria are recognized for their role in making of phytohormones. These phytohormones show a key role in altering the mechanism of plants to ecological stresses (Potters et al. 2007). The foremost important hormone produced by these rhizosphere bacteria is IAA, and treatment of plants with such bacterial species results augmented root growth and increases in the creation of horizontal roots and root hairs (Khan et al. 2017, 2018b). This increase in the growth of roots is responsible for a greater root surface and thus enhances water absorption and uptake of essential nutrients.

Another extensive distinct character used by endophytes and rhizosphere bacteria is the assembly of ACC deaminase, as ACC deaminase is chief mechanism of rhizobacteria for imparting useful effects on abiotic ally-stressed plants (Naseem et al. 2018; Saleem et al. 2007). Bacteria owning this enzyme can employ the instant ethylene precursor ACC as a source of nitrogen. ACC deaminase activity not only leads to reduction in ethylene level but also causes variations in root morphology

(Molina-Favero et al. 2008). This decrease in the production of ethylene modifies the overall stress eminence of the plant, as ethylene shows a crucial role in stress-related signal transduction pathways. Like ethylene, proline is also produced by certain PGPR species interaction to plentiful abiotic and biotic stresses (Hare and Cress 1997). Proline production has been noted to increase in stressed plants inoculated with *Burkholderia*, Arthrobacter and *Bacillus* (Sziderics et al. 2007).

Rhizobacteria are also known for the induction of induced systematic resistance (ISR) in plants (Chakraborty et al. 2006) which is another mechanism of increased lenience to abiotic stresses. In addition, reasonable vagaries in gene expression in *Arabidopsis thaliana*, treated with *P. polymyxa* under drought or infected with *E. carotovora*, support the supposition that genes intricate with plant responses to different stresses may be co-regulated by PGPR (Timmusk and Wagner 1999). In connection with this, the expression of *Osmyb4* gene encodes for transcription factor in rice that complicate in cold acclimation instigated by higher lenience of transgenic *A. thaliana* to both abiotic and biotic stresses. Xiong and Yang (2003) demonstrated that resistance to plant diseases and abiotic stresses in rice plant are controlled by an ABA-inducible mitogen-activated protein kinase (MAPK). This MAPK is attracted by both abiotic and biotic stressors and increases tolerance environmental stresses when present in higher concentration (Yang et al. 2009a, b).

Studies on plant-microbe interactions at various levels (i.e. biochemical, physiological and molecular) documented that plant-microbial associations generally direct plant reactions towards stresses. For studying profounder interactive mechanisms and linking the distinctions at molecular level with the leniency retorts against stresses, biological data was generated based on multi-omics approaches. The data generation and examination were sustained by the improvements in the high-end instrumentation and computational addition which assisted to decode separate signal molecules, proteins, genes and gene cascades to relate them with the gene network pathways for their function depiction. Technological extensions also alleviated the knowledge of gene-editing systems and metabolic profiling to unveil enormous molecular information that abetted in purifying our understanding of microbe interceded stress mitigation strategies. Multi-omics methodologies have ascended as a complete and combined investigative approaches for the partition of one of the most composite and active living systems of microbial interactions with plants and moderating the significances in the plants to benefit them by overcoming different environmental stresses (Belimov et al. 2007).

4.1.5 Physiological Mechanism Mediated by Rhizobacteria Against Salinity

Rhizospheric zone has greatest diversity of microorganisms and serves as an essential ecological niche where utmost microbial activities take place as compared to rest of bulk of soil. Diverse mechanisms have been adopted by PGPR that promote plant growth under saline condition. These include N_2-fixation, solubilization of insoluble phosphates and production of siderophores (Khan et al. 2018a, b, c).

Stress alleviation is ensured through antagonistic activity against pathogens, degradation of organic pollutant and bioremediation of heavy metal toxicity (Chowdhury et al. 2015; Smith et al. 2015).

4.1.5.1 Osmotic Balance and Ion Homeostasis

Marulanda et al. (2010) performed an experiment on maize plant inoculated with *Bacillus megaterium*. He noticed that root hydraulic conductivity was increased in inoculated maize compared to uninoculated plants when exposed to 2.59 dS m^{-1} salinity level. In another study it was reported that wheat inoculated *Bacillus aquimaris* strains showed increased total soluble and reducing sugars under saline condition (ECe 5.2 dS m^{-1}). In field conditions it was observed that inoculation of *Bacillus aquimaris* resulted in higher shoot biomass, nitrogen, phosphorous and potassium accumulation and reduced Na content in leaves (Upadhyay and Singh 2015). PGPR maintain ion homeostasis by reducing Na and Cl accumulation in leaves and their exclusion via root. Rojas-Tapias et al. conducted pot experiment to evaluate the role of PGPR on amelioration of saline stress in maize. The two most tolerant PGPR candidates, *Azotobacter strains* C5 (auxin producing) and C9, were selected. After 4 weeks ion uptake was evaluated. Inoculation of bacteria in maize plants under salt stress improved K uptake and Na exclusion, thereby enhancing K/Na ratio in maize.

4.1.5.2 Phytohormone Signalling

Under stress saline plant-microbe interaction is regulated by phytohormone signalling which contribute to increased salt tolerance. One of the most commonly studied bacterial signalling molecules is IAA produced by PGPR. It has been reported that IAA producing PGPR could produce siderophores and can solubilize inorganic phosphate, thus promoting the growth of tomato under 2% NaCl, respectively (Tank and Saraf 2010).

ABA is a stress hormone and its accumulation in plants under saline condition increases and hampers growth of plant. It was investigated that *Pseudomonas putida* Rs-198 inoculated to cotton plant (*Gossypium hirsutum*) showed increase concentration of endogenous IAA level and reduced ABA accumulation (Yao et al. 2010). In another study it was reported that *Arthrobacter protophormiae* SA3 and *B. subtilis* LDR2 when inoculated to wheat plants showed increase concentration of IAA, while ABA and ACC deamiase activity were reduced at 100 mM NaCl (Barnawal et al. 2017).

Glick et al. investigated that PGPR could lower plant endogenous ethylene level under saline stress and enhance the production of IAA to promote plant growth. *Pseudomonas fluorescens* and *Enterobacter* spp., both are ACC deaminase producing bacteria. These bacteria were inoculated to maize grown in salt-affected soil and data related to Na, K and P was recorded. Higher K/Na ratio and NPK uptake was recorded in inoculated maize compared to control plants (Nadeem et al. 2009). Another bacterium known as *Pantoea dispersa* PSB naturally produces IAA and ACC deaminase enzyme. Upon inoculation to *Cicer arietinum* cv. GPF2, it was observed that plant biomass, pod number, pod weight, seed number and seed weight were significantly increased under 150 mM NaCl. Similarly, a significant increase in leaf relative water and chlorophyll content as well reduction in NaCl uptake and electrolyte leakage was also recorded (Panwar et al. 2016).

4.1.5.3 Extracellular Molecules

A variety of extracellular compounds are secreted by PGPR to manipulate signal pathways under salt stress. These include exopolysaccharide, proteins, polyamines (PAs), volatile organic compounds (VOCs), etc. Exopolysaccharides secreted by bacteria are responsible for maintenance of soil structures and increased water holding capacity and cation exchange capacity (Upadhay et al. 2011).

Cicer arietinum var. CM-98 inoculated with *Halomonas variabilis* HT1 and *Planococcus rifietoensis* RT4 showed enhanced growth under 200 mM Nacl salt concentrations (Qurashi and Sabri 2012). Lipochito-oligosaccharides are extracellular substances secreted by PGPR and induce seed germination and root organogenesis in barley (Miransari and Smith 2009). PGPR produce and secrete antimicrobial peptides known as bacteriocins. Thuricin 17, a bacteriocin isolated from *B. thurengensis* NEB17, enhanced physiological tolerance of *Arabidopsis* to 250 m MNaCl (Subramanian et al. 2016). Polyamines are low molecular weight organic compounds that have pronounced effect on antioxidant activity under salt stress (Balal et al. 2017). Examples are spermidine, spermine, putrescine, etc. Inoculation of spermidine-producing *Bacillus megaterium* BOFC 15 to *Arabidopsis thaliana* resulted in greater biomass and enhanced antioxidant activity and robust root architecture (Zhou et al. 2016).

4.1.6 Role of PGPR in Alleviation of Heavy Metal Stress

PGPR have developed several mechanisms to overcome metal toxicity. These are discussed below.

4.1.6.1 Metal Detoxification

Rhizoremediation is the uses of inocula of natural and recombinant PGPR to remediate heavy metals from polluted soil (Dixit et al. 2015). Bioaccumulation and biosorption are two basic strategies adopted by microbes to alleviate heavy metal toxicity (Ahemad 2014; Ma et al. 2011). They secrete low molecular weight chelators called siderophore. Chelators form complexes with metals such as cadmium, copper, lead, iron and zinc (Schalk et al. 2011). Under stressful environment of heavy metal contamination, microbes experience a high level of metal toxicity. In such circumstances, PGPR have adopted different mechanisms. Some are listed below.

4.1.6.2 Biosorption

Biosorption is a cost-effective, environmentally friendly approach and possesses excellent adsorbability. Two decades ago, heavy metal-contaminated soil exceeded in volume due to industrial and agriculture sources. It is reversible and passive (faster) metabolic independent process that does not require energy.

Several genera of PGPR have been tested as potential biosorbents. They possess chemosorption sites in their cell wall and associated functional groups. These receptors have tendency for biosorption of Cd, Cu, Hg and Pb concentration. In the second step, these metals are detoxified, sequestered or compartmentalized in different subcellular organelles. It has been reported by Ayangbenro and Babola that different compounds belonging to extracellular polymeric substances are secreted by rhizospheric bacteria and play a key role in the removal or recovery of metals from contaminated site. Extrapolymeric substances include polysaccharides, glycoprotein, lipopolysaccharide and soluble peptide. They also act as biosurfactants and are commonly used to remediate heavy metals.

4.1.6.3 Bioaccumulation

Bioaccumulation mediated by PGPR is the process in which intracellular accumulation of metals occurs either passively (physical and chemical absorption) or through active biosorption (Chojnacka 2010). The former one don't require any energy it is achieved either through physical absorption or chemical adsorption, while the latter one is an energy-dependent process that requires energy for the accumulation and formation of metal complexes. It also assists in precipitation of metals intracellular or extracellular or their sequestration. In one study it has been reported that bioaccumulation is dependent on metal concentration, as high concentration of heavy metals adversely affects the bacterial growth and their accumulation capacity (Chojnacka 2010).

4.1.6.4 Bioleaching

Roy and Roy (2015) reported that PGPR is used to eliminate heavy metals from contaminated area through acidification as well as solubilization of heavy metals. Examples include *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thioosidans* and *Bacillus circulans*. Yang et al. (2009a, b) investigated that acidophilic and neutrophilic microbes have the potential to remediate heavy metals from sludge, sediment and municipal solids. Sulphate-reducing bacteria such as *Desulfovibrio desulfuricans* are a sulphur-reducing bacteria and could convert sulphate to hydrogen sulphate which then reacts with heavy metals and form their insoluble sulphides (Chibuike and Obiora 2014). Citric acid, gluconic acid, fumaric acid, lactic acid and malic acids are groups of organic acids secreted by PGPR. They interact with heavy metals forming metallo-organic complexes, thus chelate them or solubilize metal compounds and ease leaching from their surfaces.

4.1.6.5 Bioexclusion

It is the process in which essential and non-essential metals such as cadmium, copper and arsenic are effluxed from the cytoplasm through transporter proteins. Different kinds of heavy metal exporting proteins are present in microbes, which mediate efflux of toxic metals from cytoplasm. These include ATPase, cation diffusion facilitator proteins, chromate proteins and NreB- and CnrT-like resistance factors, respectively.

4.1.6.6 Metal Solubilization

Phytoextraction is directly related to the solubilization capacity of heavy metals (Ma et al. 2011). There are some PGPR that remediate heavy metal toxicity by releasing acids, proton or metal-binding compounds known as chelators.

4.1.6.7 Acidification

The solubility and mobility of metals is dependent on soil pH. Plant roots exudates and microbial secondary metabolites which are composed of low molecular weight organic compounds (LMWOAs) significantly reduce soil pH by twofold over that in bulk soil (Boddy et al. 2007; Khan et al. 2018). Acidic pH enhances the mobility and bioavailability of heavy metals in soil solution (Khan et al. 2019b).

4.1.6.8 Protonation

PGPR residing in the rhizosphere secrete protons that replace heavy metal cation sites for binding. Such explanation was supported by Naseem et al. (2018) who performed an experiment on *Rhodobacter sphaeroides* that contain carboxylate moieties on surface. Analysis was based on protonation-induced ATR-FTIR spectroscopy. It was inferred from results that carboxylate meioties play a significant role in extracellular biosorption of Ni^{2+} by establishing a weak coordinate bond.

4.1.6.9 Chelation

Several organic acids have been identified as chelator agent that are secreted by PGPR. These include glycolic acid, tartaric acid, piscidic acid, oxalic acid, etc. (Panhwar et al. 2013). Similarly, there are some metal-binding compounds, acid anions, biosurfactants, siderophores and metallophoers secreted by microbes that enable the incorporation of mineral cation into their complex ring structure. Schalk et al. (2011) reported three different types of siderophores, namely, hydroxamate siderophores, chatecholate siderophores and carboxylate siderophores that possess iron-chelating ability.

4.1.7 Metal Immobilization

4.1.7.1 Precipitation and Complexation

PGPR secrete metal-binding compounds that help in precipitation and complexation of heavy metals which may be dependent or independent on cellular metabolism of microbes (Comte et al. 2008). The secretion of exopolysaccharides by PGPR is another strategy to protect microbes from damaging effects of metals due to their metal-binding properties (Fang et al. 2010). The metal-binding behaviour of exopolysaccharide helps in adsorption and precipitation. Gupta and Diwan (2017) reported that biofilm formation was induced by exopolysaccharides that act as a protective sheath in response to heavy metal toxicity.

4.1.7.2 Metal Transformation

Redox reactions carried out by microbes play a crucial role in transforming heavy metals into bioavailable state necessary for phytotransformation (Amstaetter et al. 2010). Heavy metals such as copper and mercury are more soluble in their lower oxidation state as compared to their higher oxidation state. *Bacillus* sp. and *Geobacillus* sp. isolated from arsenic-contaminated site possess the ability to convert more toxic and mobile form of arsenic (As^{+3}) to immobile less toxic form (As^{+5}) (Khan and Bano 2016). Other heavy metals such as chromium, mercury, manganese and selenium have been reported to be immobilized and less toxified through this mechanism (Olegario et al. 2010).

4.2 Conclusion

It is concluded that PGPR are very effective in enhancing drought tolerance in plants, alone or in consortium, which is mediated through the production of exopolysaccharides and induction of new protein. The production of ACC deaminase enzymes by PGPR plays a pivotal role in alleviation of abiotic stress. Besides this, PGPR have also been revealed to encourage systematic resistance (ISR) and promote root growth under abiotic stress condition.

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Plant Growth-Promoting Rhizobacteria: Benign and Useful Substitute for Mitigation of Biotic and Abiotic Stresses

5

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Abstract

An incessant increase in global population along with a continuous augmentation in abiotic stress conditions, such as temperature, pH, salinity, etc., and limitation of natural resources has posed a serious threat to developing nations in terms of food security and enhanced nutritional value of the yield. Substantial crop losses in both qualitative and quantitative aspects due to the several prevalent phytopathogens are adding severity to the existing trouble. Confrontation with this ongoing problem initially led to the application of chemical fertilizers. However, hazardous aftereffects of the chemical fertilizers on the ecosystem have instigated a demand for a promising eco-friendly substitute that deals with both biotic and abiotic stresses. Rhizospheric microorganisms can be utilized as an effective alternative because they reside in soil and have the intrinsic property of upholding balanced ecosystem. These plant growth-promoting rhizobacteria (PGPRs) enhance plant growth even in poor and stressed environmental conditions by the formation of beneficial associations with the host through biological nitrogen fixation, phosphate solubilization, siderophore and hormone production, etc. They can also trigger host defense mechanism through induced systemic resistance (ISR). These PGPRs are also helpful for phytoremediation by various processes such as direct absorption, accumulation, etc. PGPRs are utilized in the fields of phytostimulation, biofertilization, and biocontrol activities.

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In the current chapter, we would aim to uphold the mechanisms opted by PGPR for effective plant growth promotion and defense under various abiotic as well as biotic stress conditions. In this context, we would also aim to delve in detail about the host-PGPR cross talk during the onset of stress conditions.

Keywords
Biotic stresses · Abiotic stresses · PGPR · Phytoremediation · Biocontrol

5.1 Introduction

By observing the steep increase in population growth curve with respect to time, it is very easy to predict the upcoming demand of food, fiber, fodder, and biomass by continuously decreasing arable land due to various anthropogenic activities (Abhilash et al. 2013). With an enormously growing population and limited resources, a major problem in front of developing countries is to provide food security with ecosystem stability. Both biotic such as pathogenic microorganisms, pests, weeds, etc. and abiotic stresses including low and high temperature, drought, salinity, flooding, ultraviolet light, air pollution, heavy metals, etc. are adding pressure to the crop production. Approximately 7–15% of the crops are damaged by various soilborne fungi, oomycetes, bacteria, and nematodes through various mechanisms such as destroying and damaging of root tips and root hairs, the release of toxins, etc. (Oerke 2005; Singh et al. 2014; Mishra et al. 2015). Increasing salt level in both land and irrigating water is the main problem faced by arid and semiarid areas due to which plant shows stunted growth as the photosynthetic unit becomes unable to work properly. Similar physiological modulations can be observed in plants against other abiotic stresses which ultimately lead to crop loss. These stresses cause a noticeable decrease of 50–82% in agricultural productivity and raise hindrance for the cultivation of new crops. To cope up with the abovementioned problems of the food crisis, malnutrition, etc., producers become inclined toward the unbalanced use of agrochemicals as an economically reliable substitute for crop protection. The enormous application of these chemical agents has led to severe negative impacts which include the development of pathogen resistance against applied agents, accumulation in the ecosystem due to non-degradation of the compounds, and therefore entry into the food chain. There is an urgent need to sustainably enhance the quality of crop production to meet future requirements and also protect the remaining cultivable soil from further degradation and contamination. Further, owing to the increasing awareness among people about harmful effects of these residues as well as the unavailability of chemical solutions against some phyto ailments apart from the continuously and rampantly increasing cost of pesticides, the search for a safer and eco-friendly alternative started which gave rise to biological control measures.

Currently, biological measures are one of the most emerging and sustainable methods among both agronomist and environmentalists for integrated plant growth and nutrient management systems to ease the burden on the environment. Among the numerous practices employed, application of plant growth-promoting rhizobacteria (PGPRs) is a potential measure as it prevents the plant from various phytopathogens as well as enhances the plant growth-promoting attributes due to their strong colonization affinity.

5.2 Plant Growth-Promoting Rhizobacteria (PGPRs)

The rhizosphere upholds a variety of microorganisms which can be deleterious, neutral, or beneficial (Fig. 5.1). Among numerous microfauna present in the soil, about 2–5% of free-living and rhizosphere-competent microbes providing plant growth promotional attributes even in the presence of competing microbes and phytopathogens are known as the PGPRs (Kloepper and Schroth 1978). Along with nutrients and water uptake, the root system of the host plants also secretes a variety of compounds in the rhizosphere (Walker et al. 2003) The rhizosphere PGPRs enhance the sustainability of soil for production of crops through various biotic activities that increase the nutrient turn over which in turn improve the soil structure. The main property of the PGPR which makes them more efficient is turning over of nutrients through their mobilization which enhances the sustainability for cultivation (Ahemad et al. 2009; Chandler et al. 2008). Further, several reports justify the sequestration of heavy metals and degradation of xenobiotics such as herbicides, pesticides, etc. by PGPRs, thereby leading to effective bioremediation (Ahemad 2012; Ahemed and Malik 2011; Hayat et al. 2010; Glick 2012). In this context, it is significant to notify the pursual of research on a global scale to yield biocontrol agents with numerous beneficial traits such as management of phytopathogens, plant growth promotion, heavy metal detoxification, abiotic stress tolerance, pesticide tolerance, etc. for the enhancement of sustainable agriculture (Chaudhary et al. 2012; Vaishnav et al. 2014). With all the promising plant growth promotional and biocontrol attributes, PGPRs can be used as an effective and

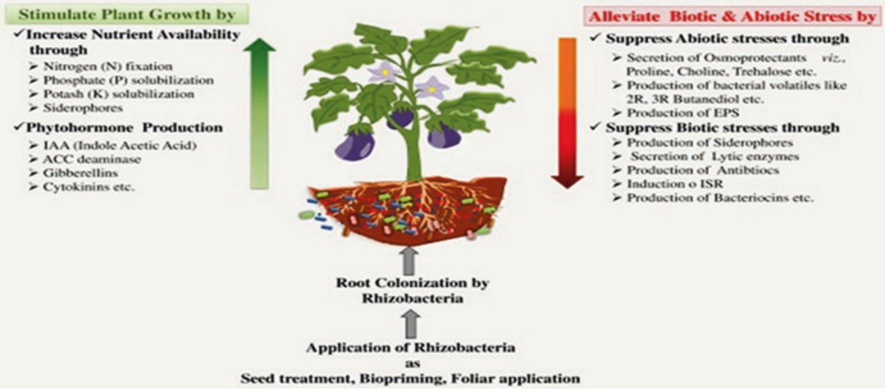

Fig. 5.1 Comparative assessment of beneficial attributes of PGPR as a respite against biotic and abiotic stress condition

eco-friendly tool for enhancing the sustainability of production, restoration of contaminated land, nutritional and food security, carbon sequestration, phytoremediation of heavily contaminated soils, and biofuel and biomass production. Presently numerous symbiotic microbes such as *Rhizobium* spp. and *Bradyrhizobium* spp. as well as nonsymbiotic microbes including *Pseudomonas*, *Bacillus*, *Azotobacter*, *Azospirillum*, and *Alcaligenes* are known globally for their application as inoculants possessing plant growth and stress-tolerant attributes (Ma et al. 2011a, b; Wani and Khan 2010; Mayak et al. 2004; Ray et al. 2016a, b, 2018b).

5.3 Mechanisms Implicated by PGPR

5.3.1 Root Colonization

A significant drawback consistently associated with PGPRs is their poor field performance owing to the inconsistency of rhizosphere colonization, particularly under field conditions (Schroth and Hancock 1981; Thomashow 1996a, b). Efficient root colonization is the primary step for effective proliferation and survival in the presence of other rhizospheric microflora as well as for establishing competence that provides effective biocontrol, plant-microbe cross talk, and enhanced PGPR efficiency (Parke 1991; Wipps 1997; Lugtenberg and Dekkers 1999). As the rhizospheric soil behave as sink for nutrients, plants release root exudates with diverse chemical compounds such as specific sugars, organic acids, amino acids, etc. which act as chemoattractants for numerous active soil microbes and synchronize the microbial presence in close proximity of root surface (Rovira 1965; Welbaum et al. 2004; Dakora and Phillips 2002). Due to the presence of these exudates, the symbiotic association takes place with the nearby rhizospheric microbial communities that promote plant growth and in turn obtaining major nutrients, such as carbon, nitrogen, phosphorus, etc., through the chemical compounds released by roots and root hairs (Nardi et al. 2000). When the PGPRs reach the root through their motile structures in response to the exudates which is known as rhizospheric effect (Hiltner 1904), some of them colonize the surface of roots and root hairs without causing harmful effects, thereby inhibiting the invasion of phytopathogens by means of nutrient and niche competition, whereas many of them have the ability to enter endodermis after crossing the barrier and exist as endophytes in different organs of the host plant (Hallman et al. 1997; Duffy 2001; Turnbull et al. 2001; Compant et al. 2005; Gray and Smith 2005; Ray et al. 2018a).

5.3.2 Growth-Promoting Attributes

Post-effective establishment and colonization, PGPRs enhance the growth and increase the productivity of host plant through various direct and indirect methods such as nutrient acquisition, regulating plant hormone and synthesis of various beneficial metabolites (Glick 2012).

5.3.2.1 Biological Nitrogen Fixation

With 78% of the fraction in the atmosphere, nitrogen is the most essential macromolecule required for plant growth and development which is fixed in plant utilizable forms through biological nitrogen fixation (BNF). In this process, atmospheric nitrogen is converted to ammonia with the help of microorganism borne nitrogenase enzyme system (Kim and Rees 1994). Nitrogenase is a two-component complex metalloenzyme system comprising of dinitrogenase reductase as iron protein and dinitrogenase as a metal cofactor, and on their basis, three different nitrogen-fixing systems have been reported, namely, Mo-nitrogenase, V-nitrogenase, and Fe-nitrogenase (Dean and Jacobson 1992; Kim and Rees 1994). Majority of BNF is performed by Mo-nitrogenase present in most of the PGPRs carrying nitrogen fixation in nonleguminous plants through the establishment of nonobligate interaction (Glick et al. 1999; Bishop and Jorerger 1990). Microorganism involved in BNF can be broadly divided into (a) symbiotic association with leguminous and (b) nonleguminous plants and (c) free-living as well as associate nonsymbiotic endophytes such as *Acetobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, etc. which fix a minor portion of atmospheric nitrogen. Majority of unavailable atmospheric nitrogen is fixed through symbiotic nitrogen fixers such as *Rhizobia* in leguminous and *Frankia* in the nonleguminous plant (Saxena and Tilak 1998; Bhattacharya and Jha 2012; Glick 2012). A number of studies revealed two third biological fixation of atmospheric nitrogen globally, and remaining requirements are fulfilled by the Haber-Bosch method (Rubio and Ludden 2008). Treatment of plants and soil with PGPRs having the nitrogen-fixing ability is an economical and ecologically sustainable substitute of chemical fertilizers (Ladha et al. 1997).

5.3.2.2 Phosphate Solubilization Activity

The soil is the most abundant reservoir of both organic and inorganic form of phosphorus, the most essential macronutrient for plant growth promotion after nitrogen (Khan et al. 2009). Regardless of such an enormous reservoir, plants, in general, face scarcity of phosphorus as the roots only absorb monobasic and dibasic forms of the ion, while a major portion of phosphorus present in insoluble forms such as inositol phosphate, phosphomonoester, and triesters remain unutilized (Bhattacharya and Jha 2012). To deal with unavailability, farmers apply numerous phosphatic fertilizers, but only a little amount is absorbed by the plant with the remaining portion being turned into insoluble complexes (Mckenzie and Roberts 1990). Among numerous rhizospheric microflora, phosphate-solubilizing microorganisms (PSM) including *Bacillus*, *Enterobacter*, *Pseudomonas*, *Burkholderia*, *Flavobacterium*, *Rhizobium*, *Microbacterium*, *Serratia*, etc. can be applied as a substitute for sustainable agriculture since they can convert unavailable form of phosphorus to available form through the activity of low molecular weight organic acids produced by PSM (Zaidi et al. 2009). These PSM also synthesize numerous phosphatases for mineralization of organic phosphorus through phosphoric ester hydrolysis (Glick 2012). Numerous beneficial effects such as mineralization, enhanced efficiency of BNF through nodule formation, increased uptake of trace elements, etc. have been observed in the host plants treated with single or amalgamated PGPRs having phosphate-solubilizing property (Ahemad and Khan 2012; Vikramal and Hamzehzarghani 2008; Zaidi et al. 2009; Ahmad et al. 2008).

5.3.2.3 Production of Phytohormones

Plant hormones are the organic compounds which act as chemical messengers generated through various metabolic processes in one portion and get distributed all over the system. They are concentration and target specifically for optimum growth and development of a plant in different environmental conditions and therefore also termed as a plant growth regulator. On the basis of previous studies, phytohormones have been classified into five major classes: auxins, cytokinins, gibberellin, abscisic acid, and ethylene. Among these, IAA is the supreme indigenous auxin which regulates cellular processes (such as division, expansion, and differentiation), regulation of genes, organ development, pigment formation, metabolite synthesis, stress resistance, and several tropic responses (Ryu and Patten 2008; Ashrafuzzaman et al. 2009). Previous studies have reported the production and release of IAA by approximately 80% of rhizospheric microorganism as their secondary metabolite which may alter the intrinsic production of phytohormone and also change the permeability of plant cell wall for enhanced release of root exudates (Glick 2012; Spaepen et al. 2007). Apart from growth and development processes, IAA is also involved in defense mechanism and plant-microbe interaction (Santner and Estelle 2009; Spaepen and Vanderleyden 2011). Numerous microflora such as *Pseudomonas*, *Mycobacterium*, *Rhizobium*, *Bacillus*, and *Rhizobia* uphold the ability to produce IAA and influence the numerous processes of host plant ranging from phytostimulation to pathogenesis (Mandal et al. 2007). PGPRs with IAA-producing abilities can be applied as biofertilizer and/or bioenhancers as they elevate root expansion through lateral and adventitious root formation, thereby increasing surface area for increased uptake of nutrient and water. Apart from regulating cellular processes, IAA also stimulates vascular bundle formation and nodule formation (Glick 2012). Enhancement in seed germination and physio-morphological changes have been reported in the orchids which were treated with IAA-producing PGPRs such as *Azospirillum brasilense* and *Bradyrhizobium japonicum* (Cassa'na et al. 2009).

5.3.2.4 ACC Deaminase

As a plant growth hormone, ethylene is a crucial metabolite generated endogenously by almost all plants and involved in conventional growth and development of host plant. Besides being involved in growth, ethylene is also confirmed as stress hormone as it affects plant growth through defoliation and other noticeable changes mainly in seedlings during biotic and/or abiotic stress conditions (Saleem et al. 2007; Bhattarcharya and Jha 2012). Numerous PGPRs including *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, etc. enhance plant growth through ACC deaminase activity. ACC deaminase is a pyridoxal 5-phosphate (PLP)-dependent polymeric enzyme which was initially reported in soil bacterium *Pseudomonas* (Honma and Shimomura 1978). A remarkable amount of ACC is released by the plant as root exudates in the soil to maintain the endogenous and external balance which in turn is utilized by PGPRs having ACC deaminase activity, thereby enhancing their proliferation (Glick et al. 1998). The enzyme utilizes the immediate precursor of ethylene and 1-aminocyclopropane-1-carboxylate and hydrolyzes it to α-ketobutyrate and ammonia which is further consumed as carbon and nitrogen sources by PGPRs (Arshad et al. 2007; Glick et al. 1998; Honma and Shimomura 1978). Further, according to Glick (2005), ACC deaminase activity varies in different organisms, and those with high activity bind inclusively to plant surfaces. Due to ACC deaminase activity of PGPRs, the endogenous level of ethylene reduces which in turn provides resistance against several stresses such as drought, salinity, flooding, high temperature, heavy metals, aromatic hydrocarbons, high radiations, wounding, insect predation, phytopathogens, etc. (Glick 2012; Lugtenberg and Kamilova 2009). Root elongation, shoot growth promotion, enhanced uptake of NPK, and increased nodulation with mycorrhizal colonization are some of the observable changes seen in plants inoculated with PGPRs (Nadeem et al. 2007, 2009; Glick 2014; Kumari et al. 2016).

5.3.3 Synthesis of Allelochemicals

Along with the growth promotion, PGPRs provide biocontrol activity through the secretion of allelochemicals which includes antibiotics, siderophores, biocidal volatiles, lytic enzymes, etc. (Bais et al. 2004; Glick 1995; Sturz and Christie 2003; Vaishnav et al. 2015, 2017).

5.3.3.1 Siderophore Production

Iron is an essential nutrient for all living forms with certain exceptions (Neiland 1995). In the rhizospheric region under aerobic environment, the ferric form of iron gets converted into insoluble hydroxides and oxyhydroxides, thereby raising the problem of iron scarcity (Rajkumar et al. 2010). Under limiting and competitive environment, rhizospheric microorganisms synthesize intra- and extracellular water-soluble peptidic iron chelator of low molecular weight, i.e., siderophore with different side chains and functional groups behaving as ligands with a different affinity (Crosa and Walsh 2002). Different edaphic and environmental factors such as amount and type of iron, pH of the soil, availability of macronutrients, the concentration of trace elements, etc. can regulate the synthesis of siderophores (Duffy and Defago 2000). These molecules can be classified into three major groups, namely, catecholates, hydroxamates, and carboxylates, on the basis of ligands utilized in ferric ion chelation (Xie et al. 2006). The efficiency of siderophore depends on the association constant of their complex formation with ferric ions. Rhizospheric siderophores uphold the higher value of association constant, thereby generating a severe iron-deficient condition for the pathogenic microorganism. Siderophores function as solubilizing agents for iron under limiting condition by reducing ferric ions to a ferrous ion which are further transported to cell interior through the gated membrane system. After this phenomenon, siderophores either get recycled or destroyed (Indiragandhi et al. 2008; Rajkumaret al. 2010; Neilands 1995). Along with iron sequestration, siderophores uphold the ability to form stable complexes with hazardous heavy metals such as Pb, Cd, Zn, Cu, Al, and Ga and radionuclide such as U, Np, etc. which are of alarming concern to the environment (Neubauer et al. 2000; Kiss and Farkas 1998).

5.3.3.2 Lytic Enzymes

Production and secretion of numerous enzymes from rhizospheric microorganism are involved in disrupting pathogenic membranes through hyperparasitic activity (Chernin and Chet 2002). Previous studies revealed that different enzymes including hydrolase, chitinase, lipases, pectinase, etc. attack pathogenic microorganisms through different mechanisms. Chitinase inhibits further spread of pathogen through hindering elongation of germ tube and spore germination (Frankwoski et al. 2001; Ordentlich et al. 1988). Some of the specific enzymes such as laminarinase are released by PGPRs alone or in combination with other enzymes to restrict specific pathogenic microorganism (Lim et al. 1991). Certain forms of glucanase, i.e., β1–3, β1–4, and β1–6, along with certain proteases directly target the glucans present in the fungal cell wall and destroy its integrity (Valois et al. 1996; Simons et al. 1997; Frankowski et al. 2001; Kamensky et al. 2003).

5.3.3.3 Antibiotic Production

Among the various methods applied by rhizospheric microorganisms to check proliferation of phytopathogens, antibiosis including the production and secretion of antibiotics is most commonly applied (Glick et al. 2007a, b; Lugtenberg and Kamilova 2009; Whipps 2001). Antibiotics are low molecular weight heterogeneous organic compounds, or metabolites primarily governed by nutrient availability and other environmental factors (Thomashow 1996; Duffy 2001). Even at low concentrations, these metabolites possess antimicrobial, antiviral, insecticidal, cytotoxic, antioxidant, antitumor, antihelminthic, and plant growth-promoting properties (de Bruijn et al. 2007; Raaijmaker et al. 2010). Broadly, these antibiotics can be classified into volatile and nonvolatile compounds which are further grouped into various subclasses. Nonvolatile antibiotics include polyketides, heterocyclic nitrogenous compounds, phenylpyrrole, cyclic lipopeptides, lipopeptide, and amino polyols, whereas hydrogen cyanide, aldehydes, alcohols, ketones, and sulfides are grouped under volatile antibiotics (Defago 1993; de Souza et al. 2003; Nielsen and Sorensen 2003; Raaijmakerset al. 2002). *Pseudomonas*, *Bacillus*, *Streptomyces*, *Burkholderia*, *Brevibacterium*, and several other microorganisms have been reported to produce and secrete antibiotics of a broad spectrum range (Keel et al. 1997; Haas and Keel 2003; Bender et al. 1999; Sutherland et al. 1985; Anjaiah et al. 1998).

5.4 PGPR Resistance to Biotic and Abiotic Stresses

A thorough understanding of the various mechanisms undertaken by PGPRs, particularly to resist biotic or abiotic stresses, is of paramount importance, more so because of the congregative nature of stress imposition. This would include not only the molecular identification of the bacterial strains involved but also the physiological as well as molecular mechanisms employed during the host-PGPR interaction.

5.4.1 Biotic Stress

Rhizospheric microbiota, particularly PGPRs, enable the augmentation of the inherent ability of plants to defend themselves against phytopathogens, apart from being a suitable alternative against chemical fertilizers (Sarma et al. 2015; Jain et al. 2012; Spence et al. 2014). In this context, management of phytopathogens through a microbial consortium or the use of endophytes has shown much promise. While endophytes have the inherent ability to provide plant protection and immunity enhancement due to their tendency of remaining sheltered within the plant interior (Ray et al. 2018a, b), microbial consortia remain in the vicinity of environmental stress but a strong promise to combat phytopathogens (Whipps 2001; Gossen et al. 2001; Stockwell et al. 2011). Several reports justify the plant growth promotional and improved disease resisting potential of PGPRs, such as *Pseudomonas* spp., *Trichoderma* spp., *Bacillus* spp., etc., on a variety of host plants, such as chickpea, pea, pigeon pea, okra, radish, tomato, wheat, pepper, *Arabidopsis*, etc. (Duffy et al. 1996; Rudresh et al. 2005; Jetiyanon 2007; Kannan and Sureendar 2009; Jain et al. 2012; Singh et al, 2013; Chauhan and Bagyaraj 2015).

The chief mechanism behind stimulation of the innate defense response of host plants by PGPRs is through induction of induced systemic resistance, operating in response to a microbial elicitor (Shoresh et al. 2010). In this context, Jain et al. (2012) reported enhancement of defense enzymes, particularly peroxidase, polyphenol oxidase, superoxide dismutase, glucanase, chitinase, etc., as well as phenol accumulation and lignin deposition in response to priming with a consortial mixture of PGPRs. In another study by Jain et al. (2015), the microbial consortia have been reported to recuperate the oxidative burst pathway inhibited by oxalic acid, the chief pathogenic factor of *Sclerotium rolfsii*/*Sclerotinia sclerotiorum*. Thus, the above studies clearly justify that PGPRs not only induce an augmented form of defense response within the host but also enable the quenching of factors responsible for induction of oxidative stress response within the host (Hammerschmidt 2005; Singh et al. 2013).

5.4.2 Abiotic Stress

Stress in nature is not a single phenomenon but a cumulative effect of various minor and major factors acting in togetherness (Mahajan and Tuteja 2005). While several natural stresses, such as drought, salt, flooding, and high/low temperature, have resulted in lowering of plant growth, certain anthropogenic activities have led to an additional confrontation with heavy metal stress, thereby declining crop yield and productivity by a significant level (Ramegowda and Senthil-Kumar 2015). Further, heavy metals sediment in soils and lead to groundwater contamination, thereby causing human health hazards. In other words, abiotic stresses may be considered as a root cause of loss of yield of several major crops (Bray 2004).

5.4.2.1 Drought Stress

Incessant reduction of rainfall year after year has led to a significant lowering of soil moisture content. Currently, even temperate regions are devising novel strategies to enhance the use of soil moisture content (Bray 2004; Farooq et al. 2009; Azcon et al. 2013; Panwar et al. 2014). Plant photosynthesis and nutrient uptake depend on a large scale on water availability in soil. Drastic reduction of soil moisture content or appearance of drought conditions severely hampers the basic requirements of the plant. For instance, water scarcity simultaneously increases the solute concentration within the plant cells, or a reduction in water potential, which in turn affect shoot and root elongation of plants. Further, water deficiency lowers carbon dioxide access by plants, thereby resulting in reactive oxygen species formation, such as superoxide, peroxide, and hydroxyl radical within plant cells, which in turn leads to apoptotic cell death of the plant (Sgherri et al. 2000).

In the above context, PGPR, such as *Pseudomonas mendocina* and *Glomus intraradices* or *G. mosseae*, was reported to release catalase enzyme and quench ROS produced within lettuce plants grown under severe drought conditions (Kohler et al. 2008). Thus PGPR may be considered as augmentation of defense enzymes in plants, such as peroxidase, polyphenol oxidase, etc. which further lead to protection of plant cell membrane and genomic DNA from oxidative damage (Bowler et al. 1992). Apart from individual PGPR, microbial consortia play a greater role in redemption from drought stress and in the improvement of plant growth. For instance, according to Figueiredo et al. (2008), a consortial mixture of beneficial PGPRs improved the overall health and nodulation of *Phaseolus vulgaris* under drought conditions as compared to inoculation with *Rhizobium* only. While report suggested PGPR treatment recuperated leaf water potential, biomass content, as well as sugar, proline, and amino acid content and loss of electrolyte leakage from plants (Sandhya et al. 2010; Vaishnav et al. 2018), treatment with consortial mixture of PGPR (*Bacillus lentus*, *Pseudomonadales* sp., and *Azospirillum brasilense*) augmented antioxidant activity as well as photosynthetic capacity along with the aforementioned properties in *Ocimum basilicum* (Heidari and Golpayengani 2012). Moreover, according to Stefan et al. (2013), consortial inoculation of PGPR improved superoxide dismutase and peroxidase activity in runner bean.

5.4.2.2 Salinity Stress

Presence of excessive amount of cations, such Na^+, K^+, Ca^{2+}, Mg^{2+}, etc., as well as anions, such as Cl^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, and HCO_3^-, in agricultural soils may be defined as saline stress (Yadav et al. 2011). As per the US Department of Agriculture (USDA) standards, soil having an electrical conductivity (EC) 4 dS m^{-1} or higher may be considered as saline soil (Seidahmed et al. 2013). Numerous reports imply saline stress as the chief cause of (a) development of drought-like situation on owing to shortage of water; (b) development of the payment of high ionic content in plants, thereby perturbing the normal physiological pathway; and (c) unavailability of other soil nutrients due to high salt concentration (Vaishnav et al. 2016). Munns (2002) reported stunted growth in plants exposed to salt stress due to lowering of water content with a simultaneous elevation in salt content. Further, accumulation of Na^+ ion content within host tissues led to additional necrosis (Parida and Das 2005) apart from interfering with the root cell plasma membrane, thereby causing stunted root growth and nutrient uptake (Yadav et al. 2011).

In the above context, priming of plants with PGPRs offers a plausible respite against salt stress (Kumari et al. 2015). Han and Lee (2005) reported that priming of lettuce plants with *Serratia* sp. and *Rhizobium* sp. did not adversely affect the growth and physiological parameters of the plant under salt stress conditions. Similarly, an enhanced nodule formation was observed in common bean and soybean at 25 mM salt concentrations upon priming with a consortial mixture of *R. tropici* (CIAT899) or *R. etli* (ISP42) and *Ensifer fredii* (*Sinorhizobium*) SMH12 and HH103 with *Chryseobacterium balustinum* Aur9 (Estevezi et al. 2009). In another report by Bano and Fatima (2009), priming of maize varieties with *Pseudomonas* sp. and *Rhizobium* sp. augmented plant growth promotional parameters even under salt stress. Similarly, a significant increase in growth promotional parameters of wheat plants under salinity stress was observed upon priming with a consortium of *Pseudomonas fluorescens*, *Enterobacter cloacae*, *Serratia ficaria*, and *P. putida* (Nadeem et al. 2013a, b).

5.4.2.3 Heavy Metal Stress

The industrial revolution, as well as some of the anthropogenic activities, has resulted in a significant increase in heavy metals and radionuclides in the soil. Few among these such as molybdenum (Mo), iron (Fe), and manganese (Mn) are reported to be essential for the photosystem, yet others, such as cadmium (Cd), mercury (Hg), chromium (Cr) etc., are particularly considered as nonessential elements. Extreme accumulation of particularly the nonessential elements not only affects the soil microflora (Oliveira and Pampulha 2006; Wani and Khan 2010; Cheng 2003) but also get translocated to different photo organelles, thereby causing disruption of membranes and simultaneous disintegration of cell organelles as well as a complete collapse of the essential physiological functions, such as photosynthesis, protein synthesis, etc. (Bray 2004; Morsy et al. 2013). Various studies have particularly focused on PGPR as effective bioremediation as well as enhancers of plant growth (He and Yang 2007; Madhaiyan et al. 2007). Dary et al. (2010) suggested augmented yield, biomass, as well as nitrogen content in plants treated with consortia of *Bradyrhizobium* sp., *Ochrobactrum cytisi*, and metal-tolerant *Pseudomonas* sp. In yet another report by Singh et al. (2010), mung bean treated with metal-tolerant PGPR exhibited augmentation in growth and biomass when grown in cadmium-infected soil. Similarly, Marques et al. (2013) reported lower metal accumulation within tissues of *Helianthus annuus* treated with *Ralstonia eutropha* and *Chryseobacterium hispalense* when grown in Cd- and Zn-infected soil.

5.5 Application and Future Prospects

Application of PGPR such as *Pseudomonas* spp., *Bacillus* spp. *Rhizobium* spp., *Mesorhizobium*, *Bradyrhizobium*, *Azospirillum*, *Azotobacter*, etc. has been reported to increase seed weight, yield, plant height, leaf area, shoot dry weight, and root growth significantly in several crops, such as maize, mung bean, soybean, wheat, groundnut, chickpea, cotton, and *Brassica* spp. (Ahemad and Khan 2010; Ahemad and Kibret 2014; Gholami et al. 2009; Zahir et al. 2010). Mechanisms, such as nitrogen fixation, phosphate solubilization, potassium solubilization, siderophore biosynthesis, IAA production, ACC deaminase synthesis, cytokinin, and gibberellin production, are responsible for plant growth promotion and enhanced crop yield (Bashan and Holguin 1997). Plant disease management mediated by PGPR will curtail the pesticide load and reduce disease in an eco-friendly manner, particularly by posing competition for nutrients, induced systemic resistance, metabolites production, etc. (Lugtenberg and Kamilova 2009). Accumulation of hazardous substances possesses a major threat to the environment. Phytoremediation involves the use of plants or plant product to degrade hazardous substances accumulated in the environment (Cunningham et al. 1995). The compromised growth of plants at contaminated sites can be overcome by application of PGPR (Burd et al. 2000). PGPRs, such as *Agrobacterium radiobacter*, *Azospirillum* spp., *Pseudomonas* spp., *Enterobacter* spp., have been reported to speed up detoxification of contaminants, including cadmium, lead, nickel, chromium, and zinc by increased uptake as well as promotion of growth and biomass accumulation in barley, maize, rye, canola, and tomato grown on contaminated site (Belimov et al. 1998; Belimov and Dietz 2000; Hoflich and Metz 1997; Burd et al. 1998; Lucy et al. 2004). Further, PGPR can survive and promote plant growth in a colder climate with the help of antifreeze proteins and aid in survival under salinity and drought stress by ACC deaminase mediated lowering of ethylene level (De Freitas and Germida 1990; Hamaoui et al. 2001; Vaishnav et al. 2016). PGPR has the ability to promote plant growth under abiotic stresses such as drought, flood, extreme temperature, high light, the presence of toxic metals and organic contaminants, and radiation and biotic stresses: insect predation, the nematodes, fungi, bacteria, and viruses (Glick 2012). Thus the above property of PGPR equips it as potential biofertilizer, biocontrol agent, psychostimulant, and phytoremediator.

Continuously increasing demand for food grain production, the simultaneous buildup of chemical residue in the food chain has led to environmental pollution. The shift toward environmental friendly methods of disease management has thus become the need of the hour. In this context, according to Tewari and Arora (2013), future research needs to be directed toward bioengineering of rhizospheric biology to achieve the desired level of crop yield by manipulating microbes as well as their microclimate. Development of ready-to-use formulation of microbial consortia could be quiet effective over its single products in plant stress reduction. Researches need to be focused on optimizing shelf life, conditions for growth, enhanced crop yield, tolerance to unfavorable environmental conditions, and development of cost-effective PGPR products affordable to farmers. The molecular and biotechnological approaches need to be exploited to explore the rhizospheric biology and attain the desired level of microbial disease control. Bioinoculants of higher efficacy need to be developed for high-value crops such as flowers, fruits, and vegetables. Further, according to Nadeem et al. (2013), the low-temperature stress may be recuperated by exploiting ice-nucleating plant growth-promoting rhizobacteria (Nadeem et al. 2013). In addition, researches need to be focused on potassium-solubilizing plant growth-promoting rhizobacteria for an augmented utilization of potassium, the third most essential macronutrient after nitrogen and phosphorus. A better understanding of plant growth-promoting rhizobacteria needs to be developed regarding the mechanism of action, plant growth promotion, ecology, and growth-stimulating effect on the plant. These will help us in the identification, screening, and development of potential commercial formulations to combat phytopathogens and maintain a sustainable agroecosystem (Nelson 2004; Gupta et al. 2015).

5.6 Conclusion

After having a glance of applications and future prospects, we can conclude that PGPRs have a multidimensional approach in favor of living organisms and the environment. Their efficiency can further be enhanced through their optimization and acclimatization in the provided space. Different inoculation system can be applied on PGPR to maintain their establishment and improve their efficiency. After the competency test, strains with the different feature can be used in combination to survive diverse and extreme environmental condition. Further detailed studies will come up with a more potent rhizobacterial strain to survive diverse ecological situations. Studies at the genetic level can provide us with a next-generation solution through forward or reverse genetics. On a precise note, PGPRs either in combination or alone could be a better and safer alternative to the chemical means.

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Rhizospheric Microflora: A Natural Alleviator of Drought Stress in Agricultural Crops

6

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Abstract

Global climate change is one of the most serious challenges facing us today. Plant growth promotion and productivity are affected due to abiotic stresses which are specifically critical in arid and semiarid regions of the world. Abiotic stresses such as drought, salinity, metal toxicity, etc. are affecting adversely the agricultural crops. The major abiotic stresses in India are drought stress and soil moisture stress. Various abiotic stress management procedures are implemented to reduce these stresses. However, as such strategies are long and costly, there is a need to develop simple and low-cost biological methods for managing drought stress. Plant growth-promoting rhizobacteria (PGPR) manage these stresses by various mechanisms, viz., tolerance to stresses, adaptations, and response mechanisms that can be subsequently engineered into plants to deal with climate change-induced stresses. These affect almost two-thirds of the farming areas of the arid and semiarid ecosystems. Production of indole acetic acid (IAA), gibberellins, and certain unknown determining factors by rhizospheric microflora results in enhanced root length, surface area, and number of root tips, leading to improved uptake of nutrients, thereby enhancing plant health under drought environments. Rhizospheric microflora enhances plant stress tolerance through 1-aminocyclopropane-1-carboxylate (ACC) deaminase and provides protection to agricultural crops from the damage caused by drought stress. These rhizospheric bacteria enhance plant resistance to various biotic and abiotic stresses. Plant growth-promoting rhizobacteria mitigate the influence of drought on crops through a process called induced systemic resistance (ISR), which comprises (a) cytokinin production, (b) antioxidant production, and (c) ACC degradation by bacterial ACC deaminase. Implementation of the rhizospheric microorganisms

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together with novel technologies for their monitoring and risk assessments can contribute to solve food security problems caused by climate change. Present review captures the recent work on the function of microorganisms in helping plants to deal with drought stress which is the major stress caused by climate change.

Keywords
Abiotic stress · Drought · PGPR · ACC · Agricultural crops

6.1 Introduction

The world population is increasing at a faster rate, and sufficient food production is a major challenge for the twenty-first century (Kaushal and Wani 2016). However, chemical fertilizers used in agriculture to increase crop productivity create critical environmental and health hazards (Kaushal and Wani 2016). This is even more intensified by climate change that causes environmental stresses such as drought and salinity which are major restraints to plant growth responsible for decreased agricultural productivity (Kaushal and Wani 2016, Zhang et al. 2010b). Drought can be defined as a prolonged period of dry weather when an area gets less rain than normal, which is a crucial problem to promoting plant growth and increasing productivity in many parts of the world (Ngumbi and Kloepper 2016, Vinocur and Altman 2005). Drought may range from moderate and short to very severe and prolonged periods, restricting plant yields (Vurukonda et al. 2016). Drought stress has a main impact on plant growth limiting crop production worldwide. It has been estimated that almost one-third of soils are too dry to support normal plant development and productivity (Calvo-Polanco et al. 2016, Golldack et al. 2014). Drought is predicted to cause serious plant growth problems on more than 50% of the earth's arable lands by 2050 (Vinocur and Altman 2005). Moreover, global climate change is spreading the problem of water scarcity to regions where drought was negligible in the past (Calvo-Polanco et al. 2016; Trenberth et al. 2014).

Drought is a major abiotic stress that adversely affects plant growth and yield potential (Tiwari et al. 2016). Water deficit caused by drought decreases soil water potential, causing cell dehydration and ultimately inhibiting cell expansion and cell division (Kaushal and Wani 2016). In addition, reactive oxygen species (ROS) produced during drought causes oxidative stress in plants (Kaushal and Wani 2016). There is a requirement to find solutions that enhance plant tolerance to drought stress and allow the productivity of crops that satisfy food demands under limited water condition (Ngumbi and Kloepper 2016; Mancosu et al. 2015).

Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria which naturally occur in the soil that colonize plant roots and promote plant growth and yield. PGPR are generally known to improve plant growth and maintaining sustainability in harmless environments. PGPR genera having plant growth-promoting attributes are *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Azospirillum*, etc. (Singh 2013).

PGPR seems to promote growth by one of the following mechanisms:

- Destruction of plant diseases (bio-protectants).
- Improved nutrient procurement (bio-fertilizers).
- Production of phytohormones (bio-stimulants).

Crop growth promotion rhizobacterial activities have been reported during drought stress in cucumber (Wang et al. 2012), mung bean (Sarma and Saikia 2014), and maize (Vardharajula et al. 2011), as well as during salinity stress in tomato, maize (Bano et al. 2013), (Mayak et al. 2004), and wheat (Tiwari et al. 2011, Kaushal and Wani 2016). Plant growth-enhancing rhizobacteria bring about drought stress tolerance in plants by the induced systemic tolerance (IST) process that involves certain physiological and biochemical changes (Kaushal and Wani 2016; Yang et al. 2009). It involves phytohormone production (Liu et al. 2013; Cohen et al. 2015), defense through antioxidant production (Wang et al. 2012), osmolyte production (Sarma and Saikia 2014), stress-related enzymes (Kim et al. 2012), bacterial exopolysaccharide (EPS) (Vardharajula et al. 2011; Timmusk et al. 2014), volatile organic compounds (Zhang et al. 2010a), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and HCN that can recover stress tolerance in plants (Fig. 6.1). The present chapter is an effort to provide insight into the mechanism shown by rhizobacteria that promote plant productivity and growth by enhanced drought tolerance.

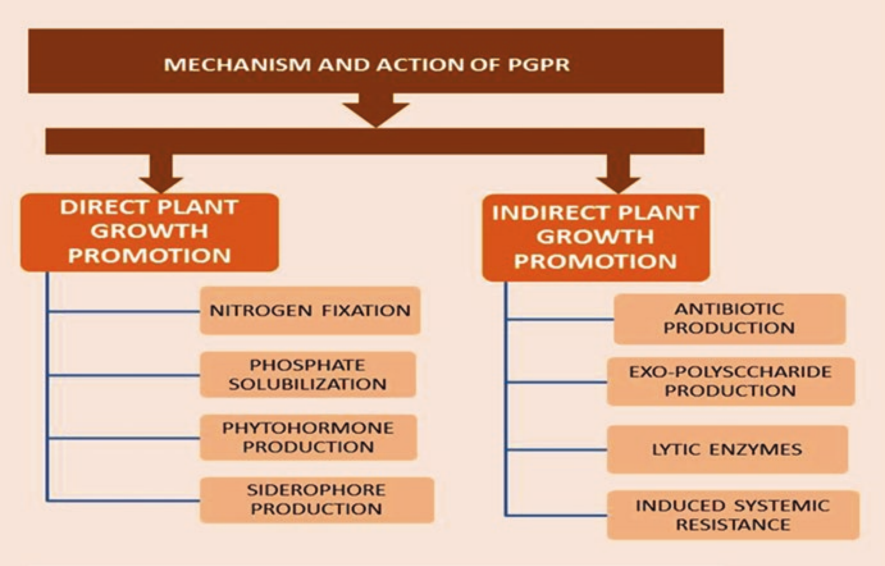

Fig. 6.1 Mechanism revealed by rhizobacteria which enhances growth of plants

Drought affects plant water potential which disturbs the typical functions, changing physiological and morphological characters in plants (Vurukonda et al. 2016). Drought is a multidimensional stress which affects the various compartments of cells or the whole plant (Vurukonda et al. 2016; Rahdari and Hosseini 2012; Rahdari et al. 2012). Growth decrease under drought stress has been studied in some plants, viz., rice, maize, and wheat (Vurukonda et al. 2016). Moreover, drought stress affects the bioavailability and transport of soil nutrients to the roots of plants by water. In this manner, drought stress lowers diffusion of water-soluble nutrients such as nitrate, sulfate, calcium, magnesium, and silicon (Vurukonda et al. 2016, Selvakumar et al. 2012). The formation of plant-associated bacterial communities in the rhizosphere is altered in response to abiotic stress, which improves resistance against stressors to promote stress (drought) tolerance of plants (Schmidt et al. 2014; Cherif et al. 2015; Vurukonda et al. 2016). Drought also influences free radicals for antioxidant defenses and reactive oxygen species like superoxide radicals, hydroxyl radicals, and hydrogen peroxide radicals (Vurukonda et al. 2016). When the reactive oxygen species (ROS) level is high, it can damage certain systems, viz., lipid peroxidation, membrane deterioration, and degradation of proteins, lipids, and nucleic acids in plants (Vurukonda et al. 2016).

Drought stress is one of the main limitations for production of food worldwide, and it is predicted to decrease cereal production by 9–10% (Lesk et al. 2016). Worldwide extensive research is being carried out to develop strategies to cope with drought stress, and most of these technologies are expensive (Khan et al. 2018). Nowadays, microbes are helpful for plants to deal with various (drought) stresses (Venkateswarlu and Shanker 2009).

6.2 Consequence of Drought Stress on Plants and Their Adaptation

Water scarcity is a serious environmental problem that affects crop growth, development, and productivity (Kiranmai et al. 2018). Drought causes high evapotranspiration. Drought stress conditions cause gathering of salts and ions in the first layer of the soil around the roots that leads to osmotic stress and ion toxicity (Fathi and Barari 2016). The size and number of leaves and fruits also decrease during such conditions. Water scarcity causes discoloration of leaf, stomatal closure on the leaf surface, and shrinkage of root and shoot of the plant. Nitrogen metabolism, photosynthetic rate, and protein synthesis in the plant are negatively affected by drought stress.

Resistance to drought is the ability of crops to grow and survive during periods of drought stress (Huang et al. 2014). Plants develop certain mechanisms to deal with drought stress. During this condition, the plant root sends a signal to the whole plant. This signal is the production of abscisic acid (ABA) at the root tip. This includes optimization of water resources, osmotic adjustment, morphological adaptations, and antioxidant systems that reduce the harmful effects of ROS linked with drought stress-responsive genes and proteins (Ngumbi and Kloepper 2016, Farooq et al. 2009).

Adaptations of plants are as below.

1. Drought escape, in which the plant completes its life cycle before the start of drought and undergoes inactivity before beginning of the dry season (Ngumbi and Kloepper 2016).
2. Drought avoidance and phenotypic flexibility, which is the capability of a plant to tolerate its normal water status under drought conditions (Blum 2005). This can be fulfilled when the plant obtains more water from the soil or decreases water loss via transpiration (Ngumbi and Kloepper 2016).
3. Drought tolerance, which occurs when normal growth of the plant and metabolic activities are maintained even under water stress conditions (Ngumbi and Kloepper 2016).

Adaptation of the plant in response to drought includes molecular, morphological, and cellular modifications to avoid damage (Fig. 6.2). (a) Certain major causes alter the physiology of plant which may reduce vegetative growth of crops under water stress. (b) Molecular mechanisms regulate the expression of stress-reactive genes of the plant under abiotic stress (Dos Reis et al. 2016). In plants, water scarcity triggered by drought decreases development and growth, arising from reduction of the water level, reduced water potential, leaf turgor loss, stomatal closure, and reduction in cell enlargement and growth (Jaleel et al. 2009). Response of a plant to abiotic stresses, which involves the extracellular stress signal by receptors of the plant cell, after that many stress regulatory networks which includes signal transduction and transcriptional regulation of stress-reactive gene expression that result in physiological response of resistance to the plant stress (Huang et al. 2012).

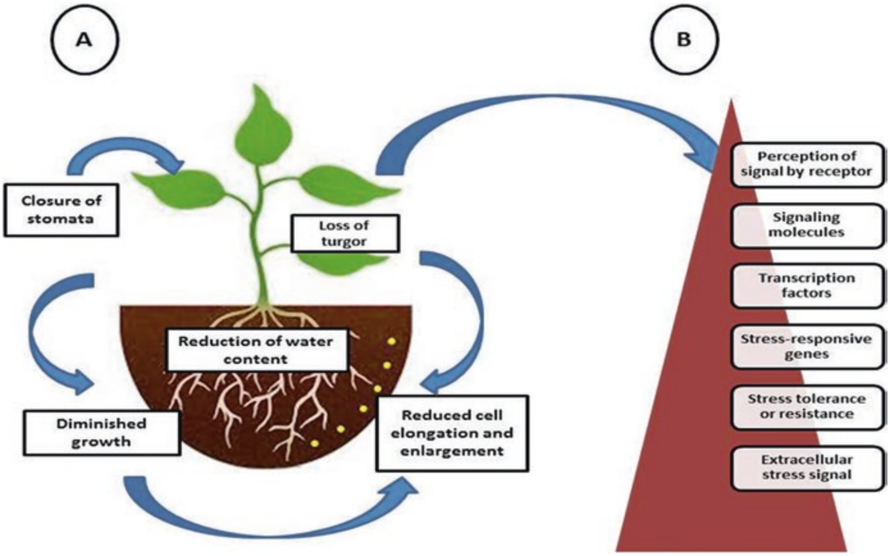

Fig. 6.2 Adaptation of plants to drought stress

6.3 Effective Function of Plant Growth-Promoting Rhizobacteria for Protecting Plants Against Drought Stress

Soil microbes including beneficial soil bacteria experience drought (Ngumbi and Kleopper 2016; Schimel et al. 2007). Drought stress disturbs soil bacteria via osmotic stress and source competition (Chodak et al. 2015) and can result in nucleic acid compensations that may occur through chemical modifications, cross-linking, and base removal (Ngumbi and Kleopper 2016). Tolerance to this stress is assessable in nature and includes accretion of many stress metabolites, like proline, polysugars, abscisic acid, and glycine betaine, and upregulation in the synthesis of enzymatic and nonenzymatic antioxidants, viz., superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, ascorbic acid, α-tocopherol, and glutathione (Gouda et al. 2018). Drought stress creates accumulation of free radicals due to changes in the conformational protein, efficiency of restricted enzymes, and electron transport chain (Ngumbi and Kleopper 2016; Berard et al. 2015). Gathering of free radicals induces protein denaturation and lipid peroxidation that finally leads to lysis of cells (Ngumbi and Kleopper 2016).

Indole acetic acid (IAA) is a synthetic hormone formed in the plant stem and transported down to the root ends (Dimpka et al. 2009). If IAA is produced in low concentrations in the root, it will enhance cell elongation, which will result in increased root growth. In addition, IAA is involved in the promotion of growth of lateral roots. Nevertheless, high amounts of IAA in the root ends have a preventive effect on root development. This prevention could be either directly or indirectly associated with ethylene synthesis associated with auxin (Dimpka et al. 2009). For instance, various studies specified the association between IAA and the ethylene precursor 1- aminocyclopropane-1-carboxylic acid (Glick 2003). By looking through the many literature, a sufficient number of cases where Root-associated PGPR may enhance the growth of plant root exudates, by producing IAA. The different plant species, which were inoculated with such PGPR, displayed root growth enhancement and improved formation of lateral roots and root, thereby promoting more tolerance against drought stress (Table 6.1).

Table 6.1 Microbially facilitated plant tolerance to abiotic (drought) stress

Microbial inoculum	Plant species	Reference
Azospirillum	Wheat (*Triticum aestivum*)	Creus et al. (2004)
Azospirillum brasilense	Common bean (*Phaseolus vulgaris*)	German et al. (2000)
Azospirillum brasilense	Maize (*Zea mays*)	Casanovas et al. (2002)
Achromobacter piechaudii	Tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*)	Mayak et al. (2004)
Osmotolerant bacteria (not completely characterized)	Rice (*Oryza sativa*)	Yuwono et al. (2005)

Furthermore, drought stress can induce conformational changes in proteins and disturb the membrane characteristics of microbes through phospholipid fatty acid composition changes (Berard et al. 2015). Soil microorganisms are small and in close contact with soil water and have semipermeable membranes (Schimel et al. 2007). As water potentials decrease and soils dry because of drought, cells have to accumulate solutes to lower their internal water potential to avoid dehydration and death (Ngumbi and Kleopper 2016). To survive in drought condition and protect cell structures and organelles, the soil bacteria employ a variety of physiological mechanisms including accumulation of compatible solutes, EPS production, and spore production (Schimel et al. 2007; Berard et al. 2015). Gathering of compatible solutes such as proline, glycine betaine, and trehalose enhances thermo-tolerance of enzymes, inhibits thermal denaturation of proteins, and helps in maintaining membrane integrity (Ngumbi and Kleopper 2016).

