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# About the Book

This book will provide a thorough state of art on the secondary metabolites and volatiles produced by plant-growth promoting rhizobacteria (PGPR). The book encompasses the plant's multiple benefits from PGPR and discusses the significant roles of PGPR in soil nutrient mobilization, improvement in soil fertility, and secretion of various volatile organic compounds (VOCs). It outlines the various VOCs and their significant role in plant communication, plant growth promotion, biological control, and above- and below-ground interactions between plants and the surrounding organisms.

The book benefits from bringing together professionals with a broad interdisciplinary expertise in PGPR and value-chain perspective and will be a welcome source of knowledge to facilitate the use of PGPR in agriculture. This book is a good compilation of research from leading scientists from across the globe, linking the translation of fundamental knowledge and emerging ideas in the field of VOCs. The book focuses on three important aspects: (1) understanding the secondary metabolites produced by PGPR, the signaling mechanisms, and how they affect plant growth; (2) the plausible role of volatile organic compounds produced by PGPR, their role, and the signaling mechanism for plant growth promotion; and (3) applications of VOCs and secondary metabolites of PGPR for seed germination, plant growth promotion, stress tolerance, and in-plant health and immunity.

# Contents

# About the Editors

**R. Z. Sayyed** is a professor and head of the Department of Microbiology, PSGVP Mandal's Arts, Science College, Shahada, India. He has over 25 years of teaching and 20 years of research experience in microbiology and biotechnology. Currently, he serves as the president of the India Chapter of the Asian PGPR Society for Sustainable Agriculture. He is associate editor of *Environmental Sustainability* (Springer); guest editor of *Sustainability* (MDPI), *Frontiers in Microbiology*, and *Frontiers in Sustainable Food System*; and academic editor of *PeerJ* and *PLOS One*. To his credit, he has received the Fellowship of the Indian Phytopathological Society (2021), Prof. M M Sharma Award (2020), Springer-Society Award (2020), Best Teachers Award (2018), Award for Excellence in PGPR Research (2017, 2018, and 2019), and Young Scientist Award (2005, 2008, and 2012). He has authored more than 250 research papers in high IF international journals; 27 books with Springer, Wiley, CRC-Taylor and Francis, and Cambridge Press; and 43 book chapters in reputed edited books. He has delivered many invited talks in several Southeast Asian and European countries. He has trained several graduates, postgraduate, and research students and produced seven PhDs under his guidance.

**Virgilio Gavicho Uarrota** from Mozambique, is an agronomic engineer at Eduardo Mondlane University (Mozambique) with an MSc in biotechnology and biosciences and a PhD in plant genetic resources from the Federal University of Santa Catarina (Brazil). Dr. Uarrota took up his third postdoctoral position in the Department of Postharvest and Industrialization, Faculty of Agronomy, Pontificia Universidad Catolica de Valparaiso, Quillota, Chile. His first postdoctoral position was in biotechnology and biosciences with

focus on bioinformatics and chemometrics, and his second postdoctoral position was in crop production with research line in physiology of crop plants. He has over 8 years of experience in teaching mathematics, biology, and physics and 13 years of research experience in plant biochemistry, metabolomics, and chemometrics. Dr. Virgilio Gavicho Uarrota has participated as collaborator professor in plant biochemistry, crop production physiology, and physiology and biochemistry of seed development, and, currently, in applied multivariate statistics at Universidad Tecnica de Manabí (Ecuador). Dr. Uarrota has received the Twas-Associateship Scheme Project as visiting researcher (2013 and 2017) at Unisa and at the International Centre of Tropical Agriculture (CIAT). He is a reviewer of many international journals, and has authored and co-authored over 40 research papers in high impact factor international journals, and more than 8 books and chapters with Springer, CRC, and Burleigh Dodds publishing. He has co-supervised 1 MSc student and participated in more than 10 examination committees of PhD and MSc students. Dr. Uarrota has delivered many short courses in data mining and an introduction to multivariate statistics, and he is an editorial board member of the journal *Computational Biology and Bioinformatics*.