Plant-associated microbes can function as drought tolerance (Fig. 6.3) through improved root system architecture, improved biological processes, improved physiological processes, and modification of plant growth substances (Ngumbi and Kleopper 2016). Microorganisms also synthesize heat shock proteins (HSPs) that identify with and bind to other proteins if they are in non-native conformations (Ngumbi and Kleopper 2016).

Alternatively, some microbes store high amounts of ribosomes, which permit them to respond with rapid synthesis of proteins when the stress is released (Placella

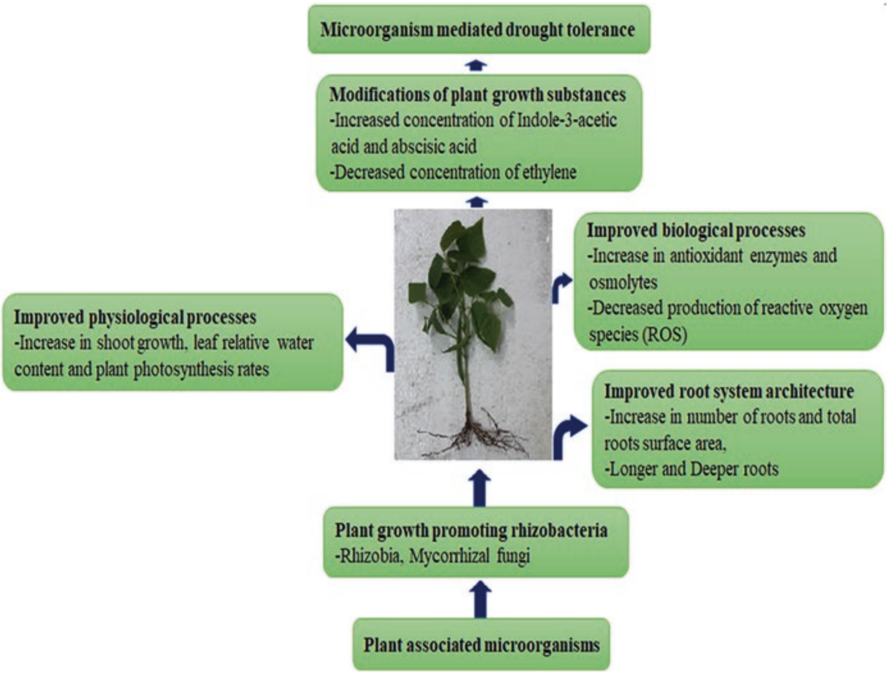

Fig. 6.3 PGPR and drought stress tolerance mechanism

et al. 2012). Other mechanisms that benefit bacteria to deal with water stress are increased efficiency of source use and reallocation within microbial cells and the production of exopolysaccharide (EPS). Exopolysaccharide serves to defend the cell as well as the local environment in which the cell is embedded (Ngumbi and Kleopper 2016).

The strategies used by soil microbes to endure drought stress have also been reported as some of the key adaptation strategies that are employed by plants to survive drought. Many of the compatible solutes that are helpful to bacteria to deal with drought stress also help plants to have drought stress tolerance (Ngumbi and Kleopper 2016).

6.4 Function of ACC Deaminase-Producing Rhizobacteria in Tolerance to Drought Stress

Activities of plants are regulated by ethylene levels. Ethylene biosynthesis is regulated by biotic and abiotic stresses (Hardoim et al. 2008). In the biosynthetic pathway of ethylene, S-adenosyl methionine (S-AdoMet) is converted by 1-aminocyclopropane-1-carboxylate synthase (ACS) further into 1-aminocyclopropane-1-carboxylate (ACC), the precursor of ethylene. Under stress environments, ethylene, the plant hormone, endogenously controls plant homeostasis resulting in decreased root development and shoot growth. Plant ACC is requisitioned and degraded by ACC deaminase-producing microbes to provide nitrogen and energy. Moreover, by removing ACC, bacteria reduce the deleterious effect of ethylene, amending plant stress and enhancing plant growth (Vurukonda et al. 2016). ACC deaminase-producing plant growth-enhancing rhizobacteria *Achromobacter piechaudii* ARV8 significantly improved both the fresh weights and dry weights of tomato seedlings and reduced the production of ethylene under water stress (Mayak et al. 2004). Under water-deficit condition, rhizobacterial growth in dry regions is likely to be major stress-adapting and enhances the growth of plants than those bacterial population where water resources are ample. The seedlings treated with *Achromobacter piechaudii* ARV8, isolated from an arid region, exhibited significantly better growth than the seedlings treated with strain *Pseudomonas putida* GR12–2 that was initially isolated from the grass rhizosphere in the high Canadian Arctic areas where water is rich (Vurukonda et al. 2016). There are certain ACC deaminase-producing PGPR which are used to mitigate drought stress in plants (Table 6.2).

The rhizobacterial presence of ACC deaminase enzyme on the roots decreases the quick synthesis of endogenous ethylene and thus enhances plant growth and yield. Seed inoculation with competitive rhizobacteria having ACC deaminase could be the most effective method for growth promotion of seedlings under control environment conditions. Since the bacterial enzyme ACC deaminase decreases the ethylene level in roots, therefore, inoculation with rhizobacteria having ACC deaminase might be an efficacious tool for the elevation of growth in crops. The use of PGPR containing ACC deaminase can be beneficial in developing strategies to amplify plant growth under drought environments.

Table 6.2 ACC deaminase-producing PGPR in mitigating plant drought stresses

ACC deaminase-producing PGPR	Plant species	Effect	Reference
ACC deaminase-producing rhizobacteria	Wheat	Increased root and shoot length, biomass, and lateral root number	Shakir et al. (2012)
Achromobacter piechaudii ARV8	Tomato and pepper	Reduced production of ethylene and improved fresh weight and dry weight	Mayak et al. (2004)
Bacillus licheniformis K11	Pepper	Higher gene expression of Cadhn, VA, sHSP, and CaPR-10	Hui and Kim (2013)
Enterobacter cloacae, *Enterobacter cancerogenus*	*Jatropha curcas*	Lower levels of ACC resulting in lower levels of endogenous ethylene, which removes the potentially inhibitory properties of stress-induced higher ethylene content or homeostasis regulation	Jha et al. (2012)

Though plant growth-enhancing bacteria use a large variety of mechanisms to promote plant growth, the bacterial attribute which is key in amplifying plant growth is the tenure of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick 2013). This enzyme is responsible for the breakdown of the plant ethylene precursor, ACC, into ammonia and α-ketobutyrate (Honma and Shimomura 1978). By decreasing ACC levels in plants, ACC deaminase-producing organisms decrease plant ethylene levels. Ethylene when present in high concentrations can cause plant growth inhibition (Nascimento et al. 2014).

In response to the occurrence of tryptophan and other small molecules in the root exudates of plants, the attached bacteria produce and release the phytohormone IAA, which is used by plants. It can stimulate plant cell enlargement, and it can induce the transcription of plant enzyme ACC synthase that catalyzes the construction of ACC (Glick 2013). ACC deaminase containing PGPR can lower the ethylene level in plant development following a varied range of biotic and abiotic stresses. ACC oxidase has a greater attraction for ACC than does ACC deaminase. When ACC deaminase-producing bacteria are existing, plant ethylene levels are dependent upon the fraction of ACC oxidase to ACC deaminase. As an outcome, crops can grow in association with ACC deaminase-containing plant growth-enhancing bacteria normally having longer roots and shoots and being more resistant to growth inhibition by various ethylene-inducing stresses (Glick 2013).

6.5 Conclusion

Drought stress may be serious environmental problem that affects agricultural productivity. Plant growth-enhancing rhizobacteria play a crucial role in drought stress tolerance of plants and have the potential in resolving future food security issues. PGPR elicit various mechanisms in confirming plant survival under drought stress.

The development of drought-tolerant agricultural plant varieties via genetic engineering and plant breeding is important, but it is a lengthy process, whereas application of PGPR on plants could improve tolerance to drought stresses in dry land agroecosystem. Taking into account the current stats available, intensive future research is required in terms of identifying the proper solution (technique) for field examination of potent microorganisms for drought stress tolerance of plants. PGPR plays a vital role in improving plant development and can change plant-microbe interactions by altering microbial ecology and bio-geochemical cycles. It is summed up for this chapter that rhizobacteria generally adapt very diverse and multiple approaches to ameliorate drought tolerance in plants like production of phytohormones like auxin, cytokinin, gibberellin, ABA, etc., active enzymes such as 1-amin ocyclopropane-1-carboxylate deaminase, and bacterial products like exopolysaccharide, biofilm, and volatile organic compounds; activation of antioxidants; and increased accumulation of osmolytes like proline, betaine, etc. Application of recent tools and techniques can improve PGPR's role in sustainable agriculture by enhancing soil fertility, plant tolerance, plant productivity, and preserving a balanced nutrient cycle. More studies on selecting suitable rhizobacteria and functional biological strategies can provide new creations and opportunities with huge potential. However, there is still a need to understand the exact mechanism of rhizobacteria-mediated tolerance to abiotic stresses and its intricacy.

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Quorum Sensing Molecules of Rhizobacteria: A Trigger for Developing Systemic Resistance in Plants

7

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Abstract

Induced systemic resistance (ISR) is a widespread phenomenon by which plants develop resistance against various pathogens. A number of plant growth-promoting rhizobacteria are reported to evoke ISR in plants through their surface components, secretion of metabolites, or production of volatile compounds. These compounds in return activate the signaling pathway in plant and allow plants to withstand pathogen attack. Quorum sensing (QS), which is defined as the intercellular communication process, is a crucial feature of rhizobacteria to sense the ecological niche and distribute their population. Signaling process involves the exchange of diffusible signal molecules that serve as autoinducers. The concentration of these QS molecules is a key factor in mediating the gene expression for EPS production, biofilm formation, extracellular enzyme production, etc. and helps bacteria to adapt in a particular environmental condition. In general bacteria have a conserved QS system with central components such as LuxR-type regulator and LuxI-type protein as receptors. At low population density, bacteria produce a low level of QS signals, which are then released in the environment. *N*-acyl homoserine lactones (AHLs) are the major signaling molecules in Gram-negative bacteria, and cyclic peptides serve as signaling molecules in Gram-positive bacteria. Recent studies revealed that AHL molecules

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play important role in plant growth and defense. In this chapter, we will discuss the role of different signaling molecules in inducing plant defense and their mechanism.

Keywords
Rhizobacteria · Quorum sensing · ISR · Signaling molecules

7.1 Introduction

Plants counter a number of challenges for their survival and growth. It includes both abiotic factors (extreme temperature and water conditions) and biotic factors (attack of microbial pathogens, insects, etc.). To deal with these adverse conditions, plants have evolved a well-defined defense mechanism through which they are capable to recognize and combat potential pathogens. The defense system is either host resistance system which is regulated by host R gene, and *avr* gene in pathogen, known as the gene for gene concept (Flor 1971). Another defense mechanism, effective against a wide range of pathogens, is called nonhost system (Gill et al. 2015). To deal with the primary attack, plants have a constitutive defense system that comprises physical barriers such as cell wall, cuticle, epidermis, and chemical barriers, viz., secondary metabolites, antimicrobial proteins, enzymes, etc. Once the pathogens overcome the physical and chemical barriers, inducible defense mechanism gets activated in plants through pathogen recognition and stimuli.

Based on the stimulus, plant defense system can be classified as systemic acquired resistance (SAR) activated by the virulent, avirulent, or nonpathogenic microorganism. SAR results in accumulation of pathogenesis-related protein and salicylic acid. Another type of defense mechanism is induced systemic resistance (ISR) that does not involve the accumulation of pathogenesis-related molecules but activates specific pathways in the host. ISR is generally induced by beneficial microorganism inhabitant of plant's own rhizosphere. Plant secrets root exudates that attract the beneficial microorganism. These microorganisms utilize the nutrients available in root exudates and multiply and colonize in the rhizosphere. These rhizobacteria release the antagonistic compounds that inhibit the growth of soil pathogens and also elicit systemic resistance in plants against plant pathogens, thus suppressing the disease in plants.

Soil rhizobacteria communicate with each other and also with the plants through the small signaling molecules. Bacterial cell to cell communication/signaling is known as quorum sensing (QS). It is a well-understood phenomenon which defines that some of the bacterial genes are only expressed when bacteria are densely populated (Sperandio et al. 2003). QS involve the exchange of diffusible signal molecules that act as autoinducers. Quorum sensing systems are broadly classified into different signaling systems on the basis of their autoinducer signal and mechanism used for its detection. First class of QS signalling molecules is acylated homoserine lactone (AHL), bacteria release, sense, and react to the accumulation of these molecules for synchronizing certain sets of genes that allow adaptation to the environmental changes. This system is common in Gram-negative bacteria and has been identified in more than 70 species of gram-negative bacteria (von Rad et al. 2008; Liu et al. 2012). Another class of signaling molecules is modified oligopeptides. These oligopeptides are synthesized as a precursor and used as autoinducers. These are recognized by histidine kinases sensors and process it to control transcription of quorum sensing target genes. This mechanism is well known in Gram-positive bacteria. Other types of signaling molecules are furanosyl borate autoinducer-2 (AI-2), autoinducer-3 (AI-3), polypeptides, and diffusible signal factors (DSFs), 4-hydroxy-2-alkylquinolines (HAQs), and diketopiperazines, which acts as autoinducing signals (Boyen et al. 2009; Rajput et al. 2016).

These small signaling molecules regulate various traits such as virulence factors, bioluminescence, sporulation, swarming, degradation enzymes, siderophore production, biofilm formation, and plasmid transfer. AHL molecules also help plants to attain priming state which is a self-protection mechanism of plants against microbial pathogens. In this chapter, we have discussed the various signaling compounds released by rhizobacteria and their role in plant defense.

7.2 Rhizobacteria

The area of soil which is directly influenced by plant root exudates and colonized by high microbial population is known as rhizosphere. It is a dynamic nutrient-rich habitat harboring a vast variety of microorganisms (bacteria, fungus, and others). These microbes may have neutral, advantageous, or deleterious effects on the plant growth and development (Berendsen et al. 2012). Different genera of bacteria like *Pseudomonas, Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus*, *Serratia*, and many more comprise the group of microorganisms that augment the plant growth known as plant growth-promoting rhizobacteria (PGPR). These rhizobacteria colonize either at the root surfaces or within the roots and promotes plant growth directly by providing them with the plant growth-promoting substances synthesized by the bacterium or help plant to uptake certain plant nutrients from the environment. Some of PGPR also promote plant growth indirectly through their antagonistic behavior against one or more phytopathogenic microorganisms (Schippers et al. 1987). Rhizobacteria can perform biodegradation, N2 fixation, phosphate solubilization, improve soil fertility, and plant growth promotion. Thus, rhizosphere is a village where a combination of dissimilar advantageous microbes synchronizes mutually with the rest of the environment (Bhargava et al. 2017).

Plant roots also secrete different type of organic nutrients (phytosiderophores, amino acids, vitamins, sugars, organic acid nucleosides, mucilage) and some signaling molecules that attracts microbes. These microorganisms metabolize the plant-exuded compounds and proliferate (Drogue et al. 2013). Carbon availability is one of the major growth-limiting factors for most of the soil microorganisms. Plants fix atmospheric carbon photosynthetically and transport approximately 40% of fixed carbon through their root system (Bais et al. 2006). Microbes present in close vicinity of roots utilize this carbon and proliferate faster than the microbes present in the surrounding bulk soil. This ultimately leads to many fold increase in the microbial population in the rhizosphere and results in a significantly distinct microbial population in rhizospheric soil. This phenomenon is explained as the rhizosphere effect (Bakker et al. 2013). Various application of plant growth-promoting rhizobacteria is shown in Fig. 7.1.

7.3 Activation of Induced Systemic Resistance in Plants Through Rhizobacteria

Since time immemorial, plants have continuously been exposed to the pathogen attacks. Nature has given the plants innate surveillance mechanism that makes them capable to fight against the attempted invasions. Sometimes these mechanisms fail to work when a virulent pathogen succeeds in infecting the plant as it circumvents triggering the defense mechanisms and escapes the plant immune system. To combat such incidences, nonpathogenic soilborne microorganisms, such as AMF and PGPR, come as saviors where they can revamp and boost plant performance by triggering the systemic defense responses that bestow resistance to plant pathogens and insect herbivores having very broad spectrum (Ryals et al. 1992). The inherent immune system of plants depends heavily on two interrelated branches, called PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is stimulated

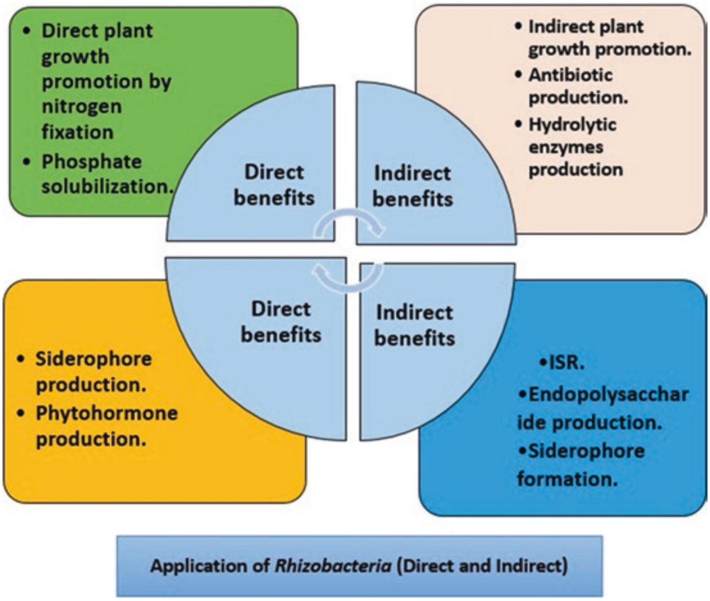

Fig. 7.1 Flowchart depicting the major benefits of rhizobacteria

by identification and acceptance of microbe- or pathogen-associated molecular patterns (MAMPs/PAMPs), which is found in almost all pathogenic and nonpathogenic microbes and act as conserved molecular signatures (Jones and Dangl 2006; Eulgem and Somssich 2007). Systemic tissues show mildly effective immune responses when activated by different microbe-associated molecular patterns (MAMPs). Recognition and identification of MAMPs by pattern recognition receptors (PRRs) at the surface of the cells trigger a pool and cascade of defense responses which confers the plant a threshold level of immunity to combat (Chisholm et al. 2006).

Systemic resistance is the latent resistance regulated by dominant single gene and exhibits broad applying spectrum including fungal, bacterial, and viral diseases. Beneficial rhizobacteria can confer ISR in plants even when the inducing microbe is far away spatially from the disease-causing pathogen. Phenotypically the ISR shows similarity to another kind of pathogen-induced immunity called systemic acquired resistance (SAR). However, rhizobacteria-induced systemic resistance (ISR) term is used for the enhanced state of resilience in plants activated and boosted by root colonization and chemical/biological inducers, which fortify non-exposed plant parts against any attack by a broad spectrum of challenging pathogenic microorganisms in the future. Induced resistance alludes to the actuation of latent combating as well as defense mechanisms that are manifested upon by a subsequent stimulus (Lugtenberg and Kamilova 2009).

The induced resistance can be either systemic or localized. When specifically the tissues which come in direct contact to the primary invader exhibit more resilience then it is termed as localized acquired resistance. Both SAR and LAR can target a range of pathogens. Studies reveal that a particular signal responsible to propagate and amplify the induced defensive capacity and spread it throughout the host is absent in LAR which differentiates it from SAR. Usually, salicylic acid (SA) also induces phenotypic responses which are similar to SAR, but studies reveal that jasmonic acid (JA) and ethylene (ET) signaling proteins are more involved in the rhizobacteria-mediated induced systemic resistance (ISR).When the studies on leaves of the induced plants inoculated by pathogens were done, it was found that there was an increase in expression of SA pathway genes on the leaves which expressed SAR, whereas leaves expressing ISR showed enhanced expression of JA-/ET-responsive genes. Therefore, both ISR and SAR work in harmony and give a synergistic effect to protect the plant against pathogens that are smart enough to resist both pathways and increase the periphery of the spectrum of protection against pathogens when compared to the conditions where only ISR or SAR was present (Van Loon 2007).

Varied factors play role in the elicitation of systemic resistance in different time frames and situations ranging from structural components like flagellin, lipopolysaccharides, and exopolysaccharides to their metabolic products like siderophores and antibiotics. Volatile organic compounds (VOCs), like acetoin and 2,3-butanediol, secreted by *Bacillus subtilis* GB03 have also been reported to activate an ISR pathway in seedlings of Arabidopsis which were inoculated with *Pectobacterium carotovorum* (syn. *Erwinia carotovora* subsp. *carotovora*) (Ryu et al. 2004; De Vleesschauwer and Hofte 2009). ISR can also be induced by certain quorum sensing (QS) molecules like acyl-homoserine lactones (AHLs), which are known to regulate the expression of those genes which are responsible for a number of physiological functions in various Gram-negative bacteria in a cell density-dependent manner. Various juxtacrine communication signals are exchanged among rhizobacteria cells, and paracrine signals are sent among other microbes be it bacteria or fungi which predominantly reside in the rhizosphere which helps the microbes to keep a check on their density and coordinate gene expression only when a quorum of cells is achieved (Zavilgelsky and Manukhov 2001; Miller and Bassler 2001; Schuhegger et al. 2006). Other bacterial signals that regulate gene expression independently of the cell density are also present.

7.4 Triggering Factors for ISR

The eliciting factors secreted by ISR-triggering rhizobacteria are different from the elicitors of pathogen as they don't cause any localized necrosis. A number of factors are responsible for the elicitation of resistance that include pathogens in avirulent forms, nonpathogenic microbes, certain chemicals, or in some cases the virulent pathogens. They act, respectively, in different time zones and places. These activators of innate immune response include dead microbial cell wall preparations; cell surface components, viz., outer membrane lipopolysaccharide (LPS) and flagella; and metabolites like siderophores which can also trigger defense-associated reactions in suspension-cultured plant cells and leaves. Different time periods are required for different sets of host plants and their elicitors, to accumulate pathogenesis-related proteins (chitinase and glucanase) and salicylic acid which helps to trigger and establish the resistance (Gómez-Gómez and Boller 2002; Erbs and Newman 2003). ISR is supported by plant growth-promoting rhizobacteria (PGPR) in a huge amount, and some characterized strains of *Pseudomonas* show the best of the results showing no visible damage to the plant's root system. Some major triggering factors (Fig. 7.2) have been discussed below.

7.4.1 Flagella

The innate immunity of plants stands tall upon the pillars of two interrelated branches, termed PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is triggered by specific conserved molecular signatures present in many pathogenic as well as nonpathogenic microbes called PAMPs/MAMPs (Eulgem and Somssich 2007). These signatures are present in bacterial flagellum and prove their worth in cells motility, virulence capacity of bacterial pathogens, and process of root colonization by rhizobacteria (Ramos et al. 2004). A conserved 15–22 amino acid stretch in Flg22 lying close to the conserved N-terminal domain of flagellin is perceived as a PAMP by the innate immune systems of many plants and animals. Flg22 is an robust elicitor in cell cultures of different plant species such as tomato, tobacco, Arabidopsis, potato, etc. (Felix et al. 1999). In the plant model of

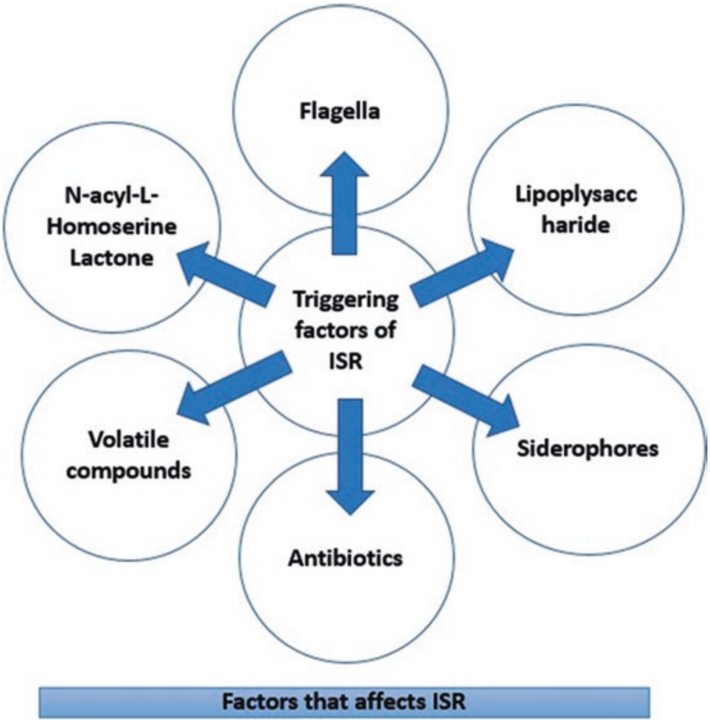

Fig. 7.2 Factors which can trigger induced systemic resistance in plants

Arabidopsis, flagellin is recognized through its direct interaction with the transmembrane leucine-rich repeat receptor kinase (LRR-RK) FLS2. Though Flg22-type sequences are universally found in flagellins in divergent families of bacteria, including *Pseudomonas putida* and *Ps. Aeruginosa*, however the flagellins of the plant-associated bacteria *Agrobacterium* and *Rhizobium* show the presence of a highly different flagellin sequence which do not have the ability to stimulate the flagellin perception system (Felix et al. 1999; Chinchilla et al. 2006).

The early response to flg22 directly involves the onset of several cascades of the JA, ET, and SA defense signaling pathways, while the late responses involve activation of SA-regulated processes (Denoux et al. 2008). The experiments done on isolated flagella and non-motile mutants of *Pseudomonas putida* strain WCS358 which lacked flagella using Arabidopsis, bean, and tomato as hosts also established the involvement of flagella in ISR (Meziane et al. 2005). Thus flagella, among the other pathogen-associated molecular patterns (PAMPs), have a major contribution in triggering the plant defense response which leads to substantial decrease in the numbers of the pathogen in plants.

7.4.2 Lipopolysaccharides

Lipopolysaccharides (LPS) are complex molecules which can be both hydrophilic as well as lipophilic in nature. Besides constituting the major structural component giving tensile strength to the outer membrane of almost all Gram-negative bacteria, they also play a number of important roles in the interactions of bacteria with eukaryotic. They serve as a prototype role model for PAMP in almost all resistance-related experiments. They serve as important virulence factors in pathogenesis triggered in plants by nonhost and avirulent bacteria owing to their capacity to curb hypersensitive responses. Their response is called "localized induced resistance" or "response" and is visible in various plants like tobacco, pepper, turnip, and Arabidopsis. LPS also alter and signal plant tissues to respond more rapidly or to a greater extent to phytopathogenic bacteria. After adhesion to cell wall components, LPS binds to specific plasma membrane receptors which sensitize and signal a series of events for accelerated synthesis of antimicrobial hydroxycinnamoyl-tyramine conjugates, which are associated to a family of pathogenesis-related (PR) proteins (Dow et al. 2000; Erbs and Newman 2003).

LPS owe their inducing capacity to highly conserved lipid A core region which can also trigger the production of nitric oxide. Lipid A and core oligosaccharides derived from the lipooligosaccharide from *Xanthomonas campestris* pv. *campestris* show elicitation of PR1 and PR2 in Arabidopsis and prevent the hypersensitive response (HR) triggered by certain avirulent bacteria (Zeidler et al. 2004; Silipo et al. 2005). LPS-induced resistance has been observed in carnation against *Fusarium* wilt by *Pseudomonas fluorescens* WCS417. Likewise, LPS of *Ps. fluorescens* strains WCS 374 and WCS 417 elicit systemic resistance in radish against *F. oxysporum* f. sp. *raphani* (Leeman et al. 1995). LPS of WCS 417r and mutant of WCS 417r lacking O-antigen side chain of LPS are shown to induce defense mechanism in Arabidopsis (Van Wees et al. 1997). Similarly, Van Loon et al. in 1998 showed that bacterial mutants lacking the O-antigen (OA) trigger systemic resistance in radish. LPS from *Burkholderia cepacia* had an insulating effect on the *Nicotiana tabacum-Phytophthora nicotianae* interaction, and LPS of the pathogen *P. solanacearum* induced systemic disease resistance in tobacco leaves (Coventry and Dubery 2001).

7.4.3 Siderophores

Siderophores are low-molecular-weight metabolites having high affinity for iron (III) (Kf> 1030), exuded by almost all aerobic and facultative anaerobic microbes. Their biosynthesis is up- or downregulated by iron levels in the environment mostly soil. They supply iron to the cell to entrap little traces of ferric iron [Fe(III)] in the surrounding niche and supply that the iron to the cell. Usually there is a shortage of iron concentration in rhizosphere, and competition for iron through the production of siderophores is used as a mechanism of bacterial antagonism against soilborne pathogens. ISR-eliciting rhizobacteria profusely produce siderophores that serve two purposes in disease suppression: one it deprives resident pathogens of iron, and second it induces systemic resistance in the plant. Most of the pathogenic microorganisms can control the regulation of siderophore production and virulence factors during process of the disease production depending on the iron status of the host. All bacterial siderophores have the capacity to sequester iron, and some of them can elicit ISR also.

One of the predominant soilborne bacterium *Pseudomonas aeruginosa* 7NSK2 produces two important siderophores, namely, pyoverdine and pyochelin. Buysens et al. in 1996 proved that pyochelin is known to reduce the damping-off disease caused by *Pythium splendens* on tomato produced by *Ps. aeruginosa* 7NSK2. In tomato seedlings, a bacterial mutant unable to produce both pyochelin and pyoverdine was found to be less resilient against disease than the WT strain. After pyochelin complements were brushed on the mutant, it regained its defensive property in tomato, indicating that pyochelin plays a major role in immunity against damping-off in tomato. These results show direct competition for iron between *Ps. aeruginosa* and *Ps. splendens*, but they also pave the path for the possibility of stimulation of defense mechanisms in plants by siderophores.

Pseudobactin (PSB), also termed pyoverdin or fluorescein, works in a complex autoregulatory manner and elicits their own synthesis and uptake in a cell density-dependent manner. They give maximum expression of the cognate synthesis and receptor genes in the presence of siderophores (Visca et al. 2007). PSB siderophore of WCS374 induces ISR against Fusarium wilt. Most reports indicate that the ISR involves ET and JA pathway proteins and exclude SA in their mechanism (Vleesschauwer and Hofte 2009). Ran et al. in 2005 proved that microbes colonizing the aerial parts of the plants can also produce siderophores as shown by *Ps. fluorescens* which can trigger ISR on *Eucalyptus urophylla* against bacterial wilt caused by *Ralstonia solanacearum*. Besides playing their crucial role as elicitors, they can also disturb the plant hormone balance and induce strong changes in heavy metal distributions in the plant.

7.4.4 Antibiotics

Antibiotics consist of a heterogeneous group of chemicals which are organic in nature and have low-molecular-weight whose function is to inhibit the growth or metabolic activities of other microorganisms (Duffy et al. 2003). The mechanism of antibiosis is based on the secretion of certain inhibitory molecules which are commonly associated with the ability of plant growth-promoting bacteria to act as antagonistic agents against phytopathogens (Glick et al. 2007).

Antibiotics are beneficial to plants as they circumvent the growth of pathogens (Weller et al. 2002). Antibiotics like 2,4-diacetylphloroglucinol (DAPG) are known to trigger the induced systemic response in Arabidopsis. The signaling route of the DAPG-induced resistance is unique in the sense that it is independent of master transcriptional regulator NPR1 and the functional JAR1 protein (Iavicoli et al. 2003). Heterocyclic N-containing blue phenazine pigment pyocyanin has been found to play a determining role in rhizobacteria-elicited ISR (Britigan et al. 1997). Audenaert et al. in 2002 showed that pyocyanin produced by the rhizobacterium *Ps. aeruginosa* 7NSK2 works synergistically with the SA-derivative pyochelin in triggering systemic resistance in bean and tomato against *Bo. cinerea*. Likewise, in Arabidopsis, this antibiotic triggers ISR in conjunction with the pyochelin siderophore, 2,4-diacetylphloroglucinol (DAPG), establishing it as an triggering determinant of *Ps. fluorescens* strains CHA0 and Q2-87 (Iavicoli et al. 2003; Weller 2007). Siddiqui and Shaukat in 2003 observed that *Ps. fluorescens* CHA0 induces resistance in tomato, against the root-knot nematode *Meloidogyne javanica*. These findings suggest that rhizobacterial DAPG can elicit ISR, opening the doors to explore more antibiotics that may be capable of eliciting ISR implants. Besides showing antagonism antibiotics also enhance the overall defensive capacity of plants.

7.4.5 Volatiles

Volatile organic compounds (VOCs) effectively promote plant growth and elicit host defense mechanisms in a number of plants. Rhizosphere emits certain VOCs in the periphery of root-colonizing bacteria as low-molecular-weight compounds (Pare' et al. 2005). Many low-molecular-weight compounds like growth-promoting volatile 2R, 3R-butanediol have been found from complex bacterial emissions of many dominant bacterial strains with the help of gas chromatographic analysis (Ryu et al. 2004). Later it was found that the production of 2R,3R-butanediol is directly proportional to rate of elicitation of systemically induced resistance against *Er. carotovora* in tobacco by *Ps. chlororaphis* O6 (Han et al. 2006).

Other volatiles produced by rhizobacteria include acetoin (3-hydroxy-2 butanone), produced by *Bacillus subtilis* and *Bacillus amyloliquefaciens* and some strains of *Streptomyces* (Farag et al. 2006; Li et al. 2010). They protect the plant by reducing the severity of disease besides curbing the proliferation of pathogens in the leaves. However they pose some serious challenges to apply in the field owing to their low efficacy and evaporative nature as compared to other chemical pesticides; nevertheless, some volatile compounds have been used successfully in the field to control plant disease (Song and Ryu 2013). Researches are in full swing to explore signaling pathways to establish the role of microbial volatiles in regulating a series of cellular processes, including plant growth and development, pathogen defense, and abiotic stress adaptation (Cho et al. 2008).

7.5 Bacterial Signaling Molecules: Acyl Homoserine Lactone

Rhizosphere comprises soil, plant roots, microorganism, and host to complex plant-microbe and microbe-microbe and plant-insect interactions. Plant roots take up nutrients from the soil and release vitamins and minerals as root exudates to maintain a nutrient-rich environment. The composition of root exudates varies among the plant's cultivar and is dependent on plant health status and their environmental conditions. Signaling molecules or secondary metabolites secreted from rhizobacteria as well as plants exudates secreted by roots are recognized and serve as communication media. The ecologic features and physical nature of the rhizosphere are defined by the root exudates released and the interactions in the rhizosphere. This interaction may be (i) bacteria-bacteria communication via bacterial-derived signaling molecules and (ii) plant-microbe communication via plant-secreted small signaling molecules or microbe-secreted signaling molecules which are sent to the host (Venturi and Keel 2016).

Among all the signaling molecules, AHLs are the well-characterized autoinducers. These are widely produced by various microbial genera such as *Agrobacterium*, *Brucella*, *Burkholderia*, *Chromobacterium*, *Enterobacter*, *Erwinia*, *Hafnia*, *Methylobacter*, *Paracoccus*, *Pseudomonas*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Rhanella*, *Serratia*, *Vibrio*, and *Yersinia* (Williams et al. 2007).

Besides rhizobacteria, many endophytes have also been reported to secret QS compounds. *Burkholderia phytofirmans* produces 3-hydroxy-C8-HSL (Sessitsch et al. 2005); *Serratia plymuthica* secretes high amount of C4–C8-HSL and also 3-oxo derivatives of HSL (Liu et al. 2011). Epiphyte (*Pantoea agglomerans*) and endophyte (*Erwinia tolerance*) of olive plant secrete AHL-like compounds which were reported to interfere with the *Pseudomonas savastanoi pv. savastanoi* virulence causing knot disease in olive plants (Hosni et al. 2011). Signaling molecules produced by general PGPR strains are summarized in Table 7.1.

The molecular structure of AHLs varies from organisms to organisms and secretes in concentration range of pM to nM. Most of the AHL-producing organisms possess a conserved QS system consists of LuxR- and LuxI-type protein and

Table 7.1 Signaling molecules produced by PGPR strains

AHL	Organism	Reference
C6-HSL, C8-HSL	*Burkholderia* sp.	Chen et al. (2013)
3-hydroxy C8HSL	*Burkholderia phytofirmans*	Ryan et al. (2015)
3-Oxo-C6-HSL	*Erwinia carotovora*	McGowan et al. (1995)
3-Oxo-C10-HSL, 3-oxo-C12-HSL	*Pseudomonas putida*	Fekete et al. (2010)
3-Oxo-C10-HSL, C6-HSL, C8-HSL	*Pseudomonas fluorescens*	Khan et al. (2005)
3-oxo-C12-HSL	*Ps. aeruginosa*	Mellbye and Schuster (2014)
C6-HSL, 3-oxo-C6-HSL, C7-HSL, C8-HSL	*Serratia marcescens*	Ryu et al. (2013)
C4-C8 HSL, 3-oxo C8 HSL	*Serratia plymuthica*	Pang et al. (2009)
C4 HSL	*S. liquefaciens*	Schuhegger et al. (2006)
3-Oxo-C8-HSL	*Agrobacterium tumefaciens*	Zhang et al. (2002)
HSL, 3-Oxo-C6-HSL	*Rhizobium* sp.	Sanchez-Contreras et al. (2007)
C8-HSL	*Mesorhizobium huakuii*	Wang et al. (2004)
C8- and C12-HSL	*Gluconacetobacter diazotrophicus*	Nieto-Peñalver et al. (2012)
3-Oxo-C16-HSL	*Sinorhizobium meliloti*	Mathesius et al. (2003)

an AHL synthase, respectively. When the concentration of AHL reaches to a threshold, it binds with the LuxR-type proteins and control transcription of target genes. All the naturally occurring AHL molecules contain homoserine lactone ring which is N-acylated with a fatty acyl group at the α-position (Chhabra et al. 2005). The chain length of AHL varies in side chain length which is consists of 4–14 carbon atoms and may also vary in saturation level. Some AHL has unsaturation at C3 atom, whereas some contain unsaturation at 5 or 7 carbon atom. Therefore, different AHL shows quite different physicochemical properties. It is reported that bacterial QS not only used for communication within the same species but QS signals are involved in cross-talk with other bacterial species (interspecies) and also with host and organisms of different kingdoms (interkingdom) (Williams 2007).

7.5.1 Role of AHL in Plant Development and Defense

In the recent past, several lines of data demonstrated that the plant development and wide communication occur between plants and naturally occurring rhizobacteria colonizing the roots of plants through signaling molecules of both the partners. Elasri et al. (2001) observed that rhizospheric soil was more populated with AHL-producing bacteria than bulk soil. This observation advocates that plants could select their microbial partners through the secretion of selective root exudates. In return, these rhizobacteria secrete QS molecules that coordinate with the plants and control several morphological traits in plants (Pierson et al. 1998; Chin-A-Woeng et al. 2003; Liu et al. 2007). The biological activity of AHLs varies with the C chain and their lipid side chains. Short-chain (4–10 carbons) AHLs improve plant growth (Gao et al. 2003; von Rad et al. 2008). Bai et al. (2012) observed the application of AHL substituted at the C3 position with ketone group (oxoC10-homoserine lactone) in mung bean which activates auxin-induced adventitious root formation using H_2O_2- and NO-dependent cyclic GMP signaling. Ortíz-Castro et al. (2008) established the fact through postembryonic root development studied in *A. thaliana* that C10-HSL influence the cell division in the meristem and affects the primary root development and lateral root formation. Furthermore, 6-μM C6-HSL treatment of seedlings for 11 days also resulted in improvement of root and shoot biomass. The modulation of a number of growth traits in *A. thaliana* was reported through the interaction of AHL with each other as well as with prokaryote domain (Zhao et al. 2013). Moreover, Palmer et al. (2014) suggested that L-homoserine is released by the degradation of AHLs by the fatty acid amide hydrolase. L-homoserine helps plants to uptake more water and minerals and consequently improves plant growth. Latest finding of Venturi and Keel (2016) revealed that the complex process of rhizospheric nitrogen mineralization and nitrogen cycling in legumes plants is also controlled by density-dependent behaviors of AHL-producing alpha-proteobacteria.

In the earlier work, it is reported that AHLs can be perceived by plants and modulate plant defense and cell growth responses, metabolism, and root and shoot growth through modification in gene expression. It is evident from several reports that AHLs induce resistance in plants and play an important role in plant immunity. The changes at the transcription level upon AHL treatment of *Medicago truncatula* roots were reported for the first time in the studies of Mathesius et al. (2003), where the author found that the treatment of *Medicago truncatula* roots with N-3-oxo-hexadecanoyl homoserine lactone (3-oxo-C16-HSL) and N-3-oxo-dodecanoyl homoserine lactone (3-oxo-C12-HSL) resulted in the modification of the quantity of 150 proteins that were related to the phytohormone production, defense and stress management, and metabolic regulation. Microarray studies conducted with AHL-treated and AHL-untreated roots of tomato plants indicate that expression of many defense-related proteins is induced in the shoots of treated plants (Hartmann et al. 2004). In another study, Barriuso et al. (2008) studied the role of AHL producing a strain of *Burkholderia graminis* M12 and M14 in plant growth promotion as well as initiation of plant defense against salt stress in tomato plants and their transgenics expressing *yen I* (short-chain AHL producer) and *LasI* (long-chain AHL producer). Results indicate that both the strains were not capable to improve plant defense and growth promotion in all the conditions, but M12 could improve plant growth in wild type and stimulate salt resistance in *LasI* transgenic line, whereas M14 enhanced plant growth only in *LasI* transgenic line and induced resistance against salt stress in wild type.

AHL-producing bacterial strains mediated induced resistance in *A. thaliana*, tomato, and barley against biotrophic, necrotrophic, and hemibiotrophic pathogens, respectively, by eliciting SA-dependent pathways which was also studied in other dicotyledonous plants (Schuhegger et al. 2006; Schikora et al. 2011; Schenk and Schikora 2015). *Serratia liquefaciens* MG1, an AHL-producing strain, provide immunity to *A. thaliana* against the fungal pathogen *Alternaria alternata* (Schuhegger et al. 2006). Another AHL-producing strain of *Pseudomonas putida* was also reported to enhance systemic resistance of tomato plants in response to the fungal leaf pathogen, *Alternaria alternate* (Schuhegger et al. 2006), while *Serratia plymuthica* reduced the symptoms of gray mold disease in tomato and bean plants (Pang et al. 2009) and also stimulated defense reactions in cucumber plants against the damping-off disease caused by *Pythium aphanidermatum.* Similar findings were reported in the studies of Benhamou et al. (2000), where endophytic bacterium *Serratia plymuthica* containing cucumber seedlings could prevent fungal attack more efficiently than the untreated control indicating the activation of induced resistance in cucumber seedlings. Liu et al. 2007 isolated antibiotic pyrrolnitrin producing. *S. plymuthica* HRO-C48 strain producing C4-/C6- and OHC4-/OHC6 from the rhizosphere of oilseed rape confers ISR-like systemic protection of bean and tomato plants against the fungal leaf pathogen *Botrytis cinerea.*

Schikora et al. (2011) reported that AHL-induced resistance is mediated through modified activation of *MPK6*, the mitogen-activated protein kinases MPK3. It was observed that root exposure to C12- and C14 N-acyl-AHLs, activated MPK6 which induced higher resistance in *A. thaliana* and barley toward obligate biotrophic fungus *Golovinomyces orontii* and *Blumeria graminis* f. sp. *hordei*, respectively. Further studies also revealed increased expression of the defense-related transcription factorsWRKY26 and WRKY29 as well as the PR1gene in the *A. thaliana*.

Schenk et al.(2012) concluded that short and medium side chain AHL affects the root development, whereas long side chain AHLs elicit systemic resistance in *A. thaliana.*

In transgenic tobacco plants, Ryu et al. (2013) reported the *Serratia marcescens* 90–166 mediated modification in the induced systemic resistance during constitutive expression of QS genes. Differential regulation of the cytoskeleton and defense-related 53 proteins were also observed in oxo-C8-HSL-treated Arabidopsis seedlings (Miao et al. 2012). Nieto-Peñalver et al. (2012) demonstrated that sugarcane endophyte *Gluconacetobacter diazotrophicus* produces C8- and C12-HSL that could be involved in enhancing the resistance in sugarcane against *Xanthomonas albilineans.* Root treatment of *Hordeum vulgare* with C8- and C12-HSL resulted in a systemic reduction of the biotrophic pathogen *Xanthomonas translucent* in leaves compared to controls. It was found that application of short- and long-chain HSL initially showed slow response, but after 4 h of incubation, the highest of SA level was attained, thus confirming the enhanced AHL-mediated systemic resistance against the pathogen. Zarkani et al. (2013) also reported the inhibition of tomato bacterial speck caused by *Pseudomonas syringae* pv. *tomato* DC3000 by oxo-C14-HSL-producing *Sinorhizobium meliloti* Rm2011 strain (Table 7.2).

7.5.2 Mechanism of AHL-Induced Resistance in Plants

The physiological stage of plants in which they act more efficiently to activate their defense response against abiotic or biotic stress is known in the primed stage of plants, and the mechanism through which plants enter to the primed stage is priming. The modified response does not require the activation of a complex cascade of genes, but it is a process of augmentation of defense-related signals (Conrath 2009; Slaughter et al. 2012). A variety of factors, viz., colonization of plant roots with beneficial rhizobacteria, infection with pathogens, or application of some natural or synthetic organic volatile compounds, can induce priming in plants (Conrath et al. 2006). However priming is not completely understood at molecular level, two mechanisms have been postulated. According to Beckers et al. (2009) inactive mitogen that activated protein kinases plays as important role in priming of plants. Inactive mitogen-activated protein kinases get activated through secondary stimulation and induce priming. Another report claimed the acetylation (H3K9, H4K5, H4K8, and H4K12) and methylation (H3K4me3 and H3K4me2) of histone protein in the promoter regions of the defense-associated (WRKY6, WRKY26, and WRKY53) transcription factors (Jaskiewicz et al. 2011). The priming state can be divided into pre-challenged, post-challenged, and transgenerational primed state (Balmer et al. 2015).

During the last decades, several reports claimed that AHLs that serve as signaling molecules in bacteria enhance plant defense response against forthcoming pathogens through priming (Schikora et al. 2011; Pieterse et al. 2014). Plants have a well-defined defense mechanism to defend the pathogen attack. These mechanism used by the plants is either jasmonic acid (JA) and ethylene (ET) dependent or

Table 7.2 AHL molecules and their role in different plants

AHL type	Plant reaction	Plant species	References
C6-HSL	Primary root elongation, transcriptional and metabolism regulation	*A. thaliana*	von Rad et al. (2008)
C6-HSL	Upregulation of defense genes	*Lycopersicon esculentum*	Schuhegger et al. (2006)
Oxo-C6, oxo-C8	Root growth and development	*A. thaliana*	Liu et al. (2012)
3-oxo-C6 (*Serratia plymuthica*)	Triggering plant immunity	*Cucumis sativa* *Lycopersicon esculentum*	Pang et al. (2009)
C6, C8, C10	Root and shoot growth	*Hordeum vulgare*	Götz et al. (2007)
3-O-C10	Adventitious root formation	*Vignaradiata*	Bai et al. (2012)
C12- HSL	Root hair development	*A. thaliana*	Ortíz-Castro et al. (2008)
3-oxo-C16-HSL, 3-oxo-C12-HSL	Defense and stress management	*Medicago truncatula*	Mathesius et al. (2003)
C4-/C6-andOHC4- /OHC6	ISR-like systemic protection	Bean and tomato	Liu et al. (2007)
C12- and C14-*N*-acyl-AHLs	Induced resistance against pathogen	*A. thaliana* and Barley	Schikora et al. (2011)
Oxo-C8-HSL	Regulation of cytoskeleton and defense-related proteins	*A. thaliana*	Miao et al. (2012)
C8-C10, C12, C14-HSL (endophyte *Gluconacetobacter diazotrophicus)*	Induced resistance	Sugarcane	Nieto-Peñalver et al. (2012)
oxo-C14-HSL	Inhibition of tomato bacterial speck	Tomato	Zarkani et al. (2013)
C8- and C12-HSL	Root elongation, defense	Barley	Rankl et al. (2016)
3-oxo-C14-HSL,	Increase in root nodulation	*Medicago truncatula*	Veliz-Vellajos et al. (2014)
C6-C8, C10-HSL	Modulate activity of glutathione *S transferase* and *dehydroascorbate reductase*	Barley	Gotz-Rosch et al. (2015)

salicylic acid (SA) dependent based on the pathogens. Involvement of SA-dependent defense in AHL-mediated priming was reported in tomato plants where AHL-producing *Serratia liquefaciens* strain MG1 enhances the accumulation of SA in plants upon pathogen attack (Hartmann et al. 2004; Schuhegger et al. 2006). These results were further supported when treatment of tomato plants with pure C6- and C4-HSL leads to the higher expression of two *chitinase* genes and *pathogenesis-related 1a* (*PR1a*) gene. It is important to mention that expression of PR1a is regulated by SA and ET. Similar results were also reported in Rankl et al. (2016), where exposure of barley roots with C8- and C12-HSL results in the accumulation of SA and ABA barley leaves, whereas JA and isoleucine contents were unaffected. During the study, another interesting observation made was the accumulation of nitric oxide (NO) in HSL-treated roots. It was interpreted that NO could be the second messenger leading to SA accumulation in leaves. Therefore, it was concluded that AHL induced systemic resistance via defense gene priming in response to biotrophic pathogen *Xanthomonas translucens* pv. *cerealis* in barley (Fig. 7.3).

Joseph and Phillips (2003) observed that treatment of roots of bean plants with 10 nM of homoserine lactone improves the stomatal conductance and rate of transpiration up to 30% in the shoot of the plant. This increased transpiration could be due to the increased flow of water and nutrients from the bulk soil to the rhizosphere. Schikora et al. (2011) found the better formation of papillae in oxo-C14-HSL-pretreated barley plants than the untreated plant when challenged with pathogenic fungus *Blumeria graminis* sp. *hordei*. These results were further supported by Schenk et al. (2012), where a researcher found that AHL pretreatment in *A. thaliana* increases the callose deposition, lignins, and accumulation of phenolic compounds in the cell wall, and the expression of genes in the cell wall and

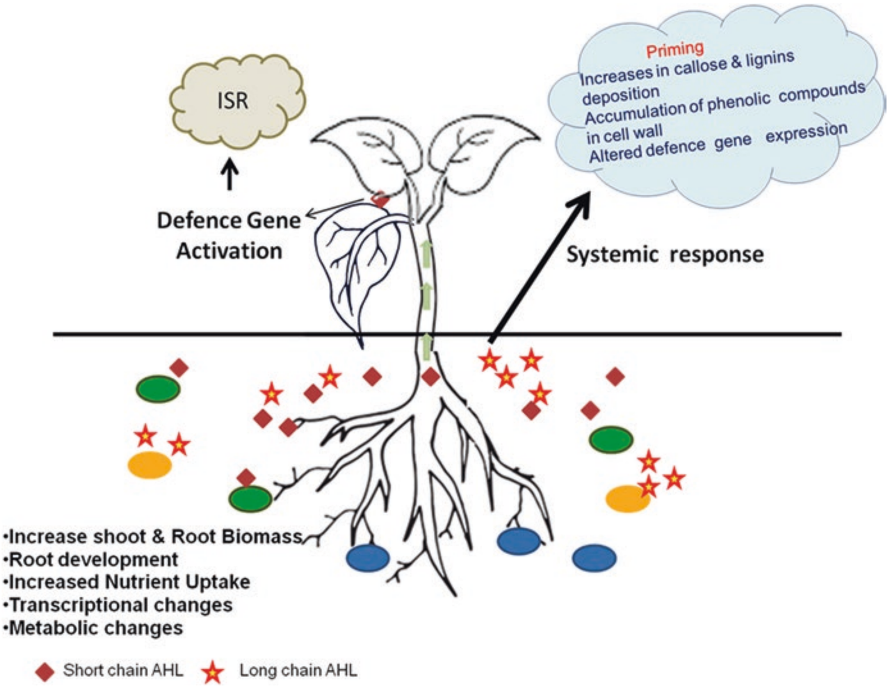

Fig. 7.3 Role of AHL in plant defense. Short- and long-chain AHL molecules secreted from bacteria are recognized by plants and enhanced nutrients uptake, shoot and root biomass, and root development. AHL signals leading to priming effects on plants and enhanced defense response. Short-chain AHLs are transported also to the distal part of plants and activate defense pathway

glycoprotein functional categories was also enhanced. Moreover, a higher number of closed stomata and reduction in open stomata indicated SA-dependent pathways activate the stomatal defense response. These results concluded that AHL application primed plants for cell wall reinforcement (nAHL prime plant for cell wall).

7.6 Concluding Remarks

The mutualistic relationship between plant and microorganisms in rhizosphere has been the major area of study and interest for both plants and microbiologist. Use of various microorganisms in the latest agricultural applications is the outcome of these researches. Research carried out in the last two decades in the area of plant-microbe interaction have shown that soil organisms communicate with each other through intercellular signaling and with the host by interkingdom signaling. It is an established fact that plants are able to shape their own microbiome through root exudates that attract various microorganisms (bacteria, fungi, and viruses), nematodes, etc.; on the other hand, rhizobacteria secretes small signaling molecules that are recognized by the plants. Plants respond to the bacterial signals and establish a relationship.

Quorum sensing is a well-known phenomenon for bacterial communication, where AHL molecules are released from bacteria and act as a communication medium. It is evident from the results that application of N-acyl-D/L-homoserine lactones (AHLs) plays a vital role in nutrition assimilation, development, and also inactivation of the defense system in various plants, but the exact mechanism is still unrevealed. Therefore, there is great need to study and understand the role of QS molecules in plant-microbe interaction and root microbe dynamics which will open the new avenues to design custom-made consortia and help to improve agriculture productivity.

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Zinc-Solubilizing Bacteria: A Boon for Sustainable Agriculture

8

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Abstract

The continuous rise in world population requires more food to feed people. To fulfill this demand, farmers apply different agrochemicals, especially fertilizers, in indiscriminate quantity in fields to increase production per unit time per unit area. Blind and imbalanced doses of fertilizers cause various adverse effects on environmental conditions through the accumulation of various minerals and biomagnifications in different ecosystems. Generally, all macroelements are applied through high-analysis fertilizers. But micronutrients are neglected, not directly involved in yield expansion, and zinc (Zn) is one of them. Zinc (Zn) is a key micronutrient, required for all living forms including plants, humans, and microorganisms for their development. Humans and other living organisms require zinc in their lives in little amounts for proper physiological functions. Zinc is a crucial micronutrient for plants which plays various important functions in their life cycle. The deficiency of zinc in the soil is one of the very common

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micronutrient deficiencies and results in decreased crop production. Majority of the agricultural soil is either zinc deficient or contains zinc in a fixed form which is unavailable to plants, as a result reflecting zinc deficiency in plants and soils. Therefore, to solve the above problem, there is a requirement for alternative and eco-friendly technology such as plant growth-promoting rhizobacteria (PGPR) and organic farming practices to enhance zinc solubilization and its availability to plants. Zinc-solubilizing bacteria (Zn-SB) are promising bacteria to use for sustainable agriculture. Zn-SB have various plant growth-promoting (PGP) properties such as Zn solubilization, P solubilization, K solubilization, nitrogen fixation, and production of phytohormones like kinetin, indole-3-acetic acid (IAA), and gibberellic acid, besides production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and siderophores, hydrogen cyanide, and ammonia. Zn-SB secrete different organic acids that solubilize the fixed form of zinc to available form, which enhances plant growth promotion, yield, and fertility status of the soil. This chapter covers the efficient application of Zn-SB, the Zn solubilization mechanism, and their application to increase crop production. The indigenous Zn-SB have proved their effectiveness over exogenous ones in the various cropping systems or crop rotations for which they are intended.

Keywords
Zinc · PGPR · Phytohormones · Sustainable agriculture · Crop yield

8.1 Introduction

Zinc is a very crucial micronutrient for crops that plays various important functions in their life cycle (Hirschi 2008). Plant growth, development, maturity, vigor, and yield are directly or indirectly affected by zinc. Humans and other living organisms require zinc in their lives in very little amounts to maintain their proper physiological functions. Zn is a vital mineral for biological and public health (Hambidge and Krebs 2007). After iron, it is the second most abundant metal in living organisms and also appears in all the enzyme classes (King 2006; Broadley et al. 2007). Therefore, its consumption through various foods is essential. Biofortification is a process of enriching zinc nutritional values in grains through agronomical and biotechnological breeding programs. Stein (2010) reported the increased availability of Zn and Fe in the staple crops through biofortification. The deficiency of Zn is a very common problem in developing countries, and it can be resolved by biofortification (Cakmak 2008). The basic aim of biofortification is to produce plants which have an augmented content of bioavailable nutrients in their edible portions (Abaid-Ullah et al. 2015). It is grown in about 8.26 Mha with production being 19.3 Mt. (Ministry of Agriculture, Government of India). There are various reasons for zinc deficiency in soil such as poor recycling of crop residues, excessive fertilization, high-yielding crop cultivars, less organic matter, and intensive cropping pattern (Hafeez et al. 2013). Zinc is among the deficient metals in human beings that negatively influences up to one-third of the worldwide population mainly preschool children and women (Hotz and Brown 2004; Zhang et al. 2012; Stein 2010). Therefore, increasing zinc content through various sources is becoming an important phenomenon. Mostly, fertilizer is the most common source of zinc, but it’s not ecological to apply with its chemicals; therefore, an alternative source are zinc-enriching microbes which would play an important role in biofortification. The use of these bacteria through the intermediation of different compounds, biofertilizers, and, most commonly, rhizobacteria in soils is cheap, sustainable, and eco-friendly for zinc availability, either through solubilizing native zinc or making available zinc from a remote area of the rhizosphere.

PGPR have plant growth promotion properties which play an important role in plant growth and yield through direct and indirect mechanisms (Glick 1995). Direct mechanisms of plant growth promotion include zinc solubilization, solubilization of insoluble phosphates, potassium solubilization, fixation of atmospheric nitrogen, and secretion of hormones such as IAA, gibberellic acid, and kinetin, besides 1-aminocyclopropane-1-carboxylate (ACC) deaminase production. Indirect mechanisms include induced systemic resistance (ISR), parasitism, competition for nutrients, antibiosis, and production of metabolites (hydrogen cyanide, siderophores) that suppress deleterious rhizobacteria and enhance plant growth. Zinc-solubilizing bacteria (Zn-SB) may convert the insoluble form of zinc in the soil to a soluble form making it easily bioavailable to plants for their growth, development, and final yield while well maintaining soil health and fertility for yielding in a sustainable way. The solubility of Zn in the soil has been mainly dependent upon soil pH and moisture. In the Indian agroecosystems, the arid and semiarid regions are often zinc deficient. The nature and amount of various organic acids produced through different soil microorganisms are mainly dependent upon the medium pH, carbon source, and buffering capacity (Mattey 1992). Zinc-solubilizing microorganisms produce various organic acids through acidification in the soil that sequester zinc cations and consequently decrease the pH in the nearby soil (Alexander 1997). Moreover, it is found that anions can also chelate zinc and increase zinc solubility (Jones and Darrah 1994). Zinc solubilization includes the siderophores production (Saravanan et al. 2011) and proton, oxidoreductive systems on cell membranes and chelating ligands (Chang et al. 2005; Wakatsuki 1995). Several beneficial bacterial strains are reported to cause solubilization of zinc on laboratory scale like *Pseudomonas aeruginosa* (Fasim et al. 2002), *Pseudomonas striata*, *Burkholderia cenocepacia*, *Bacillus* spp., *Pseudomonas fluorescens* (Pawar et al. 2015), *Gluconacetobacter diazotrophicus* (Saravanan et al. 2007), *Bacillus thuringiensis*, *S. marcescens*, and *Serratia liquefaciens* (Abaid-Ullah et al. 2015).

Wheat is an important staple food in various developing countries and provides up to 45 mg kg-1 in grains which is used as a supplement for Zn source in the diet of a human being (Stein 2010; Zou et al. 2012; Cakmak 2008; Zhang et al. 2012). Enhancement of Zn content in the Zn deficiency wheat grain has been due to the involvement of different transgenic and breeding approaches in cereals. However, political and socioeconomic issues and long time span make difficult its adaptation in field conditions from the trial room. The use of chemical fertilizers is a very fast method to increase the content of zinc in grains, but it is non-sustainable and expensive (Bulut 2013; White and Broadley 2011; Bahrani et al. 2010; Hafeez et al. 2013; Cakmak et al. 2010). The biofortification approach is one of the important strategies that would be a socially acceptable and economically viable tool for increasing Zn and Fe content in the major crops (Stein 2010). In biofortification, the major drawbacks are the root or shoot barriers and the process of grain filling. A major understanding of the zinc mechanism is needed to have insight in increasing grain quality and alleviating any accumulation of hazardous elements (Upadhyay and Srivastava 2014).

In this chapter, the results are summarized highlighting the efficient application of Zn-SB, the Zn solubilization mechanism, and their use to increase sustainable crop production. Eradication of Zn malnutrition and undernutrition from humans, plants, animals, and soils would be possible through Zn-SB through better bioavailability of zinc and other important micronutrients with their eco-friendly, beneficial, and economical nature. Indigenous Zn-SB are very effective in the various cropping systems under sustainable agriculture.

8.2 Zinc Status of Soil and its Availability to Plants

Around 50% of the agricultural soils in China have been deficient in zinc. At the same time, Indian zinc-deficient soils have covered almost 50% of the agricultural part, and the same situation has been observed in Turkey (FAO WHO 2002). About 70% of agricultural land has been recorded as Zn deficient in Pakistan (Hamid and Ahmad 2001; Kauser et al. 2001). Zinc is an essential component of plants and responsible for their metabolic processes which are very important for their development. The deficiency of Zn micronutrient is very common in plants resulting in major loss in crop production. Application of zinc fertilizers may not be profitable in controlling zinc deficiency and increasing crop yield and for sustainable crop production. There is an underutilization of Zn fertilizers in many countries, despite the widespread occurrence of zinc-deficient soils; and it may because they are not directly involved in yield expansion, compared to high-analysis fertilizers of nitrogen and phosphate. Majority of the soils are either deficient in Zn or with Zn in unavailable form to plants. Approximately 50% of the soils have inadequate Zn (FAO WHO 2002). Zn deficiency is occurring often in paddy soils, calcareous and neutral soils, diligently harvested soils and improperly drained soils, saline and sodic soils, peat soils, soils with a raised level of phosphorus and silicon, highly weathered acidic soils, sandy soils, and coarse-textured soils (Sillanpaa 1982; Alloway 2008). Deficiency of Zn may also be linked to the properties of the soil, such as being calcareous; Zn^{2+} may present as low, and this can reduce crop growth (Hacisalihoglu and Kochian 2003). The occurrence of Zn in soil is found as zincite (ZnO), zinkosite (ZnSO4), hopeite [Zn3(PO4)2•4H2O], sphalerite (ZnS), franklinite (ZnFe2O4), and smithsonite (ZnCO3); however, availability of Zn from these sources depends on various factors. The natural sources of zinc involve (a) physical and chemical weathering of parent rocks (Alloway 1995) and (b) atmospheric contribution of zinc to soils (e.g., forest fires, volcanoes, and surface dust) (Friedland 1990; International Zinc Association 2011). The primary step of Zn uptake from the rhizosphere is its accumulation in plants before its transfer to the seeds (Giehl et al. 2009). Plant roots uptake Zn in the form of Zn^{2+} cation which is the component of synthetic and organic compounds (Havlin et al. 2005; Oliveira and Nascimento 2006). Plants absorb available zinc in a reactive form from the soil solution. Available amount of zinc to plants is controlled by soil factors, e.g., total zinc concentration, organic matter, pH, clay, redox conditions, calcium carbonate, microbial activity in the rhizosphere, soil moisture, concentrations of other trace elements, and concentrations of macronutrients, especially climate and phosphorus (Alloway 2008). Supply of Zn is mostly affected by the soil pH in soil pools, on account of the fact that this element is easily adsorbed in cation exchange sites at over neutral pH and made available at low pH values (Broadley et al. 2007; Havlin et al. 2005). Cereal grains have very less concentration of Zn in contrast to animal-based foods or pulses. Presently, Indian soils are Zn deficient especially in wheat cropping systems, and grain Zn concentration will again decrease in cereals (Prasad 2005; Gupta 2005). In general, a lower concentration of Zn is found in cereal grains due to the presence of anti-nutrition factor phytic acid (PA) which decreases mineral bioavailability (Pahlvan-Rad and Pressaraki 2009). The lower bioavailability of zinc in soil directly affects zinc content in grains and human health. These Zn-deficient soils do not promote growth of various crops like sugarcane, wheat, corn, and rice. The deficiency of Zn in soil affects wheat yield, and this deficiency is overcome through the use of zinc fertilizer (Joy et al. 2017; Ahmad et al. 2012; Khan et al. 2009). However, the application of this Zn fertilizer threatens the public health and environment and puts farmers' livelihood in jeopardy. Therefore, in many countries, the application of chemical fertilizers has declined, and growers return to practices for sustainable agriculture.

To solve the problem of Zn deficiency, micronutrient biofortification of grain crops has gained interest in developing countries (Zhao and McGrath 2009; Cakmak 2008; Bouis and Welch 2010). Several approaches have been framed and performed for the fortification of cereals (Bouis 2003; Pfeiffer and McClafferty 2007). Improving concentration of Zn in cereal grains has been identified as an approach in dealing with human Zn deficiency (Pahlvan-Rad and Pressaraki 2009). Plant scientists are formulating different methodologies to solve the Zn deficiency problem in crops by application of fertilizers and/or by plant breeding approaches to increase absorption and/or bioavailability of Zn in grain crops (Cakmak 2008; White and Broadley 2009). Various dietary factors like amino acids (histidine and methionine), organic acids (citrate), and chelators (viz., EDTA) appear to support the bioavailability of zinc, whereas fibers and some minerals such as copper, iron, and calcium may reduce it in some situations (Lonnerdal 2000). Recent studies have also revealed that enhanced Zn bioavailability diminishes phosphorus and phytic acid concentrations in grains (Cakmak et al. 2010). Absorption of Zn can be enhanced by various organic acids such as citric acid, lactic acid, malic acid, and ascorbic acid. Zn-EDTA is formed through insoluble phytate-Zn and EDTA. HarvestPlus (2012) reported a 25 ppm enhancement of Zn content in wheat through fortification in Pakistan. It

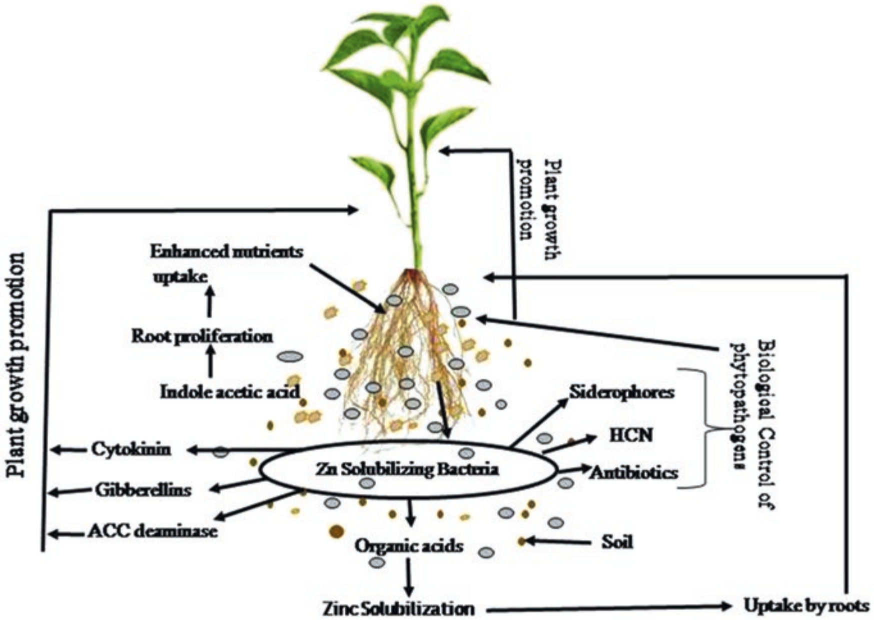

Fig. 8.1 Plant growth promotion and biological control properties of zinc-solubilizing bacteria

includes two strategies: agronomic and genetic biofortification. Soil-borne zinc-solubilizing bacteria colonize toward the rhizosphere region, multiply and compete with other rhizobacteria, and consequently enhance plant growth and yield (Kloepper and Okon 1994). Glick (2012) reported the use of PGPR which promotes plant growth through releasing phytohormones, solubilization and assistance in nutrient acquisition, and biocontrol agents to protect plants from different pathogens (Fig. 8.1). The different PGPR have been found to be very effective in zinc efficiency. These efficient PGPR improve plant growth promotion via colonizing the rhizosphere and by solubilizing the insoluble complex zinc compounds into simpler ones, thus making very easily available to the crops.

8.3 Role of Plant Growth-Promoting Rhizobacteria as Zn Mobilizers

Plant growth-promoting rhizobacteria have multi-roles to play in sustainable agriculture. They are a distinct group of bacteria that can be noticed in the rhizosphere on root surfaces as well as in association with roots (Maheshwari et al. 2012; Ahmad et al. 2008). These bacteria move around from the bulk soil to the living plant rhizosphere and antagonistically colonize toward the rhizosphere region and roots of the crops (Hafeez et al. 2005). PGPR can be divided into two groups based on their relationship with the plants, i.e., symbiotic bacteria and free-living rhizobacteria (Khan 2005). PGPR consist of beneficial microorganisms naturally occurring in the soil that make nutrients available to plants by several mechanisms such as fixing atmospheric nitrogen, solubilizing the nutrients fixed in the soil, and releasing phytohormones (Siddiqui et al. 2008; Hafeez et al. 2005; Yao et al. 2008). Besides phosphate mobilization, they also play a pivotal role in carrying out the bioavailability of soil phosphorus, potassium, zinc, iron, and silicate to plant roots (Tariq et al. 2007; Ahmad 2007; Saravanan et al. 2011; Abaid-Ullah et al. 2011). Tariq et al. (2007) reported the effect of Zn-mobilizing PGPR which significantly reduced the deficiency symptoms of Zn and constantly increased the total biomass, grain yield, and harvest index including Zn concentration in rice grains. Ahmad (2007) screened out 50 strains of Zn-mobilizing PGPR from the maize rhizosphere which have been very efficient strains on the basis of a clear transparent zone formation on respective Petri plates. Similar work has been accomplished by Yasmin (2011), who determined the effectiveness of Zn-solubilizing *Pseudomonas* sp. Z5, isolated from the rhizosphere region of rice crops. Abaid-Ullah et al. (2011) selected 9 out of 50 Zn-solubilizing PGPR qualitatively and quantitatively on various insoluble Zn ores such as ZnO, ZnS, $Zn(CO_3)_2$, and $Zn(PO_4)_3$. A positive correlation in Zn solubilization was observed between the qualitative and quantitative testing of *Serratia* sp. Similarly, higher Zn solubilization was recognized with ZnO as compared to other insoluble ores. Efficient Zn mobilizer *Serratia* sp. was tested in vivo for its beneficial effect which significantly maximized the yield and yield attributes of wheat crops. PGPR are vitally involved in the solubilization of many important minerals such as potassium, iron, phosphorous, zinc, etc., thereby increasing the bioavailability of these important nutrients to crops (Glick 1995). Penrose and Glick (2003) reported that PGPR have the ability to enhance plant growth via improving nutrient solubilization and releasing siderophore hormones, resulting in enhanced nutrient uptake by the crops (Fig. 8.1). There are various efficient PGPR strains that have shown to increase the growth and zinc content when inoculated in crops. These include *Bacillus* sp. (Hussain et al. 2015), *Pseudomonas*, *Rhizobium* (Deepak et al. 2013; Naz et al. 2016), and *Bacillus aryabhattai* strains (Ramesh et al. 2014). Different authors have reported the zinc solubilization ability of bacteria on lab scale which include *Bacillus* sp., *S. marcescens*, *Pseudomonas striata*, *Burkholderia cenocepacia*, *Pseudomonas fluorescens*, *Bacillus thuringiensis* and *Serratia liquefaciens* (Abaid-Ullah et al. 2015; Pawar et al. 2015), *Gluconacetobacter diazotrophicus* (Saravanan et al. 2007), and *Pseudomonas aeruginosa* (Fasim et al. 2002). Gadd (2007) reported that zinc-solubilizing microorganisms like *Acinetobacter*, *Bacillus*, *Gluconacetobacter*, and *Pseudomonas* have the ability to solubilize zinc from organic and inorganic pools of total soil zinc and also can be utilized to enhance zinc bioavailability to crops.

8.4 Mechanism of Zinc Solubilization by Zinc-Solubilizing PGPR

Zn is present in an insoluble form in the soil and is unavailable for plant uptake (Barber 1995). Solubilization of metal salts is an important feature of PGPR as the mobilized compound becomes available to plants. Bacterial comparative and functional genomics research has unlocked new ways for approaching these underlying mechanisms at the molecular and biochemical level. Many studies have been performed to examine the mechanisms of Zn-solubilizing PGPR. PGPR have different mechanisms to solubilize nutrients in the soil, namely, through exchange reactions, chelation, release of organic acids, and acidification (Chung et al. 2005; Hafeez et al. 2005). The mechanism of mobilization of iron and zinc likely involves siderophore formation (Tariq et al. 2007; Burd et al. 2000; Wani et al. 2007; Saravanan et al. 2011) and production of gluconate or the derivatives of gluconic acid, e.g., 2-keto-gluconic acid (Fasim et al. 2002), 5-keto-gluconic acid (Saravanan et al. 2007a, b), and many other organic acids, by PGPR (Wani et al. 2007; Di Simine et al. 1998; Tariq et al. 2007). The most preferred mechanism of zinc-solubilizing microorganisms is acidification.

8.5 Plant Growth Promotion Properties of Zinc-Solubilizing PGPR

The term PGPR was coined three decades ago when they were nothing but non-pathogenic, quick root-colonizing microbes on the surface of roots of plants which promote plant yield by different mechanisms (Agbodjato et al. 2016). Soil microbes which directly or indirectly promote plant growth are called PGPR (Akhtar et al. 2012). They are multiple groups of microbes which are found in the rhizosphere on plant root surfaces as well as in association with roots (Desai et al. 2012). These microbes move around from the bulk soil to the living plant rhizosphere region and antagonistically colonize in the rhizosphere region of plants (Islam et al. 2014). According to a study, various species of soil bacteria that thrive in the rhizosphere region of plants, but which may grow in, on, or around plant tissues and which stimulate plant growth through a superfluity of mechanisms, are collectively known as PGPR (Fig. 8.1) (Usha Rani and Reddy 2012). Recently, studies reveal that the PGPR associations range in the degree of microbial proximity to the root and intimacy of association. In general, they can be intracellular (iPGPR), which are present inside root cells, particularly the nodular regions, and extracellular (ePGPR), which exist in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex (Gopalakrishnan et al. 2014). PGPR are nothing but naturally occurring beneficial microorganisms in soil which make available different nutrients to plants by several mechanisms like fixing atmospheric nitrogen, solubilizing the nutrients fixed in soil, and producing phytohormones such as kinetin, IAA, and GA, besides ACC deaminase production which helps in the regulation of ethylene or enzyme production such as chitinase and cellulase (Saleem et al. 2007; Siddiqui and Shaukat 2004). PGPR influence direct growth promotion of plants by induced systemic resistance (ISR), competition for nutrients, antibiosis, parasitism, and production of metabolites (hydrogen cyanide, siderophore) that suppress deleterious rhizobacteria. These mechanisms ultimately are useful and beneficial in plant growth. PGPR can be estranged into two groups according to their association with plants: symbiotic bacteria and free-living rhizobacteria (Saraf et al. 1994). PGPR have also an important role in phosphate dissolution and in bioavailability of soil phosphorus, potassium, iron, and silicate to plant roots (Abaid-Ullah et al. 2015). Many studies have reported that inoculation with a potent strain of zinc-mobilizing rhizobacteria resulted in higher yield of field crops such as rice, wheat, maize, and barley. A recent study describes the effect of Zn-mobilizing PGPR which significantly overcome the deficiency symptoms of Zn and also regularly increase the total biomass and grain yield (Tariq and Ashraf 2016).

Conventional application of inorganic zinc partially fulfills the plant needs in that 96–99% of applied Zn is transformed into different insoluble forms based on the soil types and physicochemical reactions (Saravanan et al. 2004). Microbes are a potential alternative that could cater to plant Zn requirement by solubilizing the complex Zn in the soil. Various types of genera of rhizobacteria including *Bacillus* and *Pseudomonas* are reported to be used for solubilizing zinc. Microbes solubilize the metal forms by protons, chelating ligands, and oxidoreductive systems present on cell surfaces and membranes (Crane et al. 1985; Hughes and Poole 1991; Wakatsuki 1995). These bacteria also have other beneficial traits for crops like production of phytohormones, antibiotics, siderophores, vitamins, antifungal substances, and hydrogen cyanide (Fig. 8.1) (Rodriguez and Fraga 1999). The results of the studies indicated that a *Bacillus* sp. (Zn-solubilizing bacteria) can be utilized as biofertilizer for zinc in soils where native zinc is uplifted or in combination with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc sulfide (ZnS), and zinc carbonate (ZnCO3), as an option from costly zinc sulfate (Mahdi et al. 2010a, b). Consequent studies on PGPR concluded that several best strains are multitasking and PGPR traits are regularly spread among various different species and genera of microorganisms, a great number of which are native members of the soil microbial community. Broadly, individual strains vary significantly in performance. Native PGPR can influence the performance of introduced PGPR inoculants comparatively. Accordingly, knowledge and information all about the background of PGPR and their function is essential; otherwise, it's hard to estimate the response to soil inoculations with different PGPR. A number of PGPR frequently solubilize nutrients (phosphorus, zinc, iron, silicate, etc.), release auxins which encourage root development, and produce siderophores and antibiotics that may reduce root infection. Over the course of environmental stress, plants release ethylene or hydrogen cyanide and reactive oxygen species (ROS) that may be reduced by substances (enzymes) secreted by these PGPR in the soil environment.

8.6 Effect of Zinc-Solubilizing Bacteria for Plant Growth Promotion

Zinc, being an essential micronutrient for the growth of plants, human beings, and animals, has a vital role in crop nutrition as required for carrying out various enzymatic reactions, metabolic processes, and oxidation-reduction reactions smoothly. Zinc plays an important role in RNA and DNA synthesis and is a constituent of many zinc-containing enzymes critical to cellular growth and differentiation. Deficiency of this micronutrient causes malnutrition to more than half of the worldwide population, especially in developing countries, due to lack of knowledge and technology. To overcome zinc malnutrition, there is an exploitation of soil microorganisms that can mobilize unavailable zinc, increase zinc assimilation, and promote plant growth (Rana et al. 2012). The use of biofertilizers in combination with chemical fertilizers not only improved crop productivity but nutrient efficacy also. A various range of bacteria species including *Bacillus*, *Enterobacter*, *Pseudomonas*, *Azospirillum*, *Klebsiella*, *Rhizobium*, *Azotobacter*, and *Burkholderia* have been shown to promote plant growth; and these can be used as biofertilizer and biocontrol agents. Zn-mobilizing PGPR inoculants are used as biofertilizers which can accelerate the regeneration of degraded land and improve the fertility status of the soil. They also enhance the survival and growth rate of plants, maximize grain yield, reduce malnutrition rates, and control dependence on chemical fertilizers (Hafeez et al. 2001). Using Zn-SB together with other chemical fertilizers will be a key advantage for the formulation of efficient biofertilizers (Zaidi and Mohammad 2006; Gull et al. 2004). This is probably the first report on zinc-solubilizing *B. aryabhattai* strains, isolated from the rhizosphere of soil of soybean cultivated in Vertisols of central India. The use of zinc-solubilizing bacteria belonging to genera *Bacillus* has reportedly increased plant growth parameters. The effectiveness of zinc-mobilizing PGPR has an optimistic impact on the length of the root, root dry matter, root area, volume of the root, shoot dry matter, and panicle emergence index. PGPR work with the symbiotic association of microbes with plants. He et al. (2010) observed that inoculation with zinc-mobilizing bacteria, especially *Bacillus* genera, maximizes growth parameters (Zhao et al. 2011). Similar increment is recorded in zinc acquisition and dry matter accumulation, through the inoculation with PGPR (Rana et al. 2012). Practical efficient PGPR have been studied, and high-quality sympathetic use of microbial interactions is needed in plant growth increment, which will enhance the favorable outcome of field application (Usha Rani and Reddy 2012). Table 8.1 shows plant growth promotion activities of different crops through various Zn-SB strains.

8.7 Conclusions

It is apparent that the application of chemical fertilizers, pesticides, and agronomic practices and development of transgenic plants for the enhancement of Zn content in food crops have potential, although these engaged practices have elevated the cost, environmental pollution, and various socioeconomic and political issues.

Table 8.1 Inoculation effect of various strains of Zn-SB on crops

Zn solubilizer	Crops	Enhanced parameters	References
Zn-mobilizing PGPR	Rice	Enhanced the Zn content in the grain, total biomass, root area, grain yield, root weight, root length, root volume, and shoot weight	Tariq et al. (2007)
Rhizobium spp. RL9	Lentil	Improvement of dry matter, nodule number, seed yield, nodule dry mass, leghemoglobin, and grain protein	Wani et al. (2008)
Zn-mobilizing PGPR	Wheat	Enhanced root volume, root weight, root length, root area, and shoot weight	Kutman et al. 2010).
Zn-solubilizing bacterial Isolates (U, 8 M, 36, 102, and 111)	Mung bean	Improved shoot length and root length, fresh weight, and dry weight observed in seedlings	Iqbal et al. (2010)
Pseudomonas spp. P17 and *Bacillus* spp. B40	Maize	Increased the total dry mass and uptake of N, K, Mn, and Zn	Goteti et al. (2013)
Bacillus aryabhattai strains MDSR7, MDSR11, and MDSR14	Soybean and wheat	Increased shoot dry weight, plant height, root dry weight, and zinc assimilation in seeds	Ramesh et al. (2014)
Burkholderia And *Acinetobacter*	Rice	Increased mean dry matter, number of panicles, number of grains, grain yield, and straw yield and enhanced total Zn uptake	Vaid et al. (2014)
Serratia liquefaciens FA-2, *Bacillus thuringiensis* FA-3, *Serratia marcescens* FA-4	Wheat	Improvement of grain yield and Zn content of wheat tillers plant^{-1}, grains spike^{-1}, grain yield, total biomass, and dry straw weight	Abaid-Ullah et al. (2015)
Pseudomonas fragi, *Pantoea dispersa*, and *Pantoea agglomerans*	Wheat	Increased the plant growth promotion and Zn content	Kamran et al. (2017)
Bacillus strains	Soybean and wheat	Modulated growth, yield, and zinc biofortification	Khande et al. (2017).

Consequently, zinc-solubilizing bacteria must target improvements in zinc nutrient deficiency. This approach is promising due to its eco-friendly, economic, and ecological nature. It is important to have a better understanding of the interactions between plants and microbes. Zinc-solubilizing bacteria have efficient plant growth-promoting properties. The inoculation of efficient Zn-SB strains will be effective for plant growth promotion, soil health, and soil fertility for sustainable agriculture.

8.8 Future Remarks

A combination of breeding strategies and zinc fertilizer application is an important and complementary approach to alleviate zinc deficiency-related problems in human nutrition. It is crucial for people to be aware about the use of the agronomic biofortification approach for solving the zinc deficiency problem in developing countries or regions since farmers alone cannot afford expensive micronutrient fertilizers. At this condition, the plant breeding approach would become a high-priority approach to this problem. Multiple indigenous strain combinations of Zn-SB may be more suitable for local crop production and enhanced zinc content in plants. Therefore, it can be concluded that beneficial biofertilizers applied in combination are a better choice for farmers to reduce the use of chemical fertilizers for sustainable crop production. The application of different microbial technologies in agriculture is presently growing very rapidly and popularly with the recognition of novel bacterial strains, which are additionally effective in plant growth and yield. Multifunctional PGPR such as P, K, and Zn solubilizers prove to be effective biofertilizers. Various new interventions and technologies are needed to ultimately transfer genetically modified soil- and region-specific Zn-SB to the farmers' fields in a relatively short time. There is a search for new efficient strains of Zn-SB as biofertilizers for development of microbial diversity for any region. Co-inoculation with other synergistically beneficial bacterial strains is being devised, and many recent investigations show a promising trend in the field of inoculation technology. Various beneficial Zn-SB isolated from plants growing on normal soil are perfectly able to promote plant growth under many stresses. Future work is planned to study mixtures of the selected efficient Zn-SB strains for biocontrol against multiple plant pathogens in bioassays. Genotypic study of the Zn-SB strains and molecular characterization of the plant parts are necessary to easily understand plant mechanisms of zinc absorption and its requirement in plants. Furthermore, scientists need to address certain issues, like how to synergistically make co-inoculation of phosphate, potassium, and zinc solubilizers and improve the biofertilizer efficacy, what should be an ideal delivery system, how to stabilize these microbe consortia in soil systems, and how nutritional and root exudation aspects could be controlled in order to get most benefits from co-inoculation application.

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Rhizobacteria as Bioprotectants Against Stress Conditions

9

F. Pereira

Abstract

The area around the plant which is under the influence of plant roots, known as the rhizosphere, is an attractive habitat for soil microorganisms. However, although a variety of root-colonizing bacteria exist, the beneficial bacteria also called plant growth-promoting bacteria (PGPR) or rhizobacteria essentially serve as bioprotectants against stress conditions. Environmental abiotic stresses such as drought, salinity, and metal contamination, as well as biotic stresses from opportunistic pathogens, present a major challenge as it reduces the potential yields of food production. Rhizobacteria are of immense interest because they compete with indigenous bacteria and increase plant resistance against stress conditions. These bacteria have a number of traits that contribute to root colonization such as the presence of specific cell surface components, pili, fimbriae, chemotaxis toward plant exudates, ability to use specific components of plant exudates, protein secretion property, ability to form biofilms, and quorum sensing. The production of biologically active metabolites and the regulation of ACC deaminase are some of the principal mechanisms by which rhizobacteria modify the rhizosphere environment thereby enhancing plant growth. This article seeks to give an overview of mechanisms in rhizobacteria proposed to enhance stress tolerance conditions.

Keywords

Rhizobacteria · Abiotic stress · Stress tolerance · Plant growth

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9.1 Introduction

The ever-increasing rise in world population has made the task of feeding the global population extremely difficult. Biotic stresses such as phytopathogens and herbivory and abiotic stresses such as flooding, drought, heating, freezing, radiations, and salinity variations due to global warming and climate change further challenge the agriculture industry. Environmental stresses are believed to reduce the potential yields by as much as 70% in crop plants and therefore constitute a major problem for sustainable food production. According to the current scenario, it has been estimated that food production needs to be increased as much as 50% by 2030. However, since food production is sensitive to environmental conditions, alternate strategies such as the systematic identification of bacterial strains that can help farmers reduce the anticipated adverse impacts of multiple stressors from global warming are highly valuable for agricultural production. Although a variety of bacteria exist, the root-colonizing nonpathogenic bacteria also called as plant growth-promoting bacteria (PGPR) or rhizobacteria hold promise, as they can increase plant resistance to biotic and abiotic stress factors, thereby essentially serving as bioprotectants against stress conditions. Here, we present an overview of current progress on the use of rhizobacteria under stress conditions and the modes of action of these bacteria in the mitigation of abiotic stresses.

9.2 Soil as an Ecosystem

The soil is a dynamic living matrix whose complexity is determined by the interplay of physical, chemical, and biological components, which is a manifestation of environmental conditions prevalent at that time (Buscot 2005). The soil matrix together with the physical properties like texture, porosity, and moisture holding capacity and chemical properties such as the amount of organic matter in the soil, its pH, and redox conditions influences the dynamics of structure and function of the microbial communities in soils (Lombard et al. 2011). Soil ecosystem is directly influenced by climate changes. Any increase in temperature would result in an increase in the microbial activity resulting in an altered microbial community. Another important factor which influences microbial community structure is the water content. Turnover of organic matter due to microbial activity is directly influenced by the water potential (Thomsen et al. 1999). Even the rate of respiration in soil depends upon the soil moisture content, temperature, and organic matter. Therefore any changes in temperature, salinity, and an increase in metal content due to pollution would result in manifesting as a stressful environment for plants. Under stress, there is a reallocation of resources from growth pathways in microorganisms to producing protective molecules, which could also benefit plants. However, ecologically it results in substantial amounts of C and N being vulnerable to loss (Schimel et al. 2007).

9.3 Root Exudates and Microbial Community Structure

Soil having more vegetation harbors a greater microbial population, compared to soil devoid of plants. The higher number of bacteria in the rhizosphere (the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms) and rhizoplane (the external surface of roots together with closely adhering soil particles and debris) is because the rhizosphere region is rich in total organic carbon from root exudates and sloughed-off root cells and tissues (Barber and Martin 1976). Roots have been estimated to release between 10 and 250 mg C/g or about 10–40% of the total photosynthetically fixed carbon (Newman 1985). The products released by the roots in the surrounding soil are called rhizodeposits (McNear 2013). Rovira (1969) defined rhizodeposits based on their chemical composition and mode of release and function to include sloughed-off root cap and border cells, mucilage, and exudates. According to Walker et al. (2003), plant roots are not passive targets for soil organisms. This is evidenced as soon as a seed starts to germinate; the plant roots nurture a tremendous diversity of microbes via exudation of a wide variety of compounds such as carbohydrates, amino acids and proteins, organic acid anions, phytosiderophores, vitamins, purines, nucleosides, phenolics, and mucilage, which serve as chemical attractants and repellents in the rhizosphere (Bais et al. 2001; Estabrook and Yoder 1998; Stintzi and Browse 2000; Stotz et al. 2000). Del Gallo and Fendrik (1994) and Bell et al. (2013) attributed the regulation of soil microbial community to direct conflict and competition between the different species for nutrient acquisition and colonization, and differential growth patterns of the large population of microorganisms attracted to the exudates in the vicinity of the roots. According to Bais et al. (2004), root exudates promote beneficial microbial colonization on root surfaces (e.g., *Bacillus subtilis*, *Pseudomonas fluorescens*). Recently, the root-specific transcription factor MYB72 which regulates the excretion of the coumarin scopoletin, an iron-mobilizing phenolic compound with selective antimicrobial activity, has been reported by Stringlis et al. (2018) to shape the root-associated microbial community.

Dakora and Phillips (2002) explained how in low-nutrient environments, root exudates act as mediators of mineral acquisition by releasing extracellular enzymes such as acid phosphatases which mobilize phosphorus from organic compounds; as also molecules such as phytosiderophores increase iron availability through chelation. Organic acids such as malic and citric acids released into the rhizosphere from root exudates have been shown to effectively reduce the rhizosphere pH and solubilize unavailable soil Ca, Fe, and Al phosphates. Similarly, inorganic ions (e.g., HCO_3^-, OH^-, H^+) and gaseous molecules (CO_2, H_2) from root exudates could also modify the rhizosphere pH (Dakora and Phillips 2002). The mode of alteration of rhizosphere pH is linked to the form of nitrogen available in the soil. Plants respond differently when nitrogen in the form of ammonium is present. Since ammonium has a positive charge, the plant expels one proton (H^+) for every NH_4^+ taken up resulting in a reduction in rhizosphere pH; but when nitrogen in the form of NO_3^- is present, there is an increase in the rhizosphere pH due to the release of bicarbonate (HCO_3^-). Such alterations in pH can influence the availability of essential micronutrients such as Mg, Zn, and Ca for plants (McNear 2013).

Root exudates are also beneficial as chemical signals for the attraction of symbiotic partners such as *Rhizobia*. Peters et al. (1986) reported that flavonoids present in the root exudates of legumes were responsible for activating *Rhizobium meliloti* genes that help in nodulation. Where N_2 is reduced to ammonia, aldonic acids and phenolics exuded by roots of N_2-fixing legumes have been reported to serve as signals to *Rhizobiaceae* bacteria for the formation of root nodules.

Root exudates also serve as defense mechanisms against pathogenic microorganisms in the rhizosphere. Brigham et al. (1999) and Bais et al. (2002) reported biologically active compounds having antimicrobial activity in the root exudates of hairy root cultures, such as naphthoquinones of *Lithospermum erythrorhizon* and rosmarinic acid (RA) of sweet basil (*Ocimum basilicum*), respectively. Walker et al. (2003) and Doornbos et al. (2012) suggested that it is also possible that roots may develop defense strategies by secreting compounds into the rhizosphere that interfere with bacterial quorum-sensing responses such as signal blockers, signal mimics, and signal-degrading enzymes. Flores et al. (1999) attributed the survival of delicate unprotected root cells which are continuously under attack by pathogenic microorganisms to the secretion of defense proteins, phytoalexins, and other unknown chemicals. Thus, the plant may be in a positive or negative association with its microbial community, based on factors prevalent in the rhizosphere and the symbiotic or defensive role played by root secretions.

9.4 Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria were first defined by Kloepper and Schroth (1978) as organisms that, after being inoculated on seeds, could successfully colonize plant roots and positively enhance plant growth (McNear 2013). Plant growth-promoting rhizobacteria (PGPR) are nonpathogenic, free-living soil and root-inhabiting bacteria that colonize seeds and root tissue (endophytic/epiphytic) or enhance production of root thereby promoting plant growth. Root-colonizing bacteria establish on or in the root or rhizosphere to multiply, survive, and colonize along the growing root in the presence of the indigenous microflora, thereby exerting beneficial traits on plant growth and development. Some examples of rhizosphere bacteria that have been found to have beneficial effects on various plants include species of the genera *Acinetobacter*, *Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Alcaligenes*, *Azospirillum*, *Acetobacter*, *Actinoplanes*, *Bacillus*, *Bradyrhizobium*, *Cellulomonas*, *Clostridium*, *Enterobacter*, *Erwinia*, *Frankia*, *Flavobacterium*, *Pasteuria*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Thiobacillus*, *Xanthomonas*, and others (Gray and Smith 2005), as well as *Streptomyces* spp. (Tokala et al. 2002; Dimkpa et al. 2008a, 2009b). To be an effective PGPR, bacteria must be able to colonize roots because bacteria need to establish themselves in the rhizosphere at population densities sufficient to produce the beneficial effects. However, the beneficial effect of the bacterial strains of a particular genus and species is not always the same for all plants and may even be negligible in some plants. According to Glick et al. (1999), the mechanism by which the bacteria promote plant growth may be directed by facilitating uptake of nutrients or by supplying a particular growth promoting substance synthesized by the bacteria, for example, volatile compounds of *Bacillus methylotrophicus* M4-96 increased shoot biomass and chlorophyll content (Pérez-Flores et al. 2017), or indirectly by acting as biocontrol agents against phytopathogens. Effective rhizosphere colonization by bacteria can be beneficial to plants to control diseases by producing antifungal factors; it can bring about phytostimulation by the production of phytohormones, fertilization by increasing the availability of nutrients, bioremediation of hazardous chemicals in the environment (Dekkers et al. 1999), and assist in mitigating stress.

9.5 Role of Microorganisms in the Rhizosphere

Microbial communities can be considered as architects of soils (Rajendhran and Gunasekaran 2008). There is a dynamic interaction between soilborne microorganisms, plant roots, and soil constituents at the root-soil interface. Root exudates and decaying plant material provide sources of carbon compounds for the heterotrophic biota (Barea et al. 2005; Bisseling et al. 2009), while in turn, the probiotic root microbiome members such as the rhizobacteria modulate their metabolism to optimize the acquisition of nutrients, protect the host plant against pests and pathogens, encourage beneficial symbioses, change the chemical and physical properties of the soil, inhibit the growth of competing plant species, and promote plant growth (Nardi et al. 2000; Hardoim et al. 2008). For the bacteria to establish themselves in the rhizosphere, certain cell surface structures such as pili, fimbriae, and flagella facilitate movement, attachment, and colonization on root surfaces (Merritt et al. 2007; Fernàndez and Berenguer 2000). Persello-Cartieaux et al. (2003) showed that bacterial flagella possess adhesive properties, but Tokala et al. (2002) observed that rhizobacteria such as *Streptomyces* spp. that do not possess flagella could still establish beneficial interactions with plants via development of hyphae in plant tissues.

Other traits that are useful for bacteria for competitive colonization are the O-antigen of lipopolysaccharide, amino acids, and vitamin B1, and root mucilage as a source of carbon (Dekkers et al. 1999).

9.6 Mitigation of Stress

Under stress conditions, plants recruit the help of microorganisms to change the chemical environment of the rhizosphere and alter the root morphology. They differentially recognize pathogenic or beneficial rhizobacteria by detecting diffusible substances, such as the quorum-sensing compounds such as N-acyl-L-homoserine lactones, which induces changes in the root secretion profile (Mathesius et al. 2003; Ortiz-Castro et al. 2011) and activates plant immunity (Schuhegger et al. 2006).

The presence of EPS from microorganisms has been implicated in symbiosis, protection from predation, biofilm formation, and stress conditions. After establishing themselves in the rhizosphere, bacteria form a mutualistic relationship with the plant roots. Although the exact mechanisms of plant growth stimulation under stress conditions remain largely speculative, it is known that they differ between bacterial strains and most certainly depend on the various compounds released by the different microorganisms. One of the mechanisms by which they stimulate plant growth is by phosphate solubilization. Rhizosphere bacteria from the genera *Pseudomonas*, *Bacillus*, and *Rhizobium* are among the most powerful phosphate solubilizers. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorus in the soil. Stress-induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere was reported by Banerjee et al. (2010).

Another mechanism by which rhizobacteria can promote plant growth is by nitrogen fixation. Plant growth-promoting rhizobacteria (PGPR) strains *Serratia liquefaciens* and *Serratia proteamaculans* were reported to increase nodulation, nitrogen fixation, and total nitrogen yield in two soybean cultivars in a short season area (Dashti et al. 1998). Sharma and Johri (2003) reported that maize seeds inoculated with siderophore-producing pseudomonads were better suited for iron uptake under iron-stressed conditions.

Rhizobacteria are known to stimulate plant growth (Patten and Glick 2002; Joo et al. 2005; Ryu et al. 2005; Aslantaş et al. 2007; Dimkpa et al. 2009a) via secretion of phytohormones – auxins, cytokinins, gibberellins, abscisic acid (ABA), and ethylene (Arkhipova et al. 2007; Dobbelaere et al. 2003; Forchetti et al. 2007; Perrig et al. 2007), which act directly or in concert with other bacterial secondary metabolites. In low concentrations auxins, specifically indole acetic acid (IAA), are reported to be produced in the plant shoot and transported basipetally to the root tips (Martin and Elliott 1984), where they enhance cell elongation, resulting in enhanced root growth and the initiation of lateral roots. Promotion of root growth results in a larger root surface, and can therefore have positive effects on water acquisition and nutrient uptake. However, when the concentrations of auxin are too high in the root tips, they are said to have an inhibitory effect on root growth. When the plant is exposed to different types of stress, ethylene synthesis is reported to increase, and it plays a key role in stress-related signal transduction pathways (Wang et al. 2002).

Haas and Défago (2005) showed that the production of siderophores confers competitive advantages to rhizobacteria, excluding other microorganisms from this ecological niche. A pseudobactin siderophore produced by *P. putida* B10 strain was reported by Kloepper et al. (1980) to suppress *Fusarium oxysporum* in soil deficient in iron. Under highly competitive conditions, the ability to acquire iron via siderophores may finally determine the survival of microorganisms competing for different carbon sources from rhizodeposition (Crowley 2006).

9.7 Role of Rhizobacteria in Mitigating Abiotic Stresses

Environmental stress plays a crucial role in productivity, survival, and reproductive biology of plants. Plants are subjected to many forms of environmental stress, which can be categorized into two broad areas: abiotic (e.g., physical stress due to drought, high temperature, chilling and freezing, UV-B radiation, salinity, and heavy metals) and biotic stress (e.g., pathogen, herbivores). Plant growth-promoting rhizobacteria (PGPR) could play a significant role in the alleviation of stress in plants (Table 9.1).

9.7.1 Water Stress

In order to survive, plants require a certain amount of water. Too much water (flooding stress) may cause cells to swell and burst, whereas too little water (drought stress) can cause the plant to dry up. Drought stress results in various physiological and biological changes in plants (Rahdari et al. 2012) because it influences the availability and transport of soil nutrients, water being the medium by which nutrients are carried through the roots (Selvakumar et al. 2012). Drought also induces free radicals formation such as hydroxyl radicals, hydrogen peroxide, and superoxide, which at high concentrations cause damage at various levels of organization (Smirnoff 1993). Deterioration of plant cell membrane, lipid peroxidation, and

Table 9.1 Mitigation of various stresses

Stress	Stress
Salinity	Drought
ACC deaminase reducing ethylene production	ACC deaminase reducing ethylene production
Increased P, Ca^{2+}, and K^{+} uptake	Increased osmolyte production
Increased water use efficiency	IAA stimulated root growth
Removal of salt suppression of photosynthesis	Nitric oxide stimulated root growth
IAA stimulated root growth	Induced changes in root cell wall/cell membrane
Nitric oxide stimulated root growth	EPS production
Osmolyte production	Synthesis of antioxidative enzymes
EPS production	Change in transpiration rate
Synthesis of antioxidative enzymes	Improved nutrient uptake
Ion homeostasis	Reproductive delay
Improved nutrient uptake	Improved photosynthetic pigment
	Production of volatile organic compounds
Temperature stress	Heavy metal stress
ACC deaminase reducing ethylene production	ACC deaminase reducing ethylene production
Induced changes in root cell wall/cell membrane	IAA stimulated root growth
Upregulation of stress genes	Nitric oxide stimulated root growth

degradation of proteins, lipids, and nucleic acids are some of the effects of oxidative stress reported by Hendry (2005), Nair et al. (2008), and Sgherri et al. (2000). Marulanda et al. (2010) studied the possibility of increasing drought tolerance of plants growing in arid or semiarid areas by inoculation of plants with native beneficial microorganisms. Beneficial microorganisms such as *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus thuringiensis*, *Paenibacillus favisporus*, and *Bacillus subtilis* were found to colonize the rhizosphere of plants and promote growth under stress conditions. Heidari and Golpayegani (2012) showed that inoculation with rhizobacteria effectively improved plant growth by increasing antioxidant status especially of glutathione peroxidase and ascorbate peroxidase, and photosynthetic pigments in basil *(Ocimum basilicum)*. Another mechanism by which rhizobacteria are reported to impart drought tolerance is by producing exopolysaccharides (EPS). *Bacillus* spp. secrete conspicuous amounts of EPS under stress conditions (Vardharajula et al. 2010). EPS forms an organo-mineral sheath around the cells, favoring increased macroaggregate formation, which helps in the survival of plants under drought stress by increasing water stable aggregates and root-adhering soil per root tissue (RAS/RT) ratio (Alami et al. 2000). PGPR play a significant role in alleviating environmental biotic-abiotic stress conditions by the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Figueiredo et al. (2008) reported a link between ACC deaminase activity of the rhizobacterium *Rhizobium tropici* and reduction in drought stress in *Phaseolus vulgaris* L. Similar instances of enhanced drought tolerance were reported in tomato plant due to the PGPR *Bacillus cereus* AR156 (Chun Juan et al. 2012) and in *Arabidopsis thaliana* by *Paenibacillus polymyxa* (Timmusk and Wagner 1999). ACC serves as the precursor of the plant hormone ethylene synthesized in plant tissues during stressful conditions. PGPR also colonize the rhizosphere/endo-rhizosphere of plants and impart drought tolerance by producing volatile compounds (Naznin et al. 2012), inducing accumulation of osmolytes, upregulation, or downregulation of stress-responsive genes and alteration in root morphology. IAA stimulates stress tolerance because of physical and chemical changes in the plant caused by these PGPR (Marulanda et al. 2009) (Table 9.2).

9.7.2 Temperature Stress

Freezing stress can affect the amount and rate of uptake of water and nutrients, leading to cell desiccation, starvation, and death. Intense heat can cause plant cell protein denaturation or affect cell wall and membrane permeability. Inoculation of *Pseudomonas* sp. strain AKM-P6 and *P. putida* strain AKM-P7 enhanced the tolerance of sorghum and wheat seedlings to high-temperature stress due to the synthesis of high-molecular-weight proteins and also improved the levels of cellular metabolites (Ali et al. 2009, 2011). Production of ACC deaminase by rhizobacteria also helped the plant to withstand extreme temperatures (Table 9.3).

Table 9.2 Role of PGPR in ameliorating water stress

Stress condition	Rhizobacterial inoculant	Plant species	Mechanisms	References
Water stress	*Acinetobacter* sp. and *Pseudomonas* sp.	Grapevine	Siderophore release and solubilization of inorganic phosphate compounds, EPS production	Rolli et al. (2015)
Water stress	*Citrobacter freundii* J118	Tomato	Increased uptake of nutrients	Ullah et al. (2016)
Water stress	*Brevibacillus brevis*	Cotton	Phosphate solubilization, IAA production, acetylene reduction, and antifungal activity	Nehra et al. (2016)
Water stress	*Pseudomonas putida*	*Arabidopsis thaliana*	Auxin secretion	Shah et al. (2017)
Water stress	*Bacillus megaterium BOFC15*	*Arabidopsis thaliana*	Spermidine secretion (a type of polyamine)	Zhou et al. (2016)
Water stress	*Acinetobacter pittii* JD-14	Alfalfa	Improved the relative water content; chlorophyll a; chlorophyll b; carotenoid contents; nitrogen (N), phosphorus, and potassium contents	Daur et al. (2018)

Table 9.3 Role of PGPR in ameliorating temperature stress

Stress condition	Rhizobacterial inoculant	Plant species	Mechanisms	References
Temperature stress	*Burkholderia phytofirmans* strain *PsJN* (*Bp PsJN*)	*Arabidopsis thaliana*	Differential accumulation of pigments; cell wall strengthening in the mesophyll	Su et al. (2015)
Temperature stress	*Burkholderia phytofirmans* strain PsJN	Potato	ACC deaminase	Bensalim et al. (1998)
Temperature stress	*Bacillus safensis* and *Ochrobactrum pseudogrignonense*	Wheat	Increased redox enzyme activity and accumulated osmolytes like proline and glycine betaine; maintained cell viability, restored chloroplast structure	Sarkar et al. (2018a, b)
Temperature stress	*P. putida*	Canola	ACC deaminase	Cheng et al. (2007)

9.7.3 Salinity Stress

High amounts of salt taken up by a plant can lead to cell desiccation, adversely affecting biochemical processes. Mahmood et al. (2016) showed that inoculation of mung bean with EPS-producing *Enterobacter cloacae* P6 and *Bacillus drentensis* P16 increased water and nutrient availability to crops due to the formation of biofilm on the root surface. Bacterial exopolysaccharide (EPS) produced by plant growth-promoting rhizobacteria was reported to help mitigate salinity stress by reducing the content of sodium available for plant uptake (Upadhyay et al. 2011) while increasing the uptake of phosphorus and potassium. Ashraf and McNeilly (2004) explained how EPS reduces sodium uptake and transfer to leaves thereby alleviating salt stress. Kasotia et al. (2016) proposed that plant-microbe biofilm on the surface of seedlings resulted in a lesser flow of sodium to the steel, insulating from NaCl toxicity. *Enterobacter* sp. P23 was shown to promote rice seedling growth under salt stress, and this effect was correlated with a decrease in antioxidant enzymes and stress-induced ethylene production. These bacteria possessed ACC deaminase activity and therefore could use ACC as a nitrogen source preventing ethylene production since ACC is a precursor of ethylene (Sarkar et al. 2018a, b).

Bacterial IAA is also shown to stimulate (ACC) deaminase activity for degradation of the ethylene precursor ACC (Glick 2005). Bianco and Defez (2011) also proposed that the toxic effects of salinity could be reduced by modulation of major plant hormones such as IAA. Mansour (2000), Hare and Cress (1997), Kavi Kishor et al. (2005), and Verbruggen and Hermans (2008) attributed salinity tolerance to the accumulation of nitrogen-containing compounds such as the amino acid proline in plants. The activities of the antioxidative enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX), glutathione reductase (GR), and superoxide dismutase (SOD) increase under salt stress in plants, and a correlation between these enzyme levels and salt tolerance has been described (Apel and Hirt 2004). PGPR strains producing IAA showed high antioxidant enzyme activity in *Medicago* plants which were found to enhance their protection against salt stress (Bianco and Defez 2009). Wang et al. (2016) reported that *V. paradoxus* 5C-2 mitigated salt stress by improving water relations, ion homeostasis, and photosynthesis in pea plants. Rhizobacteria were also found to increase the water use efficiency in saline environments and to help alleviate salt suppression of photosynthesis (Table 9.4).

9.7.4 Ultraviolet-B Radiation Stress

UV-B radiation can affect the production of secondary metabolites such as flavonoids (Takshak and Agrawal 2014b), tannins, and lignins which act as defense compounds in plants. It is also known to impact metabolism and cause morphogenetic defects, such as a reduction in IAA oxidase and the cumulative antioxidative potential (CAP), protein and chlorophyll content, and various enzymes of the phenylpropanoid pathway. Supplementary UV-B has been known to cause an increase in the concentrations of reactive oxygen species (ROS) in leaves but a decrease in roots of

Table 9.4 Role of PGPR in ameliorating salinity stress

Stress condition	Rhizobacterial inoculant	Plant species	Mechanisms	References
Salinity	*Pseudomonas fluorescens* MSP-393	Pea	Osmolyte synthesis, alanine serine, threonine, aspartic acid, glycine, glutamic acid; osmoprotectants, protein stabilizing	Paul and Nair (2008)
Salinity	*Pseudomonas fluorescens* MSP-393	Rice	Protein stabilization and alteration of cell envelope composition; altered expression of proteins, periplasmic glucans and capsular, exo- and lipopolysaccharides	Paul et al. (2006)
Salinity	*Pseudomonas putida* FBKV2	Maize	Production of exopolysaccharides; enhancing water retention and regulating the diffusion of carbon sources	Vurukonda (2016a, b)
Salinity	*Dietzia natronolimnaea* STR1	Wheat	Modulation of transcriptional machinery of stress-related antioxidant genes	Bharti et al. (2016)
Salinity	*Bacillus megaterium*	Maize	Increased ability of the root to absorb water	Marulanda et al. (2010)
Salinity	*Pseudomonas* sp.	Pistachio	Increased protein content, antioxidant activity, ACC deaminase activity	Azar et al. (2016)
Salinity	*Enterobacter cloacae* and *Bacillus drentensis*	Mung bean	Modified stomatal conductance, transpiration rate, water relations, and synthesis of photosynthetic pigments	Mahmood et al. (2016)
Salinity	*P. pseudoalcaligenes* and *Bacillus pumilus*	Rice	Increased concentration of glycine betaine-like quaternary compounds	Jha et al. (2011)
Salinity	*Enterobacter* sp.UPMR18	Okra	ROS scavenging enzymes	Sheikh et al. (2016)
Salinity stress	*Arthrobacter protophormiae (SA3) and Dietzia natronolimnaea (STR1)*	Wheat	Enhance photosynthetic efficiency; increase indole-3-acetic acid; modulating expression of a regulatory component (CTR1) of the ethylene signaling pathway and DREB2 transcription factor	Barnawal et al. (2017)

(continued)

Table 9.4 (continued)

Stress condition	Rhizobacterial inoculant	Plant species	Mechanisms	References
Salinity stress	*Achromobacter piechaudii* and *Bacillus subtilis*	Tomato (*Lycopersicon esculentum*)	Degradation of reactive oxygen species	Mayak et al. (2004), Zhang et al. (2008), and Yang et al. (2009)
Salinity	*Enterobacter* sp. (MN17) *Bacillus* sp. (MN54)	*Chenopodium quinoa*	ACC deaminase activity, exopolysaccharide secretion, and auxin production	Yang et al. (2016)

Table 9.5 Role of PGPR in ameliorating oxidative stress

Stress condition	Rhizobacterial inoculant	Plant species	Mechanisms	References
Oxidative stress	*Azospirillum* spp.	Maize	Phytohormone production and induction of plant-stress tolerance and defense genes	Fukami et al. (2017)
Oxidative stress	*P. agglomerans* RSO6 and RS07 *B. aryabhattai* RSO25	*Spartina densiflora*	Regulation of antioxidant enzyme activity	Paredes-Páliz et al. (2018)
Oxidative stress	Rhizobacteria	*Oryza sativa*	Enzyme activities related to oxidative stress induced such as ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, superoxide dismutase	García-Cristobal et al. (2015)
Oxidative stress	*Burkholderia cepacia* SE4, *Promicromonospora* sp. SE188, and *Acinetobacter calcoaceticus* SE370	*Cucumis sativus*	Reduced activities of catalase, peroxidase, polyphenol oxidase	Kang et al. (2014)

Withania somnifera (an indigenous medicinal plant); however, the enzymatic antioxidant activity increases under UV-B treatment (Takshak and Agrawal 2014a). The adverse effects of UV-B radiation on growth characteristics of *Brassica campestris* were said to be reduced upon application of IAA at 10^{-7} M (Lal et al. 2011). However, IAA is reported to be susceptible to UV-B-induced damage via direct photodegradation and enhanced activity of IAA oxidase enzyme (Huang et al. 1997) (Table 9.5).

9.7.5 Heavy Metal Stress

Heavy metals like lead, nickel, cadmium, copper, cobalt, chromium, and mercury which are known as environmental pollutants cause toxic effects in plants, thereby lessening productivity. They affect the basic physiological and biochemical activities in plants such as photosynthesis. PGPR is known to precipitate and remove toxic metals from the surroundings by mechanisms such as intracellular sequestration or sorption to cell components. Rizvi and Khan (2018) showed that plant growth-promoting *Azotobacter chroococcum* produced siderophores, ammonia, and ACC deaminase under metal pressure and enhanced growth and yield of maize in the presence of both Cu and Pb. Also, the melanin extracted from *A. chroococcum* revealed metal chelating ability. *Kluyvera ascorbata* SUD165, a PGPR resistant to the toxic effects of Ni^{2+}, Pb^{2+}, Zn^{2+}, and CrO^{4-}, producing siderophore(s) and displaying ACC deaminase activity, was capable of promoting growth in plants in the presence of nickel by its ability to lower the level of stress ethylene induced by the nickel (Burd et al. 1998). Dell'Amico et al. (2008) also showed that inoculation with cadmium-resistant strains *Pseudomonas tolaasii* and *Pseudomonas*

Table 9.6 Role of PGPR in ameliorating heavy metal stress

Stress condition	Rhizobacterial inoculant	Plant species	Mechanisms	References
Metal stress	*P. tolaasii*	Canola (*Brassica napus*)	ACC deaminase activity	Dell'Amico et al. (2008)
Metal stress	*P. fluorescens*	Canola (*Brassica napus*)	Indole acetic acid (IAA) and siderophore production	Dell'Amico et al. (2008)
Metal stress	*Bacillus* sp.	*Rice*	Superoxide dismutase	Asch and Padham (2005)
Metal stress	*Kluyvera ascorbata*	Tomato, canola, and Indian mustard seeds	Siderophore production	Burd et al. (2000)
Metal stress	*Pseudomonas libanensis*	*Brassica oxyrrhina*	Production of indole-3-acetic acid, siderophore, and ACC deaminase	Ma et al. (2016)
Metal stress	*Pseudomonas fluorescens*	Maize	Production of IAA	Zerrouk et al. (2016)
Metal stress	*Pseudomonas stutzeri* A1501	*Rice*	Transcription and translation of acdS gene, ACC deaminase	Han et al. (2015)
Metal stress	*P. aeruginosa* strain OSG41	Chickpea	IAA, PO_4 solubilization, EPS production, siderophores, salicylic acid, 2,3-Dihydroxybenzoic acid, HCN, and NH_3 production	Oves et al. (2013)

fluorescens enabled *Brassica napus* to grow under cadmium stress by the production of IAA, siderophores, and ACC deaminase. Siderophores also play a key role in the regulation of auxin level in plants growing in metal-contaminated sites. Metals are known to inhibit auxin synthesis. Dimkpa et al. (2008b) proved that siderophores complexed with toxic metals, thereby decreasing the concentration of free metals and attenuating metal inhibition of auxin synthesis (Table 9.6).

9.8 Conclusion

PGPRs have the ability to act as bioprotectants under abiotic stress and can enhance plant growth. Because of their rhizoremediating and phytostimulating properties, they could be beneficial in replacing chemical fertilizers and supporting eco-friendly sustainable food production. Due to their worldwide importance and acceptance, PGPR are the future of sustainable agriculture.

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Rhizobacteria for Reducing Heavy Metal Stress in Plant and Soil

10

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Abstract

The intensity of pollution expansion is increasing day by day of which heavy metal pollution has taken the center stage of discussion since the last few decades. Heavy metals have direct detrimental effect on our ecosystem in general and on the agroecosystem in particular, thereby proving to be hazardous for plants, animals, and microbes. One of the most common, low-cost, and eco-friendly strategies that can be employed to counter this problem effectively is through bioremediation. However among several types of bioremediation, microbial bioremediation with the use of rhizobacteria is best suited for alleviating heavy metal stresses in the agroecosystem.

Keywords

Heavy metals · Rhizobacteria · PGPR · Bioremediation

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10.1 Introduction

There exists a lot of misperception over the classification and definition of heavy metals. Still scientific groups have not reached into any consensus regarding this issue. In a report published in the International Union of Pure and Applied Chemistry (IUPAC), Duffus (2002) raised questions over the usage of the term “heavy metals” and its classifications. Therefore he suggested undertaking a much broader approach while classifying heavy metals based on the periodic table. In agreement with his views, Appenroth (2010) proposed for considering three groups of elements (transition elements, rare earth metals, and borderline elements) as heavy metals from the periodic table after thoroughly studying their chemical properties.

Keeping all these discussions aside, however, the most commonly followed definition of heavy metals is “These are the elements with an atomic weight between 63.5 and 200.6 followed by a specific gravity of more than 5.0” (Srivastava and Majumder 2008). In simple terms, we can say that they are heavier than water by five times or are having an atomic density >4 g/cm^3 (Duruibe et al. 2007; Mahamood et al. 2012). They can also be defined as the block of all metals in Groups 3–16 that are present in period 4 and above, i.e., periods 5, 6, and 7 (Hawkes 1997). The term heavy metals in a broader sense are often used whenever there arises some implication for toxicity. As heavy metals are present in very minute quantity, i.e., 1 μg kg^{-1}, these are often represented as trace elements (Tchounwou et al. 2012). Some of these trace metals are beneficial for plants (Zn, Mn, Fe, Cu, B, and Mo), while others are non-beneficial (Se and Co), and the rest (As, Hg, Pb, Cr, Cd, and Ni) are toxic (He et al. 2005).

10.1.1 Current Status of Heavy Metal Pollution

Pollution of heavy metals has been seen everywhere across the earth (lithosphere, atmosphere, and hydrosphere). It has been escalated to such an extent that it can be found even on the most extreme climatic conditions on earth starting from Mount Everest (Yeo and Langley-Turnbaugh 2010) to the deep ocean floor (Humbatov et al. 2015) and also underneath the topsoil layer (Wuana and Okieimen 2011; Su et al. 2014). Bioaccumulation of these metals can be seen on food items like milk (Tunegova et al. 2016), vegetables (Agrawal et al. 2007; Mishra and Tripathi 2008), fishes (Ebrahimpour et al. 2011; Abarshi et al. 2017), and livestock (Rajaganapathy et al. 2011; Okareh and Oladipo 2015). Rampant pollution had led to their worldwide distribution across every continent. Be it Asia (Rajindiran et al. 2015; Chen et al. 2015; Ghorbani et al. 2015) or Africa (Yabe et al. 2010), their presence can be felt everywhere. Rapid industrialization has also escalated their concentration in developed portions of the world like Europe (Panagos et al. 2013; Toth et al. 2016), Australia (Hart and Lake 1987), and South America (Smolders et al. 2003; Eichler et al. 2015). However, their presence in Antarctica seems to be quite surprising as it is so far uninhabited and unexplored as compared to the rest of the world (Evans et al. 2000; Santos et al. 2005). These things reflect the true situation of heavy metal pollution, thus a much needed eye-opener for us to save our ecosystem from further destruction.

Heavy metals are also found above permissible limits in our day-to-day utility commodities like food items (Mahaffey et al. 1975), soft drinks (Bingol et al. 2010; Godwill et al. 2015), and cosmetics (Borowska and Brzoska 2015). In some of the worst affected countries like India and Bangladesh, arsenic (As) is present above permissible limits in rice grains (Sinha and Bhattacharyya 2014; Meharg and Rahman 2003). Rice being the staple food in these countries leads to direct intake of arsenic. Not only in rice but also arsenic in cereals, pulses, vegetables, and forage crops has been reported by several researchers (Sharma et al. 2007; Santra et al. 2013). A regular dietary intake of these arsenic-contaminated food items (Signes et al. 2008) is a direct threat to one's life. Therefore, different regulatory agencies like the World Health Organization (WHO), European Food Safety Authority (EFSA), and Agency for Toxic Substances and Disease Registry (ATSDR) have prescribed the maximum intake capacity of heavy metals as mentioned in Table 10.1.

Arsenic among all these heavy metals is ranked among the top ten hazardous chemicals by WHO. Besides this, it is also ranked number 1 by ATSDR (2017) on its substance priority list followed by lead and mercury. Lead till now is probably the most well-studied occupational toxin causing about 0.6% of all diseases worldwide (Gidlow 2004). More than 120 million people worldwide come under the threat lead toxicity with developing nations being the most affected (Venkatesh 2009).

The direct impact of heavy metal contamination is seen in soil and groundwater. The European Commission's report on soil contamination and their impact on human health stated that heavy metals are the most frequently occurring contaminants on soil (35%) and groundwater (31%). Soils (around 33%) all over the world are facing serious heavy metal contamination problem (Roslan et al. 2016). Say for China, around 19.40% of Chinese farmland is facing heavy metal

Table 10.1 Permissible limits of different heavy metals set by EFSA (European Food Safety Authority), WHO (World Health Organization), and ATSDR (Agency for Toxic Substances and Disease Registry)

Metals	EFSA (2006)	WHO-FAO (1995)	ATSDR (2018)
Ni	2.8 µg/kg of body weight (TDI)	<100 µg/day	0.0002 mg/m^3
Hg	1.3 µg/kg of body weight (TWI)	5 µg/kg of body weight per week	0.0002 mg/m^3
Cr	0.3 mg/kg of body weight (TDI)	33 µg/day	0.005 mg/kg/day
Cd	2.5 µg/kg of body weight (TWI)	7 µg of cadmium/kg of body weight per week	0.0005 mg/kg/day
As	<15 µg/kg of body weight (TWI)	<200 µg/day	0.005 mg/kg/day
Pb	<25 µg/kg of body weight (TWI)	25 µg/kg of body weight per week for adults	

TDI tolerable daily intake, *TWI* tolerable weekly intake pollution (Zhang et al. 2015). Due to soil pollution, a loss of more than 10 billion US dollars is being incurred from over 10 million polluted sites out of which 50% contaminants happen to be heavy metals (He et al. 2015). Agricultural pesticides are one of the main sources of arsenic contamination in soil. A total of around 80–90% arsenic produced annually finds its way into soil through these chemicals (Nriagu and Pacyna 1988). Hutton and Symon (1986) reported that annually 1637 tons of lead and 111 tons of arsenic are being deposited into the arable soils of the United Kingdom through anthropogenic sources.

Atmospheric pollution of heavy metals after soil is the next biggest concern for researchers. About 30% of mercury per annum is released from anthropogenic sources into the atmosphere of which 50% comes from Asia alone (UNEP 2013). Excessive release of mercury into the air transports them to North America by wind, accounting for 5–36% of mercury deposition in the United States (Jaffe et al. 2005). Due to its long-range transport ability, even the Arctic region is also polluted from mercury contamination (Ilyin et al. 2004). Other than mercury, cadmium also contributes significantly to atmospheric heavy metal pollution. It has been reported that Spain and France equally contribute (i.e., 16%) for cadmium emission in Europe's air (Dinis and Fiuza 2011). In recent times, Indian cities also show the presence of heavy metals in their atmosphere, often exceeding the maximum permissible limits (Chaudhari et al. 2012; Dey et al. 2014).

Groundwater heavy metal contamination is also an equally important global concern like soil and air heavy metal pollution. Among all heavy metals, arsenic contamination in groundwater is most noticed with South Asian countries like Bangladesh and India being worst affected (Ravenscroft et al. 2005; Pal et al. 2009). All over the world, nearly 130 million people come under the threat of arsenic contamination by drinking As-contaminated water, which is often above the prescribed limit (10 ppb) set by WHO (UNICEF 2008). Majority of these populations are inhabitants of two countries, i.e., Bangladesh (35–77 million) and India (12 million from the state of West Bengal alone), making them globally the worst hit countries (Smith et al. 2000; Ravenscroft et al. 2009). In India there are seven major states (West Bengal, Assam, Uttar Pradesh, Chhattisgarh, Bihar, Jharkhand, and Manipur) which find arsenic contamination in their groundwater (Chakraborti et al. 2017). The regulatory limits of heavy metals in drinking water prescribed by different agencies have been stated in Table 10.2.

10.1.2 Sources of Heavy Metal Pollution

Heavy metal contamination in the environment occurs through natural and anthropogenic means (Chen et al. 2009; INSA 2011). Heavy metals are nondegradable, due to which they are persistent in our environment and in the course of time get released into the soil, water, and air (Zaharescu et al. 2009; Aksu 2015; Van et al. 2016; Drira et al. 2017). During weathering and soil formation processes, they are released from rocks (metamorphic, sedimentary, and magmatic rocks) and minerals (oxides, hydroxides, and clay minerals) into the environment (Brad 2005). The fate

Table 10.2 Minimum prescribed limits for heavy metals in drinking water set by different regulatory agencies

Heavy metals	BIS (2012) in mgL^{-1}	WHO (2017) in mgL^{-1}	EPA (2001) in mgL^{-1}
Selenium	0.01	0.04	0.01
Cadmium	0.003	0.003	0.005
Lead	0.01	0.01	0.05
Mercury	0.001	0.006	0.001
Nickel	0.02	0.07	
Arsenic	0.01	0.01	0.05
Chromium	0.05	0.05	0.05
Antimony	–	0.02	5

of these metals on soil is governed by the type of parent materials and physiochemical properties of soil (Abdelilah et al. 2010; Roozbahani et al. 2015). Natural phenomena like volcanic eruptions, forest fires, and soil erosions play a major role in their distribution (Bielicka et al. 2005; Akpor et al. 2014). In aquatic systems, sediments are the chief storehouse of heavy metals, governing their overall distribution and transformation processes in water bodies (Wu et al. 2014). The level of heavy metals is often well regulated and rarely crosses their limits in natural environment.

However in contrast to natural sources, anthropogenic sources are more responsible for elevation of heavy metal concentration in natural environment (Xu et al. 2014). Human-driven activities like mining, intensive agricultural practices, and road constructions driven by urbanization and industrialization act as the perfect catalyst for their release into natural environment (Imperato et al. 2003; Liao et al. 2018). Intensive agricultural practices like excessive usage of pesticides and fertilizers coupled with sewage water for irrigation have led to the accumulation of heavy metals in cultivated soils (Sidhu 2016). Furthermore, runoff water passing through highways during rainfall contains heavy metals (Turer et al. 2001). Besides these, polyvinyl chloride (PVC) products, chargeable batteries, brake linings, tires, color pigments, furnace dusts, etc. are some other potential sources of heavy metals (Oves et al. 2016). Comprehensive descriptions for anthropogenic sources of heavy metals are listed in Table 10.3.

10.2 Effects of Heavy Metals on Life Forms

Due to their persistent nature, heavy metals accumulate in our body resulting in several health issues (Sharma et al. 2007; Garg et al. 2014). Heavy metals enter our body through food, air, and water. However the chief entry route of heavy metals into our body is through food (Darwish et al. 2015; Yadav et al. 2017). Regular intake of heavy metal-contaminated food can retard growth and weaken our immune system (Singh and Kalamdhad 2011). Alongside food, they can also make entry through the skin and air (Liang et al. 2017). Entry of these metals through food chain causes their bioaccumulation and paves the path for several cardiovascular,

Table 10.3 Different heavy metals and their anthropogenic sources

Heavy metals	Anthropogenic sources	References
Arsenic (As)	Herbicides, pesticides, inorganic fertilizers, coal and petroleum combustion, nonferrous metal smelting, mining, poultry litter, sewage sludge, fly ash, wood preservatives, desiccants, feed additives, pharmaceutical industries, glass industry, pigments, cigarettes, semiconductor manufacturing, cotton ginning	Bellows (2005), Hamzah et al. (2013), Arunakumara et al. (2013), Chung et al. (2014), ATSDR (2007a, b), and Rice et al. (2002)
Cadmium (Cd)	Cigarettes, fertilizers, polymer industry, varnished industry, coatings, pigments and coloring agents, stabilizers, electronic waste (e-waste), batteries, phosphate fertilizers, smelting and refining of nonferrous metals, fossil fuel combustion, liming agents, manures, sewage sludge	Hutton (1983), Sugita et al. (2001), Piade et al. (2015), and Rosemary et al. (2014)
Chromium (Cr)	Paints and pigments, leather industry, stainless steel and iron production, textile industry, porcelain and ceramics manufacturing, chrome alloy production and electroplating, wood preservatives, coal and oil combustion, chemical industry	Saha et al. (2011), ATSDR (2012a, b), and Chung et al. (2014)
Lead (Pb)	Battery, pigments, plastics, rubber industry, smelting plants, ceramics, petrol, gasoline, solid waste combustion, cigarettes	Zeitoun and Mehana (2014), Ashraf (2011), and Mielke et al. (2001)
Mercury (Hg)	Coal burning, chlor-alkali plants, cement production, nonferrous smelting, waste incineration, refining, gold mining, chemical industry, pharmaceutical industries, fungicides, fluorescent and ultraviolet lamps	Rodrigues et al. (2006) and Naja and Volesky (2009)
Nickel (Ni)	Mining and smelting, ferrous and nonferrous metals production, battery, chemical industry, electroplating, petroleum processing, cement manufacturing, sewage sludge incineration, coal and oil combustion, nickel matte refining, steel production, nickel alloy production, vehicle emissions, fertilizer and organic manures, cement production, disinfectants manufacture	ATSDR (2005a, b)
Selenium (Se)	Coal and oil combustion, glass industry, semiconductor manufacturing, paint industry, mining and smelting, ceramics, refining, sewage sludge, photo cells, vulcanization of rubber, pharmaceutical industries, insecticides, herbicides, lubricants, xerography (photocopiers), animal feed additives, manufacture of inorganic pigments, phosphate fertilizers	ATSDR (2003)

nervous, kidney, and bone diseases (Rani and Goel 2009; Ji-yun et al. 2016). Health issues occurring due to heavy metals are enlisted in Table 10.4.

The term heavy metal is often used in context of toxicity, but it should be noted that not all heavy metals (like Mn, Cu, Zn, Fe, etc.) are harmful (Flora et al. 2008).

Table 10.4 Impact of various heavy metals on human health

Heavy metals	Impact on human health	References
Arsenic	Arsenicosis (chronic arsenic toxicity), arteriosclerosis, laryngitis, respiratory diseases, nausea, vomiting, proteinuria, diarrhea, abdominal pain, anorexia, weight loss, pigmentation, neuritis, skin lesions, keratosis, melanosis, dermatosis, hypertension, bronchitis, oliguria, renal failure, affects heme biosynthesis, Anemia, leucopenia, low IQ in children, cancer (lungs, skin, kidney, bladder, liver, colon and nasal cancer), gastroenteritis, diabetes, neurobehavioral changes and abnormalities, peripheral neuropathy, increases fetal mortality rate, polyneuropathies, hallucinations, increases stillbirth, weakness and fatigue, edema, Bowen's disease	ATSDR (2007a, b), Singh et al. (2007), Hughes et al. (2011), Mazumder (2008), Pierce et al. (2010), Tchounwou et al. (2003), Silva et al. (2005), Florea and Busselberg (2006), and Rossman (2003)
Nickel	Severe lung damage, giddiness, headache, diarrhea, hematuria, allergic dermatitis, emphysema, nausea, pulmonary fibrosis, vomiting, vertigo, kidney problems, mucosal irritation, tachycardia, abdominal pain, muscular pain, asthma, bronchitis, dyspnea , cyanosis, cancer (lungs, nasal cavity, kidney, prostate, bone and laryngeal cancer)	ATSDR (2005a, b), Al-Fartusie and Mohssan (2017), and Das et al. (2008)
Cadmium	Hypertension, osteoporosis and osteomalacia, emphysema, testicular atrophy, muscular weakness, bronchiolitis, renal failure, olfactory dysfunction, increases fetal mortality, abdominal cramps, anosmia, memory loss, lymphocytosis, eosinophilia, nausea, vomiting, itai-itai disease, glucosuria, proteinuria, myocardial infarction, chronic rhinitis, cancer (kidney, lung, pancreas, urinary bladder, endometrium, breast, and prostate cancer)	ATSDR (2012a, b), Ayangbenro and Babalola (2017), Sharma et al. (2014), Notarachille et al. (2014), Singh and Kalamdhad (2011), and Wu et al. (2016a, b)
Lead	Headaches, hypertension, vomiting, nausea, depression, anxiety, reduced fertility and miscarriages, renal failure, hallucinations, Anemia, abdominal pain, gastrointestinal problems, high blood pressure, encephalopathy, hemoglobinuria, loss of appetite, loss of memory, intellectual disorders, behavioral problems, diarrhea, low IQ in children, constipation, lethargy, impairment of neurological development (ataxia), growth and mental retardation, cancer (lung, brain, kidney, and stomach cancer)	ATSDR (2007a, b), Sharp and Brabander (2017), Mamtani et al. (2011), Jan et al. (2015), Lee et al. (2018), Rousseau et al. (2007), Qu et al. (2018), and Patocka and Kuca (2016)

(continued)

Table 10.4 (continued)

Heavy metals	Impact on human health	References
Mercury	Prenatal toxicity and damage, impaired sexual functions, proteinuria, edema, dermatitis, pneumonitis, gingivitis, insomnia, respiratory failure, deafness, mental retardation, blindness, dysarthria, cough, dyspnea, mercurial erythrism, insomnia, weight loss, renal tubular dysfunction and kidney failure, neuropsychiatry disorders, infertility, miscarriage, neuropsychiatry disorders, memory loss	ATSDR (1999), Clarkson (1992), Maqbool et al. (2017), Golding et al. (2013), and Eqani et al. (2016)
Chromium	Irritation to the nasal cavity, asthma and cough, dermatitis, epistaxis, pneumoconiosis, gastrointestinal problems, kidney and liver problems, hypochromic anemia, decrease in sperm count, hyperplasia, postnatal hemorrhage, abdominal pain, bloody diarrhea, cancer (lung and nasal cavity), renal failure, skin ulcers	ATSDR (2012a, b), Jomova and Valko (2011), and Ding and Shi (2002)
Selenium	Nausea, vomiting, tachycardia, diarrhea, selenosis (high level of se in blood), fatigue, hair loss, irritability, dermal and neurological effects	ATSDR (2003) and Fraga (2005)
Thallium	Hair loss (alopecia), vomiting, diarrhea, constipation, palmar erythema, anorexia, blindness, affects menstrual cycle, high blood pressure, joint pain, tachycardia, polyneuropathy, muscle weakness, disturbance in vision, paraesthesia, psychosis, depression, behavioral abnormalities, gastroenteritis, may cause death also. Affects respiratory, gastrointestinal, cardiovascular and male reproductive system	ATSDR (1992), Achparaki and Thessalonikeos (2012), Peter and Viraraghavan (2005), Cvjetko et al. (2010), Xiao et al. (2012), and Li et al. (2015)
Copper	Nausea, vomiting, diarrhea, severe headache, abdominal pain, hair loss, anemia, male infertility, coughing, sneezing, insomnia, convulsion, arthritis, attention deficit disorder, pulmonary fibrosis, jaundice, autism, prostatitis, renal failure, gastrointestinal problems, hypotension, bronze diabetes, liver damage	ATSDR (2004) and Ashish et al. (2013)
Zinc	Nausea, vomiting, respiratory disorder, diarrhea, coughing, abdominal pain, anemia, leukopenia, dyspnea, renal failure, gastroenteritis, conjunctivitis, skin damage (blisters and ulcers), hypertension, acute pneumonitis, pulmonary fibrosis, constipation, headache, insomnia, pharyngitis	ATSDR (2005a, b) and Plum et al. (2010)

Some of these heavy metals are part of several metabolic pathways, while the rest are toxic to our body (Mahurpawar 2015; Al-Fartusie and Mohssan 2017). Chromium, for instance, has dual functions in our body. In its low concentration, it is used in a number of metabolic processes (like fat and protein metabolism), while excess exposure causes several respiratory diseases (Sathawara et al. 2004).

In a similar way, copper is used for iron absorption and signaling. However when present in excess amount, it causes liver and kidney dysfunctions (Ashish et al. 2013). More often than not, we consider Zn (Zinc) as an essential element as it is part of numerous proteins and metalloenzymes. It is observed that excessive amount of Zn in our body may result in nausea and vomiting while its deficiency leads to neural disorders (Plum et al. 2010). Heavy metals are mutagenic and carcinogenic in nature (Silva et al. 2005; Fernandez-Luqueno et al. 2013). Heavy metal contamination causes a wide range of health issues related to developmental, gastrointestinal, dermal, respiratory, cardiovascular, immunological, and reproductive systems (Liu et al. 2013).

Heavy metals lead to oxidative stress, and to neutralize this effect, the cell produces antioxidants (catalase and superoxide dismutase) in its response. This balance is always maintained in our body, and any imbalances lead to altered gene expression, activation of signaling pathways, and production of cytokines (Salnikow et al. 2000; Leonard et al. 2004). Activation of metal-induced signaling pathways affects several signaling components (G-proteins, MAP kinases, tyrosine kinases, growth factor receptors, and nuclear transcription factors), thereby disrupting the normal functioning of the cell (Harris and Shi 2003; Flora et al. 2008). Researchers have also reported that heavy metal stresses induce apoptosis in cells (Wang and Shi 2001). Heavy metals cause cancer and are thus labeled as carcinogens (Galaris and Evangelou 2002). These metals damage DNA and cause mutation which leads to cancer (Durham and Snow 2006; Jadoon and Malik 2017), with lung and skin cancers being the most common among them (Harris and Shi 2003). However they also cause several other cancers like liver, kidney, bladder, prostate, lymphoma, leukemia, and breast (Pourahmad et al. 2003). The central nervous system (CNS) and hematopoietic system are also affected by the presence of these metals (Florea and Busselberg 2006). It has been reported that these metals are related to a wide range of neurological diseases like Wilson's disease (Cu), Parkinson's disease (Fe, Mn, and Cu), Alzheimer's disease (Cd), Hallervorden-Spatz disease (Fe), multiple sclerosis, polycythemia, Minamata disease (Hg), muscular dystrophy, sideroblastic anemia, itai-itai disease (Cd), and blackfoot disease (As), among others (Montgomery 1995; Khan et al. 2013; Jaishankar et al. 2014; Draszawka-Bolzan 2014; Min and Min 2016). Metal toxins alter the functioning of neurotransmitters like catecholamines and bring about behavioral changes in humans (Shukla and Singhal 1984; Inoue 2013). Premature aging can occur due to heavy metal toxicity, thus paving the path for occurrence of numerous diseases (Mudgal et al. 2010).

Like humans, plants too uptake heavy metals, and their entry points are root and leaves. They get deposited in the cell wall, plasma membrane, or cytoplasm after traveling through xylem by means of apoplastic and symplastic pathways (Shahid et al. 2015; Clemens and Ma 2016). Uptake of heavy metal by plants is greatly influenced by the type of plant species and the defense mechanisms followed by them to overcome its toxicity (Alves et al. 2016). In agriculture, there are a lot of crop plants which show phytotoxicity to these metals (Forster 1954; Benzarti et al. 2008). The attributes that are hampered by heavy metal toxicity are seed germination, yield, nutrient uptake, and nitrogen fixation (Athar and Ahmad 2002; Guala et al. 2010;

Sethy and Ghosh 2013). It has been observed that sometimes heavy metals besides competing with each other also try to compete with several other essential elements for their uptake, both at the cellular level and in the soil system (Krupa et al. 2002; Israr et al. 2011). For example, a certain concentration of As (arsenic) helps in the uptake of Mn, Cu, Fe, and P; however with its further increase in concentration, uptake of these metals decreases (Farnese et al. 2014).

There is significant reduction in the photosynthetic rate of plants due to heavy metal toxicity. This is due to the fact that these metals affect the enzymes of photosystem I and II which causes lower biomass production (Oves et al. 2016). Physiological and biochemical activities of plants like respiration, translocation, transcription, translation, mineral metabolisms, cell signaling, and cell cycle along with some developmental processes like flowering and embryogenesis are also affected (Ovecka and Takac 2014). Due to the presence of abiotic stresses (i.e., from heavy metals), lower root and shoot growth is observed in several crop plants which can be correlated with decrease in chlorophyll and protein content in these plants (Manios et al. 2002; John et al. 2009). Furthermore, heavy metal toxicity is dependent on plant growth stages (Cheng 2003; Peralta-Video et al. 2004).

Like humans, plants also produce reactive oxygen species (ROS) like H_2O_2, OH^-, $^1O_2$, and O_2^- and reactive nitrogen species (RNS) like nitric oxide and peroxynitrite $ONOO^-$ and free radicals in response to oxidative stress caused by heavy metals (Zengin and Munzuroglu 2005; Moller et al. 2007). Oxidative stress results in cellular toxicity and leads to oxidative degradation of biomolecules like carbohydrates, proteins, lipids, and nucleic acids (Aras et al. 2012). Arsenic toxicity displays a variety of symptoms in plant like leaf defoliation, chlorosis, necrosis, reduced fertility, stunted growth, and senescence and under severe condition may also cause death (Gulz et al. 2005; Abbas et al. 2018). Phosphate metabolism in plants gets affected by arsenic as arsenate mimics phosphate ion and can get substituted in its place (Kaur et al. 2011). Besides this, magnesium ion in chlorophyll molecule may also be substituted by other heavy metals (Zurek et al. 2014). Likewise, cadmium also interferes with several plant processes like photosynthesis, transpiration, mineral nutrition (N, K, Ca, Mg, P, and Fe), stomatal opening, and antioxidant metabolism (Benavides et al. 2005; Nazar et al. 2012). Nickel plays an essential role in nitrogen metabolism and seed germination. However, Ni toxicity results in chlorosis and yellowing of leaves which finally affect the normal functioning of plant (Selvaraj 2018). In some cases, it is interesting to see that two heavy metals have additive effects on their toxicity in plants. For instance, in barley plant, it has been observed that the combined effect of copper and cadmium resulted in lower root and shoot growth (Zaltauskaite and Sliumpaite 2013).

10.3 Heavy Metals and Microorganisms

Microorganisms also are subjected to heavy metal stress like any other life forms. Microbes (diatoms and microalgae) are often used for heavy metal pollution assessment and act as bioindicators (Sbihi et al. 2012; Djukic and Mandic 2018). Microbes are very sensitive to heavy metals and exhibit this sensitivity even at species and strain level (Giller et al. 1998). Microbes from different habitats and groups exhibit varied level of heavy metal tolerance (Sadler and Trudinger 1967). Generally, fungi are said to be more tolerant than bacteria to these metals (Rajapaksha et al. 2004).

Soil when exposed with heavy metals for a prolonged period of time resulted in decreased microbial biomass and reduced microbial diversity and activity with further change in their genetic composition (Chen et al. 2014; Kuzniar et al. 2018). These metals also considerably influence the bacterial community structure as revealed from metagenomic studies (Yao et al. 2017). These metals enhance microbial growth in its lower concentration while when present in excess quantity are harmful for the cell by affecting its membrane integrity, destroying its cellular organelles, and damaging its genetic materials (Sengor et al. 2009). Furthermore, an increase in lag time brings about reduction in growth of microbial cells (Gikas et al. 2009). Physiological activities like respiration and metabolism are affected due to heavy metals resulting in lower production of soil enzymes (Xie et al. 2016). Further, reproduction of several fungal species also gets influenced by the presence of these metals. Baldrian (2003) reported that the reproductive stages of saprophytic and mycorrhizal fungus were more affected as compared to their vegetative stages.

Microbes growing in the presence of heavy metals show certain morphological changes like transformation from one form to another. Certain bacteria change their shape from rod to spherical in copper's presence (Sadler and Trudinger 1967). Similar findings have been reported in fungi where heavy metal induces certain morphological changes in fungal hyphae (Ali 2007). Soil-inhabiting fungus is also affected from these metals. Fungi play an important role in biodegradation process and biogeochemical cycles while influenced by the presence of heavy metals (Hartikainen et al. 2012; Khan et al. 2013). Nitrification, which is a crucial step in nitrogen cycle, is significantly inhibited by the presence of these metals (Park and Ely 2008; Hamsa et al. 2017). Microorganisms have the ability of uptaking heavy metals through certain metabolic or physiochemical pathways known as microbial biosorption. This metal uptake rate depends upon a wide array of factors like physiological state of cell, nature of growth medium, and type of microbes growing (Vijayadeep and Sastry 2014). By effectively utilizing this property, microbes can serve as a tool for alleviating heavy metal stress from the environment (Yamaji et al. 2016).

10.3.1 Bioremediation of Heavy Metal by Rhizobacteria

Soil pollutants can be extracted from the soil by employing several bioremediation techniques. Plant growth-promoting rhizobacteria (PGPR) are one of the better prospects for bioremediation of heavy metals in the rhizosphere. Rhizobacteria in combination with plants are more fruitful and provide better efficiency for bioremediation of heavy metals (Whiting et al. 2001). Upon exposure to heavy metal stress, rhizobacteria alter plant metabolism, due to which plants are able to withstand high concentrations of metals (Welbaum et al. 2004). The use of rhizobacteria in phytoremediation has therefore recently gained some momentum (de Souza et al. 1999). The symbiotic effectiveness of bacteria-plant system for the restoration of polluted soil from chromium and cadmium contamination was studied by Sobariu et al. (2017) where they utilized rhizospheric *Azotobacter* bacteria and *Lepidium sativum* plant for completing this task. They observed that the ability of heavy metal tolerance by plant improved under symbiotic condition. Furthermore, bacterial consortia native to heavy metal-contaminated soil, consisting of *Bacillus mycoides* and *Micrococcus roseus*, were found effective for phytoextraction and phytostabilization of Cd (Malekzadeh et al. 2012). Bioremediation of zinc was mediated by rhizobacteria (*Bacillus megaterium* and *Pseudomonas aeruginosa*) isolated from weed (*Suaeda nudiflora*) growing in chemically polluted site (Jha et al. 2017).

Important genera of cadmium-resistant rhizobacteria reported from some food crops (wheat, maize, barley, mustard, mung bean, black gram, and pumpkin) are *Pseudomonas* spp., *Burkholderia* sp., *Flavobacterium* sp., and *Arthrobacter mysorens* (Belimov and Dietz 2000; Ganesan 2008; Sinha and Mukherjee 2008; Kuffner et al. 2010; Xu et al. 2012; Saluja and Sharma 2014). Similarly, some arsenic-resistant gram-positive rhizobacteria are *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Arthrobacter globiformis*, and *Staphylococcus lentus*, while gram-negative rhizobacteria include *Rhizobium radiobacter*, *Rhizobium rhizogenes*, *Enterobacter asburiae*, *Agrobacterium radiobacter*, *Sphingomonas paucimobilis*, and *Pantoea* spp. (Wang et al. 2011; Titah et al. 2014; Lampis et al. 2015; Singh et al. 2015; Mesa et al. 2017). Rafique et al. (2015) reported some bacterial genera (*Bacillus*, *Pseudomonas*, and *Cronobacter*) capable of showing dual functions, i.e., simultaneously showing resistance for mercury, as well as capable of nitrogen fixation. Likewise, rhizobacteria capable of tolerating chromium are *Pseudomonas*, *Ochrobactrum*, *Mesorhizobium*, *Bacillus*, *Paenibacillus*, *Cellulosimicrobium*, and *Rhodococcus* (Faisal and Hasnain 2006; Trivedi et al. 2007; Chatterjee et al. 2009; Khan et al. 2012; Hemambika et al. 2013; Upadhyay et al. 2017).

10.3.2 Mechanisms of Heavy Metal Tolerance in Bacteria

Microbes are persistently able to survive in heavy metal-polluted environment by using a number of methods like biosorption, biomineralization, bioaccumulation, and biotransformation. Bioaccumulation is a process by which bacteria accumulate heavy metals in its cell which is influenced by various physical, chemical, and biological mechanisms operating inside its cell (Ayangbenro and Babalola 2017). Similarly, biosorption is defined as the passive uptake of metals by microbes (Malik 2004; Gadd 2009). Biomineralization is the process by which microbes form minerals. Likewise, biotransformation is another way of showing resistance toward heavy metals by microbes. It is the process of chemical alteration of chemicals such as nutrients, amino acids, toxins, and drugs by an organism. The two important factors involved in the biotransformation of heavy metals in soil are pH and carbon sources. Biotransformations of heavy metals are demonstrated in algae, fungi, and prokaryotes which convert these metals into metal sulfides. However, being insoluble in nature, these metal sulfides have reduced bioavailability (Scarano and Morelli 2003; Ayyasamy and Lee 2012). Further, microbial biofilms have the ability of accumulating or sequestering heavy metals by producing EPS (exopolysaccharides) which bind with these metals (Teitzel and Parsek 2003; Meliani and Bensoltane 2016).

Due to the presence of anionic structures, microbes have a net negative charge on their surface. This negative charge enables them to bind with metal cations. Furthermore, the polarized groups of the bacterial cell wall or the capsule enable them to bind with metal ions (El-Helow et al. 2000). Binding of these metal ions to the cell wall is governed by several attractive forces like van der Waals forces, electrostatic interactions, covalent binding, and alterations in redox potential. De et al. (2008) reported that *Pseudomonas aeruginosa* contains cysteine-rich transport proteins located in their cell membrane which enabled them to adsorb exceptionally high amount of mercury, i.e., up to 400 mg Hg g^{-1} dry cell mass. Microorganisms produce several organic and inorganic acids which help them in extracting metals from solid substrates.

10.4 Our Lead

Since the last 20 years, our group is pursuing a lot of studies related to bioremediation of heavy metals. In the case of microbial bioremediation of arsenic, we observed that the presence of a similar mechanism of resistance in the two bacterial strains isolated from two different sources may be due to horizontal gene transfer of the arsenic gene *ars* C from soil to water system and vice versa which is an alarming situation for global concern (Saluja et al. 2011). Gupta et al. (2002) developed heavy metal-resistant mutants of phosphate-solubilizing *Pseudomonas* sp. Similarly, Tripathi et al. (2004) characterize siderophore-producing lead- and cadmium-resistant *Pseudomonas putida* KNP9 strain. Gupta et al. (2005) did an in situ characterization of mercury-resistant growth-promoting fluorescent *Pseudomonads*. However, we also characterize some other cadmium-resistant strains (Rani and Goel 2009; Kumar et al. 2019). Rani et al. (2008) reported some rhizobacteria responsible for the decline of copper toxicity in pigeon pea and soil system. Besides this, our group has also reviewed several studies related to rhizobacterial detoxification of heavy metals for crop improvement and has compiled them for readers of scientific communities to comprehend its knowledge in a simpler way (Rani and Goel 2009; Goel et al. 2017; Saluja et al. 2011; Khan et al. 2011).

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11

Pesticide Residues in the Soil Cause Cross-Resistance Among Soil Bacteria

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Abstract

Multi-drug resistance among bacterial pathogens remains a serious problem worldwide. There is no clear and complete understanding about the multi-drug resistance mechanism even though the field is attaining continuous growth. Indiscriminate use of pesticides enabling the bacterial population to acquire multidrug resistance has been revived in this paper. Pesticide residues impose a bacterial system adopted for the stress due to the presence of xenobiotics. The natural evolutionary mutation mechanisms occurring randomly in the core gene sequences responsible for catabolizing complex substrates are the major reasons behind microbial resistance. Mutated gene products produced pose lesser substrate specificity than a wild enzyme. Organophosphorus hydrolase (OPH) or formaldehyde dehydrogenase and laccase are the few enzymes able to degrade many other similar xenobiotics. It has been extrapolated that degradation of many antibiotics by organophosphorus hydrolase is a kind of nonspecific degradation. Organisms growing in metal-polluted sites produce enzymes with different metal ions in their binding sites differing in specificity and conferring cross-resistance to antibiotics.

Keywords

Pesticide degradation · Cross-resistance · Soil bacteria ·
pMK-07 (plasmid_Murugan_Kirubakaran)

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11.1 Introduction

Pesticide is a chemical intended to control the pest population. It includes herbicides, insecticides, nematicides, termiticides, molluscicides, piscicides, avicides, rodenticides, predacides, bactericides, insect repellents, animal repellents, antimicrobials, fungicides, disinfectants, and sanitizers (US EPA 2005; Waili et al. 2012). Pesticide is meant to be effective against target pests and has been introduced in the agriculture sector in the mid-1950s (US EPA 2005; Ramakrishnan et al. 2011). Pesticides are widely used throughout the world and over 400 types of pesticides are commonly used. Most pesticides belong to the class of semi-volatile organic compounds and may occur in all environmental compartments, not only in the agricultural soils but also in the agriculture products (Cofie et al. 2003; Seo et al. 2007). Many pesticides are organophosphorus insecticides with nonspecific systemic or acaricide actions. They constitute heterogeneous compounds of monocrotophos and phosphoric acid that share a phosphoric acid derivative chemical structure (Yadav et al. 2015). The organic derivatives of inorganic phosphorus acids are known as organophosphorus. These insecticides are known inhibitors of acetylcholinesterase activity and affect the central nervous system of insects (Gundi and Reddy 2006).

From decades ago, to control the pests affecting various vegetable crops (cotton, sugarcane, groundnut, tobacco, maize, rice, soybeans, apple, etc.), pesticides are widely used (Vig et al. 2001; Bhadbhade et al. 2002). Modern agriculture farming uses a huge amount of herbicides as an alternative to increasing labor charges. Consequently, much of the pesticides have been sprayed on the soil without analyzing their effect on the various life forms. However, these pesticides undergo degradation leading to the formation of new compounds which are deleterious to plants (Ruifu et al. 2005). These various complex nonbiodegradable chemicals reach the ecosystem causing deterioration of the natural environment (soil and crops and livestock) (Zhang et al. 2005; Ortiz-Hernandez and Sanchez-Salinas 2010; Shah and Devkota 2009).

Pesticide residues have been identified in agriculture products worldwide (Cofie et al. 2003; Seo et al. 2007). Leaching of pesticide may reach the groundwater, apparently changing the living forms in the water bed (Singh and Singh 2003; Bhalerao and Puranik 2009). Terrestrial ecosystems may also be contaminated with pesticide compounds (Quin 2000; Singh and Walker 2006).

11.2 The Fate of Pesticide in the Soil

Pesticide reaches the soil either by aerial or ground application in the agriculture field. The overall degradation of pesticide is in turn dependent on its physicochemical properties such as water solubility, absorptive affinity, pH, organic matter content availability, microbial biomass, and redox status and environmental conditions (e.g., temperature, moisture) (Pandit et al. 2012; Yadav et al. 2015); and soil properties potentially influence the behavior of pesticide in the soil (Yadav et al. 2015; Beigel and Di Pietro 1999). Being water soluble, monocrotophos binds weakly to soil particles and quickly penetrates into plant tissues (Tomlin 1995). Indiscriminate

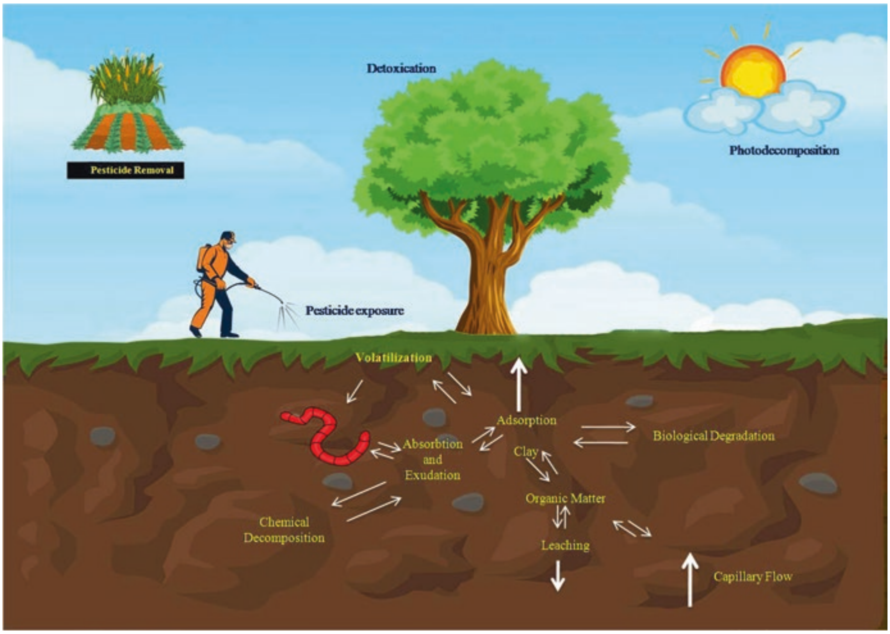

Fig. 11.1 The fate of the pesticide in the agricultural soil ecosystem

use of pesticides causes pesticides to reach soils and affect plant growth-promoting soil bacteria as follows:

(a) Adverse effects on soil fertility and crop productivity.
(b) Drastic change in the soil microflora creating serious ecological debate.
(c) Continued application of large quantities of pesticides causing everlasting changes in the soil microflora.
(d) Inhibition of N_2-fixing soil microorganisms and phosphate-solubilizing microorganisms.
(e) Suppression of nitrifying bacteria and ammonification.
(f) Adverse effects on mycorrhizal symbioses in plants and nodulation in legumes.
(g) Alterations in the rhizosphere microflora, both quantitatively and qualitatively.

Deprivation of pesticides in soil and water atmosphere may occur by various means such as photo, chemical, or biological process (Fig. 11.1).

11.3 Mechanism of Pesticide Degradation

Soil bacteria produce extracellular enzymes that act on inorganic compounds for food intake or defense purposes (Bass and Field 2011; Riya and Jagapati 2012; Scott et al. 2008). Metabolism of pesticides can be mediated through oxidation, reduction, hydrolysis, and peroxidase and oxygenase mechanisms (Ortiz-Hernandez and Sanchez-Salinas 2010; Ramakrishnan et al. 2011; Eerd et al. 2003). Detoxification of organophosphorus hydrolysis by hydrolase enzyme (Chen et al. 2014; Lan et al. 2006) causes a breakdown of pesticide into many compounds. It involves different kinds of mechanisms, namely, (i) photodegradation, (ii) chemical degradation, and (iii) biological degradation.

11.3.1 Photodegradation

Different layers of soil strata comprising pesticides are exposed to the photodegradation process (Cynthia and Muller 1998; Blatchley et al. 1998). Pesticides commonly get eliminated from soil particles through photodegradation. However, the low-volatile pesticide may remain in the soil and slowly become part of the aerosol. The pesticide is revived into the atmosphere, and it gets absorbed down into deeper layers of the soil (Meallier et al. 1994; Benitez et al. 1995). The pesticide returns back to the surface during hot and dry periods, exposed to and degraded by solar radiation.

Photodegradation will be high for a volatile fraction of the pesticide (de Bertrand et al. 1991; Kopf and Schwack 1995), and available hydroxyl radical (HO) in the soil particle could induce photodegradation (Prammer 1998). But rare evidence of hydroxyl radical-mediated degradation has been reported, and little information is available on the reaction mechanisms involved (Grover and Cessna 1991). Pesticides may get activated by the photons (Meallier et al. 1994); and excited molecules may undergo either (i) homolysis, (ii) heterolysis, or (iii) photoionization.

11.3.2 Chemical Degradation

Chemical degradation of pesticides may occur through oxidation, reduction, hydrolysis, peroxidation, and dehalogenation mechanisms (Ortiz-Hernandez and Sanchez-Salinas 2010; Ramakrishnan et al. 2011; Eerd et al. 2003). Several reports on the degradation of chlorpyrifos through oxidation and hydrolysis exist. Chlorinated 3,5,6-Trichloro-2-pyridinol (Li et al. 2010) causes broke down by the release of three chlorine atoms by a sequential dechlorination of 3,5,6-Trichloro-2-pyridinol by one oxidative and two hydrolytic leading to the formation of 3,6-dihydroxy pyridine-2,5-dione (Li et al. 2010; Ramakrishnan et al. 2011). 2–4-Dichlorophenoxy acetic acid is chemically modified by two different pathways. In one pathway, the sixth carbon is oxidized by the addition of an OH group yielding 6-OH-2,4-D. This reaction is followed by removal of acetate resulting in the formation of 3,5-dichlorocatechol (Sander et al. 1991).

11.3.3 Biological Degradation

The role of microorganisms in the dissipation of pesticides, especially in the soil, has long been recognized. Relative to the extended evolutionary period of microorganisms in nature, agriculture has only been around for more than 1000 years. Several microorganisms that have been isolated are known to utilize pesticides as a source of energy (Rangaswamy and Venkateswarlu 1992). However, most evidence suggests that soil bacteria are responsible for enhanced biodegradation of pesticides (Walker and Roberts 1993). Microorganisms capable of degrading monocrotophos have been reported from various geographical and ecological sites (Bhalerao and Puranik 2009). Leaching of monocrotophos may pollute the groundwater, ultimately resulting in adverse effects on biological systems (Singh and Singh 2003). With its high mammalian toxicity, monocrotophos is an ideal compound for decontamination and detoxification.

Organic materials are contained within the solid phase controlling chemical and physical processes in the soil. Predominant constitution of soil is humus, dominated by acidic functional groups capable of these large polymers possess a three-dimensional conformation that creates hydrophobic regions important in retaining nonionic synthetic organic compounds such as pesticides. Nonionic pesticides partition into these hydrophobic regions, decreasing off-site movement and biological availability. Humic materials will interact with nonionic pesticides by partitioning them into hydrophobic regions within the large polymeric molecule. Neither clays nor humic materials have a strong affinity for a weak acid pesticide containing phenolic hydroxyls, carboxyl groups, or aminosulfonyl linkages (Nyle and Ray 2002; Daniel 1991).

Pseudomonas aeruginosa F10B and *Clavibacter michiganensis* subsp. *insidiosum* SBL 11 are known pesticide degraders (Singh and Singh 2003). *Rhodococcus phenolics* strain G2PT utilizes a wide range of pesticides and their derivatives, chlorobenzene, and chlorobenzoic acid as sources of carbon (Marc and James 2005). Degradation of pesticides is catalyzed by an array of oxygenases, peroxidases, and hydrolases. Among these enzymes, hydrolase is studied for its efficacy to break down pesticide.

11.3.4 Organophosphorus Hydrolase (OPH)

Organophosphorus hydrolase (OPH) enzyme is used by several bacteria for degrading pesticide (Serdar et al. 1982; Mulbry and Karns 1989). OPH is a dimer of two identical subunits containing 336 amino acid residues (Dumas et al. 1989) that folds into an (α/β)8-barrel motif with a molecular weight of 72 kDa (Gerlt and Raushel 2003). Each subunit comprises binuclear zinc situated at the C-terminal portion. The two zinc atoms are separated by about 3.4 A° and are linked to the protein through the side chain of His 55, His 57, His 201, His 230, Asp 301, and a carboxylated Lys 169. Both the Lys 169 and the water molecule (or hydroxide ion) act to bridge the two zinc ions together (Benning et al. 2001).

Metal ion in the active site of OPH increases the electrophilicity of the phosphorus center through coordination with the non-ester oxygen atom of the substrate metal ion (Efrmenko and Sergeeva 2001; Raushel 2002). It hydrolyzes P–O, P–F, and P–S bonds to have wide-range substrate specificity. Metal ion substitution can alter catalytic activity of OPH metal ions including Co, Cd, Cu, Fe, Mn, and Ni (DiSioudi et al. 1999; Benning et al. 2001; Omburo et al. 1992).

Possibility mechanism of developing novel activity specificity from acquire by alteration of only one amino acid (Shim et al. 1998; Raushel 2002). Interestingly, this superfamily also includes atrazine chlorohydrolase. A similar enzyme, OPDA, has been isolated from *A. radiobacter* and was found to have 90% homology to OPH at the amino acid level and similar overall secondary structure (Horne et al. 2002; Yang et al. 2003). There are reports suggesting partial purification of this enzyme from *Pseudomonas* sp. GLC11 (Selvapandiyan and Bhatnagar 1994). Evolutionary adaptations of bacterial cultures have developed wide substrate specificity. Bacteria that are often exposed to xenobiotics develop novel metabolic enzymes with broad substrate specificity (Russell et al. 2013).

11.4 Loss of Specificity/Broad Substrate Specificity

Prolonged exposures to pesticide residues have favored the development of enzymes with broad substrate specificity. A larger group of enzymes with similar active site architecture has been identified (Holm and Sander 1997). Interestingly, this superfamily also includes atrazine chlorohydrolase. A similar enzyme, OPDA, has been isolated from *A. radiobacter* and was found to have 90% homology to OPH at the amino acid level and similar overall secondary structure (Horne et al. 2002; Yang et al. 2003). Similarly, bacteria that are often exposed to xenobiotics develop novel metabolic enzymes with broad substrate specificity (Russell et al. 2013). Such alteration may lead to enzyme production of lesser substrate specificity (Fig. 11.2).

11.5 Multi-drug Resistance

Continuous application of pesticide in the agricultural field, even at a low level, could exert selective pressure toward a selection of bacteria. Bacterial enzymes are responsible for the development of cross-resistance to antibiotics. This has been proven in our study: removing plasmid from the selected isolates resulted in loss of ability to use the pesticide (Stevenson et al. 2017; Lorenz and Wackernagel 1994).

Multidrug-resistant populations are quite common among the pesticide-degrading soil floras due to self-transmissible genes that can jump between plasmids and chromosomes (Shafiani and Malik 2003). Resistant gene cassettes have been found for the most classes of pesticides, and the gene products are involved in various resistance mechanisms. Integrons movements allow transfer of the gene cluster associated resistance gene from one DNA replicon to another. The resistance

Fig. 11.2 Decreased substrate specificity due to confirmation of a change in the enzyme by mutation

gene encoding gene establishes a resistance gene cluster, which represents a potential source for gene transfer between bacteria (Fig. 11.3).

Excessive exposure to pesticides adversely affects active sites of enzymes often responsible for antibiotic resistance. Presence of both antibiotic and pesticide select for resistance in bacteria and insects often acquire resistance in the same way, through a single mutation. Overproduction of an enzyme that breaks down dichlorodiphenyltrichloroethane (DDT) has been reported for multidrug resistance (Orzech and Nichter 2008; Bergman 2003).

The mechanism of drug resistance among soil populations is found to be different from the normal mechanism of bacterial drug resistance (Zhang et al. 2005; Heinemann 2000). Drug resistance may be due to overproduction of an enzyme that breaks down pesticide (Quin 2000; Gonzalez-Lopez et al. 1993). Similarly, it was reported *E. coli* and *Salmonella* sp. developed resistance to herbicides and antibiotics. Bacteria exposed to herbicides also developed resistance to antibiotics by cross-resistance (Kurenbach et al. 2015).

The enzyme is nonspecific for low-molecular-weight aldehydes, and this may explain the cross-resistance to other aldehydes or formaldehyde-releasing compounds (Henry et al. 2015; Ding and He 2010). Bacteria also pose multidrug resistance by producing higher copies of target molecules so that the previous antibiotic concentration is no longer sufficient for metabolic process. Mere transfer of transposable elements or plasmid simply stretches out the cytoplasm and has contributed

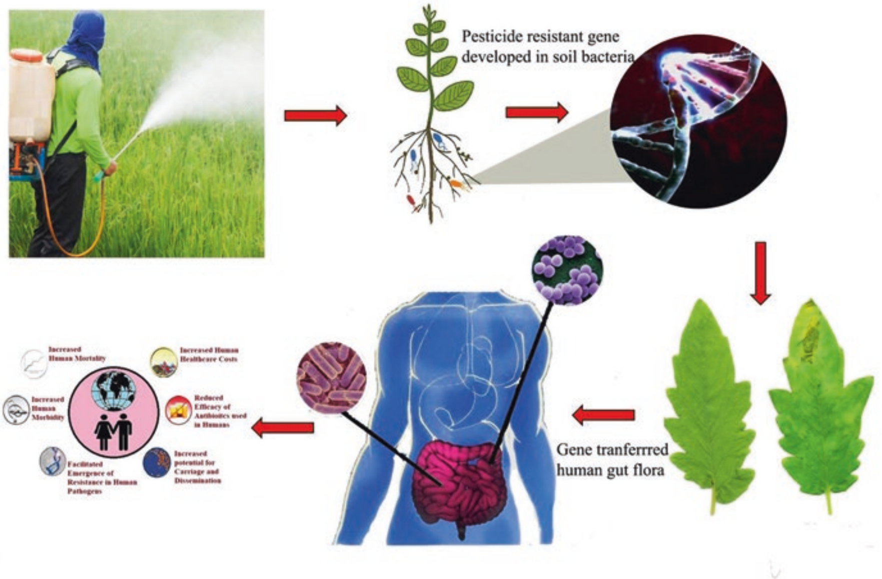

Fig. 11.3 The proposed mechanism of multidrug resistance among the human gut flora

to multidrug resistance of many other bacteria habituating the soil atmosphere. Bacterial strains exposed to pesticide continuously have evolved in a gene with broad-spectrum degradation specificity. Comparison between plasmid-containing and plasmid-cured cells proved the loss of drug resistance among plasmid-cured bacterial cells (Kirubakaran et al. 2017a).

Similar incidence is recorded where formaldehyde-resistant *Enterobacteriaceae* has been believed to be responsible for providing multidrug resistance through non-specific formaldehyde dehydrogenase activity (Chen et al. 2014; Livingstone 1998). Development of antibiotic and pesticide resistance is often presented as a modern example of evolution by mutations (Bergman 2003; Chen et al. 2014). The risks associated with the use of certain pesticides and antibiotics have a direct and indirect effect on development of multidrug resistance among bacteria (Tu et al. 2010; Anjum and Krakat 2016).

Parathion hydrolase was composed of a single subunit of approximately 43 kDa (Mulbry 1992). Another hydrolase from strain SC was membrane bound and was composed of four identical subunits of 67 kDa. While having some common features such as constitutive production and similar temperature optimal around 40 °C, it was found that the substrate specificity and structure of these enzymes differed from one another and also from the other known OPHs (Mulbry and Karns 1989).

Laccase was observed to be capable of complete rapid degradation of VX and Russian VX (Amitai et al. 1998). Several white-rot fungi are capable of organophosphorus degradation. A novel organophosphorus-degrading fungal enzyme (A-OPH) was isolated from *A. niger* ZHY256 that could hydrolyze a range of P–S bonds containing organophosphorus compounds (Liu et al. 2008). A-OPH does not require divalent cations for activation; however, Cu21 was found to activate its activity. Another novel organophosphorus-hydrolyzing enzyme was purified from *Penicillium lilacinum* BP303. Interestingly, this *Penicillium* OPH (P-OPH) was found to degrade various organophosphorus compounds by cleaving both P–O and P–S linkages (Liu et al. 2004).

Organophosphorus compounds bind to the binuclear metal center within the active site via coordination of the phosphoryl oxygen to the b-metal ion. This interaction weakens the binding of the linking hydroxide to the b-metal. The metal–oxygen interaction polarizes the phosphoryl oxygen bond and creates a more electrophilic phosphorus center. Subsequent nucleophilic attack by the bound hydroxide is aided by proton abstraction from Asp 301 mentioned by Raushel (2002).

Pesticide residues can induce cross-resistance creating multiple-drug resistance among the soil community (Kirubakaran et al. 2017b). Many of the soil bacteria able to develop multidrug resistance by the nonspecific cleavage of antibiotics by the pesticide-degrading bacterial enzymes were studied (Kruger et al. 2014). Excess use of a pesticide may lead to the evolution of pesticide degradation pathways along with genetic mutation and ribosome point mutation. Organophosphate hydrolase soil floras have contributed to the mechanism of cross-resistance (Kruger et al. 2014). Binding of pesticide to the ribosome leads to the synthesis of receptors which do not allow drugs into a bacterial cell, thereby contributing to multidrug resistance (Kazanjian et al. 2000; Bainy 2000).

Continuous application of pesticide causes multidrug (chloramphenicol, ampicillin, cefotaxime, streptomycin, and tetracycline) organophosphorus resistance among soil bacterial plasmids as pMK-07 (Kirubakaran et al. 2018a, b). Constant pressure on the gene allows the particular gene to develop multidrug resistance. The prolonged application of disinfectants at suboptimal concentrations provokes the adaption of microorganisms to those subconcentrations and possibly the development of true resistance (Tu et al. 2010; Anjum and Krakat 2015).

The recent finding on encoding genes in a field soil which have never been exposed to this group of pesticides supports this hypothesis (Singh et al. 2003). Another possibility is that this enzyme has evolved a new substrate specificity from preexisting enzymes as it has been shown that OPH (phosphotriesterase) could acquire phosphodiesterase activity by alteration of only one amino acid (Shim et al. 1998). Urease has been found to have carbamylated lysine as a bridging ligand with binuclear Ni at the active site (Hausinger and Fukumori 1995). The binuclear centers of urease and OPH were found to be remarkably similar. However, the chemical nature of the active sites of these enzymes is quite different (Raushel 2002). A larger group of enzymes with similar active site architecture has been identified (Holm and Sander 1997).

Alteration in the active site of OPH gave substrate specificity and represented the progressive natural evolution of the enzyme from organophosphorus hydrolase (OPH) to organophosphorus dehydrogenase A (OPDA) (Yang et al. 2003). A highly active OPAA from *Alteromonas undina* was isolated and purified and is comprised of a single polypeptide with a molecular weight of 53 kDa (Cheng et al. 1993). OPAA isolated from *Alteromonas* sp. JD6.5 is composed of 517 amino acids with a molecular weight of 60 kDa. However, one from *Alteromonas haloplanktis* contains a similar enzyme with 440 amino acids with molecular weight of 50 kDa (Cheng et al. 1996, 1997). OPAAs from different species of *Alteromonas* that have proved to show wide variations in catalytic activity are varying one or two amino acids (DeFrank and White 2002). Hence, the gene continuously exposed to pesticide/antibiotics would evolve to degrade similar other many xenobiotics.

11.6 Conclusion

Multidrug resistance among soil bacteria is very common. The natural selection process in the soil ecosystem enables the microorganism to adapt to pesticide applied in the soil. Hence, the presence of pesticide residues favors populations which are able to grow in the presence of both pesticides and antibiotics. The prolonged application of pesticides/antibiotics at suboptimal concentrations provokes the adaption of microorganisms and possibly the development of true resistance. Persistence of pesticide in the agriculture field enriches pesticide-utilizing bacteria and pesticide-utilizing genes despite the bio-accumulation problem.

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Psychrotrophic Microbes: Biodiversity, Mechanisms of Adaptation, and Biotechnological Implications in Alleviation of Cold Stress in Plants

12

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Abstract

Psychrotrophic microbes from the cold habitats have been reported worldwide. The psychrotrophic microbes from diverse cold habitats have biotechnological potential applications in agriculture as they can possess different direct and indirect plant growth-promoting (PGP) attributes such as solubilization of micronutrients (P, K, and Zn), 1-aminocyclopropane-1-carboxylate deaminase production, Fe-chelating compounds, indole-3-acetic acid, and bioactive compounds. Psychrophilic and psychrotrophic microbes are ubiquitous in nature and have been reported worldwide from various cold environments. The microbial communities from cold deserts have been reported using both culture-dependent techniques and metagenomic techniques, which belong to diverse major groups, viz., *Verrucomicrobia*, *Thaumarchaeota*, *Spirochaetes*, *Proteobacteria*, *Planctomycetes*, *Nitrospirae*, *Mucoromycota*, *Gemmatimonadetes*, *Firmicutes*,

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Euryarchaeota, *Cyanobacteria*, *Chloroflexi*, *Chlamydiae*, *Basidiomycota*, *Bacteroidetes*, *Ascomycota*, and *Actinobacteria*. Cold-adapted microbes, isolated from the low-temperature condition, are belonging to different genera such as *Arthrobacter*, *Bacillus*, *Exiguobacterium*, *Paenibacillus*, *Providencia*, *Pseudomonas*, and *Serratia*. On review of different research, it was found that inoculation with psychrotrophic strains significantly enhanced plant growth, crop yield, and soil fertility. The present book chapter deals with the biodiversity of psychrotrophic or cold-adapted microbes from diverse cold habitats, and their potential biotechnological applications in agriculture have been discussed.

Keywords

Adaptation · Cold alleviation · Diversity · Plant growth promotion · Psychrotrophic

12.1 Introduction

The extreme environment of abiotic stresses like flooding, salinity, drought, low or high temperature (Mina et al. 2017; Tomer et al. 2015), and acidic or alkaline pH has severely affected the productivity of several cereal crops worldwide (Malyan et al. 2016). Among different abiotic stresses, low temperature is one of the major abiotic stresses acting as the limiting factor affecting the agricultural productivity as 20% of the Earth's surfaces is covered with frozen soils (permafrost), glaciers, ice sheets, and snow. The perspective's loss of crop productivity due to the severity of abiotic stresses becomes more significant than the chemical fertilizers. The diversity of microbes inhabiting low-temperature habitats has been extensively investigated in the past few years with a focus on culture-dependent and culture-independent techniques (Fruhling et al. 2002; Kishore et al. 2010; Mayilraj et al. 2006a; Shivaji et al. 2005b; Verma et al. 2015b; Yadav 2015).

There are many reports on psychrophilic microbes from different cold habitats (Antarctica, glaciers, high altitude, ice cap cores, mountains, sea, subglacial lakes, and plant growing at low-temperature conditions) including *Acinetobacter, Arthrobacter, Bacillus, Desemzia, Exiguobacterium, Janthinobacterium,*

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Jeotgalicoccus, Flavobacterium, Methylobacterium, Micrococcus, Planococcus, Planomicrobium, Pontibacillus, Pseudomonas, Psychrobacter, Rhodococcus, Paenibacillus, Sphingobacterium, Sporosarcina, Staphylococcus, and *Virgibacillus.* The psychrotrophic microbes have been reported isolated from diverse cold habitats belonging to phyla *Verrucomicrobia*, *Thaumarchaeota*, *Spirochaetes*, *Proteobacteria*, *Planctomycetes*, *Nitrospirae*, *Mucoromycota*, *Gemmatimonadetes*, *Firmicutes*, *Euryarchaeota*, *Cyanobacteria*, *Chloroflexi*, *Chlamydiae*, *Basidiomycota*, *Bacteroidetes*, *Ascomycota*, and *Actinobacteria* (Fruhling et al. 2002; Kishore et al. 2010; Mayilraj et al. 2006a; Shivaji et al. 2005b; Yadav et al. 2017a, b, 2018a, b, 2017c).

The novel microbes have been isolated, characterized, and identified from cold habitats worldwide including *Flavobacterium phocarum,* SE14^{T} (Zhou et al. 2018); *Hymenobacter rubripertinctus,* NY03-3-30^{T} (Jiang et al. 2018); *Psychrobacter pocilloporae,* S6-60^{T} (Zachariah et al. 2017); *Glaciimonas frigoris,* N1-38^{T} (Margesin et al. 2016); *Massiliaeury psychrophila,* B528-3^{T} (Shen et al. 2015); *Azospirillum himalayense,* ptl-3^{T} (Tyagi and Singh 2014); *Exiguobacterium himgiriensis,* K22–26^{T}(Singh et al. 2013); *Flavobacterium urumqiense,* Sr25^{T} (Dong et al. 2012); *Pedobacter arcticus,* A12^{T} (Zhou et al. 2012); *Pseudomonas deceptionensis,* M1^{T} (Carrión et al. 2011); *Dioszegia antarctica,* ANT-03-116^{T} (Connell et al. 2010); *Paenibacillus glacialis,* KFC91^{T} (Kishore et al. 2010); *Arthrobacter psychrochitiniphilus,* GP3^{T} (Wang et al. 2009); *Exiguobacterium soli,* DVS 3Y^{T} (Chaturvedi et al. 2008); *Bacillus lehensis,* MLB2^{T} (Ghosh et al. 2007); *Agrococcus lahaulensis,* K22-21^{T} (Mayilraj et al. 2006e); *Actinoalloteichus spitiensis,* RMV-378^{T} (Singla et al. 2005); *Geopsychrobacter electrodiphilus,* A1^{T} (Holmes et al. 2004); *Flavobacterium omnivorum,* ZF-8^{T} (Zhu et al. 2003); *Flavobacterium frigidarium,* A2i^{T} (Humphry et al. 2001); *Sulfitobacter brevis,* EL-162^{T} (Labrenz et al. 2000); *Glaciecola pallidula,* ACAM 615^{T} (Bowman et al. 1998a); *Gelidibacter algens,* ACAM 536 (Bowman et al. 1997a); *Desulforhopalus vacuolatus,* ltk10 (Isaksen and Teske 1996); *Sphingobacterium antarcticus,* 4BY (Shivaji et al. 1992); *Halobacterium lacusprofundi,* ACAM 32^{T} (Franzmann et al. 1988); and *Nocardiopsis antarcticus* (Abyzov et al. 1983).

Prospecting the low-temperature environments has led to the isolation and identification of a great diversity of psychrotrophic microbiomes which has been found in cold habitats and reported worldwide. The microbiomes of cold habitats have been studied using different techniques including phospholipid fatty acid analysis, nucleic acid techniques, clone library method, genetic fingerprinting techniques, DNA microarray, and whole community analysis approaches (Yadav 2015; Yadav et al. 2015a, 2017b). There are many reports on complete genome sequences to know the different genes responsible for diverse attributes including *Colwellia chukchiensi* (Zhang et al. 2018), *Exiguobacterium oxidotolerans* (Cai et al. 2017), *Arthrobacter agilis* (Singh et al. 2016), *Paenibacillus* sp. (Dhar et al. 2016), *Clavibacter* sp. (Du et al. 2015), *Planomicrobium glaciei* (Salwan et al. 2014), *Octadecabacter antarcticus* (Vollmers et al. 2013), *Exiguobacterium antarcticum*

(Carneiro et al. 2012), *Rheinheimera* sp. (Gupta et al. 2011a), *Methanococcoides burtonii* (Allen et al. 2009), *Exiguobacterium sibiricum* (Rodrigues et al. 2008), *Cenarchaeum symbiosum* (Hallam et al. 2006), and *Colwellia psychrerythraea* (Methé et al. 2005).

The psychrotrophic microbes from diverse cold habitats have biotechnological potential applications in medicine, industry, agriculture, and allied sectors. The psychrotrophic microbes exhibited multifarious plant growth-promoting (PGP) attributes such as 1-aminocyclopropane-1-carboxylate (ACC) activities, solubilization of micronutrients (phosphorus, potassium, and zinc), biological N_2 fixation, deaminase activity, and production of different bioactive compounds such as ammonia, hydrogen cyanide, indole-3-acetic acid, and Fe-chelating compounds. The psychrotrophic microbe promotes growth as well as showed biocontrol activity against plant pathogenic microbes. The microbes having ACC deaminase activity help plant to alleviate cold stress (Verma et al. 2015b; Yadav 2009; Yadav 2017; Yadav et al. 2016a, 2017d). The psychrotrophic microbes attracted the attention of the scientific community due to their aptitude in plant growth, an adaptation of plants at low-temperature conditions. The present book chapter describes the biodiversity and biotechnological application of psychrophilic and psychrotolerant microbes in agriculture for PGP and mitigation of cold stress in crops.

12.2 Diversity and Distributions of Psychrotrophic Microbes

The extreme environments of low temperature are the prosperous source of psychrophilic and psychrotrophic microbiomes (archaea, bacteria, and eukarya). The cold habitats represent hot spots of microbial biodiversity for psychrophilic and psychrotolerant microbes with potential biotechnological application in diverse sectors. Diverse groups of psychrophilic microbes have been sorted out, characterized, and identified using diverse methods and from diverse cold environments including Antarctica, plant microbiomes (plant growing at low-temperature conditions), glaciers, subglacial lakes, cold desert of Himalayas, ice-coped revivers, and subalpine regions worldwide (Saxena et al. 2016; Yadav and Saxena 2018; Yadav et al. 2017b).

12.2.1 Microbial Biodiversity

The diversity of psychrophilic and psychrotolerant microbes inhabiting low-temperature habitats has been extensively investigated in the past few decades with a focus on culture-independent and culture-dependent techniques. The different groups of microbes have been reported from all three domains archaea, bacteria, and eukarya, which included different phyla, mainly *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, *Euryarchaeota*, *Ascomycota*, *Thaumarchaeota*, *Cyanobacteria*, *Basidiomycota*, *Chlamydiae*, *Firmicutes*, *Mucoromycota*, *Spirochaetes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia* (Fig. 12.1). Overall member of microbes belonging to the *Proteobacteria* has been reported as most dominant

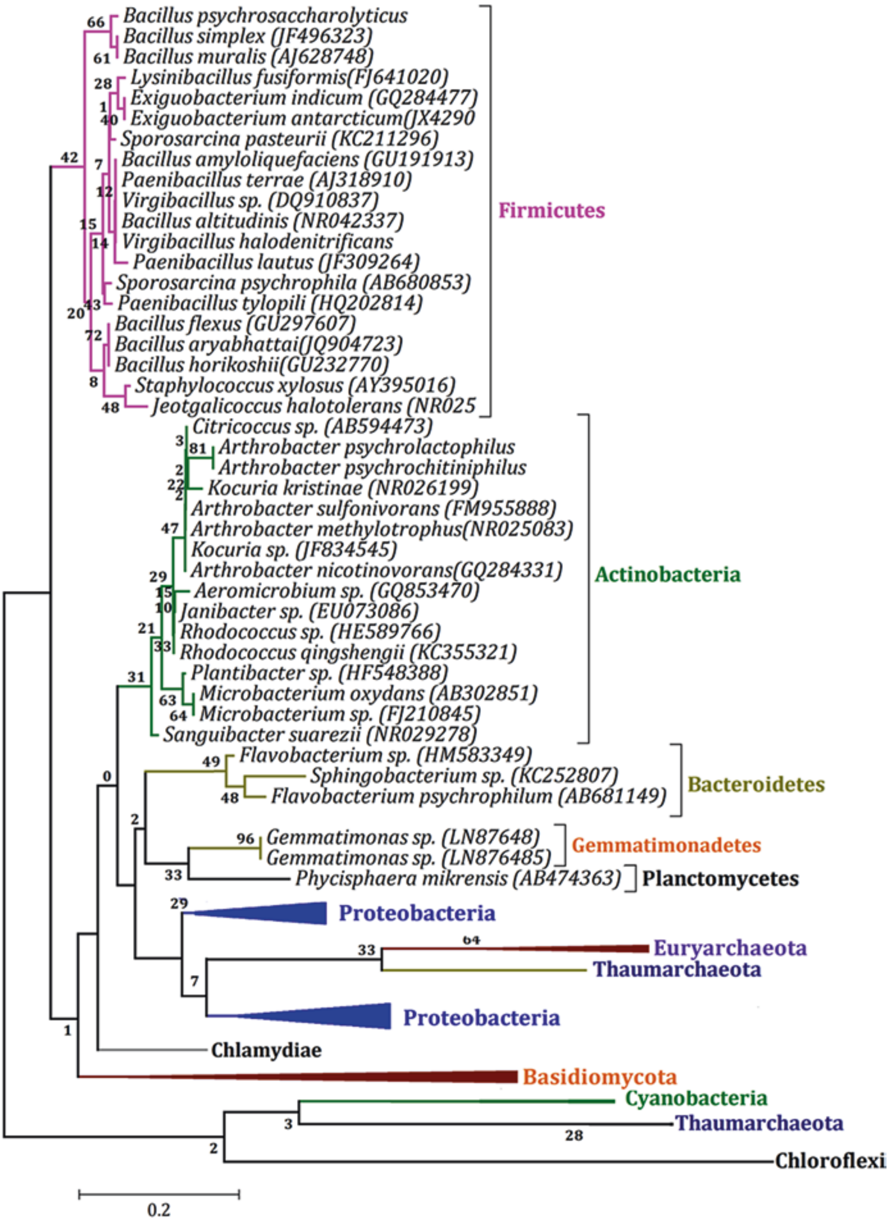

Fig. 12.1 Phylogenetic tree showed the relationship between psychrophilic and psychrotolerant microbes, isolated from diverse cold habitats worldwide

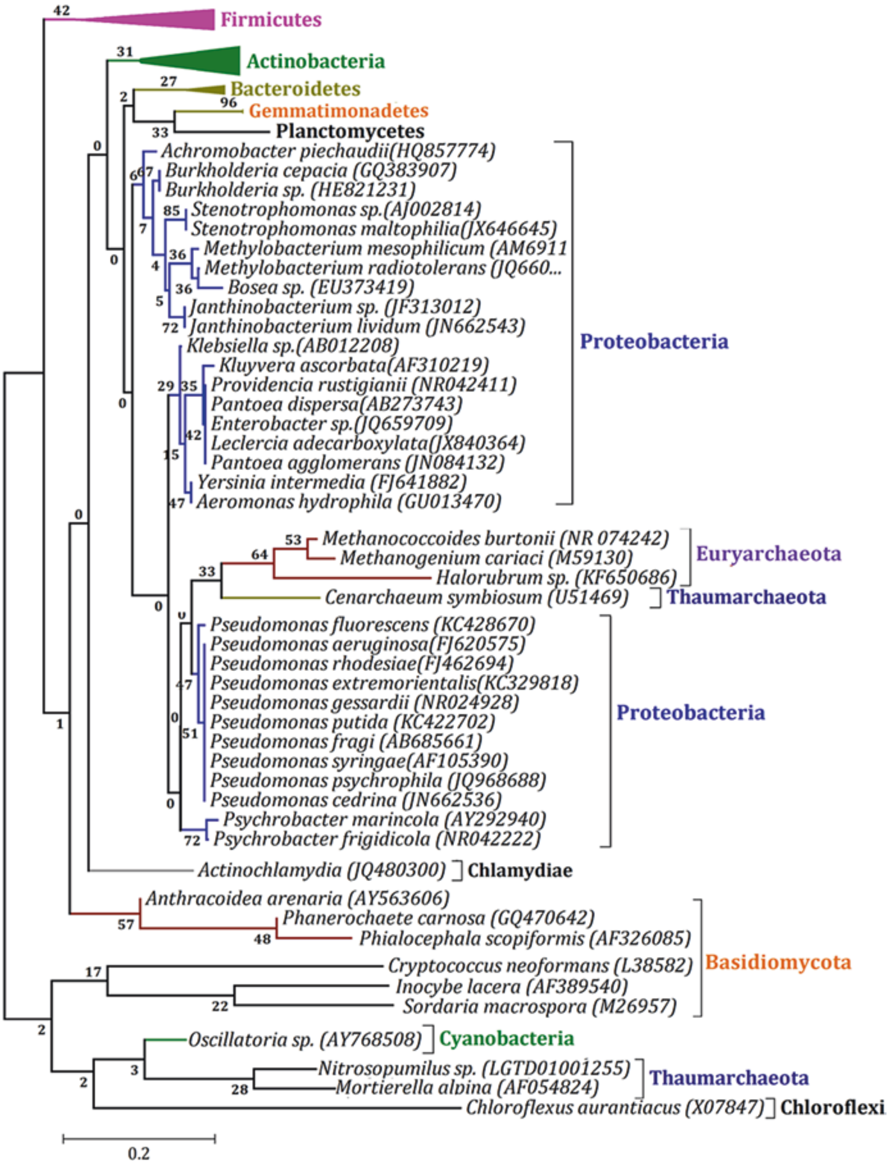

Fig. 12.1 (continued)

followed by *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Basidiomycota* (Fig. 12.2). The percentage of different phyla included *Spirochaetes* (0.36), *Chlamydiae*, *Chloroflexi*, *Verrucomicrobia* (0.60), *Gemmatimonadetes*, *Mucoromycota*, *Nitrospirae*, *Thaumarchaeota*, *Planctomycetes* (0.72), *Cyanobacteria* (0.84), *Ascomycota* (1.44), *Euryarchaeota* (2.54), *Basidiomycota* (4.208), *Bacteroidetes*

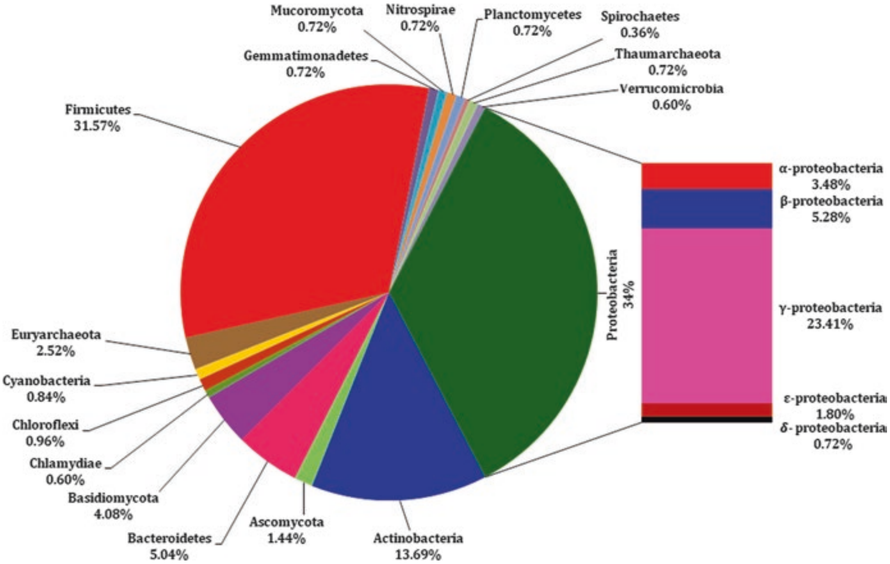

Fig. 12.2 Distribution of different phylum and groups of psychrophilic and psychrotolerant microbes, isolated from diverse cold habitats worldwide

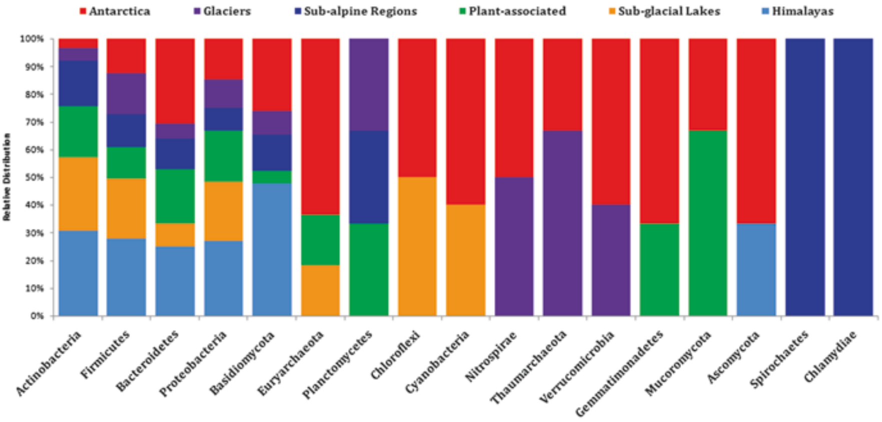

Fig. 12.3 Relative distribution of different phylum of psychrophilic and psychrotolerant microbes, isolated from diverse cold habitats worldwide

(5.04), *Actinobacteria* (13.69), *Firmicutes* (31.57), and *Proteobacteria* (32.0) [α-3.48, β-5.28, ϒ-23.41, ε-1.80, and δ0.72] (Fig. 12.2).

On review of different extreme cold habitats, it can be concluded that there are some niche-specific microbes (Fig. 12.3), as well as some common and most dominant psychrophilic microbes have been reported (Fig. 12.4). Along with common and ubiquitous microbes, some niche-specific microbes have been sorted out from

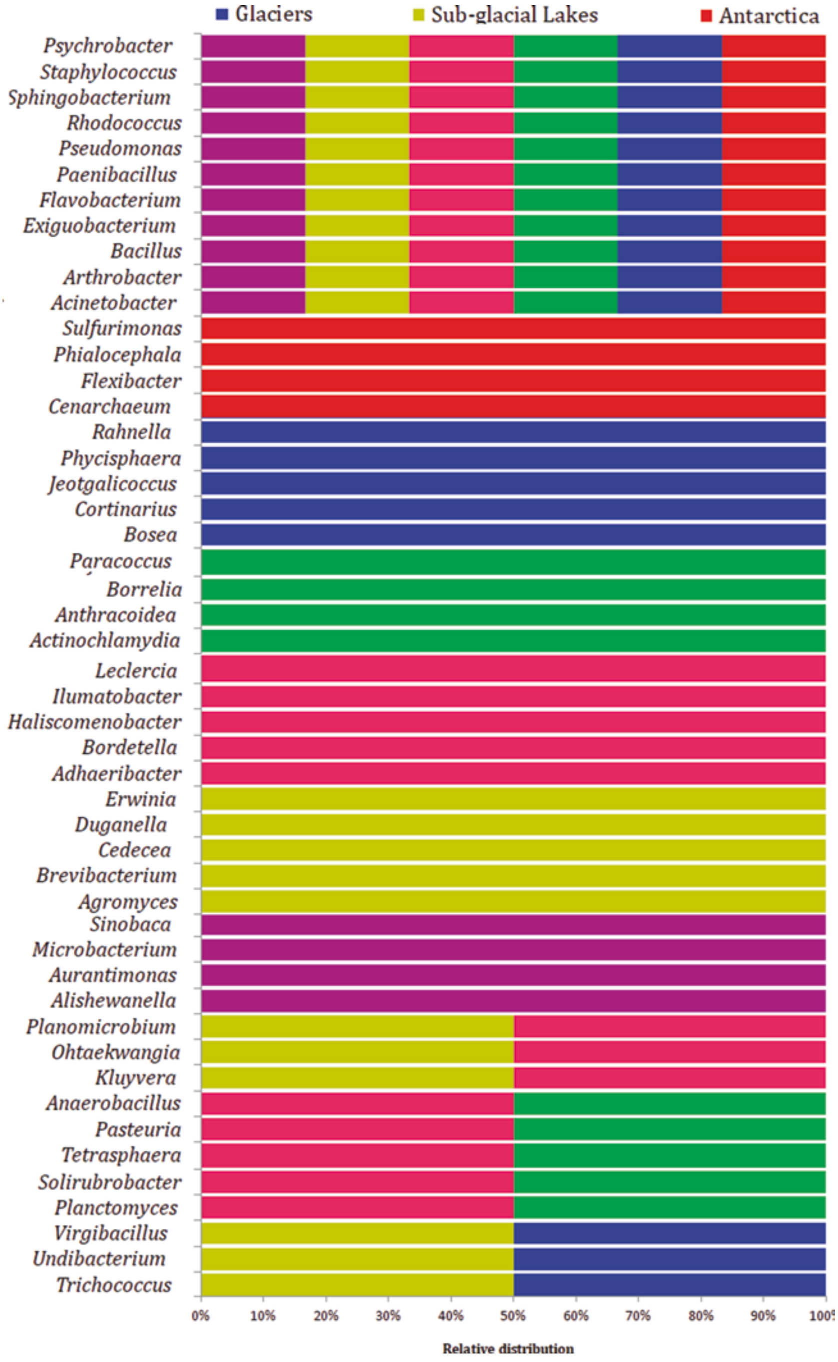

Fig. 12.4 Relative distribution of different genera of psychrophilic and psychrotolerant microbe, isolated from diverse cold habitats worldwide

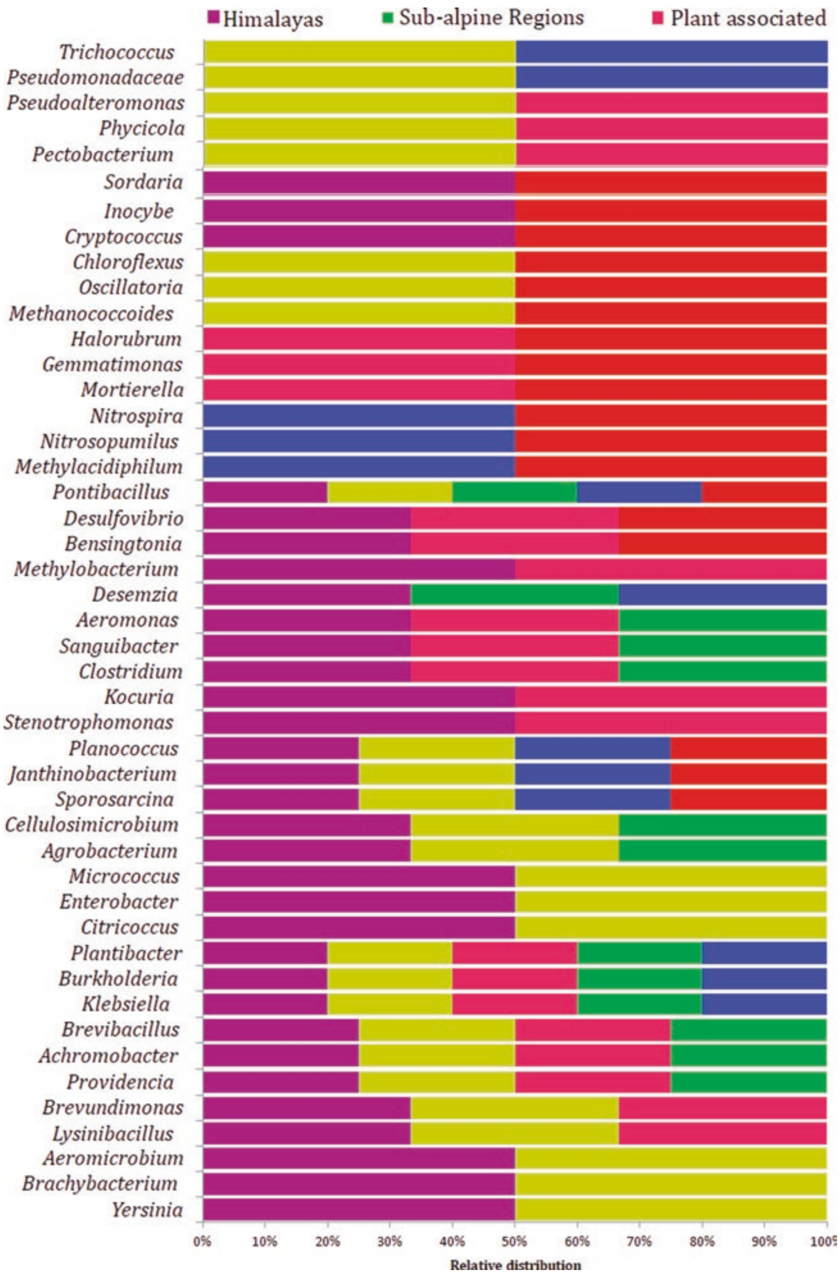

Fig. 12.4 (continued)

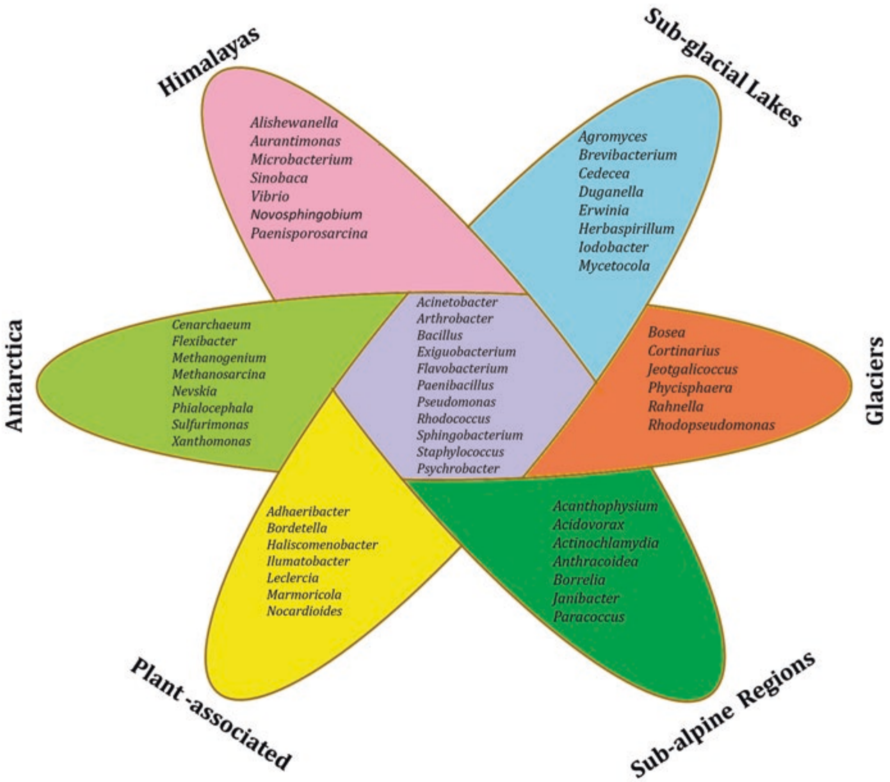

Fig. 12.5 Diversity and distribution of niche-specific psychrophilic and psychrotolerant microbes from diverse cold habitats worldwide

different cold habitats, e.g., the member of phyla *Alishewanella, Aurantimonas, Microbacterium, Novosphingobium, Paenisporosarcina, Sinobaca, and Vibrio* from different sites of Himalayas; *Agromyces, Brevibacterium, Cedecea, Duganella, Erwinia, Herbaspirillum, Iodobacter, and Mycetocola* from subglacial lakes; *Adhaeribacter, Bordetella*, *Haliscomenobacter, Ilumatobacter, Leclercia, Marmoricola,* and *Nocardioides* from plants growing in cold habitats; *Acanthophysium, Acidovorax, Actinochlamydia, Anthracoidea, Borrelia, Janibacter,* and *Paracoccus* from subalpine regions; *Bosea, Cortinarius, Jeotgalicoccus, Phycisphaera, Rahnella,* and *Rhodopseudomonas* from glaciers; and the member of microbial genera *Cenarchaeum, Flexibacter, Methanogenium, Methanosarcina, Nevskia, Phialocephala, Sulfurimonas,* and *Xanthomonas* from Antarctica only (Fig. 12.5). Microorganisms inhabit cold habitats including the Antarctic, Arctic glacier, permanently ice-covered seas, the deep sea permafrost, and Himalayan and mountain lakes (Cavicchioli et al. 2011; Chaturvedi et al. 2008; Chaturvedi and Shivaji 2006; Cheng and Foght 2007; Foght et al. 2004; Panicker et al. 2002; Pradhan et al. 2010; Prasad et al. 2014; Sahay et al. 2013, 2017; Saul et al. 2005; Shivaji et al. 2011; Srinivas et al. 2011).

12.2.2 Novel Microbes from Diverse Cold Habitats

Huge numbers of microbes belonging to different phyla and genera have been sorted out from diverse cold habitats worldwide, and it is found that all isolated microbiomes from cold deserts are psychrophilic and psychrotolerant in nature. The many microbes from the sea have been reported worldwide and are psychropiezophilic in nature. The novel microbes from cold habitats have been reported from the last four decades. The list of novel microbial isolates and the site from which samples were collected is given in Table 12.1.

The novel psychrotrophic/psychrophilic microbes have been isolated and characterized from different cold habitats worldwide including *Sphingomonas glacialis,* C16y^{T}*,* and *Pedobacter cryoconitis,* A37^{T}, from alpine glacier (Margesin et al. 2003; Zhang et al. 2011); *Lacinutrix jangbogonensis,* PAMC 27137^{T}*, Pseudomonas extremaustralis,* 14-3^{T}*, Oleispira antarctica,* RB-8^{T}*, Hymenobacter roseosalivarius,* AA-718^{T}*, Methylosphaera hansonii,* ACAM 549^{T}*,* and *Polaromonas vacuolata,* 34-P^{T}, from Antarctic (Bowman et al. 1997c; Hirsch et al. 1998; Irgens et al. 1996; Lee et al. 2014; Lopez et al. 2009; Yakimov et al. 2003); *Halohasta litorea,* R30^{T}*, Halohasta litchfieldiae,* tADL^{T}*, Halobacterium lacusprofundi,* ACAM 32^{T}*, Flavobacterium fryxellicola,* LMG 22022^{T}*, Flavobacterium psychrolimnae,* 22018^{T}*, Flavobacterium degerlachei,* LMG 21915^{T}, and *Flavobacterium frigoris,* LMG 21922^{T}, from Antarctic subglacial lakes (Franzmann et al. 1988; Mou et al. 2012; Van Trappen et al. 2004; Van Trappen et al. 2005); *Flavobacterium phocarum,* SE14^{T}*, Hymenobacter rubripertinctus,* NY03-3-30^{T}*, Pseudomonas deceptionensis,* M1^{T}*, Arthrobacter psychrochitiniphilus,* GP3^{T}*, Exiguobacterium soli,* DVS 3Y^{T}*, Flavobacterium frigidarium,* A2i^{T}*, Arthrobacter flavus,* CMS 19Y^{T}*, Cellulophaga algicola*, ACAM 630^{T}*, Glaciecol apallidula,* ACAM 615^{T}*, Glaciecol apunicea,* ACAM 611^{T}*, Methanogenium frigidum,* Ace-2^{T}*, Octadecabacter antarcticus,* 307^{T}*, Octadecabacter arcticus,* 238^{T}*, Psychrobacter glacincola,* ACAM 521^{T}*,* and *Sphingobacterium antarcticus,* 4BY, from Antarctica (Bowman 2000; Bowman et al. 1998a; Bowman et al. 1997d; Carrión et al. 2011; Chaturvedi et al. 2008; Franzmann et al. 1997; Gosink et al. 1997; Humphry et al. 2001; Jiang et al. 2018; Reddy et al. 2000; Shivaji et al. 1992; Wang et al. 2009; Zhou et al. 2018); *Cryobacterium psychrotolerans,* 0549^{T}*, Flavobacterium glaciei,* 0499^{T}*, Flavobacterium omnivorum,* ZF-8^{T}*, Flavobacterium xinjiangense,* ZF-6^{T}*, Flavobacterium urumqiense,* Sr25^{T}*, Flavobacterium xueshanense,* Sr22^{T}*, Dyadobacter hamtensis,* HHS 11^{T}*, Pedobacter himalayensis,* HHS 22^{T}*, Paenibacillus glacialis,* KFC91^{T}*,* and *Leifsonia kafniensis,* KFC-22^{T}, from glaciers (China no. 1 glacier, glacier ice, Hamta glacier, and Kafni glacier) (Chaturvedi et al. 2005; Dong et al. 2012; Kishore et al. 2010; Pindi et al. 2009; Shivaji et al. 2005a; Zhang et al. 2006, 2007; Zhu et al. 2003); *Rufibacter immobilis,* MCC P1^{T}*, Cryobacterium roopkundense,* RuGl7^{T}, and *Rhodotorula himalayensis,* 3A^{T}, from subglacial lakes (Polkade et al. 2015; Reddy et al. 2010; Shivaji et al. 2008); and *Agrococcus lahaulensis,* K22-21^{T}*, Bacillus lehensis,* MLB2^{T}*, Exiguobacterium himgiriensis,* K22–26^{T}*, Kocuria himachalensis,* K07-05^{T}*, Ornithinimicrobium*

Table 12.1 Biodiversity of novel psychrophilic and psychrotrophic microbes from diverse cold habitats worldwide

Novel microbes	Location	References
Flavobacterium phocarum, SE14^T	Antarctica	Zhou et al. (2018)
Hymenobacter rubripertinctus, NY03-3-30^T	Antarctica	Jiang et al. (2018)
Psychrobacter pocilloporae, S6-60^T	The Andaman Sea	Zachariah et al. (2017)
Psychromicrobium silvestre, AK20-18^T	Italian Alps	Schumann et al. (2017)
Glaciimonas frigoris, N1-38^T	Siberian permafrost	Margesin et al. (2016)
Methylovulum psychrotolerans, Sph1^T	West Siberia	Oshkin et al. (2016)
Sphingomonas qilianensis, X1^T	Qilian Mountains	Piao et al. (2016)
Massilia eurypsychrophila, B528-3^T	Muztagh glacier	Shen et al. (2015)
Rufibacter immobilis, MCC P1^T	Pangong Lake	Polkade et al. (2015)
Azospirillum himalayense, ptl-3^T	Chamba Valley	Tyagi and Singh (2014)
Lacinutrix jangbogonensis, PAMC 27137^T	Antarctic	Lee et al. (2014)
Exiguobacterium himgiriensis, K22–26^T	Spiti Valley	Singh et al. (2013)
Paenisporosarcina indica, PN2^T	Pindari glacier	Reddy et al. (2013)
Rhodomicrobium udaipurense, JA643^T	Udaipur, HP	Ramana et al. (2013)
Sphingobacterium psychroaquaticum, L-1^T	Michigan Lake	Albert et al. (2013)
Staphylococcus lipolyticus, SS-33^T	Bay of Bengal	Arora (2013)
Halohasta litorea, R30^T	Antarctic lake	Mou et al. (2012)
Halohasta litchfieldiae, adult	Antarctic lake	Mou et al. (2012)
Flavobacterium urumqiense, Sr25^T	Glacier ice	Dong et al. (2012)
Flavobacterium xueshanense, Sr22^T	Glacier ice	Dong et al. (2012)
Pedobacter arcticus, A12^T	Arctic	Zhou et al. (2012)
Chryseomicrobium imtechense, MW 10^T	Bay of Bengal	Arora et al. (2011)
Pseudomonas deceptionensis, M1^T	Antarctica	Carrión et al. (2011)
Sphingomonas glacialis, C16y^T	Alpine glacier	Zhang et al. (2011)
Cryobacterium roopkundense, RuGl7^T	Roopkund Lake	Reddy et al. (2010)
Dioszegia antarctica,ANT-03-116^T	Taylor Valley	Connell et al. (2010)
Dioszegia cryoxerica, ANT-03-071^T	Taylor Valley	Connell et al. (2010)
Luteimonas terricola, BZ92r^T	Innsbruck, Austria	Zhang et al. (2010)
Mucilaginibacter frigoritolerans, FT22^T	Finnish Lapland	Männistö et al. (2010)
Mucilaginibacter lappiensis, ANJLI2^T	Finnish Lapland	Männistö et al. (2010)
Mucilaginibacter mallensis, MP1X4^T	Finnish Lapland	Männistö et al. (2010)
Paenibacillus glacialis, KFC91^T	Kafni glacier	Kishore et al. (2010)
Arthrobacter psychrochitiniphilus, GP3^T	Antarctica	Wang et al. (2009)
Leifsonia kafniensis, KFC-22^T	Kafni glacier	Pindi et al. (2009)
Pseudomonas extremaustralis, 14-3^T	Antarctic	Lopez et al. (2009)
Bacillus cecembensis, PN5^T	Pindari glacier	Reddy et al. (2008b)
Exiguobacterium soli, DVS 3Y^T	Antarctica	Chaturvedi et al. (2008)
Leifsonia pindariensis, PON10T	Pindari glacier	Reddy et al. (2008a)
Marinobacter psychrophilus, 20041^T	Arctic	Zhang et al. (2008)
Psychromonas aquimarina, JAMM 0404^T	Kagoshima, Japan	Miyazaki et al. (2008)
Psychromonas japonica, JAMM 0394^T	Kagoshima, Japan	Miyazaki et al. (2008)

(continued)

Table 12.1 (continued)

Novel microbes	Location	References
Psychromonas macrocephali, JAMM 0415^T	Kagoshima, Japan	Miyazaki et al. (2008)
Psychromonas ossibalaenae, JAMM 0738^T	Kagoshima, Japan	Miyazaki et al. (2008)
Rhodotorula himalayensis, 3A^T	Roopkund Lake	Shivaji et al. (2008)
Bacillus lehensis, MLB2^T	Leh, JK	Ghosh et al. (2007)
Cryobacterium psychrotolerans, 0549^T	China no. 1 glacier	Zhang et al. (2007)
Rhodobacter changlensis, JA139^T	Changla Pass HP	Anil Kumar et al. (2007)
Agrococcus lahaulensis, K22-21^T	Lahaul-Spiti Valley	Mayilraj et al. (2006e)
Dietzia kunjamensis, K30-10^T	Kunjam Pass, HP	Mayilraj et al. (2006d)
Flavobacterium glaciei, 0499^T	China no. 1 glacier	Zhang et al. (2006)
Kocuria himachalensis, K07-05^T	Spiti Valley	Mayilraj et al. (2006b)
Ornithinimicrobium kibberense, K22-20^T	Spiti Valley	Mayilraj et al. (2006c)
Psychromonas ingrahamii, 37^T	Arctic polar sea ice	Auman et al. (2006)
Rhodococcus kroppenstedtii, K07-23^T	Spiti Valley	Mayilraj et al. (2006a)
Actinoalloteichus spitiensis, RMV-378^T	Spiti Valley	Singla et al. (2005)
Dyadobacter hamtensis, HHS 11^T	Hamta glacier	Chaturvedi et al. (2005)
Flavobacterium fryxellicola, LMG 22022^T	Antarctic lakes	Van Trappen et al. (2005)
Flavobacterium psychrolimnae, 22018^T	Antarctic lakes	Van Trappen et al. (2005)
Pedobacter himalayensis, HHS 22^T	Hamta glacier	Shivaji et al. (2005a)
Planococcus stackebrandtii, K22-03^T	Spiti Valley	Mayilraj et al. (2005)
Geopsychrobacter electrodiphilus, A1^T	Marine sediment	Holmes et al. (2004)
Flavobacterium degerlachei, LMG 21915^T	Antarctic lakes	Van Trappen et al. (2004)
Flavobacterium frigoris, LMG 21922^T	Antarctic lakes	Van Trappen et al. (2004)
Flavobacterium micromati, LMG 21919^T	Antarctic lakes	Van Trappen et al. (2004)
Flavobacterium omnivorum, ZF-8^T	China no. 1 glacier	Zhu et al. (2003)
Flavobacterium xinjiangense, ZF-6^T	China no. 1 glacier	Zhu et al. (2003)
Oleispira antarctica, RB-8^T	Antarctic	Yakimov et al. (2003)
Pedobacter cryoconitis, A37^T	Alpine glacier	Margesin et al. (2003)
Flavobacterium frigidarium, A2i^T	Antarctica	Humphry et al. (2001)
Arthrobacter flavus, CMS 19Y^T	Antarctica	Reddy et al. (2000)
*Cellulophaga algicola,*ACAM 630^T	Antarctica	Bowman (2000)
Staleya guttiformis, EL-38^T	Ekho Lake	Labrenz et al. (2000)
Sulfitobacter brevis, EL-162 T	Ekho Lake	Labrenz et al. (2000)
Glaciecola pallidula, ACAM 615^T	Antarctica	Bowman et al. (1998a)
Glaciecola punicea, ACAM 611^T	Antarctica	Bowman et al. (1998a)
Hymenobacter roseosalivarius, AA-718^T	Antarctic	Hirsch et al. (1998)
Psychroflexus torquis, ACAM 623^T	Sea ice, Antarctica	Bowman et al. (1998b)
Gelidibacter algens, ACAM 536	Burton Lake	Bowman et al. (1997a)
Methanogenium frigidum, Ace-2^T	Antarctica	Franzmann et al. (1997)
Methylosphaera hansonii, ACAM 549^T	Antarctic	Bowman et al. (1997c)
Octadecabacter antarcticus, 307^T	Antarctica	Gosink et al. (1997)
Octadecabacter arcticus, 238^T	Antarctica	Gosink et al. (1997)
Psychrobacter glacincola, ACAM 521^T	Antarctica	Bowman et al. (1997d)
Psychroserpens burtonensis, ACAM 188	Burton Lake	Bowman et al. (1997a)

(continued)

Table 12.1 (continued)

Novel microbes	Location	References
Shewanella frigidimarina, ACAM 591	Antarctic sea ice	Bowman et al. (1997b)
Shewanella gelidimarina, ACAM 456	Antarctic sea ice	Bowman et al. (1997b)
Desulforhopalus vacuolatus, ltk10	Kysing Fjord	Isaksen and Teske (1996)
Cenarchaeum symbiosum, Fosmid 4B7	Sponge symbiotic	Preston et al. (1996)
Polaromonas vacuolata, 34-P^T	Antarctic	Irgens et al. (1996)
Sphingobacterium antarcticus, 4BY	Antarctica	Shivaji et al. (1992)
Halobacterium lacusprofundi, ACAM 32 ^{T}	Antarctic lake	Franzmann et al. (1988)
Nocardiopsis antarcticus,	Antarctica	Abyzov et al. (1983)

kibberense, K22-20^T, *Rhodococcus kroppenstedtii,* K07-23^T, *Actinoalloteichus spitiensis,* RMV-378^T, and *Planococcus stackebrandtii,* K22-03^T, from Himalayas (Ghosh et al. 2007; Mayilraj et al. 2005, 2006a, b, c, e; Singh et al. 2013; Singla et al. 2005).

12.2.3 Genome Sequencing of Psychrotrophic Microbes

In the last few decades, the draft genome sequencing has been done of microbes isolated from cold environments worldwide (Table 12.2). The whole genome sequences of psychrotrophic microbes are available such as *Zhihengliuella sp., Arthrobacter agilis, Idiomarina* sp., and *Rheinheimera* sp. isolated from Pangong Lake, a subglacial lake in the Himalayas (Gupta et al. 2011a, b; Mishra et al. 2018; Singh et al. 2016); *Arthrobacter* sp., *Hymenobacter* sp., *and Methanococcoides burtonii* isolated from Antarctica (Allen et al. 2009; Koo et al. 2014; Sastre et al. 2017); *Cryobacterium roopkundensis, Acinetobacter* sp., *Paenibacillus* sp., and *Pseudomonas trivialis* (Dhar et al. 2015, 2016; Gulati et al. 2015; Pal et al. 2015; Reddy et al. 2014; Swarnkar et al. 2014); *Exiguobacterium sibiricum* and *Nesterenkonia* sp. from Himalayas; and *Arthrobacter alpines* from subalpine regions. Along with psychrophilic whole genomes, there are many whole genomes of psychropiezophilic microbes available. These include *Colwellia chukchiensi, Octadecabacter antarcticus, Cenarchaeum symbiosum,* and *Colwellia psychrerythraea* isolated from sea habitats (Zhang et al. 2018; Hallam et al. 2006; Methé et al. 2005; Vollmers et al. 2013). The whole genome sequences of psychrophilic and psychrotolerant microbes help to understand about different gene responsible for different attributes of microbes and their adaptation at low-temperature conditions. Along with the presence of genes required for various basic physiology and metabolic processes, the sequenced genomes of psychrotrophic microbes from the cold habitats also have gene related to survival under the extremely low-temperature conditions. The genes for various cold adaptations and cold shock proteins, the genes for DNA repair system, the genes for carotenoid/terpenoids biosynthesis pathway, and a group of chaperone proteins have been reported from different cold-adaptive microbes (Table 12.3).

Table 12.2 Genome sequencing of psychrophilic and psychrotrophic microbes isolated from diverse cold habitats worldwide

Microbes	Source	Size (Mb)	G + C (%)	CDS	References
Colwellia chukchiensi	Chukchi Sea	4.04	41.9	3477	Zhang et al. (2018)
Colwellia polaris	Canada	4.43	37.5	3686	Zhang et al. (2018)
Zhihengliuella sp.	Pangong Lake	3.53	69.84	3363	Mishra et al. (2018)
Exiguobacterium oxidotolerans	Cold marine	3.03	46.80	2989	Cai et al. (2017)
Nesterenkonia sp.	Permafrost	3.70	69.50	2886	Singh et al. (2017)
Arthrobacter sp.	Antarctic	4.13	60.7	3616	Sastre et al. (2017)
Arthrobacter agilis	Pangong Lake	3.60	69.79	3316	Singh et al. (2016)
Arthrobacter alpines	Sikkim	4.30	60.64	4154	Kumar et al. (2016)
Microterricola viridarii	Glacier	3.70	68.70	3456	Swarnkar et al. (2016)
Paenibacillus sp.	Lahaul-Spiti	5.88	46.83	6093	Dhar et al. (2016)
Arthrobacter sp.	Glacier	4.03	65. 3	4623	Kumar et al. (2015b)
Arthrobacter sp.	Chandra Taal	3.60	58.97	3454	Kiran et al. (2015)
Paenibacillus	Lahaul-Spiti	8.44	50.77	7335	Dhar et al. (2015)
Paenibacillus sp.	Kunzum Pass	5.77	41.33	5638	Pal et al. (2015)
Pseudomonas trivialis	Lahaul-Spiti	6.45	59.91	6032	Gulati et al. (2015)
Saccharomyces eubayanus	Cold habitat	1.27	39.60	589	Baker et al. (2015)
Arsukibacterium ikkense	Cold habitat	4.13	49.7	3605	Lylloff et al. (2015)
Clavibacter sp.	Mongolia	3.12	73.5	2888	Du et al. (2015)
Acinetobacter sp.	Lahaul-Spiti	4.31	40.75	4017	Swarnkar et al. (2014)
Cryobacterium roopkundensis	Roopkund	4.36	65.30	4048	Reddy et al. (2014)
Planomicrobium glaciei	Chandra River	3.90	46.97	3934	Salwan et al. (2014)
Hymenobacter sp.	Antarctica	5.26	60.7	4328	Koo et al. (2014)
Octadecabacter antarcticus	Sea ice, Arctic	4.81	54.60	4428	Vollmers et al. (2013)
Exiguobacterium antarcticum	Lake Fryxell	2.82	47.50	2746	Carneiro et al. (2012)
Idiomarina sp.	Pangong Lake	2.59	45.50	2299	Gupta et al. (2011b)
Rheinheimera sp.	Pangong Lake	4.52	46.23	3942	Gupta et al. (2011a)
Methanococcoides burtonii	Ace Lake	2.54	44.08	2406	Allen et al. (2009)
Exiguobacterium sibiricum	Permafrost	3.03	47.70	2981	Rodrigues et al. (2008)
Cenarchaeum symbiosum	Marine	2.05	57.40	2017	Hallam et al. (2006)
Colwellia psychrerythraea	Sea ice, Arctic	5.37	38.00	4634	Methé et al. (2005)

Table 12.3 Gene and gene products of cold-inducible proteins from psychrophilic and psychrotolerant microbes

Gene	Gene product	Description	Functions	References
cspA	cspA-D	Cold shock protein	Cold adaptation	Yadav (2015)
cspB	capB	Cold acclimation proteins	Cold adaptation	Yadav (2015)
yfiA	pY	Associated with 30S ribosomal subunit	Translational (A-site) inhibitor	Di Pietro et al. (2013)
cspA	–	RNA/ssDNA chaperone	–	Kaufman-Szymczyk et al. (2009)
cspG	CspG	Cold shock protein homolog	–	Gualerzi et al. (2003)
cspI	CspI	Cold shock-inducible	–	Gualerzi et al. (2003)
gyrA	GyrA	DNA gyrase, subunit A	DNA-binding subunit of gyrase	Gualerzi et al. (2003)
infA	IF1	Protein chain initiation factor IF1	Translation initiation	Gualerzi et al. (2003)
infC	IF3	Protein chain initiation factor IF3	Translation initiation stimulates mRNA translation	Gualerzi et al. (2003)
hupB	HUβ	Nucleoid protein	DNA supercoiling	Giangrossi et al. (2002)
otsA	OtsA	Trehalose phosphate synthase	Critical for viability at low temperatures	Kandror et al. (2002)
otsB	OtsB	Trehalose phosphatase	Critical for viability at low temperatures	Kandror et al. (2002)
tig	Trigger factor	Multiple stress protein	Ribosome binding	Kandror et al. (2002)
cspE	CspE	RNA chaperone	Transcriptional antiterminator	Feng et al. (2001)
deaD	DeaD	ATP-dependent RNA helicase	Facilitates translation	Beran and Simons (2001)
pnp	PNPase	3′-5′ exoribonuclease, component of the RNA degradosome	Cold shock protein required for growth at low temperatures	Yamanaka and Inouye (2001)
infB	IF2	Protein chaperone, protein chain initiation factor IF2	Translation initiation, fMet-tRNA binding	Caldas et al. (2000)
Bc-Csp	–	Cold shock protein	–	Mueller et al. (2000)
cspA	CspA	Cold shock-inducible	–	Brandi et al. (1999)
dnaA	DnaA	Global transcription regulator	DNA binding and replication initiation	Atlung and Hansen (1999)
lpxP	–	Cold-inducible	Lipid A synthesis	Carty et al. (1999)

(continued)

Table 12.3 (continued)

Gene	Gene product	Description	Functions	References
Tm Csp	–	Cold shock protein	–	Welker et al. (1999)
rbfA	RbfA	Cold shock adaptation protein	Ribosome assembly/ maturation	Bylund et al. (1998)
has	H-NS	Nucleoid protein	Transcriptional repressor	Mojica and Higgins (1997)
cspB	–	Cold shock-inducible	–	Chapot-Chartier et al. (1997)
Csps	–	Cold shock protein	–	Berger et al. (1996)
Caps	–	–	Cold adaptation	Berger et al. (1996)
hscA	Hsc66	DnaK-like chaperone	–	Lelivelt and Kawula (1995)
hscB	HscB	DnaJ-like chaperone for HscA	–	Lelivelt and Kawula (1995)
aceE	AceE	Pyruvate dehydrogenase, decarboxylase	–	Jones and Inouye (1994)
aceF	AceF	Pyruvate dehydrogenase	Transcriptional enhancer	Jones and Inouye (1994)
nusA	NusA	–	Transcription termination elongation	Jones and Inouye (1994)
recA	RecA	–	Homologous recombination	Jones and Inouye (1994)
cspB	–	–	Affects cell viability at low temperature	Willimsky et al. (1992)

12.3 Mechanisms of Adaptation of Microbes at Low Temperature

Survival of microbes at low temperatures aggravates scientific interest due to several reasons, including potential applications of cold-active enzymes in diverse filed. Cold adaptation at low temperatures can unravel the mysteries of life science to know about how the machinery of life operates at extremely low temperatures. During the last few decades, a number of researchers from world investigations have been performed involving some cold-adapted microbial strains. Adaptation at low temperatures may be due to the role of cold shock and antifreeze proteins, role of cryoprotectants, maintenance of membrane fluidity, and role of hydrolytic enzymes (Chattopadhyay 2000, 2006; Chattopadhyay and Jagannadham 2001; Di Pietro et al. 2013; Horn et al. 2007; Phadtare 2012; Saxena et al. 2015; Suman et al. 2016; Yadav et al. 2019a).

Cold shock proteins (CSPs) are a group of ubiquitously occurring proteins, which are believed to protect the producer organism from cold stress. These cold shock proteins have been found to occur in psychrophilic/psychrotrophic bacteria such as *Arthrobacter sulfureus, Bacillus licheniformis, Exiguobacterium undae, Janthinobacterium lividum, Pseudomonas stutzeri, Psychrobacter marincola,* and *Sporosarcina pasteurii* (Yadav 2015). The cold acclimation proteins (Caps), another class of cold stress proteins, have been reported from psychrotrophic bacteria *Arthrobacter, Exiguobacterium, Janthinobacterium,* and *Pseudomonas* (Yadav et al. 2019b). Homologs of the *cspA* gene were detected in several Antarctic bacteria. There are many cold and heat shock proteins responsible for cold adaption in many bacteria (Bae et al. 2000; Cairrão et al. 2003; Carty et al. 1999; Di Pietro et al. 2013; Giangrossi et al. 2002; Lelivelt and Kawula 1995; Moll et al. 2002; Yamanaka et al. 1998).

The role of antifreezing proteins (AFPs) in bacterial cold adaptation has been reported from 11 bacterial isolates obtained from several Antarctic lakes (Gilbert et al. 2004). The presence of antifreezing compounds (sugars, organic acids, cryoprotectants, amino acids, antifreezing proteins) indicates their role in the survival of organisms at a subfreezing temperature (Yadav 2015). Cold-tolerant *Pseudomonas, Arthrobacter,* and *Sporosarcina* were found to protect cytoplasmic components by synthesizing antifreezing compounds/proteins, sugars, cryoprotectants, and specific intracellular/extracellular amino acids needed for cold adaptation of the microbial cells and harsh conditions of freezing. The modern "omic" technologies have an improved understanding of the adaptation of psychrophilic microbes at low-temperature conditions. Cold adaptation involves various changes in bacteria due to downshift of temperature by cold-active enzymes, antifreezing compound production, fatty acids configuration, accumulation of compatible osmotic solutes (e.g., mannitol, glycine, betaine), ice nucleating and antifreezing protein production, carotenoid pigment biosynthesis, and EPS biosynthesis (De Maayer et al. 2014).

12.4 Microbes-Mediated Cold Stress in Plants

The extreme environment of low temperature affects the productivity of several bowls of cereal and commercial crop plants. The low temperature plays a significant role in reducing plant growth and agricultural productivity worldwide. The psychrotrophic microbiomes are widely distributed in the agroecosystem of low-temperature habitats and play a variety of roles in plant growth promotion through different mechanisms of biological nitrogen fixation. The psychrophilic/psychrotrophic microbes have capabilities to solubilize the micronutrients under the low-temperature conditions. The solubilization of micronutrients such as zinc, potassium, and phosphorus may play important role in plant growth and soil health by zinc-, potassium-, and phosphorus-solubilizing microbes. These cold-adapted psychrotrophic microbes when inoculated with crop have capabilities to promote plant growth and also help in the alleviation of cold stress in the plant under the cold environmental conditions. There are many reports on the microbial diversity of micronutrient-solubilizing microbes from cold habitats and their applications for mitigation of stress under the harsh conditions. The microbes such as *Arthrobacter*, *Bacillus* and *Bacillus*-derived genera, *Curtobacterium*, *Flavobacterium*, *Kluyvera*, *Pseudomonas*, *and Serratia* have been isolated and evaluated as P-solubilizing microbes which are responsible for the mobilization of P to plant and also play important role in adaptation and mitigation under the extreme cold habitats (Yadav et al. 2016a, 2017c, 2019b).

Sustainable agriculture agroecosystems require the use of different strategies to increase or maintain the current rate of crop and food production (Pathak et al. 2014; Bainsla et al. 2018), and it is only possible to use microbial bioinoculants/biofertilizers as plant growth promoters having diverse multifunctional PGP attributes such as biological nitrogen fixation by nitrogen-fixing microbes present associated with plants as plant microbiomes (rhizospheric, endophytic, and epiphytic) as well as microbes present in soil as soil microbiomes. Nitrogen is one of the major limiting factors for plant growth and crop yield, and microbes having the capability to fix nitrogen help crops to get nitrogen from the atmosphere as well as from soil (Fagodiya et al. 2017; Pathak et al. 2016). The use of N_2-fixing microbiomes as biofertilizers/bioinoculants in single form and as jointly in the form of the microbial consortium is a sustainable method to increase plant growth and enhance crop yield under the normal as well as abiotic stress condition of cold stress. A variety of biological nitrogen-fixing microbes like *Serratia, Rhizobium, Pseudomonas*, *Klebsiella*, *Herbaspirillum*, *Gluconoacetobacter, Enterobacter*, *Bacillus*, *Azotobacter*, *Azospirillum*, *Azoarcus*, and *Arthrobacter* have been reported to fix N_2 under the low-temperature conditions (Kumar et al. 2019; Rana et al. 2016, 2017; Verma et al. 2015b, 2016; Yadav 2015).

The microbes produce the hormone which helps in plant growth and adaptation in extremely stressful habitats, e.g., stress-induced plant hormone. The psychrotrophic microbes can lower the level of C_2H_2 in the plant by a precursor 1-aminocyclo propane-1-carboxylate (ACC) of plant-produced ethylene. ACC deaminase producing psychrophilic/psychrotrophic microbes associated with different crops may play a role in regulating ethylene levels which help the plant to adapt under the cold stress habitats. The psychrotrophic/psychrotolerant microbial strains exhibiting ACC deaminase activity have been isolated, characterized, and evaluated for plant growth and adaption under the low-temperature conditions belonging to different genera such as *Serratia, Rhizobium, Ralstonia, Pseudomonas, Enterobacter, Burkholderia, Bacillus, Azospirillum, Alcaligenes, Agrobacterium, Acinetobacter*, and *Achromobacter* (Khalid et al. 2006; Verma et al. 2014, 2015b; Xu et al. 2014).

The plant growth promotion through indirect mechanism occurs when psychrophilic and psychrotrophic microbes prevent the detrimental effects of pathogens by producing diverse groups of bioactive compounds such as β-1, 3-glucanase, Fe-chelating compounds, fluorescent pigment, cyanide production, chitinases, and antibiotics. The microbes having the production of hydrolytic enzymes play important role in different industrial and agricultural processes (Rastegari et al. 2019; Rana et al. 2019; Sharma et al. 2019). There are many reports on microbial bioresources used as biocontrol agents which are inhibitory to plant pathogens (Rana et al. 2018; Kumar et al. 2015a; Yadav et al. 2019a) (Table 12.4).

Biofertilizers/bioinoculants in single form or microbial consortium help in plant growth and soil nutrient enrichment and finally in making the nutrients available to

Table 12.4 Cold-adapted psychrotrophic microbes with multifarious PGP attributes for the alleviation of cold stress in plants

Psychrotrophic microbes	P	IAA	Sid	ACC	References
Acinetobacter rhizosphaerae	785 ± 1.2	15.6 ± 1.2	+	+	Gulati et al. (2009)
Aeromonas hydrophila	31.5 ± 1.8	21.4 ± 1.0	+	–	Yadav et al. (2015a)
Arthrobacter methylotrophus	55.9 ± 1.4	21.4 ± 1.3	+	+	Verma et al. (2015c)
Arthrobacter sulfonivorans	25.6 ± 1.2	27.6 ± 0.7	+	–	Yadav et al. (2015b)
Bacillus altitudinis	43.9 ± 0.7	6.6 ± 1.0	–	–	Verma et al. (2015c)
Bacillus amyloliquefaciens	39.4 ± 2.4	14.2 ± 1.0	+	–	Yadav et al. (2015a)
Bacillus aryabhattai	45.6 ± 1.0	15.6 ± 0.7	–	–	Verma et al. (2015c)
Bacillus firmus	35.2 ± 3.3	35.2 ± 1.0	+	+	Yadav et al. (2015b)
Bacillus licheniformis	19.2 ± 1.0	13.2 ± 1.0	+		Yadav et al. (2016a)
Bacillus pumilus	36.1 ± 0.8	32.3 ± 1.2	+	–	Yadav et al. (2015b)
Bacillus subtilis	19.8 ± 0.5	27.7 ± 0.9	+	+	Yadav et al. (2015b)
Bacillus subtilis CKS1	+	–	–	–	Kumar et al. (2015a)
Bordetella bronchiseptica	48.6 ± 0.9	15.2 ± 1.1	+	–	Verma et al. (2015c)
Cellulosimicrobium cellulans	15.5 ± 1.1	18.4 ± 0.8	–	+	Yadav et al. (2015b)
Desemzia incerta	47.5 ± 1.2	28.6 ± 1.0	+	–	Yadav et al. (2015b)
Flavobacterium psychrophilum	66.0 ± 0.7	11.4 ± 1.5	+	+	Verma et al. (2015c)
Kocuria kristinae	64.0 ± 1.0	20.4 ± 1.1	+	–	Verma et al. (2015c)
Paenibacillus tylopili	48.4 ± 2.4	39.4 ± 2.4	+	–	Yadav et al. (2016a)
Pantoea agglomerans	22.0 ± 1.4	43.9 ± 1.1	+	–	Yadav et al. (2015a)
Pantoea dispersa	44.5 ± 0.2	4.4 ± 0.5	+	–	Selvakumar et al. (2008)
Providencia rustigianii	131.7 ± 1	51.0 ± 2.0	+	+	Yadav et al. (2015a)
Pseudochrobactrum kiredjianiae	–	+	+	+	Qin et al. (2017)
Pseudomonas cedrina	182.6 ± 1	9.99 ± 1.0	+	+	Yadav et al. (2015a)
Pseudomonas fluorescens	90.2 ± 1.7	9.4 ± 0.2	+	–	Mishra et al. (2011)
Pseudomonas fluorescens	768.3	–	–	–	Gulati et al. (2008)
Pseudomonas fragi	45.5 ± 1	11.3 ± 0.5	+	+	Yadav et al. (2015a)
Pseudomonas fragi CS11RH1	514.97	2.69 ± 0.3	–	–	Selvakumar et al. (2009a)
Pseudomonas geniculata	45.0 ± 1.2	66.7 ± 0.5	+	–	Verma et al. (2015c)
Pseudomonas jessani	7.9 ± 0.1	16.2 ± 0.3	+	–	Mishra et al. (2011)
Pseudomonas koreensis	97.3 ± 1.9	15.8 ± 0.3	+	–	Mishra et al. (2011)
Pseudomonas lurida	69.7 ± 1.5	9.9 ± 0.2	+	–	Mishra et al. (2011)
Pseudomonas lurida M2RH3	–	12.58 ± 0.1	+		Selvakumar et al. (2011)
Pseudomonas lurida NARs9	+	+	+	–	Mishra et al. (2009)
Pseudomonas moraviensis	44.2 ± 2.1	154.6 ± 1.	+	+	Yadav et al. (2015a)

(continued)

Table 12.4 (continued)

Psychrotrophic microbes	P	IAA	Sid	ACC	References
Pseudomonas poae	768.3	–	–	–	Gulati et al. (2008)
Pseudomonas putida	169.9 ± 3.0	10.1 ± 0.2	+	–	Mishra et al. (2011)
Pseudomonas reactans	23.23 ± 1	61.4 ± 0.5	+	–	Yadav et al. (2015a)
Pseudomonas sp.	15.7 ± 1.82	21.8 ± 0.2	+	–	Mishra et al. (2009)
Pseudomonas vancouverensis	66.3	8.33	+	–	Mishra et al. (2008)
Pseudomonas trivialis	718.0	–	–	–	Gulati et al. (2008)
Psychrobacter frigidicola	20.83 ± 1	65.9 ± 1.0	+	+	Yadav et al. (2015a)
Rahnella sp.	805.0 ± 1.	24.5 ± 1.5	+	+	Vyas et al. (2010)
Sanguibacter antarcticus	20.1 ± 0.1	9.3 ± 0.9	+	+	Yadav et al. (2015b)
Sanguibacter suarezii	18.1 ± 0.5	76.8 ± 0.3	+	+	Yadav et al. (2015a)
Stenotrophomonas maltophilia	55.7 ± 0.5	66.1 ± 0.7	+	+	Verma et al. (2015c)

the crops. Extensive work on the biofertilizers/bioinoculants is available (Kour et al. 2017; Srivastava et al. 2013; Yadav et al. 2018a). There are many reports that microbes mediated mitigation of cold stress for the plant growth and yield of different commercial crops (Fernandez et al. 2012; Ghorbanpour et al. 2018; Mishra et al. 2008, 2009, 2011; Qin et al. 2017; Selvakumar et al. 2008; 2009a, b, 2011, 2013; Srinivasan et al. 2017; Verma et al. 2015c; Wang et al. 2016; Yadav et al. 2015c, d, 2016b) (Table 12.5).

The cold-tolerant *Pseudomonas* strains (RT5RP2 and RT6RP) have been isolated from the rhizoplane of wild grass from Rudraprayag District of Uttarakhand (India) by Selvakumar et al. (2013). Both isolates showed growth at a temperature ranging between 4 and 30 °C. Kinetics of phosphate solubilization by the bacterial strains showed a nonlinear regression of the rate of P solubilization, which fitted best in the power model, and showed a declining trend across three different temperatures. In a pot experiment, bacterization of lentil seeds with *Pseudomonas* strains combined with URP as a sole source of phosphorus showed an increase in P uptake by the plants compared to the application of rock phosphate alone. Wang et al. (2016) studied the effect of a consortium of *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21 on chilling tolerance in tomato seedlings. The study indicated the increase in the survival rates in treated tomato seedlings six times more in comparison to the untreated ones. Further, the accumulation of the MDA, as well as H_2O_2, was also enhanced with the onset of the chilling stress.

A psychrotrophic *Pseudochrobactrum kiredjianiae* A4 has been isolated from cave soil by Qin et al. (2017). The strain was screened for different plant growth-promoting traits. The strain showed siderophore, IAA production, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. Further, the strain also inhibited the growth and development of *Rhizoctonia cerealis, Fusarium graminearum, Magnaporthe grisea, Fusarium oxysporum, and Botrytis cinerea* under in vitro conditions. The isolate improved the physiological parameters and reduced the defense enzymes activities of wheat (*Triticum aestivum* L.) in the presence of *R. cerealis*

Table 12.5 Microbes-mediated alleviation of cold stress in plants

Microbes	Crop	Function	References
Azospirillum brasilense	Wheat	Dry weight	Turan et al. (2012)
Bacillus megaterium	Wheat	Dry weight	Turan et al. (2012)
Bacillus subtilis	Wheat	Dry weight	Turan et al. (2012)
Bacillus amyloliquefaciens	Wheat	Cold alleviation	Verma et al. (2015a)
Burkholderia phytofirmans P	Grapevine	Physiological activity	Barka et al. (2006)
Exiguobacterium acetylicum	Pea	Germination	Selvakumar et al. (2009b)
Bacillus subtilis	Tomato	Biomass enhancement	Kumar et al. (2015a)
Pseudomonas lurida	Wheat	Nutrient uptake	Selvakumar et al. (2011)
Pseudomonas sp.	Lentil	P-uptake	Selvakumar et al. (2013)
Pseudomonas vancouverensis	Wheat	Germination	Mishra et al. (2008)
Burkholderia phytofirmans	Grapevine	Carbohydrate metabolism	Fernandez et al. (2012)
Microbial consortium[a]	Rice	Germination, growth, enzymatic activity, biomass enhancement	Kakar et al. (2016)
Microbial consortium[b]	Tomato	Soluble sugar, proline, antioxidant defense system, stress-related gene activation	Wang et al. (2016)
Pantoea dispersa	Wheat	Growth and nutrient uptake	Selvakumar et al. (2008)
Pseudochrobactrum kiredjianiae	Wheat	Physiological parameters	Qin et al. (2017)
Pseudomonas frederiksbergensis	Tomato	Proline content, antioxidant enzymes	Subramanian et al. (2016)
Pseudomonas migulae	Green gram	Biomass, chlorophyll content, and nitrate reductase activity	Suyal et al. (2014)
Pseudomonas sp.	Wheat	Chlorophyll, anthocyanin, physiologically Fe	Mishra et al. (2011)
Pseudomonas vancouverensis	Tomato	Reduced electrolyte leakage and lipid peroxidation in leaf tissues	Subramanian et al. (2016)
Serratia nematodiphila	Pepper	Improved growth	Kang et al. (2015)
Sphingomonas faeni	Finger millet	Shoot, root length, biomass, antioxidant activity	Srinivasan et al. (2017)
Sphingomonas faeni	Foxtail millet	Shoot, root length, biomass, antioxidant activity	Srinivasan et al. (2017)
Trichoderma harzianum	Tomato	Growth, relative water content, electrolyte leakage, proline content	Ghorbanpour et al. (2018)

Microbial consortium[a] [*Bacillus amyloliquefaciens* and *Brevibacillus laterosporus*]
Microbial consortium[b] [*Bacillus cereus*, *B. subtilis*, and *Serratia* sp.]

under greenhouse conditions. Statistical analysis of measured parameters well revealed that A4-inoculated treatment alleviated pathogenic stress in wheat plants. In the study of Ghorbanpour et al. (2018), the effects of *Trichoderma harzianum* AK20G strain (Th) has been demonstrated as a biocontrol agent on the tolerance of tomato (*Solanum lycopersicum* L.) plants under chilling stress. In the study, the tomato plants were exposed to low temperatures after treating them with *Trichoderma harzianum* AK20G strain (Th), and their physiological, biochemical, and molecular responses were investigated at different time courses. Results clearly reported the alleviation of the adverse effects of the cold stress in treated plants as shown by enhancement of photosynthetic as well as the growth rates. In treated plants, the reduction in lipid peroxidation rate and electrolyte leakage was evident, while increment in leaf water content and proline accumulation was observed. Further, the gene expression analysis showed the improvement in expression of *TAS14* and *P5CS* with time as the cold stress continued.

12.5 Conclusion and Future Prospect

The psychrotrophic microbes have attracted the attention of the scientific community due to their ability to promote plant growth and soil health under cold stress conditions. Due to the ability to promote crop growth under low-temperature condition, the psychrotrophic microbes have potential biotechnological applications for sustainable agriculture. The cold-adapted microbes promote plant growth under the extremely low-temperature condition by both direct and indirect plant growth-promoting mechanisms. The microbiomes from cold habitats having the capability to solubilize micronutrients, fix the atmospheric nitrogen, and produce different phytohormones are included under the direct plant growth promotion strategies, whereas psychrotrophic microbes having the capability to produce Fe-chelating compounds, cyanide, chitinases, and antibiotics are included under the indirect plant growth promotion mechanisms. Cold-tolerant microorganisms are widely distributed in the agroecosystem and play a variety of roles, extending their role in the alleviation of cold stress in plants. Though most research work conducted so far has largely focused on microbiomes from natural habitats as well as psychrophilic/psychrotolerant microbes, it is a welcome sign that many agriculturally important resourceful microbes are being described from various parts of the Earth. The genomes of more psychrophilic and psychrotolerant microbes sorted out from cold habitats should be sequenced to understand the adaptations, survival, and growth of these microbiomes under these extremely harsh conditions of low temperature.

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Drought-Tolerant Phosphorus-Solubilizing Microbes: Biodiversity and Biotechnological Applications for Alleviation of Drought Stress in Plants

13

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Abstract

Drought is one of the major abiotic stresses accepted as the main constraint for loss of the crop yield worldwide. Further, problems are created by nutrient limitations particularly low phosphorus (P). Soils though have higher concentration of total phosphorus but are actually deficient in available orthophosphate due to

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which modern agricultural systems are highly dependent on chemical fertilizers. These chemical fertilizers are neither eco-friendly nor economically feasible and sustainable. Biotechnology offers a number of sustainable solutions to mitigate these problems by using plant growth-promoting (PGP) microbes. The PGP microbes colonize the rhizospheric region, or they may be endophytic or epiphytic and are beneficial for plant growth and adaptation to abiotic stresses. These microbes help the crops to tolerate drought conditions by different mechanisms including the production of exopolysaccharide (EPS), various phytohormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and a number of volatile compounds, enhancement of nutrient uptake, induction of the accumulation of osmolytes and antioxidants, upregulation or downregulation of the stress-responsive genes, or bringing about of alterations in root morphology. Inoculating plants with PGP microbes can increase tolerance against abiotic stresses such as drought, salinity, and metal toxicity. Systematic identification of bacterial strains providing cross-protection against multiple stressors would be highly valuable for agricultural production in changing environmental conditions. Among the PGP microbes, P-solubilizing microbes play an important role in plant growth and soil health, which belong to diverse genera such as *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Lysinibacillus*, *Paenibacillus*, *Pseudomonas*, *Serratia*, and *Streptomyces*. The present chapter deals with biodiversity of P-solubilizing drought-tolerant microbes, mechanisms of plant growth promotion, and mitigation of drought stress in the plants.

Keywords
Biodiversity · PGPR · Drought · P solubilizers · ACC deaminase

13.1 Introduction

There are a number of factors which are leading to climate change such as urbanization, industrialization and agriculture is one of the most exposed sectors to such changes (Bhatia et al. 2013a; Kumar et al. 2017). Increasing human population has further added to the world's food security concern (Bainsla et al. 2018; Pathak et al. 2014). Additionally, the use of chemical fertilizers is also affecting the environment (Fagodiya et al. 2017b). The pressure of population is increasing at such an alarming rate that there is a critical need to enhance agricultural productivity in an eco-friendly manner (Bhatia et al. 2015; Mukherjee et al. 2018). Agriculture is one of the most exposed sectors to various climatic changes (Kumar et al. 2016a; Bhattacharyya et al. 2018). One of the major hurdles to increase the yield and productivity is the exposure of the crops to the drought conditions in different parts of the world (Naveed et al. 2014; Vinocur and Altman 2005). Drought is one of the major abiotic stresses acting as the limiting factor for agricultural productivity worldwide (Mina et al. 2017; Tomer et al. 2015). It has been estimated that there is approximately 9–10% reduction in the national production of the cereals due to the drought conditions (Lesk et al. 2016). Thus, there is a necessity to find ways to combat water deficit conditions and their influence on food security (Alexandratos and Bruinsma 2012), and particularly there is a need to improve the tolerance of the plants to drought so that they can grow under conditions of water stress (Mancosu et al. 2015; Sachs et al. 2010). However, plants have developed different mechanisms to survive under drought conditions such as by making alterations in the morphology, osmotic adjustments, optimization of water resources, antioxidant systems which greatly lessen the adverse effects of reactive oxygen species (Bhatia et al. 2013c), and induction of an array of stress-responsive genes and various proteins (Farooq et al. 2009), and one of the approaches that have been used for the mitigation of the adverse effects of the water stress conditions is the development of cultivars which can tolerate drought conditions (Barrow et al. 2008; Eisenstein 2013).

No doubt, conventional plant breeding techniques have surely led to the growth of high-yielding, drought-tolerant crop varieties, but there are a number of disadvantages of using this approach as it is time-consuming and labor intensive, and even using this approach can lead to the loss of some desirable traits from the host's gene pool, and breeding techniques provide benefits to a single crop species which are non-transferrable to other crop systems (Eisenstein 2013; Philippot et al. 2013). So, the major challenge is to evolve efficient, cost-effective, easily adaptable approaches for managing abiotic stress conditions. Studies up to date suggest that microorganisms can prove very efficient in supporting the growth of the plants during the water stress conditions. Plant-associated beneficial microbes are recently attaining greater attention as they play an important role in enhancing the productivity of the crops and also providing resistance against the stress conditions and are known as plant growth-promoting microbes (PGPMs) (Glick et al. 2007; Yang et al. 2009). Among PGPMs, the rhizospheric biology is the most studied one with a focus on rhizobacteria known as plant growth-promoting rhizobacteria (PGPR). The PGPR contribute to mitigating the stress conditions through diverse mechanisms (Hayat et al. 2010; Mapelli et al. 2013). The PGPR directly enhance the uptake of the micronutrients, through phytohormone production; fixing of atmospheric nitrogen; P, K, and Zn solubilization; or indirectly stimulating the immune system against various fungal pathogens by production of various compounds, enzymes, siderophores, antibiotics, and osmolytes or improving either texture or structure of the soil (Mapelli et al. 2012).

Ethylene, known as the stress hormone, is the most important plant hormone whose synthesis is accelerated whenever there are stress signals such as drought, high temperature, floods, the presence of any chemical or metals, mechanical wounding, and pathogenic infection. Ethylene plays a very important role in regulating various physiological processes including differentiation of the tissues, development of the lateral buds, emergence of the seedlings, development as well as the elongation of the root hair, synthesis of anthocyanins, ripening and degreening of the fruits, and production of various volatile compounds which are responsible for aroma in fruits if present in low concentrations but when present in high concentrations prove inhibitory causing damages to the plants leading to epinasty, development of the shorter roots, and premature senescence. As the drought conditions start appearing, the synthesis of ethylene is stimulated which then inhibits the germination of the seeds, elongation of the roots, and nodulation of the legumes (Ahamd et al. 2017). Some PGPR possess an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase which cleaves the precursor 1-aminocyclopropane-1-carboxylate (ACC) into α-ketoglutarate before it can get converted into ethylene, ultimately lowering the ethylene levels in plants during the stress conditions (Glick 2004); though there are few reports on the presence of ACC deaminase in PGPR, it plays an important role in supporting the growth as well as the development of the plants by reducing the levels of the ethylene produced during the biotic or the abiotic stress (Ali et al. 2014). The enzyme has been detected in a limited number of bacteria and plays a significant role in sustaining plant growth and development under biotic and abiotic stress conditions by reducing stress-induced ethylene production in plants. In the past few years, bacteria that have been reported to provide tolerance to host plants under different abiotic stress environments include *Achromobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Methylobacterium*, *Microbacterium*, *Mitsuaria*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, and *Rhizobium* (Curá et al. 2017; Grover et al. 2011; Huang et al. 2017; Niu et al. 2017; Shah et al. 2017).

Applied microbiology and biotechnology have opened up new possibilities for potential applications of beneficial microbes for agriculture, industry, and medicine. An understanding of microbial diversity from drought habitats and its potential application in agriculture is important and useful for plant growth, protection, and yield under the rainfed environmental conditions. The drought-tolerant microbes attracted the attention of the scientific community due to their ability to promote plant growth and adaptation under the abiotic stress of drought. Drought-tolerant microbes with novel secondary metabolite and bioactive compound production ability will be applicable in a broad range of industrial, agricultural, and medical processes. The microbes with multifunctional PGP attributes could be valuable in agriculture as bio-inoculants and biocontrol agents and for biofortification of micronutrients. The present book chapter describes the method of isolation of drought-tolerant microbes from diverse habitats, characterization, identification, biodiversity, and biotechnological applications in agriculture for the alleviation of drought stress in plants.

13.2 Characterization of Drought-Tolerant Microbes

Drought stress is one of the major problems of the agriculture sector (Malyan et al. 2016b). It greatly affects the productivity of the crops especially in arid and semi-arid regions of the world. Microbes will play a potent role in managing the stress; once these beneficial microbes are isolated, their various properties such as their potential to tolerate extremities and their genetic diversity are completely studied, and methods for their use in agriculture production are developed. Further, inoculating the plants with microbes possessing multifunctional PGP attributes will also

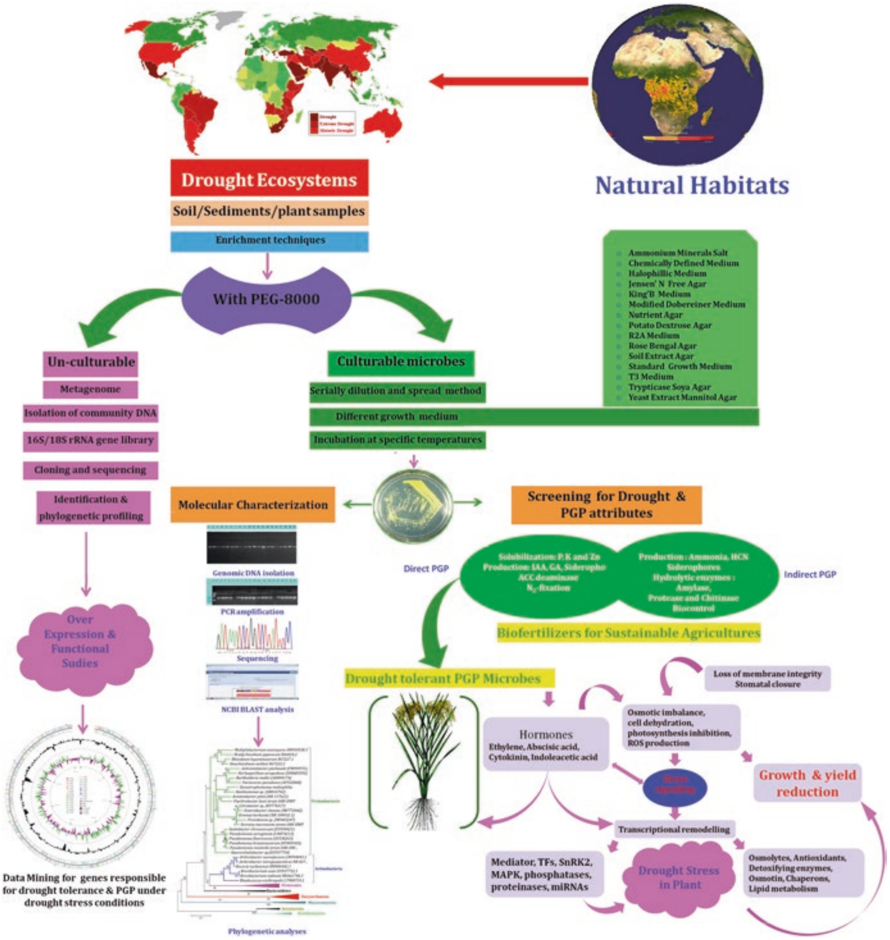

Fig. 13.1 A schematic representation of the isolation, characterization, identification, and potential application of drought-tolerant microbes

help the plants in arid or semiarid regions to overcome drought. A schematic and graphical reorientation has been given in Fig. 13.1 for the characterization, identification, and potential applications of drought-tolerant microbes.

13.2.1 Isolation and Enumeration of Drought-Tolerant Microbes

The culturable microbes from soil and rhizospheric soil can be isolated through enrichment techniques using the standard serial dilution plating methods employing different growth media for different groups of microbes, e.g., nutrient agar for heterotrophic microbes, King's B agar for *Pseudomonas* sp., Congo Red yeast mannitol agar for *Rhizobium*, Jensen N_2-free agar for N_2-fixing microbes, ammonium mineral salt agar for methylotrophs, tryptic soy agar for *Arthrobacter*, soil extract agar for soil-specific and niche-specific microbes, Luria-Bertani agar for endophytic microbes, and potato dextrose agar for fungi (Verma et al. 2017b). Among different groups of microbes, the bacteria members of *Firmicutes* have been reported as most dominant worldwide, as they can survive in very extreme environments due to their ability to form endospores under the unfavorable conditions. The bacterial member of phylum *Firmicutes* such as *Bacillus* and *Bacillus*-derived genera (BBDG) can be isolated using heat enrichment technique. A selective enrichment technique using 0.25 M and 0.75 M sodium acetate buffer with LB broth and T_3 agar can be employed for isolation of *Bacillus thuringiensis* (Yadav et al. 2015d).

For isolation of drought-tolerant endophytic microbes, the roots, shoots, and seeds should be washed in running water to remove adhering soil and surface sterilized by dipping in 0.1% of mercuric chloride for 5 min following 2% of sodium hypochlorite for 10 min. The root, stem, and seed of selected crops can be cut into 1 cm pieces and placed onto Luria-Bertani agar, nutrient agar, modified Dobereiner medium, and yeast extract mannitol agar. The drought-tolerant epiphytic microbes can be isolated from the phyllosphere of selected plants. Plant leaves (3 g) can be agitated at 150 rpm at ambient temperature for 2 h in 500 mL Erlenmeyer flasks containing 25 g of glass beads and 50 mL of phosphate buffer. After agitation, appropriate dilutions of the flask contents can be plated onto a different medium. Imprint method can be also used to isolate epiphytic microbes (Holland et al. 2000).

The plant microbiomes (epiphytic, endophytic, and rhizospheric) and microbes from a soil sample collected from the arid and semiarid region can be isolated using different growth media as in its original constitutes and with low water potential with polyethylene glycol (PEG-8000) for isolation of natural and putative drought-tolerant microbes, respectively. Along with putative drought-tolerant microbes, the polyextremophiles (microbes with more than one abiotic stress properties) can be isolated from diverse habitats using two or more abiotic stress conditions (Yadav 2015). The plates may be incubated at 4 °C–50 °C (psychrophilic, mesophilic, and thermophilic drought-tolerant microbes), and the population may be counted after 3–20 days (fast-, medium-, and slow-growing microbes). Colonies that appear may be purified by repeated streaking to obtain distinct pure colonies using respective growth medium plates. The pure cultures may be maintained at 4 °C as slants and glycerol stock (20%) at –80 °C for further use.

13.3 Molecular Characterization

The molecular diversity analysis of drought-tolerant microbes may be done using DNA isolation, quantification, PCR amplification, amplified rDNA restriction analysis (ARDRA) with different restriction enzymes, clustering analysis using the software NTSYS-2.02e package (numerical taxonomy analysis program package, Exeter Software, USA), conserved region gene sequencing, BLAST analysis, and finally taxonomical affiliation analysis using MEGA software. The genomic DNA should be extracted by the different methods. The amount of DNA extracted should be electrophoresed on 0.8% agarose gel. Amplification of the 16S rRNA gene (*Archaea* and *Bacteria*) and ITS gene sequences (*Fungi*) should be done by using the universal primers. The amplification conditions for *Archaea* (Yadav et al. 2015c), *Bacteria* (Yadav et al. 2015b), and *Fungi* (Yadav et al. 2018c) may be used for 100 μL of PCR reactions. The PCR-amplified 16S rDNA should be purified by QIA quick PCR product purification kit (Qiagen). The 100 ng purified PCR products may be digested separately with different restriction endonucleases (such as *Alu* I, *Hae* III, and *Msp* I). The clustering analysis may be done using the software NTSYS-2.02e package (numerical taxonomy analysis program package, Exeter Software, USA), and similarity among the isolates should be calculated by Jaccard's coefficient. PCR-amplified 16S rRNA and ITS genes should be purified and sequenced using universal primers. 16S rRNA and ITS gene sequences should be analyzed using codon code aligner v.4.0.4. 16S rRNA and ITS gene sequences should be aligned to those of closely related microbial species available at GenBank database using BLASTn program. Microbial isolates may be identified based on a percentage of sequence similarity (≥ 97%) with that of a prototype strain sequence in the GenBank. The phylogenetic tree should be constructed on the aligned datasets using the neighbor-joining method implemented in the program MEGA software.

13.4 Characterization of Microbes for PGP Attributes

To know the plant growth-promoting ability and other agricultural and biotechnological applications of drought-tolerant microbes, the purified microbes should be screened qualitatively for direct PGP attributes which include biological N_2 fixation (Boddey et al. 1995); production of phytohormones indole-3-acetic acid (Bric et al. 1991), gibberellic acid (Brown and Burlingham 1968), and ACC deaminase (Jacobson et al. 1994); and solubilization of phosphorus (Pikovskaya 1948), potassium (Hu et al. 2006), and zinc (Fasim et al. 2002). The microbes should be also screened qualitatively for indirect PGP attributes which include production of ammonia (Cappucino and Sherman 1992), HCN (Bakker and Schippers 1987), siderophores (Schwyn and Neilands 1987), and hydrolytic enzymes (Yadav et al. 2016b) and biocontrol against different fungal pathogens (Sijam and Dikin 2005). After qualitatively screening, the selected drought-tolerant microbes with PGP attributes should be quantitatively screened for N_2-fixing attribute by using the acetylene reduction assay (ARA) (Han and New 1998), P solubilization (Mehta and Nautiyal 2001), K solubilization (Verma et al. 2016a), and IAA production (Patten and Glick 2002). The selected drought-tolerant microbes with multifunctional PGP attributes may be evaluated under the controlled and natural conditions for their ability of plant growth promotion and alleviation of drought stress in crops.

13.5 Biodiversity and Distributions of Drought-Tolerant Microbes

Different groups of microbes have been reported as drought tolerant, and polyextremophiles associated with plants and from diverse habitats which belong to all their microbial domains *Archaea*, *Bacteria*, and *Eukarya*, which included different phyla mainly *Acidobacteria*, *Actinobacteria*, *Ascomycota*, *Bacteroidetes*, *Basidiomycota*, *Euryarchaeota*, *Firmicutes*, *Mucoromycota*, and *Proteobacteria.* The phylum *Proteobacteria* was further grouped as *α*-, *β*-, *γ*-, and *δ*-*Proteobacteria*. Overall the distribution of microbes varied in all bacterial phyla; *Proteobacteria* was most dominant followed by *Firmicutes* and *Actinobacteria* (Fig 13.2).

On review of different extreme drought environments, it was found that eight different phyla have been sorted out belonging to the different domains of *Archaea*,

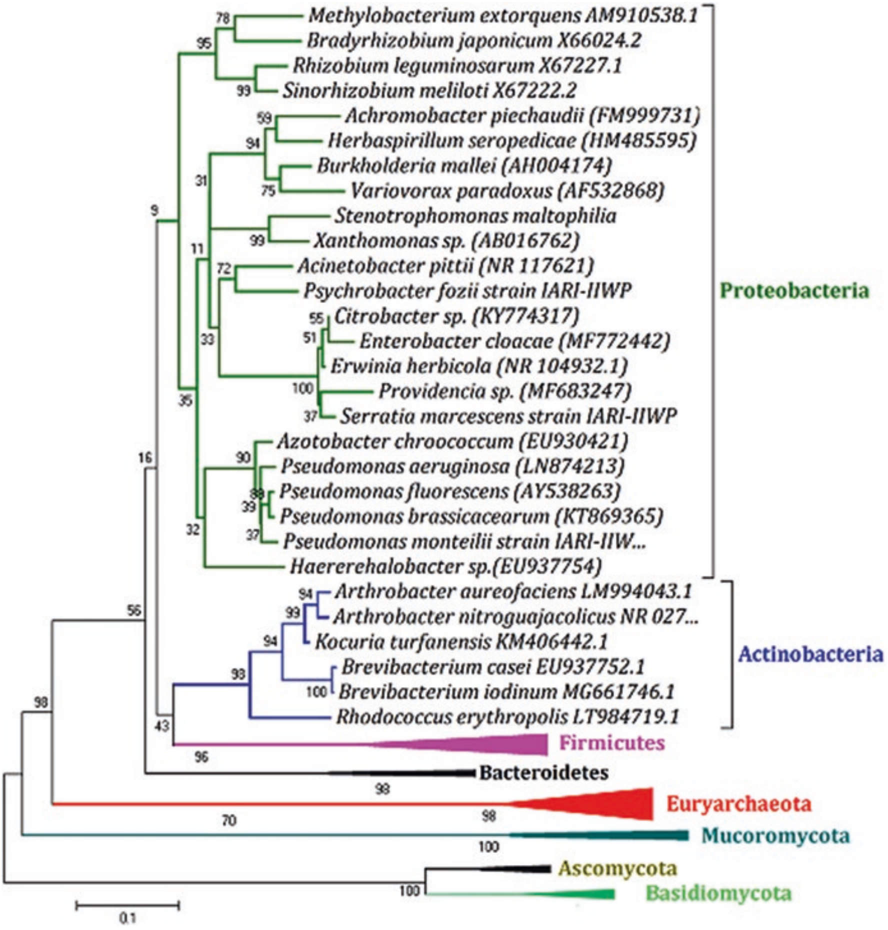

Fig. 13.2 Phylogenetic tree showing the relationship among different groups of microorganisms reported as drought tolerant from diverse habitats

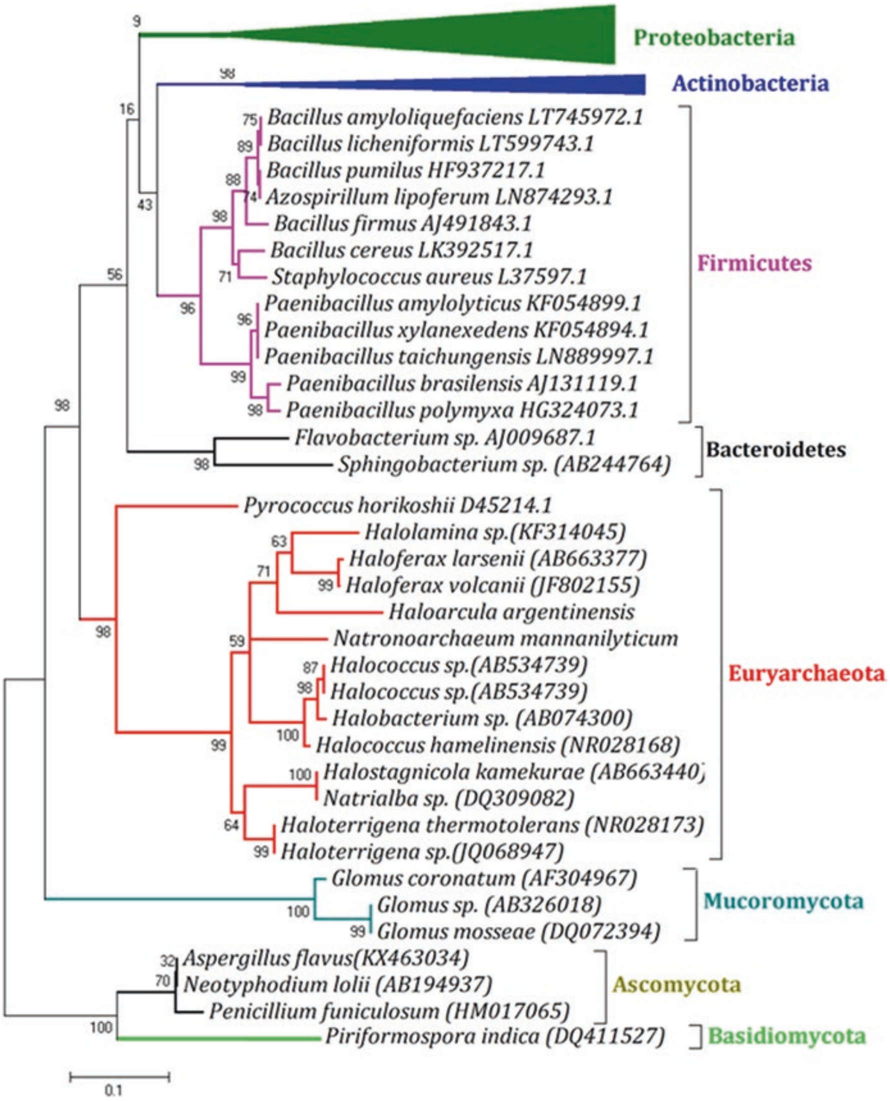

Fig. 13.2 (continued)

Bacteria, and *Fungi* (Fig 13.3). The more than 156 distinct species from different genera such as *Achromobacter*, *Acinetobacter*, *Arthrobacter*, *Aspergillus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Frankia*, *Glomus*, *Herbaspirillum*, *Methylobacterium*, *Paenibacillus*, *Penicillium*, *Piriformospora*, *Providencia*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, *Serratia*, and *Sinorhizobium* have been reported from drought habitats and characterized for their PGP attributes and finally evaluated for alleviation of drought stress in different crops (Fig. 13.4).

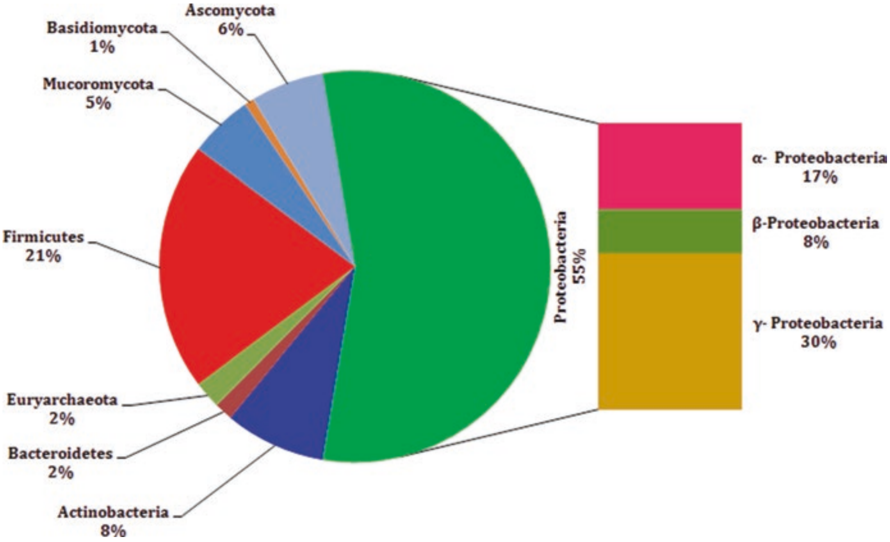

Fig. 13.3 The abundance of drought-tolerant microbes belonging to diverse phyla reported from diverse habitats

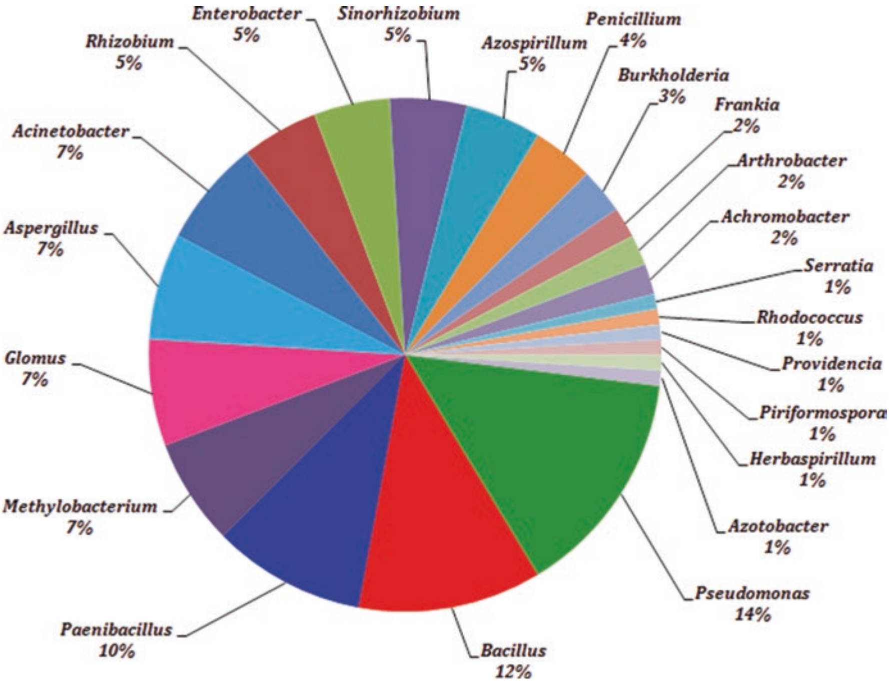

Fig. 13.4 Distribution and abundance of different predominant genera of drought-tolerant microbes

The drought-tolerant microbes have been sorted out from different sources and have been applied for plant growth promotion and alleviation of drought stress in the form of microbial strains, microbial consortium, as well as biofertilizers and biocontrol agents for replacement of chemical fertilizers as eco-friendly resources. On review of different research on microbial diversity of drought-tolerant microbes, it was found that among different domains and phyla, the drought-tolerant microbes belonging to different genera have been sorted out and characterized for PGP under abiotic stress of drought; e.g., the 11 distinct species belong to 9 genera, namely, *Arthrobacter*, *Brachybacterium*, *Brevibacterium*, *Frankia*, *Kocuria*, *Microbacterium*, *Micrococcus*, *Rhodococcus*, and *Zhihengliuela* of phylum *Actinobacteria*; the 5 distinct species belong to 2 genera *Flavobacterium* and *Sphingobacterium* of phylum *Bacteroidetes*; the 21 distinct species belong to 12 genera, namely, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Halolamina*, *Halosarcina*, *Halostagnicola*, *Haloterrigena*, *Natrialba*, *Natrinema*, *Natronoarchaeum*, and *Pyrococcus* of phylum *Euryarchaeota*; the 27 distinct species belong to 6 genera *Bacillus*, *Brevibacillus*, *Clostridium*, *Paenibacillus*, *Staphylococcus*, and *Streptococcus* of phylum *Firmicutes*; the 22 distinct species belong to 13 genera *Acetobacter*, *Agrobacterium*, *Allorhizobium*, *Azorhizobium*, *Azospirillum*, *Bradyrhizobium*, *Brevundimonas*, *Ensifer*, *Mesorhizobium*, *Methylobacterium*, *Phyllobacterium*, *Rhizobium*, and *Sinorhizobium of* phylum α-*Proteobacteria*; the 11 distinct species belong to 10 genera *Achromobacter*, *Alcaligenes*, *Azoarcus*, *Burkholderia*, *Delftia*, *Duganella*, *Herbaspirillum*, *Mitsuaria*, *Ralstonia*, and *Variovorax* of phylum *β-Proteobacteria*; and 40 distinct species belong to 19 genera *Acinetobacter*, *Aeromonas*, *Azotobacter*, *Citrobacter*, *Cronobacter*, *Enterobacter*, *Erwinia*, *Haererehalobacter*, *Halomonas*, *Klebsiella*, *Pantoea*, *Proteus*, *Providencia*, *Pseudomonas*, *Psychrobacter*, *Raoultella*, *Serratia*, *Stenotrophomona*, and *Xanthomonas* of phylum *γ-Proteobacteria*.

Microbes have been reported by both culture-dependent and culture-independent approaches. It is possible to assess only a small fraction of the microbial diversity associated with plants using the isolation methods described above because few microbial species can be cultivated using traditional laboratory methods. The sizes of microbial communities as determined using culture-independent methods might be 100–1000-fold larger than communities uncovered via traditional isolation. Many novel drought- and heat-tolerant microbes have been sorted out from diverse low water-deficient habitats and microbiomes (epiphytic, endophytic, and rhizospheric) from plants growing in drought stress conditions. There are very few reports for niche-specific microbes, but there are many reports on niche specificity of microbes from different extreme habitats (Saxena et al. 2016b; Yadav et al. 2016b, 2015a, 2017e). Among different genera, the most predominant are *Achromobacter*, *Acinetobacter*, *Allorhizobium*, *Arthrobacter*, *Aspergillus*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Frankia*, *Glomus*, *Methylobacterium*, *Paenibacillus*, *Penicillium*, *Phyllobacterium*, *Piriformospora*, *Pseudomonas*, *Rhizobium*, *Stenotrophomonas*, and *Streptococcus* (Fig. 13.5).

Drought is among one of the major abiotic stress factors responsible for the decrease in the yield of the crops in the world with the population increasing day by

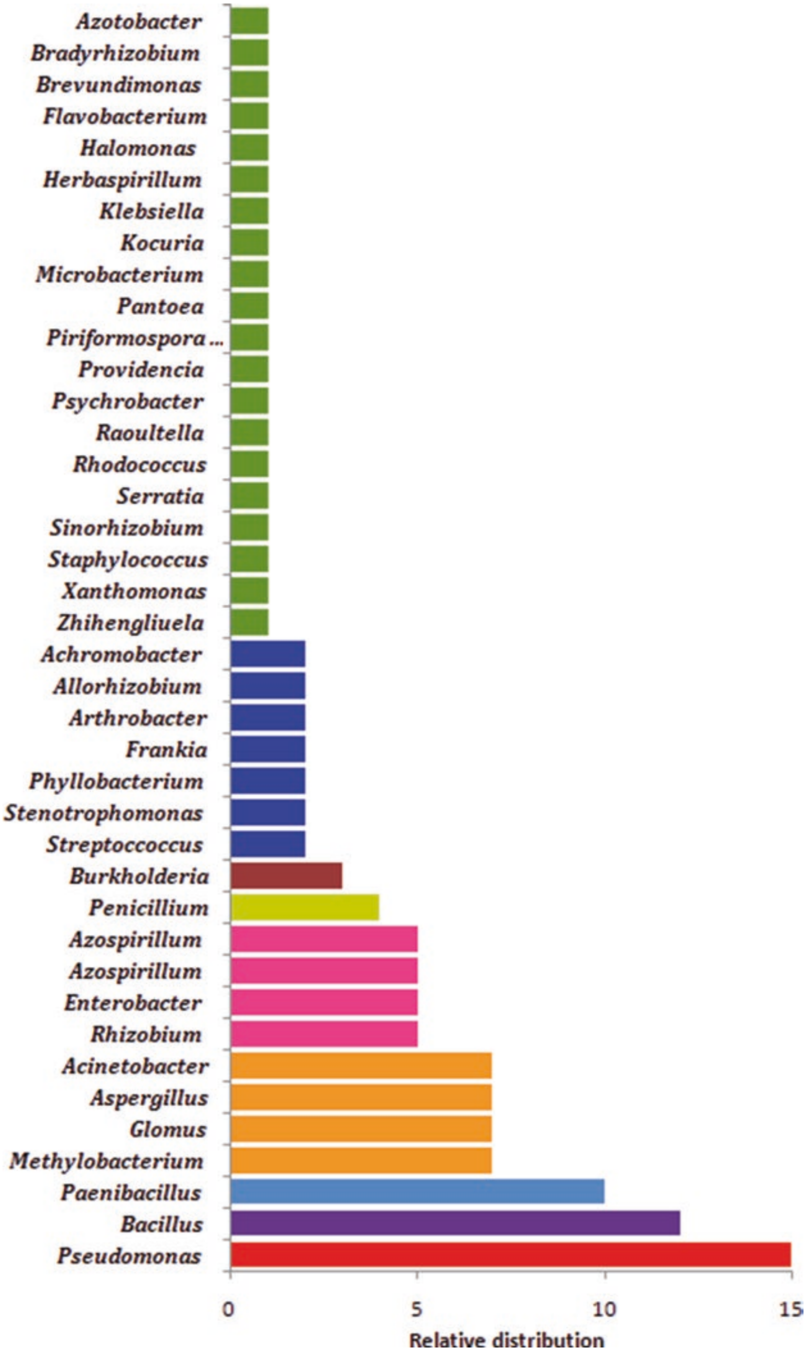

Fig. 13.5 Distribution of predominant genera of drought-tolerant microbes belonging to diverse phyla reported from diverse habitats

day. Further, problems are caused by the limitations of the nutrients particularly phosphorus due to which dependency on phosphate fertilizers is increasing. The main reason for using phosphate fertilizers is the insolubility of phosphorus and its unavailability to the plants. But using these phosphate fertilizers is not eco-friendly and safe. Thus, biotechnology offers a number of ways to alleviate these problems through the use of P-solubilizing drought-tolerant microbes. During drought, the phosphatase activity in soil could decrease and accumulation of phosphorus is expected. There are a number of reports on mitigation of the adverse effects of the drought by addition of PGP microbes (Dimkpa et al. 2009; Saxena et al. 2016a; Verma et al. 2014, 2016b; Yang et al. 2009). Chowdhury et al. (2009) isolated and characterized drought-tolerant microbes from plant *Lasiurus sindicus*, a perennial grass, endemic to the Thar Desert of Rajasthan, India. The majority of sequences belonged to Gram-positive bacteria, *Actinobacteria* being the most predominant one, closely followed by *Firmicutes*. Sandhya et al. (2009) isolated EPS-producing fluorescent pseudomonads from alfisols, vertisols, inceptisols, oxisols, and aridisols of different semiarid millet-growing regions of India. The selected microbes were screened in vitro for drought tolerance in trypticase soy broth supplemented with different concentrations of polyethylene glycol (PEG-6000). Out of 81 isolates, 26 could tolerate the maximum level of stress (−0.73 MPa) and were monitored for the number of EPS produced under the maximum level of water stress. The strain *Pseudomonas putida* GAP-P45, isolated from alfisol of sunflower rhizosphere, showed the highest level of EPS production under water stress conditions. In another study, Sandhya et al. (2010) reported five strains of *Pseudomonas* which were drought tolerant simultaneously solubilizing phosphorus and also possessing other plant growth-promoting attributes including the production of gibberellins, IAA, and siderophores and helped the inoculated maize to tolerate drought by influencing biochemical and physiological characteristics of the seedlings.

Arzanesh et al. (2011) demonstrated the increase in yield of wheat by inoculation with *Azospirillum* sp. under drought stress. The study by Vardharajula et al. (2011) reported *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus thuringiensis*, *Paenibacillus favisporus*, and *Bacillus subtilis* as drought tolerant. Ali et al. (2014) screened 32 fluorescent *Pseudomonas* sp. for drought tolerance isolated from rhizospheric and non-rhizospheric soils of different crops using polyethylene glycol 6000 (PEG 6000). Nine isolates could tolerate 15% PEG which were further screened for ACC deaminase activity, and only *Pseudomonas fluorescens* (SorgP4) showed ACC deaminase activity and also IAA, siderophore, and HCN production as well as the solubilization of phosphorus.

The diversity of plant growth-promoting bacteria was investigated from wheat grown in different sites in the central zone of India by Verma et al. (2014). Epiphytic, endophytic, and rhizospheric bacteria were isolated using different growth media. Bacterial diversity was analyzed through amplified ribosomal DNA restriction analysis (ARDRA) using three restriction enzymes *Alu* I, *Hae* III, and *Msp* I which led to the grouping of 348 isolates into 24–29 clusters at >75% similarity index. 16S rRNA gene-based phylogenetic analysis revealed that 134 strains belonged to 3 phyla, namely, *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, with 38 distinct species of 17 genera. *Bacillus* and *Pseudomonas* were dominant in the rhizosphere while *Methylobacterium* was dominant in the phyllosphere. Endophytic niche-specific bacteria were identified as *Delftia* and *Micrococcus*. Among different groups of microbes, the *Archaea* are true extremophilic as well as polyextremophilic and exhibited more than two abiotic stress tolerance activities. *Archaea* are unique microbes that are present in ecological niches of high temperature and salinity. A total of 157 *Archaea* have been isolated and characterized by heat and salt tolerances by Yadav et al. (2015c). The selected isolates have been identified as 17 distinct species of 11 genera, namely, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Halolamina*, *Halosarcina*, *Halostagnicola*, *Haloterrigena*, *Natrialba*, *Natrinema*, and *Natronoarchaeum*, using 16S rRNA gene sequencing and BLASTn analysis.

The application of plant growth-promoting rhizobacteria (PGPR) to agro-ecosystems is considered to have the potential for improving plant growth in extreme environments featured by water shortage. Niu et al. (2017) isolated bacterial strain from foxtail millet (*Setaria italica* L.), a drought-tolerant crop cultivated in semiarid regions in the northeast of China. The isolates were identified as *Pseudomonas fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae* on the basis of 16S rRNA sequence analysis. Abiotic stresses such as drought represent adverse environmental conditions that significantly damage plant growth and agricultural productivity. In the study by Barnawal et al. (2017), the mechanism of plant growth-promoting rhizobacteria in stimulating tolerance against abiotic stresses has been explored. Results suggest that PGPR strains, *Arthrobacter protophormiae* (SA3) and *Dietzia natronolimnaea* (STR1), can facilitate salt stress tolerance in wheat crop, while *Bacillus subtilis* (LDR2) can provide tolerance against drought stress in wheat. In the study by Sandhya et al. (2017), 39 endophytic bacteria have been isolated from different crops with the main focus on maize roots and seeds. Endophytes were screened for drought stress tolerance, plant growth-promoting (PGP) traits, and antifungal activity. The selected isolates have been identified using biochemical and 16S rRNA gene sequencing and confirmed as *Pseudomonas aeruginosa*, *Pseudomonas monteilii*, *Pseudomonas putida*, *Acinetobacter brumalii*, *Enterobacter asburiae*, *Sinorhizobium meliloti*, *Pseudomonas thiveralensis Pseudomonas fulva*, and *Pseudomonas lini*.

Martins et al. (2018) reported the effect of some plant-associated bacteria (PAB) on increasing soybean tolerance to drought stress, the mechanisms of the drought tolerance process, and the effect of the PAB on promoting plant growth and on the biocontrol of *Sclerotinia sclerotiorum*. PAB were isolated from soybean rhizosphere and *S. sclerotiorum* sclerotia. The strains identified as UFGS1 (*Bacillus subtilis*), UFGS2 (*Bacillus thuringiensis*), and UFGRB2 and UFGRB3 (*Bacillus cereus*) were selected on their ability to grow in media with reduced water activity. The agricultural crops are often affected by the scarcity of fresh water. Seasonal drought is a major constraint on Northeast Indian agriculture. Almost 80% of the agricultural land in this region is acidic and facing severe drought during the winter period (Saikia et al. 2018). The ACC deaminase-producing bacteria have been isolated and identified as *Ochrobactrum pseudogrignonense* RJ12, *Pseudomonas* sp. RJ15, and *Bacillus subtilis* RJ46.

13.6 Mechanisms of Plant Growth Promotion

Plants play an important role in selecting and enriching the types of microbes by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates and the corresponding ability of the microbes to utilize these as sources of energy (Malyan et al. 2016a; Bhatia et al. 2013b), the microbial community develops in the interaction as epiphytic/endophytic/rhizospheric. Microbes associated with crops are of agricultural importance as they can enhance plant growth and improve plant nutrition. Plant-associated microbes (epiphytic/endophytic/rhizospheric) stimulate the growth of the plants by different mechanisms such as production of phytohormones such as auxins, cytokinins, ethylene, and gibberellins; biological nitrogen fixations; solubilization of phosphorus, potassium, and zinc; production of siderophores and various hydrolytic enzymes such as amylases, cellulases, pectinases, and proteases; and ACC deaminase activity helping plants to overcome stress conditions. They act as biocontrol agents protecting plants against various phytopathogens. Treatment of various crops with PGPR has been reported to directly enhance the growth, seedling vigor, root and shoot growth, seed weight, biomass, early flowering, and fruit yields (Yadav 2009; Yadav et al. 2015e, 2018a, d) (Bach et al. 2016; de Bruijn et al. 1997; Ellis 2017; Errakhi et al. 2016; Haas and Défago 2005; Iniguez et al. 2004; Leong 1986; Lin and Xu 2013; Pal and Gardener 2006; Pankievicz et al. 2015; Quadt-Hallmann et al. 1997; Raaijmakers et al. 2002; Rashid et al. 2012; Suman et al. 2001, 2016; Taulé et al. 2012; Van Loon et al. 1998; Verma et al. 2017b; Yadav 2017; Yadav et al. 2018b). Sustainable agriculture requires the use of different strategies to increase or maintain the current rate of food production while reducing damage to the environment and human health (Gupta et al. 2016a; Yadav et al. 2018e). The use of microbial plant growth promoters is an alternative to conventional agricultural technologies. The plant growth-promoting microbes can affect plant growth directly by providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment or indirectly by promoting plant growth which occurs when PGP microbes decrease or prevent the deleterious effects of one or more phytopathogenic organisms.

13.6.1 Phosphorus Solubilization

Phosphorus (P) is the major macronutrient which is required by the plants for their various metabolic processes including energy transfer, signal transduction, macromolecular biosynthesis, photosynthesis, and respiration but is simultaneously the major limiting mineral nutrient for the growth of the plants due to its least availability as well as least mobility. The soil constitutes about 0.5% phosphorus; for plant absorption only a small amount of phosphorus is available and others remain as insoluble salts. Soil phosphorus is classified into two broad groups, organic and inorganic. Organic phosphorus is found in plant residues, manures, and microbial tissues. Inorganic forms of soil phosphorus consist of apatite (the original source of all phosphorus), complexes of iron and aluminum phosphates, and phosphorus absorbed onto clay particles. The inorganic phosphate reacts with cations such as aluminum (Al^{3+}), iron (Fe^{3+}), and calcium (Ca^{2+}) and forms insoluble complexes. In alkaline soils, phosphate exists as tricalcium phosphate [$Ca_3 (PO_4)_2$] and in acidic soils as $FePO_4$ and $AlPO_4$. The phosphorus in insoluble form is not easily available to plants. The replacement of soil P reserves through chemical fertilization is a common but long-term practice. There are many studies which have reported that beneficial microbes are efficient in solubilizing nutrients from soil (Hinsinger 2001; Nelsen and Safir 1982; Raghothama 1999; Son et al. 2006; Yadav and Saxena 2018; Yadav et al. 2017a, 2017d; 2017f) (Table 13.1). The solubilization of inorganic insoluble phosphate salts by microbes results in the production or release of organic acid and organic acid decreases the pH (Wakelin et al. 2004; Yadav et al. 2015c, 2016a).

Nelsen and Safir (1982) reported that onion plants (*Allium cepa* L) grown in pots and infected by the mycorrhizal fungus *Glomus etunicatum* were more drought tolerant than were non-mycorrhizal ones when exposed to several periods of soil water stress separated by periods of high water supply, which was shown by greater fresh and dry weights and higher tissue phosphorus levels in the mycorrhizal plants. The tissues of stressed, non-mycorrhizal plants were deficient in P, despite the fact that only non-mycorrhizal plants were fertilized with high levels of P (26 mg P per 440 g soil). The P nutrition of plants has been implicated in the ability of plants to tolerate drought, and it was concluded that the ability of the mycorrhizal fungus to maintain adequate P nutrition in the onions during soil water stress was a major factor in the improved drought tolerance. About 95–99% of the soil phosphorus is present in complexes and reacts with various cations such as aluminum, calcium, and iron and cannot be used up by the plants (Son et al. 2006). The concentration of phosphorus in most of the soils approximately varies from 0.1 to 10 μM, whereas the concentration required for grasses is nearly about 1–5 μM, and high-P-demand crops, for instance, tomato and pea, require about 5–60 μM (Raghothama 1999), and if phosphorus is present at the sub-optimal levels, the loss of the yield can be up to 5–15% (Hinsinger 2001). Thus, in order to fulfill the phosphorus demands of the plants, phosphate fertilizers are being used in agricultural production, yet a huge proportion of these phosphate fertilizers get converted into insoluble form leading to low fertilizer efficiency.

The phosphorus-solubilizing rhizobacteria are attracting greater attention nowadays as they are economically feasible and environment-friendly as well as possess a greater agronomic utility so that the expensive P-chemical fertilizers used can easily be compensated. Thus, there are a lot of benefits to inoculate the crops with these microbes as they are safer to use, they will not produce toxic products, and moreover, they will not get accumulated in the food chain (Elias et al. 2016). Adding more, it has also been a well-known fact that phosphorus-solubilizing bacteria used

Table 13.1 Drought-tolerant P-solubilizing microbes with multifarious PGP attributes

Drought-tolerant microbes	P	IAA	Sid	ACC	References
Acinetobacter sp. M05	+	–	+	–	Zhang et al. (2017)
Azospirillum lipoferum B3	+	+	–	+	Arzanesh et al. (2011)
Bacillus altitudinis	+	+	+	–	Sunar et al. (2015)
B. aquimaris, IARI-IHD-17	+	–	–	–	Verma et al. (2014)
B. aryabhattai, IARI-IHD-34	+	+	+	–	Verma et al. (2014)
B. halodenitrificans PU62	+	+	+	+	Ramadoss et al. (2013)
B. licheniformis BGBA 1	+	+	+	–	Pahari and Mishra (2017)
B. megaterium, IARI-IIWP-9	+	+	+	–	Verma et al. (2014)
Bacillus sp.	+	–	–	+	Hussain et al. (2013)
Bacillus sp., AW1	+	–	+	+	Rana et al. (2011)
Bacillus sp. PS-12	+	+	–	+	Hussain et al. (2013)
B. subtilis, IARI-IIWP-2	+	+	+	+	Verma et al. (2014)
Brevundimonas diminuta, AW7	+		+	+	Rana et al. (2011)
Delftia sp., IARI-IIWP-31	+	+	+	–	Verma et al. (2014)
Duganella violaceusniger, IIWP-23	+	+	+	–	Verma et al. (2014)
Flavobacterium sp. PS-41	+	+	–	+	Hussain et al. (2013)
M. mesophilicum, IIWP-45	+	+	+	–	Verma et al. (2014)
M. radiotolerans, IHD-35	+	+	+	–	Verma et al. (2014)
M. extorquens, IIWP-43	+	+	+	–	Verma et al. (2014)
Paenibacillus amylolyticus, IHD-24	+	–	–	–	Verma et al. (2014)
P. dendritiformis, IIWP-4	+	+	+	–	Verma et al. (2014)
P. durus, IARI-IIWP-40	+	–	–	+	Verma et al. (2014)
Paenibacillus sp., IARI-IHD-15	+	+	+	–	Verma et al. (2014)
P. taichungensis M10	+	+	+	–	Zhang et al. (2017)
Providencia sp., AW5	+	–	+	+	Rana et al. (2011)
P. brassicacearum E85	+	–	+	+	Aarab et al. (2015)
P. fluorescens,153	+	–	–	+	Zabihi et al. (2011)
P. fluorescens, SorgP4	+	–	–	+	Ali et al. (2014)
P. fuscovaginae, IIWP-29	+	+	+	–	Verma et al. (2014)
P. lini, IARI-IIWP-33	+	–	+	+	Verma et al. (2014)
P. monteilii, IARI-IIWP-27	+	+	+	+	Verma et al. (2014)
P. plecoglossicida, S1	+	+	+	–	Rolli et al. (2015)
P. putida	+	–	–	+	Zabihi et al. (2011)
Pseudomonas sp.	+	+	+	+	Poonguzhali et al. (2008)
P. thivervalensis, IHD-3	+	+	+	+	Verma et al. (2014)
P. tolaasii IEXb	+	+	+	–	Viruel et al. (2011)
Psychrobacter fozii, IIWP-12	+	+	+	+	Verma et al. (2014)
Serratia marcescens, IIWP-32	+	–	–	–	Verma et al. (2014)
Staphylococcus aureus 22F	+	+	+	–	Toribio-Jiménez et al. (2017)
Stenotrophomonas sp., IIWP-34	+	+	–	–	Verma et al. (2014)
Streptococcus thoraltensis 5CR-F	+	+	–	–	Toribio-Jiménez et al. (2017)

P phosphorus solubilization, *IAA* indole acetic acid, *Sid* siderophores, *ACC* 1-aminocyclopropane-1-carboxylate in combination with the phosphate fertilizers possess a very beneficial effect on the uptake of the phosphorus and eventually on the growth of the plants. The major P-solubilizers belong to genera *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Aspergillus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Haloarcula*, *Halobacterium*, *Halococcus*, *Micrococcus*, *Mycobacterium*, *Penicillium*, *Pseudomonas*, *Rhizobium*, and *Serratia* (Behera et al. 2014; Gaba et al. 2017; Goldstein 2000; Mathur et al. 2011; Singh et al. 2016; Yadav 2015; Yadav et al. 2016a, 2017b). Various mechanisms used by P solubilizers to convert the insoluble forms of the phosphorus into the soluble forms consist of acidification, chelation, exchange reactions, and production of organic acids (Chung et al. 2005; Yadav et al. 2015c).

Ramachandran et al. (2007) isolated *Pseudomonas* sp. and *Azospirillum* sp. from rhizospheric soil as well as the root cuttings of *Piper nigrum* which possessed the high capability of solubilizing phosphorus in vitro. The phosphorus-solubilizing bacteria from the rhizosphere of chickpea, mustard, and wheat have been reported by Kundu et al. (2009). These P-solubilizing bacteria belonged to genera *Aeromonas*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*. In another research by Fatima et al. (2009), the potential P-solubilizing bacteria were reported to be associated with wheat rhizospheric soil which were identified as *Azospirillum* (WPR-42, WP-3), *Pseudomonas* (WPR-61), and *Azotobacter* (WPR-51). Along with P-solubilizing bacterial isolates, fungi and their association with crops have been reported from the rhizospheric region of *Sorghum bicolor* inoculated with arbuscular mycorrhizal fungi; the P-solubilizing genera identified on the basis of the morphology and biochemical tests consisted of *Acinetobacter* sp., *Bacillus* sp., *Micrococcus* sp., *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*, respectively (Chandrasekeran and Mahalingam 2014). The P-solubilizing rhizobacteria are commonly found in association with various crops such as *Enterobacter agglomerans* with tomato (Kim et al. 1998), *Pseudomonas chlororaphis* and *Pseudomonas putida* with soybean (Cattelan et al. 1999), *Bacillus licheniformis* RC08 and *Bacillus megaterium* RC07 with wheat and spinach (Çakmakçı et al. 2007), *Bacillus megaterium* (M-3) with chickpea (Elkoca et al. 2007), and *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 with maize (Hameeda et al. 2008).

Peix et al. (2001) reported *Mesorhizobium mediterraneum* (PECA21) mobilized tricalcium phosphate when added in soil proficiently in barley and chickpea. In the study of Chen et al. (2006), *Arthrobacter aureofaciens*, *Delftia* sp., *Phyllobacterium myrsinacearum*, and *Rhodococcus erythropolis* have been reported for the first time to possess the capability to solubilize phosphorus. Liu et al. (2014) reported *Acinetobacter pittii* ASL12, *Escherichia coli* ASG34, and *Enterobacter cloacae* ADH302 as efficient P-solubilizers isolated from betel nut (*Areca catechu*) and their effects on plant growth and phosphorus mobilization in tropical soils. Rai et al. (2017) isolated PSB including *Pseudomonas putida*, *Pseudomonas* sp., and *Pseudomonas plecoglossicida* from the rhizospheric region of *Aloe vera*. Sharma et al. (2017) reported *Pseudomonas aeruginosa* to be a potent phosphorus-solubilizing strain from the rhizospheric region of apple in Trans-Himalayan region of Himachal Pradesh. Microorganisms present in soil play an important role in maintaining the ecological balance by active participation in nitrogen, phosphorus, and carbon cycles in nature. Phosphorus plays an important role in plant nutrition and has an important biochemical role in respiration, cell division, photosynthesis, cell enlargement, and several other processes in the living plant. It is one of the most important vital macronutrients requisite for the growth and development of plants. Yadav and Pandey (2018) investigated the occurrence of PSB from tomato rhizosphere soil samples collected across Jaipur, Rajasthan. The PSB isolates have been identified using 16S rRNA gene analysis and confirmed as *Bacillus* sp., *Streptomyces* sp., and *Cronobacter* sp. The study concludes that using potential phosphate-solubilizing bacteria as biofertilizers will not only enhance soil fertility and crop productivity but will also maintain and protect soil health leading to sustainable agriculture.

The P-solubilizing microbes play an important role in plant growth and soil health for sustainable agriculture. The drought-tolerant P-solubilizing microbes with multifarious PGP attributes have been reported to have the capability to solubilize phosphorus along with other plant growth-promoting attributes such as the production of phytohormone and Fe-chelating compounds and ACC deaminase activity. Figure 13.6 represents the drought-tolerant P-solubilizing microbes with multifunctional PGP attributes which may be used as biofertilizers to replace chemical fertilizers; e.g., the six strains, namely, *Bacillus halodenitrificans* PU62; *Bacillus subtilis*, IARI-IIWP-2; *Pseudomonas monteilii*, IARI-IIWP-27; *Pseudomonas* sp.; *Pseudomonas thivervalensis*, IHD-3; and *Psychrobacter fozii*, IIWP-12, exhibited multiple PGP attributes of P solubilization, IAA production, siderophore production, and ACC deaminase activity (Poonguzhali et al. 2008; Ramadoss et al. 2013; Verma et al. 2014) (Table 13.1; Fig. 13.6).

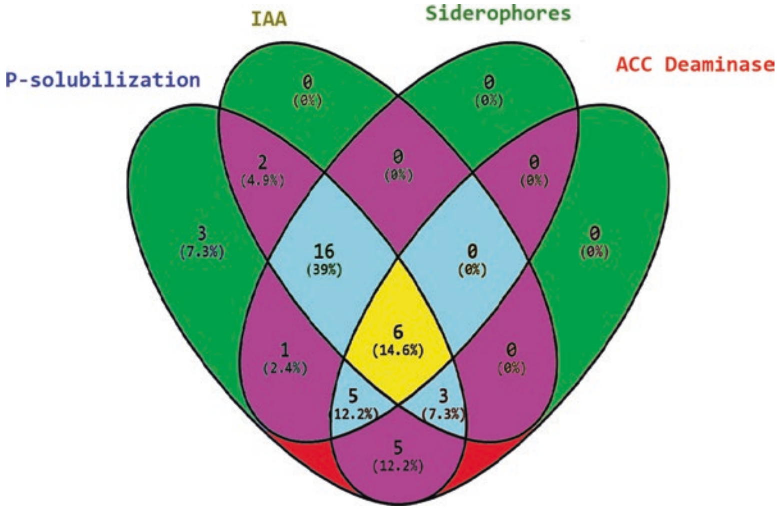

Fig. 13.6 Venn diagram showing the drought-tolerant microbes with multifarious PGP attributes

13.6.2 Production of Phytohormones

Another important role of plant growth-promoting rhizobacteria is the synthesis of various phytohormones (plant growth regulators). The well-known phytohormones include auxins (most common being indole acetic acid), cytokinins, and gibberellins. These plant growth hormones play major roles in various developmental processes of the plants ranging from cell division, cell cycle, cell elongation, and differentiation to root initiation, flowering, ripening of the fruits, and senescence. There are diverse groups of microbial species such as *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Delftia*, *Duganella*, *Exiguobacterium*, *Kocuria*, *Lysinibacillus*, *Methylobacterium*, *Micrococcus*, *Micrococcus*, *Paenibacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Psychrobacter*, *Serratia*, and *Stenotrophomonas* (Saxena et al. 2015b; Yadav et al. 2017e; Kour et al. 2017b; Rana et al. 2016; Srivastava et al. 2013; Verma et al. 2015a, c, 2017a).

13.7 Indole Acetic Acid

Indole acetic acid plays various important roles in plants: it induces cell elongation and cell division, and it also acts as the signaling molecule required for the development of plant organs as well as the coordination of growth. There are diverse pathways which are used by PGP microbes for the production of indole-3-acetic acid including L-tryptophan-dependent and L-tryptophan-independent pathways. Majority of plant growth-promoting microbes use the L-tryptophan-dependent pathway including *Azospirillum*, *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bradyrhizobium*, *Erwinia herbicola*, *Pantoea agglomerans*, *Pseudomonas syringae*, and *Rhizobium* (Burdman et al. 2000; Dobbelaere et al. 2003; Goswami et al. 2016; Saxena et al. 2015a), whereas very few use the L-tryptophan-independent pathway including *Azospirillum brasilense* (Goswami et al. 2016). Bottini et al. (2004) isolated P-solubilizing and IAA- and GA-producing *Enterobacter*, *Xanthomonas*, and *Pseudomonas* from the rhizospheric region of sorghum. Ahmad et al. (2005) reported *Azotobacter* sp. to be the potent producer of IAA producing about 7.3–32.8 mg/ml. The strains of *Rhizobium*, *Microbacterium*, *Sphingomonas*, and *Mycobacterium* isolated from the roots of the epiphytic orchid *Dendrobium moschatum* are among the most active IAA producers (Tsavkelova et al. 2007). Swain et al. (2007) used the suspension of the IAA-producing strain of *Bacillus subtilis* on the surface of *Dioscorea rotundata* and reported an increment in the root/stem ratio and also the number of the sprouts in comparison to the uninoculated plants.

In the study by Marulanda et al. (2009), it was reported that rhizosphere microorganisms can increase drought tolerance of plants growing under water-limited conditions. Three indigenous bacterial strains isolated from drought soil and identified as *Pseudomonas putida*, *Pseudomonas* sp., and *Bacillus megaterium* were able to stimulate plant growth under dry conditions. When the bacteria were grown in axenic culture at increasing osmotic stress caused by polyethylene glycol (PEG) levels (from 0 to 60%), they showed osmotic tolerance and only *Pseudomonas* sp. decreased indole acetic acid production concomitantly with an increase of osmotic stress (PEG) in the medium. *P. putida* and *B. megaterium* exhibited the highest osmotic tolerance, and both strains also showed increased proline content, involved in osmotic cellular adaptation, as much as increased osmotic stress caused by NaCl supply. These bacteria seem to have developed mechanisms to cope with drought stress. The increase in IAA production by *P. putida* and *B. megaterium* at a PEG concentration of 60% is an indication of bacterial resistance to drought. Their inoculation increased shoot and root biomass and water content under drought conditions. Bacterial IAA production under stressed conditions may explain their effectiveness in promoting plant growth and shoot water content increasing plant drought tolerance. *B. megaterium* was the most efficient bacterium under drought (in successive harvests) either applied alone or associated with the autochthonous arbuscular mycorrhizal fungi *Glomus coronatum*, *Glomus constrictum*, or *Glomus claroideum*. *Bacillus megaterium* colonized the rhizosphere and endorhizosphere zone. We can therefore say, that microbial activities of adapted strains represent a positive effect on plant development under drought conditions; IAA-producing *Enterobacter aerogenes* and *Enterobacter cloacae* promoted growth in cowpea (Deepa et al. 2010). Joseph et al. (2012) isolated and characterized rhizobacteria from chickpea, and all the identified isolates including *Bacillus*, *Pseudomonas*, and *Azotobacter* produced IAA, and about 85.7% of *Rhizobium* were capable of producing IAA. In the study of Goswami et al. (2014), the IAA producer *Kocuria turfanensis* 2M4 showed the capability to promote growth in *Arachis hypogaea*.

The diversity of plant growth-promoting bacteria was investigated from wheat grown in different sites in the semiarid region in the central zone of India (Verma et al. 2014). Bacterial diversity was analyzed through amplified ribosomal DNA restriction analysis (ARDRA) using three restriction enzymes *Alu* I, *Hae* III, and *Msp* I which led to the grouping of 348 isolates into 24–29 clusters at >75% similarity index. 16S rRNA gene-based phylogenetic analysis revealed that 134 strains belonged to three phyla, namely, *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, with 38 distinct species of 17 genera. *Bacillus* and *Pseudomonas* were dominant in the rhizosphere while *Methylobacterium* was dominant in the phyllosphere. Endophytic niche-specific bacteria were identified as *Delftia* and *Micrococcus*. A sampling of different sites showed variation in diversity indices. In vitro plant growth-promoting activities of bacteria exposed more than three beneficial traits which may act independently or concurrently. Phosphate solubilization and siderophore production were the predominant traits exhibited by these microbes. The many species of genera *Bacillus*, *Exiguobacterium*, *Micrococcus*, *Pseudomonas*, and *Psychrobacter* showed antagonistic properties against fungal pathogens *Fusarium graminearum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. These promising isolates showing a range of useful plant growth-promoting attributes insist to be explored for agricultural applications. In another study by Verma et al. (2016a), the culturable bacilli has been investigated in six wheat-cultivating agro-ecological zones of India, viz., northern hills, north-western plains, north-eastern plains, central, peninsular, and southern hills zone. These agro-ecological regions are based on the climatic conditions such as pH, salinity, drought, and temperatures. The selected bacilli have been identified using 16S rRNA sequencing which included eight genera, namely, *Bacillus*, *Exiguobacterium*, *Lysinibacillus*, *Paenibacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina*, and *Staphylococcus*. The study by Verma et al. (2016a) was the first report for the presence of *Bacillus endophyticus*, *Paenibacillus xylanexedens*, *Planococcus citreus*, *Planomicrobium okeanokoites*, *Sporosarcina* sp., and *Staphylococcus succinus* in the wheat rhizosphere that exhibit multifunctional PGP attributes. These niche-specific and multifarious PGP bacilli could serve as inoculants for crops growing in respective climatic conditions.

The agricultural crops are often affected by the scarcity of fresh water. Seasonal drought is a major constraint on Northeast Indian agriculture. Almost 80% of the agricultural land in this region is acidic and facing severe drought during the winter period (Saikia et al. 2018). The ACC deaminase-producing PGPB *Ochrobactrum pseudogrignonense* RJ12, *Pseudomonas* sp. RJ15, and *Bacillus subtilis* RJ46 offer drought stress tolerance by regulating plant ethylene levels. All the strains could produce IAA (68–85 μg ml^{-1}). The consortium treatment decreased the ACC accumulation and downregulated ACC-oxidase gene expression. This consortium could be an effective bio-formulator for crop health improvement in drought-affected acidic agricultural fields.

13.8 Gibberellins and Cytokinins

Gibberellins (GAs) are a broad group of phytohormones playing an important role in germination of seeds, elongation of the stem, flowering, and fruit setting (Hedden and Phillips 2000). They consist of nearly about 136 dissimilar structured molecules including from 128 species of plants and 7 species of fungi and only 4 including GA1, GA3, GA4, and GA20 from 7 species of bacteria (MacMillan 2001). *Bacillus* sp. rarely produces gibberellin; only two strains have been documented to possess the capability of producing gibberellins, and these are *Bacillus pumilus* and *Bacillus licheniformis* (Gutiérrez-Mañero et al. 2001). Boiero et al. (2007) well demonstrated the promotion of growth of the shoot in dwarf mutants of maize and rice by excretion of gibberellin-like substances by *Azospirillum* sp. Production of the gibberellins has also been confirmed in *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* (Bastián et al. 1998), and *Bacillus* sp. (Gutiérrez-Mañero et al. 2001) by using various physicochemical methods, including GC-MS (Jha and Saraf 2015).

Verma et al. (2014) reported drought-tolerant microbes in wheat grown in the semiarid region in the central agro-ecological zone of India. The plant microbiomes (epiphytic, endophytic, and rhizospheric) have been isolated using different growth media. 16S rRNA gene-based phylogenetic analysis revealed that 134 strains belonged to 3 phyla, namely, *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, with 38 distinct species of 17 genera. *Bacillus* and *Pseudomonas* were dominant in the rhizosphere while *Methylobacterium* was dominant in the phyllosphere. Endophytic niche-specific bacteria were identified as *Delftia* and *Micrococcus*. Phosphate solubilization and siderophore production were the predominant traits exhibited by these microbes. Among 38 distinct species, 12 bacterial strains exhibited the plant growth-promoting attributes of gibberellic acid production under the water deficit conditions, e.g., *Bacillus aquimaris*, *Bacillus subtilis*, *Bacillus tequilensis*, *Duganella violaceusniger*, *Methylobacterium radiotolerans*, *Micrococcus luteus*, *Micrococcus* sp., *Paenibacillus dendritiformis*, *Pseudomonas stutzeri*, *Pseudomonas thivervalensis Psychrobacter fozii*, and *Serratia marcescens.* These promising isolates showing a range of useful plant growth-promoting attributes insist to be explored for agricultural applications for rainfed environmental conditions. In another research by Verma et al. (2016a), the eight bacilli associated with wheat, *Bacillus amyloliquefaciens* BNE12, *Bacillus atrophaeus* BSH3, *Bacillus endophyticus* BNW9, *Bacillus fusiformis* BNW5, *Bacillus mojavensis* BPZ6, *Bacillus rigui* BSH4, *Bacillus sphaericus* BNW8, and *Bacillus subtilis* BPZ1, have been reported as GA producers.

Cytokinins are another important group of phytohormones produced by microorganisms (Persello-Cartieaux et al. 2003). They play major roles in the promotion of cell division, cell growth, and cell differentiation simultaneously affecting the apical dominance, axillary bud growth, and leaf senescence. Various genera have been reported which possess the capability to produce cytokinins including *Azospirillum*, *Bacillus*, *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Xanthomonas* (Maheshwari et al. 2015; Persello-Cartieaux et al. 2001). In the study by Sandhya et al. (2017), 39 endophytic bacteria were isolated from different crops with the main focus on maize roots and seeds. Endophytes were screened for drought stress tolerance, plant growth-promoting (PGP) traits, and antifungal activity. Out of 39 isolates, 32 showed drought tolerance up to –1.02 matric potential (MPa) and exhibited most of the plant growth-promoting traits. Among identified bacteria, nine species including *Acinetobacter brumalii* MRC12, *Enterobacter asburiae* MRC31, *Pseudomonas aeruginosa* FTR, *Pseudomonas aeruginosa* NFTR, *Pseudomonas lini* MRR2, *Pseudomonas monteilii* FMZR2, *Pseudomonas monteilii* MZ30V92, *Pseudomonas putida* FMZR9, and *Sinorhizobium meliloti* MRC33 produced gibberellic acid, and seven species including *Enterobacter asburiae* MRC31, *Pseudomonas aeruginosa* FTR, *Pseudomonas aeruginosa* NFTR, *Pseudomonas lini* MRR2, *Pseudomonas monteilii* FMZR2, *Pseudomonas putida* FMZR9, and *Sinorhizobium meliloti* MRC33 produced cytokines under the drought stress conditions. In the study by Lubna et al. (2018), an endophytic fungus, *Aspergillus niger* CSR3, was isolated from *Cannabis sativa*. The culture filtrate (CF) was initially screened for growth-promoting activities such as the presence of siderophores, phosphate solubilization, and the production of indole acetic acid and gibberellins. The growth promotion action was due to the presence of various types of gibberellins (GAs) and IAA in the endophyte CF. *Preussia* sp. has been least known to improve plant growth and produce phytohormones.

Al-Hosni et al. (2018) investigated the production of nitric oxide (NO), indole-3-acetic acid (IAA), and gibberellins (GA_4, GA_7, GA_{15}, and GA_{53}) by a novel endophytic-fungal strain *Preussia* sp. BSL-10. Production of these phytohormones was validated by RT-PCR analysis, which indicated the expression of genes encoding *tryptophan synthase* (*TRP*), *indole-3-acetamide hydrolase (IAAH)*, *tryptophan-2-monooxygenase (IAAM)*, *aldehyde dehydrogenase (ALD)*, *GA_4 desaturase (DES)*, *geranylgeranyl-diphosphate synthase (GGS2)*, *ent-desaturase oxidase (P450-4)*, *GA_{14} synthase (P450-1) and nitrite reductase (NIRK/NIRS)*, *cytochrome P450 (P450nor)*, *nitrate reductase (NR)*, *NOS-like (NOL)*, and *nitric oxide reductase (QNOR/CNOR).* In plant growth-promoting effects, the inoculation of *Preussia* sp. BSL-10 significantly increased the growth of dwarf mutant *Waito-C* and wild-type rice cultivars.

13.8.1 Production of Fe-Chelating Compounds

Iron is one of the most vital elements important for the growth of all living organisms. It acts as the cofactor for different enzymes; it is involved in the process of photosynthesis, respiration, and nitrogen fixation; and its deficiency leads to various metabolic alterations (Solano et al. 2008). Iron is present in abundance in the soil but is not available for the plants as well as the microbes present in the soil as the oxidized form of iron which is Fe^{3+} reacts forming oxides and hydroxides which is not accessible to the plants as well as the microbes. Under such iron-limiting conditions, PGPR has the capacity to produce low-molecular-weight iron-chelating compounds called siderophores for the acquisition of ferric ions (Whipps 2001). These siderophores can easily be utilized by rhizospheric bacteria and plants can also directly absorb these complexes. Siderophores are categorized into catecholates (phenolates), hydroxamates, and carboxylates. *Acinetobacter calcoaceticus* isolated from the rhizosphere of wheat produced catechol type of siderophores (Chaudhari Bhushan et al. 2009). Amplified ribosomal DNA restriction analysis (ARDRA) revealed *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Rhodococcus* sp. to be siderophore producers (Tian et al. 2009). Silva-Stenico et al. (2005) reported *Methylobacterium extorquens* from *Citrus sinensis* to be hydroxamate type of siderophore producer. Vaidehi and Sekar (2012) reported *Methylobacterium phyllosphaerae* MB-5 and CBMB-27 to produce hydroxamate type of siderophores during the limitations of iron. *Pseudomonas fluorescens* is the most common siderophore producer releasing pyochelin and pyoverdine (Solano et al. 2008). Enterobactin produced by *Escherichia coli*, bacillibactin by *Bacillus subtilis* and *Bacillus anthracis*, and vibriobactin by *Vibrio cholerae* are some of the catecholate siderophores (Saharan and Nehra 2011). In another research, *Pseudomonas chlororaphis*, a siderophore producer, enhanced seed germination as well as the root-shoot biomass. It has been well demonstrated that cold-tolerant mutant of *Pseudomonas fluorescens* possessing 17-fold enhancement in the production of the siderophores can improve the plant growth-promoting effect on mung bean (Katiyar and Goel 2004).

The PGP microbes stimulate plant growth in multiple ways, viz., production of siderophores and suppression of pathogenic organisms. PGP microbes have been reported to not only improve plant growth but also to suppress the plant pathogens, of which *Pseudomonas* and *Bacillus* were well characterized. Pink-pigmented facultative methylotrophs synthesize a variety of metabolites useful for the plants including phytohormones that promote plant growth and yield. PGP microbes are used as biocontrol agents to reduce the development of plant diseases caused by plant pathogenic fungi, bacteria, viruses, and nematodes (Verma et al. 2014). The diversity of plant growth-promoting bacteria was investigated from wheat grown in different sites in the central zone of India. 16S rRNA gene-based phylogenetic analysis revealed that 134 strains belonged to three phyla, namely, *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, with 38 distinct species of 17 genera. Among 38 distinct species, 23 of them (*Arthrobacter humicola*, *Bacillus aryabhattai*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus tequilensis*, *Bacillus thuringiensis*, *Corynebacterium callunae*, *Delftia* sp., *Duganella violaceusniger*, *Methylobacterium extorquens*, *Methylobacterium mesophilicum*, *Methylobacterium radiotolerans*, *Paenibacillus dendritiformis*, *Paenibacillus* sp., *Pantoea ananatis*, *Pseudomonas fuscovaginae*, *Pseudomonas lini*, *Pseudomonas monteilii*, *Pseudomonas stutzeri*, *Pseudomonas thivervalensis*, *Psychrobacter fozii*, and *Stenotrophomonas maltophilia*) were found to produce Fe-chelating compounds under water-deficient conditions. *Bacillus* and *Pseudomonas* were dominant in the rhizosphere while *Methylobacterium* was dominant in the phyllosphere. These promising isolates showing a range of useful plant growth-promoting attributes insist to be explored for agricultural applications.

Sandhya et al. (2017) reported 39 endophytic bacteria from different crops. Endophytes were screened for drought stress tolerance, plant growth-promoting (PGP) traits, and antifungal activity. Out of 39 isolates, 32 could show drought tolerance up to –1.02 matric potential (MPa) and exhibited most of the plant growth-promoting traits. Among 39 bacteria, isolates such as *Pseudomonas putida* strain FMZR9, *Pseudomonas aeruginosa* strain FTR, *Pseudomonas aeruginosa* strain NFTR, *Enterobacter asburiae* strain MRC12, *Pseudomonas thivervalensis* strain MRC33, and strains FGR3, FMZR7, NFRGR1, and NFMZR2 were siderophore positive. These drought-tolerant PGPMs help in plant growth and act as biocontrol agents for crops growing under drought stress conditions. The application of plant-growth-promoting bacteria is an alternative strategy for improving plant fitness under stressful conditions (Saikia et al. 2018). The ACC deaminase-producing PGPB offer drought stress tolerance by regulating plant ethylene levels. All three microbes *Ochrobactrum pseudogrignonense* RJ12, *Pseudomonas* sp. RJ15, and *Bacillus subtilis* RJ46 exhibited the ability to produce siderophore (6.2–11.32 μmol benzoic acid ml^{-1}) under conditions of drought stress. The consortium treatment significantly increased seed germination percentage, root length, shoot length, and dry weight of treated plants. Elevated production of reactive oxygen species-scavenging enzymes and cellular osmolytes, higher leaf chlorophyll content, increase in relative water content, and root recovery intension were observed after consortium treatment in comparison with the uninoculated plants under drought conditions. The consortium treatment decreased ACC accumulation and downregulated ACC-oxidase gene expression. This consortium treatment could be an effective bio-formulator for crop health improvement in drought-affected acidic agricultural fields.

13.8.2 Biological Nitrogen Fixation (BNF)

Nitrogen is a vital nutrient for the growth as well as development of the plants, but most of the soils have a deficiency of nitrogen. Therefore, application of nitrogenous fertilizers is very important to fulfill the demands of the plants so that the maximum yield could be achieved (Fagodiya et al. 2017a; Pathak et al. 2016; Gupta et al. 2015). But, the use of chemical fertilizers leads to the depletion of non-renewable sources of energy, various environmental issues, and human hazards; further the production cost is very high (Khan et al. 2019). Urea is one of the cheapest sources of nitrogen for the plants, but less than 50% of this applied urea can be used up by the plants due to NH_3 volatilization and denitrification which also pollute the environment as these processes emit various greenhouse gases and some losses occur due to leaching (Bhatia et al. 2013b; Gupta et al. 2016b). Leaching causes the toxicity of groundwater (Kumar et al. 2016b). Thus, biological nitrogen fixation is a potent and eco-friendly alternative to the use of chemical fertilizers. There are a number of rhizobacteria and endophytes which can fix the atmospheric nitrogen and make it available for the plants. In the past few years, the use of plant growth-promoting microbes has increased (Figueiredo et al. 2008), and using PGPR as bio-inoculants will surely reduce the use of chemical fertilizers.

The microbes which can fix atmospheric nitrogen are basically of three groups including symbiotic nitrogen fixers which are host specific and free-living nitrogen fixers which are not host specific (Oberson et al. 2013), and the third group includes associative symbiotic nitrogen fixers. Symbiotic nitrogen fixers include strains of *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mesorhizobium*, and *Frankia*, and free-living and associative symbiotic nitrogen fixers include the strains of *Azospirillum*, *Azotobacter*, *Acetobacter*, *Azoarcus*, *Achromobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Citrobacter*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Mycobacterium*, *Paenibacillus*, *Pseudomonas*, *Rhodobacter*, and *Serratia* (Verma et al. 2013, 2015b; Yadav et al. 2013, 2017c).

Other strains demonstrated to be nitrogen fixers include *Paenibacillus odorifer*, *Paenibacillus graminis*, *Paenibacillus peoriae*, and *Paenibacillus brasiliensis* (Berge et al. 2002; von der Weid et al. 2002). Among these strains, the species of *Azotobacter* and *Azospirillum* are the majority used for agricultural trials. *Azospirillum* has been used for various crops for the growth enhancement of wheat (Sala et al. 2007), rice (Pedraza et al. 2009), and maize (Montañez et al. 2009) through biological nitrogen fixation. *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14, and *Bacillus* OSU-142 are also potent nitrogen fixers and have also been used as biofertilizers for increasing the yield as well as the quality of spinach, sugar beet, and wheat (Çakmakçı et al. 2007). The N-fixing *Bacillus* strains and *Azospirillum brasilense* sp246 promote the growth of spring wheat and barley when cultivated in organic and low-N input agriculture (Canbolat et al. 2006). *Rhizobium leguminosarum* E11 increased root dry weight, root length, and growth of cotton (Hafeez et al. 2004). Inoculation with *Bradyrhizobium* sp. (S62 and S63) showed positive effects on growth, nodule number, and yield of soybean (Egamberdiyeva et al. 2004). The inoculation of chickpea with *Rhizobium* and

N-fixing *Bacillus subtilis* appreciably led to an increase in the nitrogen percentage (Elkoca et al. 2007). The drought-tolerant ACC deaminase bacteria *Ochrobactrum pseudogrignonense* RJ12, *Pseudomonas* sp. RJ15, and *Bacillus subtilis* RJ46 exhibited the ability to fix atmospheric nitrogen (Saikia et al. 2018).

13.9 Mechanisms of Microbes-Mediated Drought Tolerance

The elucidation of the various mechanisms by which plants respond to drought stress is very important so that stress-tolerant plants could be grown. This process is very complex as it involves various factors which are affecting and at the same time the factors which are affected. During drought, the availability of the nutrients is also affected, and this can be overcome by the use of the plant growth-promoting microbes. These PGP microbes have been in use since the past few decades, and they possess a great potential to improve the yield of the crops during the stress conditions through their complex interactions with the plants, and a large number of them have been isolated and characterized (Araujo 2008; da Silva et al. 2006; Saikia et al. 2018). Research is already going on to find out the mechanisms by which plant microbiomes help the plants to cope with and grow during conditions of drought. The most important mechanism suggested so far by different researchers is by maintaining the homeostasis in and around the plant root through the presence of the enzyme 1-aminocyclopropane-1-carboxylate deaminase in microbes which protects the plants from the damages caused due to the drought and thus is considered to be the most important signaling molecule helping the plants to combat the drought conditions. There are a number of other mechanisms that also exist in rhizobacteria helping the plants to tolerate drought including the production of various antibiotics, enzymes, nitric oxides, organic acids, osmolytes, phytohormones, and siderophores and solubilization of phosphorus. Thus, PGPR are highly precious for sustainable agriculture for survivability and efficacy under field conditions; proper techniques for applications need further research and development (Duan et al. 2009; Kour et al. 2017a, b; Saikia et al. 2018; Timmusk et al. 2013) (Table 13.2).

13.9.1 ACC (1-Aminocyclopropane-1-Carboxylate) Deaminase Activity

Ethylene is one of the most important plant hormones which is usually found in gaseous form and is produced endogenously. It is efficient at low concentrations controlling various activities such as growth, cellular metabolism, and even senescence. However, when there are stress conditions such as drought, flooding, chilling temperature, and pathogenic attack, the production of ethylene is enhanced. Ethylene when present at high concentration proves to be inhibitory for the growth of the plants. But, PGP microbes possess an enzyme ACC deaminase which converts ACC, the immediate precursor of ethylene, to α-ketoglutarate and ammonium, thus lowering the concentration of the ethylene during the stress conditions and

Table 13.2 Microbe-mediated drought tolerance in plants

Microbial inoculate	Plant	Mechanisms	References
Achromobacter piechaudii	Tomato	ACC deaminase	Mayak et al. (2004)
A. brasilense	Bean	Antioxidant	German et al. (2000)
A. lipoferum	Maize	Gibberellins/ABA	Cohen et al. (2009)
Azospirillum sp.	Wheat	IAA	Arzanesh et al. (2011)
Bacillus cereus AR156	Tomato	Photosynthetic	Wang et al. (2012b)
B. licheniformis K11	Pepper	ACC deaminase	Lim and Kim (2013)
Bacillus sp.	Maize	EPS production	Vardharajula et al. (2011)
Bacillus sp.	Lettuce	Cytokinin	Arkhipova et al. (2007)
B. subtilis	*Platycladus*	Cytokinin	Liu et al. (2013a)
B. subtilis LDR2	Wheat	ABA/ACC content	Barnawal et al. (2013)
B. thuringiensis	Wheat	Volatile compounds	Timmusk et al. (2014)
B. thuringiensis, AZP2	Pine	ACC deaminase	Timmusk et al. (2014)
Bradyrhizobium japonicum	Chickpea	Phytohormones	Bano et al. (2010)
Paenibacillus polymyxa	Rice	ACC deaminase	Timmusk et al. (2014)
Pantoea agglomerans	Wheat	EPS production	Amellal et al. (1998)
P. brassicacearum	*Arabidopsis*	Delayed transition	Bresson et al. (2013)
P. brassicacearum	*Arabidopsis*	ABA content	Bresson et al. (2013)
P. aeruginosa	Mung bean	Antioxidant	Sarma and Saikia (2014)
P. fluorescens YX2	Maize	Choline	Gou et al. (2015)
P. fluorescens, ACC-5	Pea	ACC deaminase	Zahir et al. (2008)
P. putida NBRIRA	Chickpea	miRNAs genes	Jatan et al. (2018)
P. putida P45	Sunflower	EPS production	Sandhya et al. (2009)
Pseudomonas sp.	Pea	ACC deaminase	Arshad et al. (2008)
Pseudomonas spp.	Pea	ACC deaminase	Arshad et al. (2008)
Rhizobium etli	Beans	Trehalose	Reina-Bueno et al. (2012)
Rhizobium sp.	Sunflower	EPS production	Alami et al. (2000)
Rhizobium etli	Beans	Trehalose	Suárez et al. (2008)
Sinorhizobium meliloti	Alfalfa	Cytokinin	Xu et al. (2012)
Variovorax paradoxus	Pea	ACC deaminase	Belimov et al. (2009)
MC^{1a}	Cucumber	ACC deaminase	Wang et al. (2012a)
MC^{2}	Sunflower	Enzyme activity	Singh et al. (2015)
MC^{3}	Rice	Enzyme activity	Khalilzadeh et al. (2016)

[a] *Microbial consortium* [MC^{1} (*Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21); MC^{2} (*Azotobacter chroococcum* and *Bacillus polymyxa*); MC^{3} (*Azotobacter* and *Pseudomonas*)]

stimulating the growth of the plants. ACC deaminase activity has been reported in *Achromobacter xylosoxidans*, *Agrobacterium* genomovars, *Alcaligenes*, *Azospirillum lipoferum*, *Bacillus licheniformis*, *Brachybacterium saurashtrense*, *Brevibacterium casei*, *Brevibacterium iodinum*, *Burkholderia phytofirmans*, *Cronobacter sakazakii*, *Enterobacter cloacae*, *Haererehalobacter* sp., *Halomonas* sp., *Klebsiella* sp., *Mesorhizobium* sp., *Methylobacterium fujisawaense*, *Micrococcus* sp., *Pseudomonas putida*, *Pyrococcus horikoshii*, *Ralstonia solanacearum*, *Rhizobium leguminosarum*, *Rhodococcus*, *Sinorhizobium meliloti*, *Variovorax paradoxus*, and *Zhihengliuela alba* (Jha et al. 2012; Fujino et al. 2004; Gontia et al. 2011; Madhaiyan et al. 2006; Gontia-Mishra et al. 2017). Further, the gene encoding ACC deaminase i.e., *acd*S has been demonstrated in many bacterial genera including *Agrobacterium*, *Achromobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, and *Rhizobium* (Blaha et al. 2006; Govindasamy et al. 2015); further in *Bradyrhizobium japonicum* USDA110 and *Rhizobium leguminosarum* bv. *viciae* 128C53 K, regulated by leucine-responsive regulatory protein (LRP)-like protein and a promoter r70 (Gontia-Mishra et al. 2014; Kaneko et al. 2002; Ma et al. 2003), *Enterobacter cloacae* UW4 and *Pseudomonas putida* UW4 in which the gene is under the regulation of leucine-responsive regulatory protein (LRP) (Cheng et al. 2008; Li and Glick 2001).

Inoculating plants with ACC deaminase-containing PGP microbes leads to a variety of physiological changes in the plants (Glick et al. 2007; Saleem et al. 2007) including longer roots in host plants, thereby helping in the uptake of water from deeper soil layers under water deficit conditions (Zahir et al. 2008). *Achromobacter piechaudii* ARV8 utilizing ACC decreased drought-induced ethylene evolution and improved growth of pepper and tomato seedlings (Mayak et al. 2004). In the study of Arshad et al. (2008), pea was inoculated with *Pseudomonas fluorescens* and *Pseudomonas putida* showing ACC deaminase activity so as to find their potential to mitigate the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.), and it was reported that inoculating with *Pseudomonas* sp. decreased the imposed effects of the drought stress on the growth and yield of pea. Joe et al. (2014) reported *Azospirillum brasilense* CW903 and *Methylobacterium oryzae* CBMB20 showing ACC deaminase activity which reduced ethylene levels in plants. Microbial strains possessing ACC deaminase activity have been known to be 40% more proficient in forming nitrogen-fixing nodules as compared to strains lacking this activity (Ma et al. 2004; Ma et al. 2003).

13.9.2 Production of Exopolysaccharide and Phytohormones

Production of exopolysaccharide (EPS) by PGP microbes plays a vital role in influencing the soil structure. EPS-producing microbes stimulate the water-binding capacity of soil and help in regulation of the supply of nutrients and water to roots. EPS help in irreversible attachment colonization of the microbes to the roots due to a network of fibrillar material that permanently connects the microbes to the root surfaces. Bashan et al. (2004) demonstrated the role of polysaccharide-producing *Azospirillum* in the aggregation of the soil. The production of the extracellular biofilms by PGP microbes for binding and making the water molecules in the rhizospheric region available is another strategy for alleviation of the water stress conditions (Timmusk and Nevo 2011). EPS production has been reported in *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Streptococcus mutans* (Vimala and Lalithakumari 2003). EPS-producing *Pseudomonas* sp. and *Acinetobacter* sp. conferred the drought tolerance in pepper plant by forming hydrophilic biofilms around the roots (Rolli et al. 2015).

In a study of Arkhipova et al. (2007), *Lactuca sativa* L. showed increased amount of ABA which was related to observed drought tolerance when treated with *Bacillus* sp. Cohen et al. (2008) reported that *Azospirillum brasilense* Sp245-treated *Arabidopsis* plants showed enhanced ABA levels which were linked to the observed drought tolerance. In the study of Marulanda et al. (2009), *Trifolium repens* plants were treated with *Pseudomonas putida* and *Bacillus megaterium* under drought stress; the study concluded the increase in root-shoot biomass and water content was due to IAA production. *Azospirillum* sp. and *Bacillus thuringiensis* are capable of synthesizing IAA and evidently causing the enhancement of the formation of lateral roots and root hairs, thereby helping plants to grow under water deficit conditions (Armada et al. 2014). Some strains of *Azospirillum lipoferum* producing abscisic acid (ABA) and gibberellins can prevent the loss of water in their maize plant hosts by regulating the closure of stomatal and various stress signal transduction pathways (Cohen et al. 2009). Cytokinin-producing *Bacillus subtilis* enhanced the shoot growth and also conferred drought stress tolerance in *Platycladus orientalis* (Liu et al. 2013b), and similar observations were made by Arkhipova et al. (2007) when lettuce was inoculated with cytokinin-producing *Bacillus subtilis*. In the study of Curá et al. (2017), the maize was inoculated to study the role of *Azospirillum brasilense* SP-7 and *Herbaspirillum seropedicae* Z-152 under drought stress. The report concluded enhanced biomass production; higher carbon, nitrogen, and chlorophyll content; and lower levels of abscisic acid and ethylene in the inoculated maize plants.

13.10 Physiological Characteristics of Plants to Cope with PGP Microbes

13.10.1 Changes in Root and Shoot Characteristics

The major adaptations to combat the drought stress include the changes in the root architecture and inhibition of the shoot growth. The increase in the root number with a smaller diameter, deeper root systems, and shorter shoots which limit the leaf area available for evaporation are some of the adaptive mechanisms for proper growth of the plants, but shorter shoots though will not threaten the endurance of plants but will surely interfere with the yield during the water stress conditions. The studies on different crops under drought conditions suggest that those with deeper root systems and high number of roots will be able to tolerate drought conditions more efficiently than those possessing a few roots. Thus, PGPMs by different direct as well as indirect mechanisms affect the root as well as the shoot architecture and support the growth as well as the maintenance of the productivity of the plants under drought stress. Vardharajula et al. (2011) reported that corn plants inoculated with plant growth-promoting *Bacillus* sp. under drought stress conditions showed improvement in the shoot growth as well as the dry biomass. Naseem and Bano (2014) studied the effects of strain *Alcaligenes faecalis* (AF3) on seeds in growth chamber tests and found that drought-stressed PGPR-treated plants showed an enhancement in root length by 10%, and it was concluded that development of root system was due to inoculation which enhanced the water uptake and allowed treated plants to tolerate drought stress.

Cohen et al. (2015) studied morphophysiological and biochemical responses of *Arabidopsis thaliana* Col-0 and aba2-1 mutant plants when inoculated with *Azospirillum brasilense* Sp 245 strain in well-watered and in drought conditions. The strain improved the biomass of the plants, lateral root number increased, the formation of the photosynthetic and photoprotective pigments was stimulated, ABA levels, plant sed yield, plant survival, proline content, relative water content increased, stomatal conductance and malondialdehyde content decreased. Timmusk et al. (2014) showed 78% higher biomass in wheat treated with PGP microbes under drought stress compared to untreated plants. Bresson et al. (2014) demonstrated the enhancement in lateral root length and modifications of the root architecture with PGP microbe strain *Phyllobacterium brassicacearum* STM196 which conferred observed drought tolerance. The increases in shoot and plant growth under drought stress as a result of treatment with PGP microbes have been reported in various crops including *Sorghum bicolor* L. (sorghum) (Grover et al. 2014), *Helianthus annuus* L. (sunflower) (Castillo et al. 2013), wheat (Arzanesh et al. 2011; Kasim et al. 2013), *Vigna radiata* L. (green gram) (Saravanakumar et al. 2011), and maize (Naseem and Bano 2014; Naveed et al. 2014; Sandhya et al. 2010).

13.11 Relative Water Content

Another important criterion to measure the water status of the plants is measuring the relative water content (RWC), and a decrease in the RWC results in limited cell expansion and certainly reduction in the growth of plants (Ashraf 2010; Castillo et al. 2013; Lu et al. 2010). Thus, RWC can act as one of the best parameters for assessing the capability of PGPR to ameliorate the drought stress. It has been suggested that RWC may help the plants to overcome the oxidative and osmotic stresses caused by drought stress. In fact, a number of studies carried out for the investigation of the potent PGPR which can help the plants to survive in the drought conditions have used this parameter in PGP microbe-treated and PGP microbe-untreated plants and have reported that plants treated with PGP microbes under drought conditions maintain relatively higher relative water content (Ngumbi and Kloepper 2016).

Casanovas et al. (2002) demonstrated a high RWC in maize which was treated with *Azospirillum brasilense* (BR11005), and it was concluded that the bacterial abscisic acid (ABA) caused stomatal closure and alleviated the water stress. Dodd et al. (2010) concluded that the increased RWC could be due to altered physiological processes such as stomatal closure. In the study of Grover et al. (2014), sorghum plants treated with PGPR *Bacillus* sp. strain KB 129 under drought stress showed a 24% increase in RWC.

13.12 Accumulation of Various Compatible Solutes

Further, at the cellular level, an important adaptation that helps the plants to overcome damages caused by drought is an osmotic adjustment (Blum 2005; Farooq et al. 2009). This adaptation is very important for protecting cellular organelles, enzymes, and proteins (Farooq et al. 2009; Huang et al. 2014). In response to drought stress, various compatible solutes accumulate in plants (Kiani et al. 2007), the most important being glycine betaine and non-protein amino acids, for instance, proline, and others being sugars including sucrose, polyols such as mannitol, organic acids such as malate, and various inorganic ions such as calcium (Ngumbi and Kloepper 2016). These solutes are important for maintaining turgor and also in lowering the water potential but without a decrease in the actual water content (Serraj and Sinclair 2002).

13.12.1 Proline

Proline is one of the major osmolytes accumulated in plants in response to drought (Huang et al. 2014; Verbruggen and Hermans 2008; Yoshiba et al. 1997). The increase in the proline content in plants treated with *Bacillus* strains under water stress was linked to the upregulation of gene P5CS, which is concerned with the biosynthesis of proline, and there was inhibition of expression of the gene for ProDH, which is mainly involved in the metabolism of proline (Yoshiba et al. 1997). Proline not only plays an important role in the osmotic adjustments, but it also stabilizes cellular structures such as proteins and membranes, scavenges free radicals, and buffers the cellular redox potential (Ashraf and Foolad 2007; Hayat et al. 2012). The increase in the proline content has been directly linked to the capability of the plants to tolerate drought (Sankar et al. 2007). The synthesis of proline has been demonstrated to increase in plants exposed to abiotic stress in the presence of *Burkholderia* (Barka et al. 2006), as well as *Arthrobacter* and *Bacillus* (Sziderics et al. 2007).

The transgenic plants of *Arabidopsis thaliana* introduced with *Pro*BA genes of *Bacillus subtilis* showed higher production of proline and an increase in their osmotic stress tolerance (Chen et al. 2007). Treatment with PGP microbes has been reported to increase the proline levels in potato (Gururani et al. 2013), maize (Naseem and Bano 2014; Sandhya et al. 2010; Vardharajula et al. 2011), sorghum (Grover et al. 2014), and *Arabidopsis thaliana* L. (Cohen et al. 2015). Maize seedlings treated with *Azospirillum brasilense* under water deficit conditions showed improvement in relative as well as absolute water content as compared to uninoculated plants. Further, the treated plants did not show any drop in the water potential; there was an enhancement of the root growth, foliar area, as well as aerial biomass. The proline content in the leaves and roots also showed an increment. All these results showed more significance at 75% reduction in water supply than at 50% reduction (Casanovas et al. 2002).

Wang et al. (2012a) reported a three- to fourfold increase in the proline content in leaves of *Cucumis sativus* L. (cucumber) when treated with a mixture of *Bacillus cereus* (AR156), *Bacillus subtilis* (SM21), and *Serratia* sp. (XY21) in comparison to untreated controls. It was concluded in the study that the increased proline content in the leaves protected the plants from over-dehydration thus contributing to the observed drought tolerance. Ghosh et al. (2017) studied the role of *Pseudomonas putida* GAP-P45 on the regulation of proline metabolic gene expression in *Arabidopsis thaliana* under water deficit conditions. In the inoculated plants, quantitative real-time expression analysis of proline metabolic genes under water deficit conditions showed a delay but prolonged upregulation of the expression of genes including *ornithine-Δ-aminotransferase* (*OAT*), *Δ^{1}-pyrroline-5-carboxylate synthase1* (*P5CS1*), and *Δ^{1}-pyrroline-5-carboxylate reductase* (*P5CR*), as well as proline catabolism, i.e., *proline dehydrogenase1* (*PDH1*) and *Δ^{1}-pyrroline-5-carboxylate dehydrogenase* (*P5CDH*), which are involved in proline biosynthesis. The inoculated plants showed enhancement in the growth, fresh weight, plant water content, chlorophyll content, and accumulation of endogenous proline and reduction in the primary root length.

13.12.2 Glycine Betaine

Glycine betaine is one of the major osmolytes which is accumulated in the plants in response to the stress conditions. It is known to possess a positive effect on the integrity of the membrane along with certain other adaptive roles which mediate osmotic adjustments during environmental stresses. Gou et al. (2015) evaluated the role of *Klebsiella variicola* F2, *Raoultella planticola* YL2, and *Pseudomonas fluorescens* YX2 on maize in a pot experiment under drought stress to determine their role in plant growth promotion and accumulation of choline and glycine betaine in leaves. The study well demonstrated that the PGPR strains regulated the osmotic adjustments by accumulating choline and subsequently glycine betaine thereby improving the water relations and ultimately promoting the growth under drought stress.

13.12.3 Trehalose

Trehalose is synthesized by some microorganisms and some plants which help to resist extreme abiotic stress such as desiccation (Chaplin 2006; Julca et al. 2012). Trehalose is a non-reducing disaccharide, i.e., α-D-glucopyranosyl-1, 1-α-D-glucopyranoside, that is formed by two molecules of glucose linked through their anomeric carbons. It plays a chief role in stabilizing dehydrated enzymes and proteins, providing higher levels of soluble carbohydrates, elevating capacity for photosynthesis, and protecting biological structures from damage during stress. Rodríguez-Salazar et al. (2009) studied the effect of drought stress on maize plants inoculated with genetically engineered *Azospirillum brasilense* for trehalose biosynthesis. The study conferred stress tolerance in maize plants as well as enhanced leaf and root biomass.

13.13 Antioxidant Metabolism

Another important consequence of the drought is the stimulation of the production of various reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), and the hydroxyl radical (HO-) (Cruz de Carvalho 2008), and these reactive oxygen species decrease the normal, metabolic processes of the plants by causing an oxidative damage to lipids and various proteins ultimately leading to cell death (Farooq et al. 2009; Hasanuzzaman et al. 2013). Plants possess certain enzymatic and non-enzymatic oxidants which are also referred to as the scavenging enzymes which play an efficient and supportive role to overcome the negative effects of the drought (Cruz de Carvalho 2008). Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR), and ascorbate peroxidase (APX) are among the most important enzymatic antioxidants (Cruz de Carvalho 2008; Farooq et al. 2009; Gill and Tuteja 2010; Hasanuzzaman et al. 2013). Treatment of the plants with PGP microbes under drought stress in relation to the antioxidant enzymes has been investigated in different studies, and an enhancement in the accumulation of various oxidant enzymes decreasing the oxidative injury has been well demonstrated.

Saravanakumar et al. (2011) reported that green gram plants treated with *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB showed an enhancement in catalase activity which was conferred to be directly related to the observed drought tolerance. Gururani et al. (2013) treated potato plants with *Bacillus pumilus* (DH-11) and *Bacillus firmus* (40), and the enhancement in the ROS-scavenging enzymes such as ascorbate peroxidase, catalase, and superoxide dismutase was reported to be the main mechanism for observed drought tolerance. In the study of Gusain et al. (2015), drought-tolerant (Sahbhagi) and drought-sensitive (IR-64) cultivars of rice were treated with *Pseudomonas fluorescens* (P2), *Pseudomonas jessenii* (R62), *Pseudomonas synxantha* (R81), *Bacillus cereus* BSB 38 (14B), and *Arthrobacter nitroguajacolicus* (YB3) to demonstrate their role on growth and induction of the stress-related enzymes under different levels of drought stress; the study concluded that the inoculated plants showed higher content of proline and enhanced ascorbate peroxidase, catalase, peroxidase, and superoxide dismutase activities, respectively.

Singh et al. (2015) inoculated *Helianthus annuus* seedlings with *Azotobacter chroococcum* (A+) and *Bacillus polymyxa* (B+) separately and in a consortium of the two (AB+) under water stress conditions. The maximum relative water content and seedling growth were observed in AB+-treated seedlings, increased superoxide dismutase activity was observed in A+ and AB+, and enhanced catalase activity was observed in leaves of seedlings treated with A+ and AB+. Kakar et al. (2016) studied the effect of *Bacillus amyloliquefaciens* Bk7 and *Brevibacillus laterosporus* B4 and also various biochemical elicitors such as salicylic acid and β-aminobutyric acid (SB) and their mixture for different abiotic stresses including drought stress;

after withholding water for 16 days, the treated rice plants showed 100% survival and increased seedling height and shoot number; reduction in chlorosis, wilting, necrosis, and rolling of leaves; and 3.0- and 3.6-fold enhancement in activities of antioxidant enzymes including superoxide dismutase and catalase. In the study of Khalilzadeh et al. (2016), rice was seed inoculated with *Azotobacter chrocoocum* strain 5 (F_1), *Pseudomonas putida* strain 186 (F_2), *Azotobacter* + *Pseudomonas* (F_3), and Cycocel with different water treatment levels, and an increase in catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) activities was observed.

13.14 Upregulation of Expression of Drought-Tolerant Genes

In addition to the accumulation of various osmolytes, increased relative water content, and changes in root and shoot characteristics, the upregulation in expression of certain drought stress-responsive genes has also been reported in certain studies. In the study of Lim and Kim (2013), the effect of inoculating pepper plants with *Bacillus licheniformis* K11was analyzed in relation to drought resistance. The seedlings treated with PGPR tolerated drought stress, whereas uninoculated ones died after 15 days. After 10 days of drought stress, treated pepper plants showed a total of six differentially expressed stress proteins by two-dimensional polyacrylamide gel electrophoresis and 2D-PAGE differential display PCR (DD-PCR), respectively. Among these stress proteins, specific genes of Cadhn, VA, sHSP, and CaPR-10 were expressed 1.5-fold more in pepper treated with *Bacillus licheniformis* K11 under drought conditions. Sarma and Saikia (2014) reported an increase in the production of reactive oxygen species-scavenging enzymes and cellular osmolytes, root as well as shoot length, dry weight, relative water content, and upregulation of various drought stress-responsive genes including dehydration-responsive element binding protein (DREB2A), catalase (CAT1), and dehydrin (DHN) in mung bean treated with *Pseudomonas aeruginosa* GGRJ21 as compared to the untreated plants under drought stress.

13.15 Potential Biotechnological Applications of Drought-Tolerant Microbes

The various coordinated mechanisms of PGPR affecting the growth of the plants will prove to be a very powerful tool for sustainable agriculture. The applications of the beneficial rhizobacteria on different crops under laboratory as well as greenhouse experiments are already proving to be successful. Consequently, the achievement of the industries which will produce PGPR-based bio-inoculants will depend on pioneering business management, marketing of the product, and extensive research. Further, optimization of the processes for better formulations of effectual strains of PGPR will be also required so as to introduce them in agriculture.

13.15.1 Biofertilization

Biofertilizers are defined as the products which generally contain microorganisms which by diverse direct or indirect mechanisms influence the growth of the plants even under stressed conditions such as floods, drought, low temperature, salinity, etc. and thus can open new doors for the sustainable agriculture (Kour et al. 2017a). The direct attributes include nitrogen fixation, phosphorus solubilization, production of phytohormones, showing ACC deaminase activity, and siderophore production, whereas the indirect attributes include the production of the ammonia, HCN, siderophores, and various hydrolytic enzymes such as amylases, cellulases, pectinases, phytases, proteases, and xylanases. The most studied microbes used as biofertilizers are plant growth-promoting rhizobacteria (PGPR). The well-known genera of PGP microbes include *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Azospirillum*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Azoarcus*, *Burkholderia*, *Clostridium*, *Erwinia*, *Enterobacter*, *Gluconacetobacter*, *Flavobacterium*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, *Serratia*, etc. (Sudhakar et al. 2000; Bertrand et al. 2001; Bonaterra et al. 2003; Joo et al. 2005; Murphy et al. 2003). Different bacterial genera including *Bacillus*, *Rhizobium*, *Pseudomonas*, *Paenibacillus*, *Pantoea*, *Burkholderia*, *Azospirillum*, *Achromobacter*, *Microbacterium*, *Variovorax*, *Enterobacter*, *Methylobacterium*, etc. have been known to support the growth of plants and overcome the stress conditions (Pandey et al. 2016). Diverse strains of *Bradyrhizobium japonicum* (Thal-8, Tal-620, Dulawala) which show differential response to drought conditions imparted different degrees of tolerance to water stress conditions in inoculated chickpea and also increased the root biomass, number of nodules, weight of seeds, and IAA and GA content in the leaves (Bano et al. 2010). A number of studies in glasshouse and fields have demonstrated the effects of PGPR on the enhancement of growth and productivity, and various studies have been published (Kennedy et al. 2004; Lucy et al. 2004). Among PGP microbes, *Azospirillum* has been assessed the most (Burdman et al. 2000; Dobbelaere et al. 2001; Lucy et al. 2004; Vessey 2003). Further, the species of *Pseudomonas* and *Bacillus* (Alam et al. 2001; Çakmakçı et al. 2001; Kokalis-Burelle et al. 2006) are also receiving greater attention as they are associated with the rhizosphere of many crops and also possess the capability to stimulate growth (Chelius and Triplett 2000; Dong et al. 2003; Sturz et al. 2001). Recently, PGP microbes are used in consortium rather as single strain to provide the benefits to the plants. *Pseudomonas*, *Bacillus*, and *Rhizobium* in combination is considered to be the most effective phosphate solubilizers (Adesemoye et al. 2008; Rodríguez-Díaz et al. 2008; Rodríguez and Fraga 1999). The biofertilizers are completely safe to use and are eco-friendly. Thus, it is very important to use such effective strategies in combination with the chemical fertilizers and organic manures for integrated nutrient management systems so that biological productivity and health of the soil could be easily maintained, and chiefly the farmers are unwilling to use the recommended doses of the fertilizers as their cost is very high and also due to the risk of crop failures on account of aberrant weather conditions (Choudhary 2017).

13.15.2 Bioprotectants

There are a number of fungi, bacteria, viruses, nematodes, etc. which are pathogenic to plants (Viswanathan and Samiyappan 2002). In recent times, the use of PGPR as bio-inoculant for the biological control of plant diseases is on the rise (Aliye et al. 2008; Altindag et al. 2006; Xue et al. 2009). There are a number of different mechanisms of biocontrol including the induction of the systemic resistance, production of siderophores which prevent the proliferation of pathogens, and production of various antibiotics and hydrolytic enzymes. PGPR are known to produce many antifungal compounds such as 2,4-diacetylphloroglucinol (DAPG), phenazines, pyoluteorin, pyrrolnitrin, tensin, and nicotinamide. The most widely used PGPR for biocontrol are the strains of *Bacillus subtilis* due to their capability of reducing disease and also antibiotic-producing capacity (Kokalis-Burelle et al. 2006). Fluorescent pseudomonads are also among the potent biocontrol agents suppressing various soil-borne phytopathogens by the synthesis of different antifungal compounds and sequestering iron in the rhizospheric region by producing siderophores making iron unavailable (Dwivedi and Johri 2003). Inoculating plants with diverse strains of *Pseudomonas fluorescens* leads to a decrease in mortality of seedlings caused by *Aspergillus niger* (Dey et al. 2004) and shows an inhibitory effect against *Sclerotium rolfsii* by causing reduction in the incidence of stem rot severity. *Pseudomonas fluorescens* strain WCS374 has been reported to suppress *Fusarium* wilt in radish and also to increase yield by about 40% (Bakker et al. 2007).

Kumar et al. (2009) reported the biocontrol activity of *Streptomyces* sp. *Streptomyces* has been reported to be among the major genera showing potential against various pathogens such as *Acyrthosiphon kondoi*, *Fusarium avenaceum*, *Myzus persicae*, *Rhizoctonia bataticola*, tobacco necrosis virus, tomato mottle virus, etc. PGPR when used as bio-inoculants possess efficacy for the suppression of various diseases with a simultaneous increase in the chlorophyll content and number of leaves, ultimately enhancing the overall productivity.

13.16 Conclusion and Future Prospect

The improvement of the stress tolerance and productivity of crops is the major goal of agriculture. The use of PGP microbes is an emerging field of science which is proving its potential in helping plants to combat abiotic stresses by different mechanisms including production of phytohormones, solubilization of phosphorus, production of ACC deaminase, and production of siderophores. There are reports that have been published on tolerance of stress by plants inoculated with PGP microbes, and exploitation of these beneficial bacteria will surely make a breakthrough in growing crops as well as enhancing the yield of the crops under stress conditions. Agriculture accounts for ~ 70% of all water use, and the world population is increasing annually; soon more people will need to be fed while also using less water. The use of plant-associated bacteria is an eco-friendly alternative that can increase crop water use efficiency. Sustainable agriculture requires the use of strategies to increase or maintain the current rate of food production while reducing damage to the environment and human health. The use of microbial plant growth promoters is an alternative to conventional agricultural technologies. Plant growth-promoting microbes can affect plant growth directly or indirectly. The direct promotion of plant growth by PGP microbes, for the most part, entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. The indirect promotion of plant growth occurs when PGP microbes decrease or prevent the deleterious effects of one or more phytopathogenic organisms. Future research in microbes will rely on the development of molecular and biotechnological approaches to increase our knowledge of microbes and to achieve an integrated management of populations of microbial communities. Research on ACC deaminase and P solubilization by plant growth-promoting microbes is in progress, and leads in microbe-mediated alleviation of diverse abiotic stress. The application of multifarious PGP microbes or consortium over single inoculation could be an effective approach for reducing the harmful impact of stress on plant growth under the abiotic stress conditions.

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Role of PGPR for Alleviating Aluminum Toxicity in Acidic Soil

14

Jintu Dutta and Utpal Bora

Abstract

The soil pH is a very crucial determining factor for the solubility of different metal ions, nutrient availability, and various physical properties. Among different factors, aluminum (Al) toxicity in acidic soil is considered as a limiting factor for plant growth. When soil pH falls to lower than 5, Al is solubilized into different ionic forms and causes toxicity to the plants. In acidic soils, Al limits the growth of roots either by restraining cell division, cell elongation, or both, causing stunted root growth. Moreover, Al ions also form complexes with phosphoric acid which makes phosphorus (P) unavailable to plants. In recent years, considerable efforts have been made to addressing how bacteria respond to the changing environment since the terrestrial ecosystems are increasingly under the pressure of human activities. The agricultural soil is a great example where most human interference occurred. Due to the extensive use of chemicals and pollutants, the agricultural soils gradually become acidic and less fertile. In this chapter, we are trying to include the Al chemistry in acidic soils and its toxic effects on plants at higher concentration. The chapter also includes the role of plant growth-promoting rhizobacteria (PGPR) to mitigate the Al toxicity in acidic soil.

Keywords

Al toxicity · Acidic soil · PGPR

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14.1 Introduction

The acidity of soil is a very crucial factor for the growth and yield of many crops. The soil acidity is adversely affecting the crop all over the world, and almost 50% of the arable lands of the world are covered by acidic soil (von Uexküll and Mutert 1995). In India, also it is estimated that approximately one-third of the cultivated land is affected by soil acidity (Mandal 1997). Moreover, agricultural soil is a hotspot for anthropogenic disturbance due to the intensive use of agricultural-based chemicals and pollutants resulting in significant changes in soil characteristics such as acidification and attenuation of soil fertility (Jenkins et al. 2009). There are ample of limiting factors co-exist in acidic soils including toxic levels of aluminum (Al), manganese and iron (Fe), with deficiencies of some vital elements, such as phosphorus (P), nitrogen, potassium (K), calcium (Ca), magnesium, and some micronutrients (Kochian et al. 2004). However, it is observed that Al toxicity and P deficiency are the most crucial for the plant health and growth (Kochian et al. 2004). Aluminum (Al) in soils is solubilized into ionic forms, viz., $Al(OH)_2^+$, Al(OH), and $Al(H_2O)_3^+$, especially when the soil pH drops to lower than 5, and it is found to be very toxic to the plants. These Al ions form complexes with phosphoric acid which makes phosphorus (P) unavailable to plants (Zheng 2010). Soil P is an important macronutrient for plant growth. P is one of the major components in energy metabolism and biosynthesis of nucleic acids and cell membranes with an important role in regulation of a number of enzymes. P deficiency may lead to major problem for agricultural production (Singh and Satyanarayana 2011). Al toxicity also influences the root morphology of the plants and reduces the root growth due to which it makes plants more sensitive to various abiotic stresses such as water and nutrient stress. It also reduces the ability of crop plants to acquire P from the soil and ultimately reduces crop yield (Chen et al. 2012).

In order to produce a better crop yield on acidic soils, farmers are recommended to apply alkaline materials such as lime to increase the soil pH and thus eliminate Al toxicity and to apply P fertilizer to increase the availability of P in soil. In some previous studies, it was reported that application of P could alleviate Al toxicity in plants. This Al toxicity alleviation effect is commonly based on two possible mechanisms: The application of P can directly precipitate Al by forming Al-P complex in soil and on plants (such as on root surface, in root cell walls, or within root cells), and indirectly, the application of P could alleviate Al toxicity by improving the root morphology and facilitating nutrient uptake or by secreting special root exudates.

It is still poorly understood the environmental factors that control the distribution and abundance of soil microorganisms despite the soil microbes being the dominant engines of biogeochemical cycles and major pool of living biomass in terrestrial ecosystems (Fierer et al. 2012). Soil-dwelling P-solubilization microbes were known to solubilize the insoluble complexes of P such as aluminum phosphate ($AlPO_4$) in acidic soil. Recent study conducted using a variety of molecular or biochemical approaches has started to explore the distributional patterns exhibited by soil microbial communities and the biotic or abiotic factors driving these patterns (Rousk et al. 2010). Researcher has demonstrated that the soil microbial

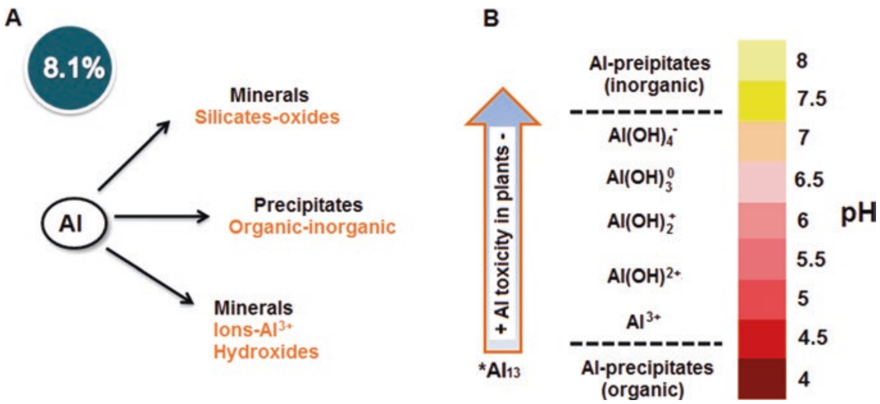

Fig. 14.1 Aluminum abundance and speciation in the earth's crust. (**a**) Different forms of Al in the soil. Al is mainly found in the mineral form such as aluminum silicates and aluminum oxides. Moreover, depending on the soil pH, Al can be found as precipitates or conjugated organic and inorganic and molecular ions. (**b**) Al speciation in the soil solution. Al concentration and the speciation of Al depend on the pH and the chemical environment of the soil solution. (Adapted from Bojórquez-Quintal et al. 2017)

communities across the diverse ecosystem are often strongly correlated with differences in soil chemistry (Frey et al. 2004; Nilsson et al. 2007; Lauber et al. 2008; Jenkins et al. 2009). In particular, it has been shown that the composition and in some cases diversity of soil bacterial communities are often strongly correlated with soil pH (Fierer and Jackson 2006; Hartman et al. 2008; Jenkins et al. 2009; Lauber et al. 2009; Wu et al. 2017). However, current emergence of promising technologies such as high-throughput sequencing is dramatically intensifying our knowledge of soil microbial diversity, linking microbial ecology and the plant-microbe interaction and functioning (Fig. 14.1).

14.2 Aluminum Chemistry in the Acidic Soil

Al is a member of boron group of chemical elements with atomic number 13. It is the most abundant metallic element in our earth crust and third most abundant of all element (after oxygen and silicon) and comprising approximately 8% by weight (FitzPatrick 1986). The oxides of aluminum, iron, and manganese particularly the poorly crystallized and microcrystalline forms are undoubtedly the most reactive components of acidic soils. Aluminum bound as oxides and form complex aluminosilicates. Since aluminum occurs exclusively in the trivalent form, only pH and complex formation affect the solubility of its oxides. Hartwell and Pember first assumed that the soluble aluminum is a major inhibitor for plant growth and development in acid soils nearly 90 years ago, but till date the precise mechanism of aluminum phytotoxicity is not fully understood (Krstic et al. 2012). The acidic soils are predominantly present in humid tropical and subtropical areas of the world and are characterized by having excess H^+, Mn^{2+}, and $A1^{3+}$ with deficiencies of Ca^{2+}, Mg^{2+}, and PO_4^{3-}. In addition, sulfur dioxide and other air pollutants cause acid soil stress in those areas other than the tropics (Foy 1984). The chemistry of Al in soil is reasonably complex, and the hydroxyl-rich aluminum compounds solubilize to an extent in the soil solution. Al has a high ionic charge and a small ionic radius, therefore having the second largest charge-to-radius ratio ($z/r = 5.9$). Therefore, Al strongly polarizes the water molecule in the hydration shell (Vitorello et al. 2005). When the pH of a solution is raised above 4.0, $A1^{3+}$ forms the mononuclear species $Al(OH)^{2+}$, $Al(OH)^{3+}$, and $Al(OH)^{4+}$ and soluble complexes with inorganic ligands such as sulfate ($Al(SO)_4^+$) and fluoride ($A1F_2^+$, AlF_3^+) and also with many organic compounds. Larger polynuclear hydroxyl aluminum species also form as metastable intermediates during $AI(OH)_3$ precipitation. The mononuclear $A1^{3+}$ species appears to be most toxic at low pH, at which it exists as an octahedral hexahydrate. With escalating pH, $Al(H_20)^{3+}$ undergoes repeated deprotonations to form insoluble $Al(OH)_3$ at pH 7.0. One of the most important polymer triskaideka aluminum, $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$, referred to as Al_{13} (Parker and Bertsch 1992), seems to be the most toxic Al species.

14.3 Aluminum Toxicity in Plants

Al toxicity is a crucial factor for limiting crop productivity in acidic soil worldwide. In acid soil with high mineral content, Al is the major cause of phytotoxicity. When the soil pH is lower than 5, Al ions are released to the soil and the plant root becomes vulnerable to Al, which enters into root tip cell and reduces root development of plant. As we know, root growth and elongation is a process of cell division, but the Al exposure in the root tip causes inhibition of cell elongation and cell division. Finally, it leads to stunting and poor development of root hair and apices accompanied by reduced water and nutrient uptake (Panda et al. 2009). Moreover, it has been reported that Al exposure in root tips causes the decrease of mitotic activity in different plant species, viz., wheat (Frantzios et al. 2001; Li et al. 2008), maize (Marienfeld et al. 2000; Doncheva et al. 2005), barley (Budikova and Durcekova 2004), and bean (Marienfeld et al. 2000). Doncheva et al. (2005) reported that cell division (decrease of S-phase cells) in the proximal meristem and apical meristem of roots was inhibited after 5 min and 10–30 minutes of Al exposure, respectively. Similarly, it has also been demonstrated that Al can accumulate in the nuclei of cells in the meristematic region of the root tip within 30 minutes (Silva et al. 2000). It was also reported that Al impedes root apex cell division and lateral roots, increases the rigidity of the cell wall by cross-linking of pectins, and reduces DNA replication because of increased rigidity of the double helix (Zhang et al. 2014; Eekhout et al. 2017). Furthermore, a number of researchers dissected the Al toxicity in the cellular level, and they found that Al can affect the constituents' symplast (calmodulin) (Tokizawa et al. 2015), apoplast (pectin matrix) (Eticha et al. 2005a, b; Delhaize et al. 2007), and DNA in cells of plant roots (Kochian et al. 2004; Sade et al. 2016).

However, among the different components present in the cell wall network, pectins have been proposed to be a critical site for Al-cell wall interactions (Blamleyet al. 1993). Interactions of Al can lead to the displacement of other cations such as Ca^{2+} which is fundamental for cell wall stability (Matsumoto et al. 1977a, b; Rincón and Gonzales 1992; Schmohl and Horst 2000; Tabuchi and Matsumoto 2001). The disruption of cytoplasmic Ca^{2+} homeostasis due to Al interference may be directly or indirectly involved in the inhibition of the cell division or root elongation. Al might disrupt Ca-dependent metabolism by maintaining Ca^{2+} levels in the cytoplasm or by preventing Ca^{2+} transients from occurring altogether (Panda et al. 2009). As a result, the strong and rapid binding of Al can alter cell wall integrity and mechanical properties, making it more rigid and leading to a decrease in the mechanical extensibility of the cell wall required for normal cell expansion (Kochian et al. 2005).

Al in low pH affects the plasma membrane and alters the function and structure of plasma membrane (Sasaki et al. 1994; Wagatsuma et al. 1995; Vitorello and Haug 1996; Ishikawa and Wagatsuma 1998; Ishikawa et al. 2001; Ofei-Manu et al. 2001; Vitorello et al. 2005). Al has a greater affinity for the choline head of phosphatidylcholines which is a lipid constituent of the plasma membrane, where Al can displace other cations, viz., Ca^{2+}, that may form bridges between the phospholipid head groups of the membrane bilayer. As a result, the phospholipid packing and fluidity of the membrane is altered (Akeson and Munns 1989; Kochian et al. 2005). Due to displacement of cations, Al stimulates the exceptional synthesis of callose (β-1, 3-glucan) on the surface of plasma membrane by β-1, 3-glucan synthase (Gupta et al. 2013). Therefore, accumulation of callose in the apoplast is considered as early symptoms of Al toxicity (Horst et al. 1997; Massot et al. 1999). Since the synthesis of callose is assisted by the presence of Ca^{2+}, therefore it has been assumed that Al-prompted displacement of Ca^{2+} from the membrane surface may increase the apoplasmic Ca^{2+} pool requisite to trigger callose synthesis (Ryan et al. 1993; Ahn et al. 2001; Gupta et al. 2013). Under Al stress, the speed of callose accumulation may further enhance and lead to cellular damage by preventing intercellular transport through plasmodesmatal connections (Sivaguru et al. 2000).

One of the most noticeable consequences of root Al exposure is an almost immediate depolarization of the plasma membrane (Lindberg et al. 1991; Papernik and Kochian 1997). The electrochemical potential of the plasma membrane has been changed due to direct and indirect interactions of Al with different number of ions transport pathways (Miyasaka et al. 1989). It has been demonstrated that the nuisance of Al can significantly degrade the activity of the plasma membrane H^+-ATPase which consequently led to the disruption of the H^+ gradient in both in vitro (e.g., membrane vesicle studies) and intact roots of several plant species (Ryan et al. 1993; Ahn et al. 2001, 2002; Ahn and Matsumoto 2006). However, the H^+ gradient in transmembrane can act as a major driving force for secondary ions transportation. Thus, the Al-prompted disruption of the H^+ gradient could certainly alter the ion homeostasis of root cells (Gupta et al. 2013). Plant exposure to Al can prevent the acquisition of several essential cations such as Ca^{2+}, Mg^{2+}, K^+, and NH_4^+ (Huang et al. 1992; Rengel and Elliott 1992; Nichol et al. 1993; Ryan and Kochian 1993; Lazof et al. 1994). In this context, the electrophysiological approaches were used to demonstrate that Al^{3+} interacts directly with several different plasma membrane channel proteins and barricades the uptake of ions such as K and Ca^{2+} (Gassmann and Schroeder 1994; Piñeros and Kochian 2001; Piñeros and Tester 1995). In addition to directly altering ion permeation through channels, extracellular Al can further modulate the transporter's activity via altering the membrane potential. For instance, Al-induced depolarizations of plasma membrane can amend voltage-dependent Ca^{2+} channel transport by indirectly altering and shifting the activation thresholds of distinct transport pathways, such as hyperpolarization-activated (Kiegle et al. 2000; Very and Davies 2000) and depolarization-activated (Piñeros and Tester 1997; Thion et al. 1996; Thuleau et al. 1994) Ca^{2+} channels.

Al tends to bind with phosphorus (P) in acidic soil and form insoluble complexes in soils and plant roots, thereby creating a P deficiency for plant growth. Soil P is an important macronutrient for plant growth. P is a crucial factor for different cellular mechanisms in plants, and its deficiency may lead to a major problem for agricultural production (Singh and Satyanarayana 2011). It also acts as a metabolite involved in energy transfer, the activation of proteins, and the regulation of metabolic processes (Marschner 1995; Franke et al. 2002). Inorganic phosphate is the primary source of P for plants. It enters into the equilibrium reactions defined by P sorption isotherm (Fox and Kamprath 1970). Even in the most fertile soils, P concentration in soil solutions rarely exceeds 8 μM (Barber et al. 1962). The plants have adapted a number of morphological and biochemical strategies to access the P in soil. The highly branched root systems with more root apices are more capable of acquiring P. It has been observed in some plant species that the surface area of roots in contact with the soil increased in diameter of roots when the plants are under P-stressed (Ma et al. 2001) and sometimes the density and length of root hairs also increased (Bates and Lynch 1996; Foehse and Jungk 1983; Smitha et al. 2002). Therefore, there is a strong correlation between Al toxicity and P deficiency. Al toxicity affects the root morphology due to which it has been seen that plant growth is adversely affected by P deficiency at the occurrence of Al toxicity. The plants cannot uptake the sufficient P, and the deficiency of phosphorus occurs in acidic soils mainly because of Al-P interaction (Table 14.1).

14.4 PGPR for Alleviating Aluminum Toxicity

14.4.1 Plant Growth-Promoting Rhizobacteria (PGPR)

Kloepper and Schroth (1978) for the first time defined an important group of microbial communities that colonized the plant roots and exerts beneficial effects and termed them as plant growth-promoting rhizobacteria (PGPR). These PGPR can improve growth, nutrition assimilation, and health of plants in different agroecosystems (Philippot et al. 2013). PGPR are sometimes also termed as plant health-promoting rhizobacteria (PHPR) or nodule-promoting rhizobacteria (NPR) according to their functions in the plant rhizosphere soil (Burr and Caesar 1984). PGPR can be divided into two groups: the PGPR which live inside the plant cells,

Table 14.1 The negative effects of aluminum (Al) on different plant species

Plant species	Effect of aluminum imposed on plant	Negative Al influence on plant	References
Wheat (*Triticum aestivum*) roots	Under Al stress condition, wheat plants experience substantial decrement in growth, pigment content, and activities of several enzymes such as GPX, (CAT) catalase, and APX and leaf structure	Antioxidant system	Li et al. (2010) and Malekzadeh et al. (2015)
	Cytoskeleton in the cells of DTZ is particularly susceptible to Al stress in apical maize root zone	Cytoskeleton	
Wheat (*Triticum aestivum*)	Callose formation or induction (1,3-*b*-D-glucan) is reported in the root of several plants subjected to Al stress; callose blocks the plasmodesmata, thereby inhibiting cell-cell trafficking in plants	Callose formation	Silva et al. (2010) and Silva (2012)
Rye (*Secale cereale*)	Due to the Al exposure, the accumulation of callose was also reported in the meristematic region		
Silver birch (*Betula pendula*)	Exposure of Al at lower concentrations (2 and 5 mg l^{-1}) accelerates the growth responses in two races of *B. pendula*, while exposure to higher concentration of Al limits the growth responses in both the races	Growth of plants	Kidd and Proctor (2000)
	Similarly, lower concentration of Al (<5 mg^{-1}) significantly enhanced the expansion of leaf, while its higher concentration (>25 mg l^{-1}) delimited the expansion of leaf	Nutrient acquisition in plants	
Wheat (*Triticum aestivum*)	Root growth inhibition is the prime response of aluminum toxicity in plants	Root growth	Marienfeld et al. (2000), Li et al. (2008), Frantzios et al. (2001), Tamás et al. (2005), Blancaflor et al. (1998), and Horst et al. (1999)
Maize (*Zea mays*)	Aluminum exposure significantly reduced the mitotic activity in the root tips of several plant species, like wheat	Plant cytoskeleton (principal target of Al toxicity	
Barley (*Hordeum vulgare*)	Besides this, Al also has inhibitory effect on microtubules and actin microfilaments, and it has been demonstrated that exposure of Al altered the microtubules and microfilaments stability, organization, as well as polymerization		
Bean (*Phaseolus vulgare*)			
Barley (*Hordeum vulgare*)	Disruption in redox homeostasis is observed in plants exposed to Al stress	Oxidative stress	Yamamoto et al. (2001), Kuo and Kao (2003), Guo et al. (2004a, b), Peixoto et al. (1999), Panda et al. (2013), and Hossain et al. (2005)
Sorghum (*Sorghum bicolor*)	Exposure to Al leads to the overexpression of reactive oxygen species which eventually results in the oxidative stress in plants (lipid peroxidation, DNA damage, etc.)	Lipid peroxidation	
Triticale rice, green gram, and wheat (*Triticum aestivum*)			

(continued)

Table 14.1 (continued)

Plant species	Effect of aluminum imposed on plant	Negative Al influence on plant	References
Arabidopsis thaliana	Reduction or disturbance is observed in cytoplasmic Ca^{2+} homeostasis in plant subjected to Al stress	Cytoplasmic Ca^{2+}	Jones et al. (1998)
Tobacco cell cultures			
Red clover (*Trifolium pratense*)	Distinct phytotoxicity symptom has been demonstrated in *Trifolium* even at submicromolar concentrations of Al	Inhibition of H^+ efflux	Dai et al. (2014), Panda et al. (2008), Panda et al. (2013), and Matsumoto (1988)
Barley (*Hordeum vulgare*)	Aluminum exposure inhibits the efflux of H^+ ion from the root of barley seedling	Reduced mitochondrial activity	
Tobacco cell	Exposure to Al inhibits the respiratory activity and redox status on cultured tobacco cell	Reduced mitochondrial respiratory function	
Pea (*Pisum sativum*) root		Altered redox status	
Cowpea (*Vigna unguiculata*)	Aluminum toxicity induces negative effect on the acquisition and accumulation of nitrogenous compounds, activity of nitrate reductase enzyme, and absorbed and accumulated level of macronutrients Ca, Mg, and K	Root system	Cruz et al. (2014)
Rice (*Oryza sativa*) cultivars	Plants exposed to Al stress experience severe root growth inhibition and intense reduction in chlorophyll content, thereby reducing photosynthetic activity	Root system	Guo et al. (2012)
	Significant increase in the level of proline and malonaldehyde contents		
	Considerable enhancement in the level of peroxidase and superoxide dismutase activities		
Norway spruce (*Picea abies*)	Synergistic effect of drought and Al stress induced several empirical impacts in plants	Reduced acquisition of water and nutrient	Prabagar et al. (2011) and Slugenová et al. (2011)
Ginseng (*Panax ginseng*)	In Al-stressed plants were observed stunted root growth and different morphology	Root growth	Farh et al. (2017)
	Yellowing symptoms were observed in the leaves which gradually developed in due time and led to the wilting of the foliage	Wilting and mass reduction of foliage	
	Declined chlorophyll content		

produce nodules, or localize inside the specialized structures are called as iPGPR (i.e., symbiotic bacteria), and the PGPR which live outside the plant cells and do not produce nodules but still promote plant growth are called as ePGPR (i.e., free-living rhizobacteria) (Gray and Smith 2005). A putative rhizobacteria qualifies as PGPR when it is able to produce a positive effect on the plant upon inoculation, and they should be competitive to survive in the existing rhizosphere communities. In rhizosphere, generally about 2–5% of bacteria are PGPR, and majority of plausible PGPR bacterial genera include *Arthrobacter, Azospirillum*, *Azotobacter*, *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Klebsiella*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Enterobacter*, *Xanthomonas*, *Chromobacterium*, *Serratia, Caulobacter, Frankia,* and *Thiobacillus* that have been documented to promote plant growth (Glick 1995; Vessey 2003; Bhattacharyya and Jha 2012; Bal et al. 2013).

In the last decade, several researches have been carried out to understand the functioning of rhizosphere as an ecological niche which eventually also gains attention in sustainable agricultural practice. PGPR are the potential tools to contribute for the development of sustainable agricultural systems (Schippers et al. 1995). Commonly, PGPR contribute to the plant health by three different ways (Glick 1995), i.e., producing particular compounds for the plants (Dobbelaere et al. 2003; Zahir et al. 2004), facilitating the uptake of certain nutrients from the soil (Lucas García et al. 2004; Çakmakçi et al. 2006), and reducing or preventing the microorganisms which can cause the plant disease (Jetiyanon and Kloepper 2002; Guo et al. 2004a, b; Saravanakumar et al. 2008). PGPR may influence the plant growth directly by fixing atmospheric nitrogen, solubilizing insoluble phosphates, and secreting phytohormones such as indole acetic acid (IAA), gibberellic acid (GA), and ACC (1-aminocyclopropane-1-carboxylic acid) deaminase which helps in regulation of ethylene. PGPR can indirectly stimulate the plant growth by siderophore production which sequesters iron an important cofactor for pathogenic bacteria to grow, induced systemic resistance (ISR), competition for nutrients, production of different types of anti-microbial metabolites (such as antibiotics) to suppressive the deleterious microbes (Glick and Bashan 1997). The concept of PGPR has now been confined to the bacterial strains that can execute at least two of the three criteria such as aggressive colonization, plant growth stimulation, and biocontrol (Weller et al. 2002; Vessey 2003). PGPR has been reported for the benefit of different agricultural crops like rice (Sudha et al. 1999), tomato (Mena-Violante and Olalde-Portugal 2007), wheat (de Freitas 2000), maize (Biari et al. 2008), canola (Naderifar and Daneshian 2012), chili (Bharathi et al. 2004), lentil (Siddiqui et al. 2007), and tea (Dutta et al. 2015; Dutta and Thakur 2017). Moreover, actinobacteria is also one of the major groups of bacteria among the rhizosphere microbial populations which plays a significant ecological role in soil nutrient cycling (Halder et al. 1991; Elliott and Lynch 1995) as well as in plant growth-promoting (PGP) activities (Merzaeva and Shirokikh 2006). Several reports are available on the actinobacteria for their promising PGP activity (Gomes et al. 2000; Sousa et al. 2008, Anwar et al. 2016). Actinobacterial strains, like *Micromonospora* sp., *Streptomyces* sp., *Streptosporangium* sp., and *Thermobifida* sp., are documented as PGPR which efficiently colonized in rhizosphere and showing biocontrol activity against a wide range of root pathogenic fungi (Franco-Correa et al. 2010). *Streptomyces* sp. isolated from *Araucaria angustifolia* rhizosphere showed PGP and act as a potential biocontrol agent against *Fusarium* and *Armillaria* pine rot (de Vasconcellos et al. 2010).

14.4.2 Role of PGPR for Alleviating Aluminum Toxicity

One of the strategies that have been considered to counter various environmental stressors is PGPR (Lugtenberg and Kamilova 2009; Hayat et al. 2010). PGPR can improve plant performance under stress and consequently enhance yield by using both direct and indirect mechanisms (Dimkpa et al. 2009). Though work on the effect of PGPR on Al stress alleviation is not sufficiently advanced, there are few efforts that have been made by researchers. Lemire et al. (2010) showed that *Pseudomonas fluorescens* can survive under Al stress by orchestrating metabolic balance to counter Al toxicity. Zerrouk et al. (2016) evaluated the effect of *P. fluorescens* strain 002 to alleviate damages caused by Al toxicity in maize roots, and they showed that inoculation of the strain *P. fluorescens* 002 in the maize root increased the biomass where roots are exposed for 6 days to 90 M $AlCl_3$ solution. Farh et al. (2017) conducted the experiment on ginseng plant and showed that the PGPR abolished the Al stress. In this experiment, three PGPR strains, i.e., *Pseudomonas simiae* N3, *Burkholderia ginsengiterrae* N11-2, and *Chryseobacterium polytrichastri* N10, were selected to treat the plants. After Al application, they monitored the morphology of the bacterized seedlings and compared with Al-stressed nonbacterized seedlings (negative control) as well as mock seedlings for 7 days. They observed the yellowing symptom in negative control seedlings which was gradually developed on the leaves part and led to completely wilting of the foliage, while leaves of mock seedlings were remaining green. The wilting rate of the negative control seedlings was also found significantly high. Subsequently, chlorophyll content and dry weight of the negative control seedlings' foliage were found to be significantly declined. Roots of negative control seedlings were also observed to be stunted and morphologically different compared to those of mock seedlings' roots. Similarly, Mora et al. (2017) carried out a study on Al-tolerant PGP bacteria isolated from the rhizosphere and the endosphere of ryegrass (*Lolium perenne*) grown in acidic Chilean volcanic soil in order to characterize a bacterial consortium capable of contributing to alleviation of Al^{3+} toxicity and supporting plant growth in Andisol. In this study, five strains, i.e., *Klebsiella* sp. RC3, *Stenotrophomonas* sp. RC5, *Klebsiella* sp. RCJ4, *Serratia* sp. RCJ6, and *Enterobacter* sp. RJAL6, were selected based on their capacity to tolerate high Al concentration (10 mM) and to exhibit multifarious PGP traits. The strains showed different PGP traits like P solubilization, IAA production, ACC deaminase activity, and exudation of organic acid anions and siderophores. They performed an experiment and tested the consortium of PGP bacteria in an assay with ryegrass plants growing in a soil with high Al saturation (24%) and showed that selected bacterial consortium was able to promote plant growth by alleviating the Al stress by forming Al^{3+}-siderophore complexes.

In addition, Al toxicity is a major concern in acidic soil which extensively limited the access of phosphorus to plant. To alleviate the P deficiency due to anthropogenic substances present in the soil especially Al, the microorganisms such as phosphate-solubilizing bacteria (PSB) could be used to provide crops with available P from barely soluble forms in the soil and physiologically active substances that trigger a higher metabolic activity once they interact with the plant (Adesemoye et al. 2009). It has been illustrated in different crops such as peanut (Wang et al. 2014) and wheat (Delfim et al. 2018) that inoculation of *Bacillus thuringiensis* improved the solubilization of sparingly soluble phosphate compounds in soils, resulting in a higher crop yield and increasing the concentration of soluble P in soil, and the supply of this nutrient in plants showed better plant growth. Panhwar et al. (2014) isolated the PSB from acid sulfate soil of Kelantan, Malaysia. Three potential strains were selected and characterized as *Burkholderia thailandensis*, *Burkholderia seminalis*, and *Sphingomonas pituitosa* on the basis of their phosphate-solubilizing ability and other PGP traits. This study illustrated that isolates have the potential to reduce Al toxicity, fix nitrogen, solubilize phosphate, and promote rice growth in the acidic soil. These studies prove that the PGPR are not only capable of alleviating Al toxicity from the acidic soil but also promote the plant growth by its diverse PGP traits.

14.5 Conclusion

The use of PGPR in the different agroecosystems is well adapted and considered as a beneficial and sustainable technology for future agriculture. The PGPR have proved their ability as plant growth promoter in different adverse and contaminated agro-ecosystems. However, much attention is needed from different stakeholders associated with PGPR to promote this branch of study. The application of PGPR to alleviate Al toxicity would be a very promising and sustainable technology especially in the acidic agricultural and P-deficient soil.

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Rhizobacteria: Legendary Soil Guards in Abiotic Stress Management

15

Afreen Khan, R. Z. Sayyed, and Sonia Seifi

Abstract

All plants are continuously subjected to various types of biotic and abiotic stress factors from the time they have been planted in the field up to the time of harvesting, transport, storage, and consumption of the plant or plant-based products. These stresses result in the negative and deleterious effects on crop health and also cause enormous losses across the globe. To reduce the intensity of the losses produced by these stress factors, researchers all across the world are involved in inventing new management practices which may include traditional genetics methodology and various techniques of plant breeding. The use of microorganisms to mitigate both abiotic and biotic stress can provide an economical, eco-friendly solution to the problem of losses due to abiotic and biotic stresses. One such category of microorganisms is root-colonizing nonpathogenic bacteria like plant growth-promoting rhizobacteria (PGPR) which can increase the plant's resistance to biotic and abiotic stress factors. PGPR is the bacteria residing in the rhizosphere region and is involved in promoting plant growth and suppressing stress components. PGPR colonize the rhizosphere for nutrition which they acquire from plant root exudates. The mechanism by which plant growth-promoting rhizobacteria can accomplish the abovementioned task includes increment in plant growth by enrichment of soil nutrients through nitrogen fixation, solubilization of phosphates, production of metal ion chelators, and elevated

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production of plant growth-promoting hormones. The mechanism also focuses on elevated protection of the plants through influencing the levels of production of cellulases and β-1,3-glucanases which result in the activation of the defense mechanism of plants against pests and pathogens. PGPR also contains useful variation for making plant tolerant to abiotic stress factors like temperature extremes, pH variations, salinity and drought, and heavy metal and pesticide pollution. Enrichment of plant rhizosphere with such potential stress-tolerating PGPR is expected to provide enhanced plant growth and high yield of plant products in stress-affected areas. This chapter summarizes the research related to PGPR and its benefits and also throws light on the involvement of PGPR in abiotic stress management.

Keywords

Rhizobacteria · Stress tolerance · Salt stress · Drought stress · Pesticide stress · Heavy metal stress

15.1 Introduction

The major limiting factor for agricultural productivity is exposure of crops to various abiotic stresses. To survive the harmful external pressure induced by various environmental conditions, plants must modify their biological mechanisms; failure in the same results in reduced plant development and productivity. The indigenous microflora of any diverse environmental niche shows extensive metabolic capabilities to alleviate abiotic stresses observed in the environment to which they belong (Kumar et al. 2018). Various types of microbial interactions are observed with plants, and they are an essential segment of the ecosystem; hence, the natural microflora is believed to regulate the local and systemic reactions of plant defense mechanism which can definitely increase the chances of survival of the plant in stress-affected area (Meena et al. 2017). Productivity in principal crops is witnessing great reduction all over the world due to increased incidences of abiotic and biotic stresses (Grover et al. 2011). Plant resistance to these biotic and abiotic stress factors can be improved by inoculation with root-colonizing pathogenic bacteria which can be applied as biofertilizers and can enhance the effectiveness of phytoremediation. Inoculation of plants with nonpathogenic bacteria can also provide "bioprotection" against biotic stresses, and some root-colonizing bacteria can increase tolerance against abiotic stresses such as drought, salinity, and metal toxicity. Any disparity in nitrogen (N) cycling and nutritional status of the soil, the occurrence of phytopathogens, alteration in climatic conditions, and occurrence of abiotic stresses are the interwoven factors for a reduction in productivity of an agricultural field. However, the rapid increase in land degradation by numerous man-made activities leads to an estimated loss of 24 billion tons of fertile soil worldwide (FAO 2011).

The early 1990s experienced heightened interest in bacterial endophytes which further increased multiple times with results that provide confirmation to the fact that inoculation of plants with nonpathogenic rhizospheric bacteria induces positive changes in plant growth and productivity. Hence currently a mixed population of bacterial inoculants is commercially available for use as protection against biotic and abiotic stresses (Dimkpa et al. 2009a, b).

Plant growth-promoting rhizobacteria (PGPR) are associated with plant roots and hence have a major role in alleviating the effects of abiotic stresses such as drought, low temperature, salinity, metal toxicity, high temperatures, etc. on plants through various mechanisms like induced production of osmoprotectants and HSPs, i.e., heat shock proteins. During the crop production, microorganisms can be used as indicators of soil biodiversity and quality and can also contribute to reducing the effects of negative stress caused in plants by abiotic factors (Milosevic et al. 2012). A range of examples of stress tolerance mediated by PGPR can be found in the previous study; the modes of action remain less elaborative, as most of the studies and results are based on the lab-scale studies and do not replicate the same effects in the agricultural fields. Some of the bacterial strains which reduce the effects of abiotic stress are also shown to provide protection against stress induced by biotic factors. Thus for sustainable agricultural systems, bacterial inoculants which provide cross-protection against both biotic and abiotic stress factors will be extremely beneficial. Inoculation of agricultural fields with stress-tolerant PGPR would become more effective with detailed information about the concept of cross-protection. Hence this chapter highlights the benefits of colonization of plant rhizosphere with PGPR in increased agricultural productivity (Dimkpa et al. 2009a, b).

15.2 Beneficial Effects of Rhizobacteria

A major part of total organic carbon (approximately 85%) in the rhizosphere comes from sloughing of the root cells and tissues. Hence indigenous microflora of the rhizosphere alters their metabolic activities for obtaining the nutrients through the exudates. In this view, it is essential to study the bacterial motility during interaction with the plant. Microorganisms are the most diverse and elemental living system on earth. As an essential living component of the rhizosphere, they are an important component of the agricultural production systems. As natural inhabitants of seeds, microorganisms aid in the proliferation of the seeds and establishment of diverse symbiotic associations (Chakraborty et al. 2015). Natural inhabitants of the plant help in supporting the plant during nutrient acquisition, providing better resistance against various plant diseases and tolerating abiotic stresses. Intrinsic metabolic activities of the rhizospheric bacteria and potent genetic capabilities make them good candidates for fighting adverse environmental conditions (Singh 2016; Singh et al. 2016). Vivid evidence to essential attributes of the plant-microbial interactions can be provided by regulation of cellular, biochemical, and molecular mechanisms which are closely related to stress tolerance (Bakker et al. 2013). Microorganisms colonize the plant rhizosphere in high density. Hence rhizospheric soil which is influenced by root composition is highly enriched with amino acids, fatty acids, nucleotides, organic acids, phenols, and phytohormones. The highly enriched nutrient composition of the soil results in colonization of the rhizospheric soil with microflora such as bacteria, fungus, algae, and protozoa. The extent of colonization in rhizospheric soil is 10–100 times more than bulk soil. Among all the natural inhabitants of the rhizosphere, bacterial influence toward better plant productivity and elevated defense is most significant. Plant rhizobacteria can be categorized based on their proximity to the roots as (1) bacteria living rhizosphere, (2) bacteria colonizing the rhizoplane, (3) bacteria found in root tissues (endophytes) which also colonize spaces between cortical cells, and (4) bacteria living inside specialized root structures (nodules) which includes the legume-associated rhizobia and the woody plant-associated *Frankia* sp. Bacteria that belong to any of the abovementioned categories and are involved in plant growth promotion directly through nitrogen fixation, phosphate solubilization, iron chelation, etc. or are involved in indirect growth promotion through suppression of plant diseases and induced resistance toward abiotic stresses are referred to as plant growth-promoting rhizobacteria (PGPR) (Gopalakrishnan et al. 2015). Specificity of interactions between plant and rhizospheric bacteria is determined by soil composition and extent of root exudates available. Rhizospheric bacteria which have exhibited beneficial effects on plants include species of the genera *Bacillus*, *Enterobacter*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Serratia*, as well as *Streptomyces* species (Dimkpa et al. 2008, 2009a). The details of definite mechanisms of plant growth promotion remain largely elusive, as it is related to bacterial strains and most importantly is based on the different compounds released by the various rhizospheric microorganisms. The studies suggest that production of the primary plant growth-promoting hormones such as auxins, cytokinin, gibberellins, abscisic acid (ABA), and ethylene has a large share in the direct promotion of plant growth. These hormones can directly, or, in combination with other bacterial secondary metabolites, stimulate plant growth usually, in a concentration-dependent manner (Patten and Glick 2002).

Rhizobacteria can be elucidated as bacteria inhabiting the rhizosphere including bacteria colonizing the root proximities, and the rhizoplane (exo-root) also incorporates the bacteria that penetrate into the root cortex (endo-root). Most of the rhizospheric bacteria that have plant growth-promoting properties are endophytic in nature (Schmidt and Baldwin 2008). *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Serratia*, and *Streptomyces* are among the most predominant rhizospheric bacteria. Endophytes are found within the roots but are also observed in other parts of the plants such as stems, seeds, tubers, and unopened flowers. Endophytic PGPR can further be differentiated as extracellular endophytic PGPR (ePGPR) and intracellular endophytic PGPR (iPGPR). iPGPR can enter inside the plant cell and are able to produce specialized structures called nodules. ePGPR are prominently found in the rhizosphere or rhizoplane or within the apoplast but are never observed inside the plant cells. According to their vicinity to the roots, ePGPR can be further divided into (a) those colonizing root zone but are not in actual contact of the roots, (b) those colonizing rhizoplane, and (c) those living in the spaces between cortical cells of the roots (Dimkpa et al. 2009a, b).

15.2.1 Induced Systemic Resistance Versus Induced Systemic Tolerance

Various plant growth-promoting activities have been associated with PGPR which affects the plant growth and promotion directly and indirectly. Plant growth promotions through direct mechanisms involve enhanced release of phytohormones and mobilization of nutrients by the PGPR strains in the rhizospheric regions which can further be absorbed by host plant, thereby positively affecting their growth. Plant growth promotions through indirect mechanisms occur when rhizobacteria prevent the effect of phytopathogens (Kloepper et al. 2004).

Few strains of PGPR can also result in suppression of plant diseases caused by a variety of pathogens through production of physical and chemical changes associated with plant defense, and this process is called induced systemic resistance (ISR) (Lucas et al. 2014). Recent reports suggest that PGPR also play a role in increasing plant response to abiotic stresses such as drought, salinity, high and low temperature, etc. This phenomenon was termed as "Induced Systemic Tolerance" (IST) (Yang et al. 2016).

15.2.2 Mechanisms of Stress Tolerance Mediated by PGPR

The key to the adaptation and survival of crop-plant and associated rhizobacteria is the establishment of fruitful interactions between both the partners. Hence induced systemic tolerance (IST) is the term applied to explain the microbe-mediated induction of abiotic stress responses (Fig. 15.1). The role played by microorganisms to reduce the deleterious effects of abiotic stresses in plants has been the area of concern from the last few decades (Sharma et al. 2016; Sirari et al. 2016; Meena et.al. 2017).

Table 15.1 summarizes a few of the examples published on beneficial effects of bacteria on plants under various abiotic stress, bacteria involved in the interaction, and the plant species to which they are applied. Common adaptation mechanisms of plants exposed to environmental stress such as water and nutrient deficiency or toxicity due to heavy metal exposure generally include changes in root morphology. The process of change or any alteration in root morphology has major involvement of phytohormones such as auxin. Auxin particularly indoleacetic acid (IAA) are produced in the plant shoot region and are then transported downward to root tips, where they result in enhancement of cell elongation which results in better root growth. Auxins also result in the promotion of the lateral root initiation. The majority of rhizobacteria that exhibit a beneficial effect on plant growth have been shown to produce elevated levels of IAA. Hence inoculation of stress-affected plant species with such bacteria will result in better growth of roots and enhanced lateral root and root hair formation (Kajic et al. 2016; Damodara et al. 2018; Dimkpa et al. 2009a, b).

Promotion of root growth results in a larger root surface and can, therefore, have positive effects on water acquisition and nutrient uptake. In addition to all the

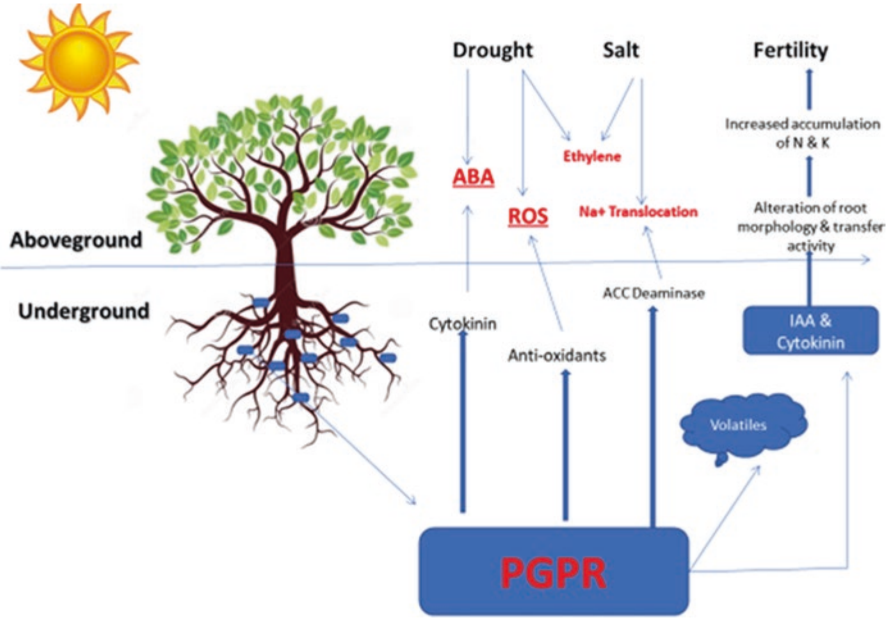

Fig. 15.1 Induced systemic tolerance (IST) elicited by PGPR against drought, salt, and fertility stresses underground (root) and aboveground (shoot)

abovementioned characteristics, rhizospheric bacteria contribute to the regulation of ACC deaminase activity which further helps in the survival and growth of crop plants under abiotic stress (Glick et al. 2007; Bargaz et al. 2015). Various mechanisms which aid in elevated tolerance against abiotic stresses will be further explained in detail.

15.3 Rhizobacteria-Mediated Salt Tolerance

Salinity is one of the most serious factors which limit the productivity of agricultural crops, with adverse effects on germination, plant vigor, and crop yield worldwide, more than 45 million hectares of irrigated land has been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in soil. High salinity affects plant in various ways which include water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, and reduction of cell division and expansion of genotoxicity.

All the vital processes such as photosynthesis, protein synthesis, and metabolic processes are majorly affected during the establishment of salt stress. During initial exposure to salinity, the first symptom which occurs is water stress experienced by crop plants which result in reduced leaf expansion. Stress due to increased salinity

Table 15.1 Beneficial effects of inoculation with selective PGPR on plant growth under abiotic stress conditions

Stress type	Bacterial inoculate	Plant species	Reference
Salt	*Azospirillum brasilense*	Pea (*Phaseolus vulgaris*)	Dardanelli et al. (2008)
Salt	*Pseudomonas syringae*	Maize (*Zea mays*)	Nadeem et al. (2007)
Salt	*P. fluorescens*	Groundnut(*Arachis hypogaea*)	Sarvana Kumar and Samiyappan (2007)
Salt	*Azospirillum*	Maize (*Z. mays*)	Hamdia et al. (2004)
Salt	*A. brasilense*	Chickpeas (*Cicer arietinum*), faba beans (*Vicia faba* L.)	Hamaoui et al. (2001)
Drought	Osmotolerant bacteria (not completely characterized)	Rice (*Oryza sativa*)	Yuwono et al. (2005)
Drought	*Achromobacter piechaudii*	Tomato (*L. esculentum*), pepper (*Capsicum annuum*)	Mayak et al. (2004b)
Drought	*Azospirillum*	Wheat (*T. aestivum*)	Cecilia et al. (2004)
Drought	*A. brasilense*	Maize (*Z. mays*)	Casanovas et al. (2002)
Temperature	*Burkholderia phytofirmans*	Grapevine (*Vitis vinifera*)	Barka et al. (2006)
Temperature	*B. phytofirmans*	Potato (*Solanum tuberosum*)	Bensalim et al. (1998)
Temperature	*Aeromonas hydrophila*, *Serratia liquefaciens*	Soy bean (*Glycine max*)	Zhang et al. (1997)
Nutrient deficiency	*Bacillus polymyxa*, *Mycobacterium phlei*	Maize (*Z. mays*)	Egamberdiyeva (2007)
Iron toxicity	*Bacillus subtilis*, *Bacillus megaterium*	Rice (*O. sativa*)	Asch and Padham (2005) and Terre et al. (2007)
	Bacillus sp.		

also results in an imbalance in osmotic pressure and further hampers cell expansion and cell division and also inhibits stomatal functioning.

With prolonged exposure to salt stress, plants experience ionic stress which further leads to early senescence of adult leaves and results in a reduction of leaf area available for photosynthesis for supporting continuous growth. Excess of Na$^+$ ions and Cl$^-$ can affect plant enzymes and leads to cell swelling, reduced energy production, and various other physiological changes. Various studies suggest that inoculation with rhizobacteria can mitigate the deleterious effects of salt stress in different plant species (Barassi et al. 2006). Many reports suggest that *Azospirillum*-inoculated seeds of lettuce (*Lactuca sativa*) showed elevated rates of germination leading to better vegetative growth than non-inoculated control plants when subjected to salinity stress (Asari 2015).

It is reported that sodium uptake remains unchanged when plants are inoculated with rhizospheric bacteria. Furthermore, the inhibition of photosynthesis was less dreadful in the plants inoculated with PGPR as compared to uninoculated variants under salinity stress (Hahm et al. 2017). For instance, tomato plants inoculated with *Achromobacter* species exhibit comparatively less serious effects of salinity stress. Though the exact mechanism remains elusive, it has been reported that other than regulation of bacterial deaminase, increased absorption of phosphates and potassium plays a key role in the management of salinity stress (Mayak et al. 2004a).

Most of the rhizospheric bacteria are the inhabitants of the root surface and are also observed in spaces between rhizodermal layers and root hairs, while few are found in rhizosphere without being in actual contact of the root system. Exudates of the roots and sloughed-off cells are enriched with flavonoids, phenolic compounds, and organic acids which play an essential role in inducing beneficial effects on stress-affected plants. PGPR contributes in growth promotion of stress-affected plants through elevated assimilation of nutrients, by regulating nitrogen fixation, and solubilization of phosphates and also controls plant pathogen through competition and antagonism (Ilangumaran and Smith 2018).

Regulation of abiotic stress can be achieved by inoculation with PGPR through direct and indirect mechanisms which further leads to the induction of systemic tolerance. Different species of PGPR have been explored for their abilities to improve plant water relations, ion homeostasis, and elevated photosynthesis efficiencies. Alleviation of stress is achieved by complex interactions between signaling events which occur during plant-microbial interactions (Smith et al. 2017). Colonization of the root surface and extracellular polysaccharide matrix by PGPR results in the formation of a protective barrier against salinity stress. Few extracellular molecules which act as signaling agents result in manipulation of phytohormone status of the crop plants. This leads to amplified root-to-shoot communication which results in the improvement of water and nutritional balance and stomatal conductance. When stimulation of osmolyte accumulation occurs, it may result in retarded leaf senescence which contributes to photosynthesis. Regulation of water potential and stomatal conductance is affected by hydraulic conductivity and rate of transpiration. For instance, few reports suggest that maize plants which were inoculated with *Bacillus megaterium* result in enhanced hydraulic conductivity compared to uninoculated plants when subjected to salt stress. Elevated hydraulic activity is shown to be connected with high expression of plasma-membrane protein—aquaporin. Rhizospheric bacteria results in the induction of enhanced osmolyte accumulation and signaling of phytohormones which contributes to the survival of the plants through initial salinity stress (Marulanda et al. 2010). PGPR restrict salt uptake of the plant by capturing cations in the exopolysaccharide matrix, resulting in alteration of root structure and further regulates expression of ion affinity transporters. The mineral nutrient acquisition of both micro- and macronutrients is enhanced due to inoculation with PGPR which mitigates the effects of the high influx of Na^+ and Cl^-. The maintenance of ion homeostasis is regulated by PGPR by reducing accumulation of Na^+ and Cl^- in leaves and other parts of the plants. PGPR also improves the activity of high-affinity K^+ transporters to alleviate salinity stress. The literature suggests that inoculation of stress-affected plants with *Azotobacter* strains results in elevated K^+ uptake and Na^+ exclusion leading to increased contents of chlorophyll, proline, and polyphenols which makes it evident that inoculation with PGPR enhances plant's response during stress (Rojas Tapias et al. 2012).

15.4 Rhizobacteria-Mediated Temperature Tolerance

Elevated temperature, a consequence of global climate change, also has an adverse effect on crop productivity. Heat stress results in a negative influence on photosynthetic rate, plant water relations, and flowering and fruiting in both tropical and temperate crops (Drigo et al. 2008). Increased water requirements and decreased yield in plants were reported due to a shift in maximum and minimum temperature. Extreme changes in temperature results in a stress condition for plants. For instance, root elongation normally takes place above species-dependent minimum temperature range and exhibits linear increase with increasing temperatures only up to specific temperatures above which the root elongation rapidly decreases resulting in stunted development of root system. The favorable effects of different PGPR strains on growth and physiological development of soybean plants under sub-optimal root zone temperatures were checked, and it was observed that stimulation of rhizobacteria is interactively dependent on the temperature of the rhizosphere. It has often been asserted that growth-promoting consequences are associated with nitrogen fixation, but the positive effects were observed and resulted in physiological changes in the plants even before the commencement of the nitrogen fixation process (Govindasamy et al. 2008).

This proves that mechanisms which function for the alleviation of temperature stress in rhizobacteria are independent of nitrogen status. The stimulation of genes in response to elevated temperature stress is regulated by heat stress transcription factors (Hsfs). Plant Hsfs have a highly composite gene family which consists of approximately more than 20 members, and the appearance of heat shock-induced Hsfs genes are reported to modulate transcription during the prolonged response to heat shock (Baniwal et al. 2004). Breeding of cultivars which are heat-tolerant or development of transgenic varieties for heat-tolerance is a time-consuming and less profitable approach (Vanaja et al. 2007). Hence an approach regarding inoculation of plants under temperature stress with rhizobacteria can be useful. Thermotolerant varieties of *Pseudomonas putida* according to Srivastava et al. (2012) are a result of overexpression of stress sigma factor σ^s and improved the formation of biofilm at high temperature. It was also demonstrated that heat shock proteins (HsPs) that stabilize the membrane are induced under stress condition and confer thermotolerance to rhizobacteria and thus the plant at elevated temperatures. A thermotolerant strain of *Pseudomonas* spp. (AKM-P6) exhibiting PGPR activities was identified by Ali et al. (2009) from the rhizosphere of pigeon pea grown under arid and semi-arid zones in India. The abovementioned strains of *Pseudomonas* sp. help sorghum seedlings to cope up with heat stress through induced biosynthesis of high-molecular-weight proteins in higher levels which results in reduced injuries to cellular membranes and enhanced contents of metabolites such as proline, chlorophyll, sugars, amino acids, and proteins. This thermotolerance indicated by *Pseudomonas* sp. AKM-P6 is predicted to be due to the production of exopolysaccharides.

Bensalim et al. (1998) also investigated the heat stress-alleviating effects of *Burkholderia phytofirmans* PsJN on 18 clones of potato plants grown under different temperature zones (20 °C day, 15 °C night, 33 °C day, 25 °C night). Results were estimated from accurate measurements of stem length, shoot, and root biomass. The abovementioned parameters of plants inoculated with high temperature-tolerant varieties suggest that colonization of the potato plants with thermotolerant strains of rhizobacteria plays a vital role in their adaptation to heat. It was found that tuberization was improved by as much as 63% in rhizobacteria-treated clones of potato. One more report suggests that inoculation of grapevine (*Vitis vinifera*) with the strains of *Burkholderia phytofirmans* PsJN results in lowering the rate of biomass reduction and leakage of osmolyte which are prominent indicators of cell membrane injury due to heat shock.

Abiotic stresses result in a range of complex stimuli that possess many different yet altered attributes, and every single stimulus provides plant cell with a different array of information. For example, stress due to low temperatures results in mechanical constraints, changes in macromolecular activity, and diminished osmotic potential in the cell. Cold stress affects the growth and development of crop plants in an unfavorable way and thereby results in reduced expression of the full genetic potential of plants by limiting metabolic receptions and proper water uptake. Membranes rigidification is one of the many ways through which plants identify chilling stress caused due to reduced fluidity of the cellular membrane (Chinnusamy et al. 2005).

Membrane rigidification results in the induction of cold-responsive (COR) genes. Expression of COR genes initiates activation of expression of CBF3, CBF 2, and CBF 1 (C-repeat binding factors) during cold acclimation which regulates singling cascade required for alleviation of cold shock. The ability of plants to cope up with the chilling stress can be enhanced, upon exposure to low but nonfreezing temperatures intermittently. Among other physiological changes induced due to cold stress is elevated contents of sugar, proline, and anthocyanin which can be observed during cold acclimation or hardening procedures. This can be confirmed by studies which report that grapevine plants inoculated with rhizobacteria (*Burkholderia phytofirmans*) accumulated marginally higher amounts of carbohydrates as compared to control plants which were uninoculated variants. In addition, plants also displayed increased levels of proline and phenols, photosynthetic rates, and deposition of starch (Barka et al. 2006). Such physiological changes are also representative indicators for ISR, and hence it is proposed that rhizobacteria-mediated tolerance to cold temperatures stress is emphatically correlated with the induction with ISR.

15.5 Rhizobacteria-Mediated Drought Tolerance

Dehydration and reduced availability of cellular water represent a common stress challenge which plants encounter under drought, salt, and cold conditions. As water is one of the most essential factors which affects the growth and survival of microorganisms. And hence water deficit is an essential abiotic factor that influences the agricultural productivity with high intensity and affects plant development-related aspects such as a decreased rate of photosynthesis and reduction in available leaf area due to premature leaf senescence. Water deficiency leads to drought stress which limits crop growth and productivity, especially in arid and semiarid regions (Hassen et al. 2016). Rhizospheric bacteria utilize different mechanisms to alleviate the effects of drought stress on the plant (Table 15.2).

Groover et al. (2001) have investigated some of the mechanisms which include (1) alleviation of soil drought impact through the production of exopolysaccharides, (2) induction of resistance genes, (3) increased circulation of water in plants, and (4) synthesis of ACC deaminase, indoleacetic acid, and proline. PGPR are involved in mitigating the impact of drought on plants through a process so-called induced systemic tolerance (IST) which includes (a) cytokinin production, (b) production of antioxidants, and (c) degradation of ethylene precursor ACC by bacterial ACC deaminase (Milosevic et al. 2012).

Drought stress also results in activation of a large army of genes which are often referred to as "stress genes." Most of the genes which are activated in response to drought stress are also responsive to other abiotic stresses such as salinity stress or chilling stress. For instance, RD 29A rhizobacteria have been shown to result in modification of the root sensitivity, growth of leaves, and also increased tolerance to soil trying evidently by influencing ethylene signaling pathway (Rubin et al. 2017). The ACC deaminase activity of *Achromobacter piechaudii* has been reported to provide tolerance against water deficit in tomato and pepper plants, resulting in a marginal improvement in fresh and dry weights of the stress-affected plants. Ethylene production was significantly reduced in the plants which were inoculated with tolerant PGPR strains. It also results in improved recovery from water-deficient soils although inoculation did not influence relative water contents at significant levels (Mayak et al. 2004a, b).

Table 15.2 Effects of rhizobacteria on mitigation of drought stress in crops

Microorganism	Crop	Mechanism
Pantoea agglomerans	Wheat	EPS production which affects the structure of rhizospheric soil
Rhizobium sp.	Sunflower	Production of EPS which affects the structure of rhizospheric soil
Pseudomonas putida P45	Sunflower	Production of EPS which affects the structure of rhizospheric soil
Azospirillum sp.	Wheat	Increased water circulation
Achromobacter piechaudii	Tomato pepper	Synthesis of ACC deaminase
ARV8		
Variovorax paradoxus	Pea	Regulation of ACC deaminase
Pseudomonas sp.	Pea	Reduced ethylene production
AM fungi	Sorghum	Enhanced water circulation
Brome mosaic virus (BMV)	Rice	Unknown
Pseudomonas mendocina and *Glomus intraradices*	Lettuce	Increased antioxidative status
Bacillus megaterium and *Glomus* sp.	Clover	Production of indoleacetic acid and proline

On exposure to drought tolerance, maize seedlings inoculated with *Azospirillum brasilense* displayed enhanced relative and absolute water contents in comparison to non-inoculated plants. Inoculation with *Azospirillum* also results in prevention of significant drop in water potential which is closely interlinked with root growth, total aerial biomass, and foliar area and is also associated with proline accumulation in leaves and roots. The effects of drought tolerance were more evident at a 75% reduction in the water supply as compared to a 50% reduction. Thus, these results suggest that PGPR play a key role in providing resistance and increasing adaptation of plants to drought stress and have a vital role in solving future food deficiency problems. It is also reported that interaction between plants and rhizobacteria under drought stress affects plants as well as leads to positively change the soil properties. The mechanisms elicited by rhizobacteria such as triggering osmotic response and induction of novel genes play a vital role in the survival of plants under drought stress. The development of drought-tolerant crop varieties through genetic engineering and plant breeding is essential, but it is a time-consuming process. PGPR inoculation to alleviate drought stress in plants opens a new chapter in the application of microorganisms in dryland agriculture (Varukonda et al. 2016).

15.6 Rhizobacteria-Mediated Pesticide Tolerance

Pesticide accumulation in soils beyond the recommended safety levels occurs either by repeated application or due to their gradual degradation rate. The effect of pesticide on plant growth occurs by an alteration in plant root's architecture. This results in the appearance of a number of root sites for infection by rhizobacteria and the transformation of ammonia into nitrates. This process of the transformation of microbial compounds to plants is made easier by the rhizobacterial infection. With the abovementioned changes in plant growth and development, the activity of free-living or symbiotic nitrogen-fixing bacteria has also been positively affected through rhizobacterial infection (Gopalakrishnan et al. 2015). Various strains of rhizobacteria have the displayed ability of pesticide degradation due to the activation of degradative genes carried by plasmids or flanked by transposons/chromosomes (Kumar et al. 1996). From the studies it was suggested that very few strains of rhizobacteria have the ability to tolerate pesticide stress under actual field conditions, and hence research on isolation, identification, and characterization of such pesticide-tolerant species of rhizobacteria needs to be pursued in detail as such rhizobacteria are essentially required in present-day conditions of ever-growing pesticide contamination in fields and considering the magnitude of pesticide residue generated.

15.7 Rhizobacteria and Heavy Metal Resistance

Various industrial operations discharge multiple types of heavy metals and upon consequent accumulation in ecological systems create a massive threat to the varied agroecosystems. When heavy metals like arsenic, mercury, cadmium, and lead which are highly toxic to plants accumulate into the soil to abnormal levels, it causes a dramatic change in microbial composition and their activities (Cheung and Gu 2007) which leads to a consequent loss in soil fertility. Once the cytosolic metal concentration in plants increases above the tolerable limit, phytotoxicity of heavy metal inhibits transpiration and photosynthesis, disturbs carbohydrate metabolism, and drives the secondary stresses like nutrition stress and oxidative stress which collectively affects the plant's growth and development (Kraemer and Clemens 2005).

For differentiation between a standard and stress response against heavy metal contamination, it is essential to characterize the minimum and maximum concentration of every metal for different varieties of soil (Carmen and Roberto 2011). Responses of rhizobacteria toward some of these heavy metals have been well-documented. Many rhizospheric bacteria release metal-chelating substances in rhizosphere such as iron-chelating siderophores. Siderophore production by bacteria has been shown to significantly affect plant uptake of metals. Hence rhizobacteria can positively affect the bioavailability of heavy metals that can prove to be extremely toxic to plants even in low concentrations (Dimkpa et al. 2009a, b). Due to variation in soil conditions, metal valences are also affected, which can be correlated to microorganism to be specific rhizobacteria which also alter the metal bioavailability by acidifying the microenvironment and by significantly affecting redox potential. Autotrophic and heterotrophic leaching of heavy metals which results in enhanced volatilization through methylation process and release of metal chelators such as siderophores can help in the mobilization of heavy metals. This way, sorption of heavy metals to cell components is essentially the result of intracellular sequestration or precipitation as insoluble organic compounds which reduce heavy metal toxicity to plants (Gadd 2004).

Barley plants which were grown on contaminated soil with high contamination of cadmium obtained 120% higher grain yield and a twofold decrease in Cd contents in grain when the plants were inoculated with commercially available PGPR *Klebsiella mobilize* CIAM 880. Stimulation of these effects was studied with a mathematical model which indicates migration of rhizobacteria from rhizoplane to rhizosphere where they form a complex with the heavy metal, making it nonavailable for the plant uptake (Pishchik et al. 2002). High intracellular carbohydrates and large cell inclusions increase the resistance of *Rhizobium leguminosarum* to cadmium, copper, nickel, and zinc, whereas production of those has also been shown to counter heavy metal-induced oxidation. In *Rhizobium*-legume symbiosis, it is usually the plant that is the limiting factor regarding tolerance to metal toxicity for metals such as aluminum, copper, iron, and cadmium. Nodules help plants survive because bacteroids counter metal stress (Balestrasse et al. 2001).

15.8 Conclusion and Future Perspective

In the present-day scenario when we are experiencing the threat of global warming, the agricultural production methodology should be designed by considering the ever-changing environmental conditions and the availability of different types of stresses. Plant growth-promoting rhizobacteria can be utilized to mitigate the biotic stresses and can confer elevated tolerance to abiotic stresses in the host plant (Tabassum et al. 2017). Thus, identification and detailed analysis of rhizobacterial strains that have the capabilities of providing cross-protection against multiple stress factors will be highly important (Dimkpa et al. 2009a, b). Induced systemic response (ISR) in the crop plants may be critically important for the ability of rhizobacteria to alleviate the effects of both biotic and abiotic stress. Thus, the information obtained from a detailed analysis of ISR against plant pathogens will be applicable in understanding signaling cascades induced by PGPR which results in elevated tolerance to abiotic stresses. The rhizosphere is a unique environmental niche which provides habitats and nutrients to rhizospheric bacteria which in return provides numerous benefits of better plant growth, defense against infections against phytopathogens, and survival of plants under different types of stress.

However, the amount of success in obtaining the benefits of PGPR tends to decrease as it moves from laboratory experiments to the greenhouse and finally to fields, which suggests that there is need of research on the various aspects of PGPR under field conditions. Therefore, generation of research data and knowledge on screening protocols and strain improvement of ideal rhizobacterial strain for rhizospheric competence and sustainability is the current need to enhance field level successes (Gopalakrishna et al. 2015). The application of PGPR to help plants cope up with the stress in the agricultural field seems laborious, yet a lot is left to be utilized (Ilangumaran and Smith 2018). As various types of abiotic stresses are serious threats to total crop yield worldwide, agricultural experts are working to find quicker and reliable solutions as annual crop production is seriously affected by higher degree from abiotic stresses. Hence at the moment, expanding the geographical area, finding new strategies for breeding for abiotic stress tolerance, and detailed analysis of rhizobacteria-mediated alleviation of abiotic stresses are essential areas of focus. Among all of this, PGPR-mediated abiotic stress management has gained enormous popularity and has attracted a lot of interest as it has the ability to serve the purpose in an economical manner. This way, indigenous microbes should be provided with prime importance for the successful achievement of the task as they have better acclimation ability over an imported strain (Sarma et al. 2012).

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Rhizobacteria–Plant Interaction, Alleviation of Abiotic Stresses

16

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Abstract

At the present scenario, climate change became the potential threat to growers with rise in temperature, inconsistent rainfall, and salinization of agricultural land. However, the microbes more specifically plant growth-promoting rhizobacteria (PGPR) play a significant role to mitigate the abiotic stresses. Rhizobacteria act as bioprotectants against drought, salt, heavy metals, high temperature, and cold stress. During drought condition, PGPR intensifies osmolytes (proline, glycine, betaine) and acts as an osmoprotectant. The drought-related enzyme ACC deaminases were regulated by the PGPR, which also regulates the stomatal physiology during the water deficit conditions. The salt stress in plants was also a complex process to understand. During salt stress condition, PGPR acts as an activator of antioxidant enzymes and polyamines and also acts as a modulator of abscisic acid. Inoculation of PGPR affects the expression of 14 genes (four upregulated and two downregulated) related to salt stress. The effect of heavy metal toxicity is also found in plants, which is due to the improper fertilizer applications, industrial waste, sludge, etc. The main site for accumulation of heavy metals is the root nodule. At present many PGPR sp., i.e., *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Enterobacter* sp., and *Rhizobium* sp., were proposed to speed up the phytoremediation process of nodules. Bacterial metallothioneins (MTs) of the family Bmt, a family with low-molecular proteins, play a significant role to absorb heavy metals. High temperature also acts as a constraint of normal plant root nodulation and rhizobial growth. The strains of PGPRs evolve during the heat stress period against the raised temperature with the production of extra LPS, EPS, and special class of proteins, i.e., heat shock proteins

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(HSPs). Cold tolerance can also be derived by PGPR as the accumulation of more carbohydrate, regulation of stress-related genes for osmolytes expression, and enhancement of specific protein synthesis, which helps plant to fight against cold stress.

Keywords
Bioprotectant · Polyamines · Phytoremediation · Transpiration · Heat shock protein

16.1 Introduction

Crop plants were suffering from various diseases, which may be due to a number of biotic and abiotic factors. The environmental condition, which is favorable for pathogen, leads to disease in the presence of susceptible host, whereas adverse environmental conditions such as drought, salt, temperature stresses, and metal toxicity to plants again affects the plant physiology. Drought, salt, and temperature stresses are major abiotic environmental factors that reduce the plant population in a particular area, limit economic yield in agriculture, and threaten food security. Climate change plays a greater role to induce abiotic and biotic stress (Fedoroff et al. 2010). Many plant breeding programs were carried on to improve the traits by which crop plants are able to fight against the abiotic stresses. These breeding programs may be very effective in the field of abiotic stress management, but these programs are more cumbersome and require the best breeding materials that could adjust with the changing environments. To overcome these problems, the beneficial microbes provide an easy, quick, and eco-friendly option for researchers to cope with the abiotic as well as biotic stresses. Among microbial population in soil, bacterial attendance is more than any other microbes, containing 10^8 cells of bacteria per gram of soil (Raynaud and Nunan 2014). Plant utilizes beneficial bacteria against other harmful microbes (biotic stress) as well as abiotic stress. They release an organic compound that improves the crop plant fitness against stress by improving plant physiology (Lynch and Whipps 1990; Barriuso et al. 2008). Isolation and inoculation of these beneficial bacteria to plants gave significant results toward the mitigation of abiotic stress (Lugtenberg et al. 2013). Recently "omics" technologies reveal the significance of the plant growth-promoting rhizobacteria (PGPR) with their mechanism of action against the stresses. PGPR became boon especially for the farmers, so they can mitigate the loss by getting benefits against abiotic stress like salt stress, drought stress, and nutrient-heavy metal toxicity (Egamberdiyeva and Islam 2008; Zahir et al. 2008; Sheng 2005).

16.2 Rhizobacteria-Mediated Abiotic Stress Tolerance in Plants

16.2.1 Tolerance to Drought Stress

During drought stress, the status of water content in the tissues of plant cell reduces rapidly, and the level of water in the leaves, i.e., leaf water potential, also decreases during the daytime. Other physiological parameters like stomatal conductance, the rate of transpiration, and osmotic adjustment are disturbed due to drought stress in plants. In the condition of severe drought, Rubisco level decreases during photosynthesis (Lawlor and Cornic 2002; Song et al. 2009; Bota et al. 2004). It is positively correlated with the relative water content and also affects the activation of inhibitors such as CO_2 and Mg^{2+} (Parry et al. 2002).

Maintenance of water potential can be regulated by rhizobacteria in the plants that intensify the production process of osmolytes during drought condition (Farooq et al. 2009). Rhizobacteria also produce glycine betaine osmo-tolerant compound that also helps in the management of plant against drought. These osmo-tolerant compounds also increase by the production of IAA and are found to be stimulated by rhizobacteria (Yuwono et al. 2005).

Several strains and species of *Pseudomonas*, viz., *P. entomophila*, *P. stutzeri*, *P. putida*, *P. syringae*, and *P. montelli*, ameliorated drought stress in maize crop (Sandhya et al., 2010). The harmone which releases durin drought stess found to down regulate by PGPR through producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase. ACC deaminase degrades the ACC, which is a primary precursor of ethylene and reduces ethylene negative effect under drought condition (Glick 2014). The effect of ACC deaminase is also seen on strawberry plant inoculated with the N_2-fixing and P-solubilizing bacteria. It has shown that strawberry plants downregulate the MDA (malondialdehyde) and H_2O_2 content, which may contribute to the activation of physiological and biochemical processes involved in the alleviation of the effect of drought stress (Erdogan et al. 2016). During stress the parts of the plant by which the water can be a lost is stomata and the regulation for stomatal water loss also seen to be less in the plants where PGPR inoculation was made than non-inoculated plants. These plants showed the increase in K content, depressing stomatal conductance and regulating proline accumulation. This type of stomatal regulation mechanism was observed in autochthonous bacterial strains of *Bacillus megaterium*, *Enterobacter* sp., *Bacillus thuringiensis*, and *Bacillus* sp. that retard water stress in *Lavandula* and *Salvia* (Armada et al. 2015). In another experiment which was conducted on foxtail millet (drought-tolerant crop), inoculated with bacterial strains *P. fluorescens* DR7 producing high level activity of ACC deaminase and EPS (exopolysaccharide) which stimulated seed germination and seedling growth under drought stress (Niu et al. 2018).

16.2.2 Tolerance to Salt Stress

The responses of the plant to soil salinity is a complex process due to salt stress; approximately 20% of irrigated land and crop yield reduce significantly (Qadir et al. 2014). When soil salinity increases, the plant comes under the osmotic stresses that lead to reduction in root pressure, i.e., reduction of the water absorption capacity of roots, and accelerated water losses from leaves. The nutrient imbalance during the salt stress induces the defense in the form of reactive oxygen species (ROS) (Munns and Tester 2008; Rahnama et al. 2010; Munns 2005), but in severe soil salinity condition, the plant cannot detoxify itself from ROS. Other ill effects of salt stress also seen in the reduction of antioxidant activity of enzymes decreased photosynthetic activity, etc. (Rahneshan et al. 2018). The eco-friendly and sustainable mechanism to cope with the salt stress is through microbial inoculation especially the PGPRs, which play a great role. The PGPR–plant interactions show several mechanisms and interactions like physiological and molecular mechanisms used for salt tolerance to crop were ion homeostasis of Na^+, synthesis of osmoprotectant by plants (proline, glycine betaine, sugar, and polyols), activation of antioxidant enzymes (SOD, CAT, GPX, and APX), polyamine synthesis (diamine putrescine, triamine spermidine, and tetra-amine spermine), and abscisic acid modulation (Hasegawa 2013, Tahir et al. 2012; Saxena et al. 2013; Gill et al. 2013; Shu et al. 2012; Keskin et al. 2010).

During plant–microbe interaction, some complex network of signaling events that occurred leads to ion homeostasis and an increase of photosynthetic efficiency in plants (Smith et al. 2017). Due to salt imbalance, PGPR induces aggregation of phytohormone, which leads to overcoming the salt stress in plants. The salt-tolerant capacity of *Bacillus amyloliquefaciens* SN13 was used against the salt stress by Nautiyal et al. (2013). Bacterial strain *Bacillus amyloliquefaciens* SN13 inoculated to the rice plant and exposed to salinity condition of NaCl 200 Mm increased the salt tolerance and plant growth. The effect of *Bacillus amyloliquefaciens* SN13 was also analyzed at the molecular level and found 14 genes correlation with salt stress. Among the 14 genes, 5 genes NADP-Me2-NADP-malic enzyme (NADP-Me2), ethylene-responsive element binding proteins (EREBP), salt overly sensitive 1 (SOS1), BADH, and somatic embryogenesis receptor-like kinase 1 (SERK1) were upregulated, and 2 genes glucose insensitive growth (GIG) and serine-threonine protein kinase (SAPK4) were downregulated. Besides salt stress, SN13 inoculation also minimizes the osmotic and ionic stress response. PGPR stimulate the osmoprotectants, i.e., proline, trehalose, and glycine betaine with a quick response when the plant was under salt stress. PGPR have been also known for the ion balancer in the cells of roots; it balances the influx of Na^+ and Cl^- ions. PGPR help to maintain ion homeostasis and high K^+/Na^+ ratios in shoots when Na^+ and Cl^- accumulate in the leaves; it leads to acceleration of Na^+ exclusion from roots, boosting the activity of high-affinity K^+ transporters. Bacteria modulated plant hormone status by releasing exogenous hormones, metabolites, and enzymes that may contribute to enhance salt tolerance.

16.2.3 Tolerance to Heavy Metal Stress

In modern agriculture, field soils of a major part of the world became contaminated by heavy metals due to unjudicious use of agrochemicals such as high doses of N, P, K fertilizers, insecticides, and fungicides; improper irrigation source; industrial waste; etc. The heavy metal toxicity misbalanced the ecosystem affecting all living and nonliving entity. The major contaminants with heavy toxic effect to the soil as well as crops are Cd, Cu, Zn, Ni, Co, Cr, Pb, and As (Passariello et al. 2002). Due to this heavy metal toxicity, plants generate the reactive oxygen species (ROS), and another most deleterious effect on plants due to heavy metal is lipid peroxidation that can directly cause biomembrane deterioration.

Use of microorganisms such as *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Enterobacter* sp., and *Rhizobium* sp. speeds up the phytoremediation process, which had been reviewed in detail by Ma et al. (2011). The phytoremediation process was boosted with the use of PGPR. The bioremediation of heavy metal can be easily done when root nodule fixes the residual metals with the help of PGPR. The growth, abundance, and nodulation ability of PGPR were adversely influenced by heavy metal contaminant present in the soil. Due to this metal toxicity condition, some gene alteration also occurs, which favors in symbiosis most probably in N_2 fixation (Vasilica et al. 2011).

The soil microbes which is beneficial for plant growth and promotion, i.e., PGPRs, symbiosis with the plants that were grown in the high metal toxicity during their molecular characterization shows the variation in their genes related to nodulation. Metallothioneins (MTs) in bacteria designated as bacterial MTs of the family Bmt are low-molecular, metal-binding protein evolved during the stress condition (Huckle et al. 1993). Recombinant bacterial strain with metallothionein was found helpful for plants to bind heavy metal from the soil and acts as a free radical scavenger (Ehsanpour et al. 2012). The enhancement of bioremediation process in metal-infested soil by plants treated with a strain of PGPRs having bacterial Mts found a positive result for the removal of heavy metals such as Cd^+ and Hg^{2+} (Sriprang et al. 2002; Murthy et al. 2011).

16.2.4 Tolerance to High Temperature

The effect of high temperature on the growth of the plant is well known: when the temperature rises, the water losses increase due to transpiration. When transpiration lasted for a prolonged condition, then the wilting of plants and ultimately death of plant occur. Acceleration in temperature impairs the nodulation process in the plants, ultimately N_2 fixation is affects and results in less plant growth, like alfalfa plant observed in desert condition, which shows less nodulation up to 5 cm of soil horizon, but extensive nodulation was observed after 5 cm of soil horizon (Munns et al. 1979).

PGPRs again play a significant role in reducing the heat stress in plant; it has the osmolyte production and carbon flux reduction property that protect the plant against the heat stress (Canarini and Dijkstra 2015). Normally the PGPR community grows in the temperature ranging 26–31 °C and is unable to grow above 37 °C, but Eida et al. (2018) isolated some microbial community from rhizosphere, which can withstand over 45 °C. Temperature range beyond the normal, i.e., 45 °C *Pseudomonas putida strain* AK MP7 was found beneficial when used in heat stress condition in wheat (Ali et al. 2011). The PGPR *Pseudomonas aeruginosa* (strain 2 CpS1), when applied as a seed treatment in wheat, was found significant during elevated heat condition (Meena et al. 2015). PGPR also secretes some polysaccharides that formed a biofilm around the root nodules, which increases the water retention capacity to many folds. The regulation of heat stress in plants is a knotty process, and the stains of PGPR that evolve during the heat stress condition play a great role, as these strains have the ability to secrete proteins, lipopolysaccharide (LPS), and exopolysaccharides (EPS) to combat with the problem of increased temperature. There were various *Rhizobium* strains which have the ability to withstand during the heat stress and also have the ability of rapid N_2 fixation has been identified; when these *Rhizobium* strains are exposed to different temperature, i.e., 30 and 40 °C, the changes in extracellular polymeric substances (EPS), LPS, and proteins are observed (Nandal et al. 2005).

Later researchers observed the exclusive class of proteins that were produced by cells in response to a stressful condition, which were called heat shock proteins (HSPs). Some HSPs are seen to be immediately involved in de novo protein biogenesis related to heat stress. These types of proteins are termed as molecular chaperons (Craig et al. 1993). These chaperons help in regaining the shape of heat shock proteins, which were denatured during the heat stress so that it can work efficiently (Hartl and Hayer-Hartl 2009). The chaperon analysis in 53 stains of *Mesorhizobium* sp. for heat stress shows increased transcripts of dnaK and groESL genes, which were related to heat stress (Alexandre and Oliveira 2011).

16.2.5 Tolerance to Cold

Low temperature also causes stresses to plant health and plays an important role in the geographical distribution of several plant species and significantly affects the yield of the most valuable crop (Theocharis et al. 2012). For improving plant tolerance to cold, plants undergo several changes to its physiological and molecular modification resulting in the process of acclimatization against cold. Modification includes accumulation of carbohydrate, osmolytes production, specific protein synthesis, and expression of stress-related genes (Ruelland et al. 2009). For survival against cold stress, enhancement of chilling resistance by the use of beneficial bodies has been reported as a new solution to induce plant defense (Theocharis et al. 2011).

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Role of Rhizobacteria in Drought Tolerance

17

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Abstract

Drought is the most destructive abiotic stress affecting the world's food security. Rhizospheric and endophytic bacteria produce range of enzymes and metabolites, which help the plants to tolerate abiotic stress. Induced systemic resistance gets developed in plants surviving in drought conditions. Drought tolerance is induced in crops due to the production of exopolysaccharides, phytohormones like gibberellic acid, cytokinins, abscisic acid, and IAA, ACC deaminase, antioxidants, osmolytes, and volatile compounds. Plants in drought conditions survive due to rhizobacteria enhancing photosynthetic activity. PGPR improves the growth, antioxidant activity, and photosynthetic activity of the crops in drought conditions. Rhizobacteria assist in resource attainment, i.e., nitrogen, phosphorus, and essential minerals by changing the root morphology, improving the soil structure, and bioremediation of the polluted soils.

Keywords

Exopolysaccharides · Phytohormones · Antioxidant · Indole-3-acetic acid · Bioremediation

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17.1 Introduction

Drought is the critical abiotic stress affecting food security. Drought stress restricts the crop yields (Bottner et al. 1995) and can cause serious plant growth problems (Kasim et al. 2013). Plants are affected by various environmental stresses. In environment plants suffered from biotic and abiotic stress. Biotic stress is induced by microorganisms, insects, as well as higher animals and abiotic stress, including water logging, heat, drought, cold, wind, and intense light (Wahid et al. 2007). Drought limits plant productivity, disturbs water relation, and also reduces water use efficiency in plants. Crop production reduces by 9–10% due to drought stress (Lesk et al. 2016). The effects of stress range from morphological to molecular levels. Plants have two strategies to cope with drought condition, i.e., drought avoidance and dehydration tolerance (Blum 2005). Microorganisms play a vital role in the survival of crops under drought conditions. Use of rhizobacteria to induce abiotic stress tolerance can help to control stress in many plants and crops (Rejeb et al. 2014). *Paenibacillus polymyxa* was first reported to alleviate drought stress in *Arabidopsis thaliana* (Timmusk et al. 2005). There is a report on the efficacy of *Pseudomonas fluorescens* strains in enhancing drought-tolerant traits in terms of chlorophyll content, photosynthesis activity, and oxidative stress (Sudhakar et al. 2013). The drought stress can be overcome by developing drought-resistant varieties, changing crop calendars, resource management, conventional breeding, and genetically improved drought-resistant plants. Current studies show that rhizobacteria enable plants to overcome drought stress. Due to water stress, plants show morphophysiological changes, namely, effects on carbon metabolism, water relations, and hormone production that regulate plant growth (Wilkinson and Davies 2010).

Rhizobacteria play a very important role in drought stress tolerance in plants. The rhizobacteria-mediated plant tolerance to drought stress is shown in Table 17.1.

17.2 The Relation Between Drought Stress and Growth of the Plant

Drought has an impact on plant-water potential and turgor (Hsiao 2000), which changes the morphological and physiological characteristics in plants (Rahdari et al. 2012). Growth was found to be affected due to drought stress in crops, namely, maize (Kamara et al. 2003), barley (Samarah 2005), rice (Lafitte et al. 2007), and

Table 17.1 Rhizobacteria-mediated plant tolerance to drought stress

Rhizobacteria	Plant species	References
Achromobacter piechaudii	Tomato (*Solanum lycopersicum*)	Mayak et al. (2004)
	Black pepper (*Piper nigrum*)	Mayak et al. (2004)
Azospirillum sp.	Wheat (*Triticum aestivum*)	Creus et al. (2005)
A. brasilense	Maize (*Zea mays*)	Casanovas et al. (2002)
A. brasilense	Common bean (*Phaseolus vulgaris*)	German et al. (2000)

wheat (Rampino et al. 2006). Growth parameters like water content and fresh weight are found to be affected due to stress imposed by drought condition (Jaleel et al. 2009). This pressure limits the diffusion of nutrients and mass flow of nutrients, which are soluble in water (Selvakumar et al. 2012). Crops grown in water scarce conditions have low chlorophyll content (Rahdari et al. 2012). Drought decreases the yield of the plants and various crops. The mechanisms for drought tolerance include the synthesis of exopolysaccharides, volatile compounds, osmolytes, antioxidants, phytohormones, and 1-aminocyclopropane-1-carboxylate.

17.3 Effect of Exopolysaccharide on Drought Tolerance in Plants

Exopolysaccharides are hydrophilic in nature, which provides protection to rhizobacteria under drought stress. EPS binds with the water in the soil, and due to this, the soil dries more slowly and also protects the bacteria from water potential fluctuations in drought conditions (Hepper 1975).

17.4 Drought Resistance in Plants Mediated by Plant Growth Hormones Produced by Rhizobacteria

Plant growth hormone indole-3-acetic acid (IAA) helps in the commencement of lateral and adventitious roots and elongation of stems and roots of the plants (Glick 1995). IAA helps plants to survive under extreme stress conditions. Plants inoculated with rhizobacteria producing IAA increase root and root hair formation, which in turn increases nutrient and water uptake by the plants. This helps the plants to overcome problem of water deficit (Egamberdieva and Kucharova 2009). Plants having a good root system sustain drought stress better than the plants having fewer roots. This is because the roots assist plants for uptaking water from the soil profile (Gowda et al. 2011). PGPR-treated clover (*Trifolium repens* L.) plants showed more shoot and root biomass and water content under drought stress. This increase was correlated with the production of IAA elicited through the application of PGPR (Marulanda et al. 2009). Also, a study has shown that *Arabidopsis* plants inoculated with *Phyllobacterium brassicacearum* strain STM196 resulted in improved lateral root length and modifications of the root architecture that led to the significant drought tolerance (Bresson et al. 2014).

Abscisic acid (ABA) has a vital role in many physiological responses in plants. It is important for drought stress tolerance (Cohen et al. 2015). Plant organs have increased production of ABA contents under drought stress, which improves plant growth (Farooq et al. 2009). Rhizobacteria increase ABA concentrations in plants to minimize drought stress conditions (Arkhipova et al. 2007). The ABA enhances the drought tolerance in plants and crops by leaf transpiration regulation, root hydraulic conductivity (Aroca et al. 2006), and aquaporins (Zhou et al. 2012).

17.5 Drought Tolerance in Plants Due to Rhizobacterial 1-Aminocyclopropane-1-Carboxylate Synthase

Ethylene is one of the plant regulators. Biosynthesis of ethylene is dependent on biotic and abiotic stresses (Hardoim et al. 2008). Ethylene precursor 1-aminocyclo propane-1-carboxylate (ACC) is synthesized from S-adenosylmethionine (S-AdoMet). In stress conditions, the plant hormone ethylene maintains homeostasis, which affects root and shoots growth. The rhizobacteria producing ACC deaminase reduces ethylene production under drought stress (Mayak et al. 2004). Rhizobacteria with the potential of ACC deaminase production is found to improve the growth, yield, and ripening of pea grown under drought stress condition (Arshad et al. 2008). Wheat plants harboring ACC deaminase producing rhizobacteria improve the root and shoot length and root-shoot mass of wheat. Developed roots help plants to uptake water and nutrients, which significantly improved the health of crops under drought stress (Shakir et al. 2012).

17.6 Antioxidants in Drought Stress Tolerance

The drought stress tolerance is increased due to reactive oxygen species (ROS (Helena and Carvalho 2008). Enzymatic antioxidants include catalase, peroxidase, superoxide dismutase, glutathione reductase, and ascorbate peroxidase. The specific antioxidant enzyme activity can be measured to assess the scavenging system. The antioxidant activity is correlated with the extent of drought tolerance (Guo et al. 2006). Rhizobacteria-treated plants have more levels of antioxidant enzymes, and the high level of antioxidant enzymes contributes to drought stress tolerance. Gururani et al. (2013) reported a considerable rise in enzyme scavengers like ascorbate peroxidase, catalase, and superoxide dismutase. The specific activity of catalase was found to be increased by 1.8 under drought stress conditions in plants treated with rhizobacteria. Saravanakumar et al. (2011) reported higher catalase activity in green gram plants with *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB.

17.7 Effect of Volatile Compounds in Drought Tolerance

The stimulation of various volatiles occurs in plants suffering from multiple stresses. Volatiles are a fast noninvasive technique to check drought stress on crops (Timmusk et al. 2014). Volatile compounds produced by rhizobacteria are 2R- and 3R-butanediol, which showed stimulation of drought tolerance in crops.

17.8 Proline, the Osmolyte for Drought Sustainability in Plants and Crops

Osmotic adjustment is the key adaptation that makes plants to tolerate drought conditions. (Farooq et al. 2009). Osmolytes protect the plant cellular organelles against the oxidative damage (Huang et al. 2014). Accumulation of compatible solutes is the osmotic adjustment (Kiani et al. 2007), in response to drought stress (Nilsen and Orcutt 1996). These solutes help the plants to maintain the water potential (Serraj and Sinclair 2002). Proline is the important osmolyte that accumulates in plants experiencing drought stress (Huang et al. 2014). In many plants and crops, there is a direct correlation between higher proline level and drought tolerance (Sankar et al. 2007). There are reports of higher proline content in pea (*Pisum sativum* L.) (Alexieva et al. 2001), chickpea (*Cicer arietinum* L.) (Mafakheri et al. 2010), rice (*Oryza sativa* L.) (Lum et al. 2014), and soybean (Silvente et al. 2012) grown under drought stress. Plants with more proline content possess the potential to tolerate drought stress. Rhizobacteria-treated crops have more proline contents. This data is reported in maize (Naseem and Bano 2014), sorghum (Grover et al. 2014), potato plants (Gururani et al. 2013), mung bean (Sarma and Saikia 2014), and *Arabidopsis* (*Arabidopsis thaliana* L.) (Cohen et al. 2015). Soluble sugars are osmolytes that help in the osmotic adjustment of plants under drought stress.

17.9 Trehalose Production by Desiccation-Tolerant Microorganisms for Drought Tolerance

Recently, a study has been done on desiccation-resistant, xeroprotectant-producing microorganisms for their potential of plant protection against drought and their role as PGPR. The trehalose production was correlated with their plant protection ability when grown under drought conditions (Vilchez et al. 2016).

17.10 Conclusion

Rhizobacteria will play a pivotal role in resistance development in plants affected by drought stress and will also solve the food security problem. Rhizobacteria can provide a better and cost-effective alternative toward drought tolerance in crop plants. Rhizobacteria can also adopt multiple approaches to ameliorate drought tolerance in plants.

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