# Chapter 1
# Plant Growth-Promoting Rhizobacteria (PGPR): An Overview


**Darshan Lobhi, Nitinkumar P. Patil, Estibaliz Sansinenea, and R. Z. Sayyed**

**Abstract** Rhizosphere is a zone of predominantly commensal and mutualistic interaction between plants and microorganisms. Plant growth-promoting rhizobacteria (PGPR) are referred to those bacteria which colonize on the root surface and aid in the enhanced development of the plant. Plant roots provide important nutritional requirements for both plant and microorganisms. PGPR exhibit great phenotypic and genotypic diversity. The apparent PGPR can be counted as PGPR when they show good comparative results on plant growth upon inoculation. In the recent years, study of PGPR has taken a peak interest because of their replacement as biofertilizers over chemical and synthetic fertilizers. Factors that affect the growth promotion are the production of phytohormones, such as indole acetic acid, gibberellic acid, etc., fixing of atmospheric nitrogen, solubilization of phosphates, etc. PGPR can also act as a biocontrol agent exhibiting various mechanisms, such as the induction of systemic resistance and antagonism.

**Keywords** Antifungal properties · Biocontrol · Mineral solubilization · PGPR · Phytohormones

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# 1 Introduction

The term ‘rhizosphere’ was first introduced in 1904 by the Scientist Lorenz Hiltner. Hiltner coined the term ‘bacteriorhiza’ specifying bacteria community associated with roots (Hartmann et al. 2008). Rhizosphere encompasses millimetres of soil surrounding the plant root where complex ecological and microbial processes occur (Bertha 2005). For a microbiologist, the soil environment is unique in several ways. It contains an array of bacteria, actinomycetes, fungi, algae and protozoa. Soil is referred to as one of the most dynamic sites where unavailable nutrients are made available through microbial agencies (Hulkoti 1981). Associated microbes can play a crucial role in the formation of or modification of soil (Lambers et al. 2009). The major applications of PGPR for plant growth improvement include agriculture, horticulture, forestry and environmental restoration. Another application of PGPR is their antagonistic activity against phytopathogenic bacteria, synthesis of fungicide enzymes, reduction in available iron to phytopathogens in soil, etc. (Glick et al. 2004).

# 2 Mechanisms of Plant Growth Promotion

## *2.1 Production of Phytohormone*

### 2.1.1 Indole-3-Acetic Acid (IAA)

The production of phytohormone is most likely the reason for the enhanced growth of the plants (Idris et al. 2007). Indole-3-acetic acid is one of the most physiologically active auxin (Shahab et al. 2009). Many plant growth-promoting rhizobacteria (PGPR) have been studied for the production of these plant hormones. L-tryptophan being the precursor is metabolized to yield IAA as a final product (Shahab et al. 2009). The chemical structure of indole-3-acetic acid is presented in Fig. 1.1.

Increasing concentration of tryptophan results in increased production of IAA. Thus, any changes in the levels of IAA can affect the root system (Bharucha et al. 2013). Important functions regulated by IAA are cell expansion, cell division,

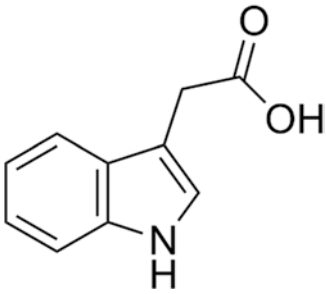


**Fig. 1.1** Structure of indole-3-acetic acid (Schutz et al. 2003)

root elongation and gene regulation. It also acts as signalling molecule and can have direct influence on plant physiology. *Rhizobium* and *Mycobacterium* genera are among the most active IAA producers which are first isolated from the roots of the epiphytic orchid *Dendrobium moschatum* (Saharan and Nehra 2011).

A majority of bacteria isolated from the rhizosphere inherit the ability of synthesizing and releasing IAA as a secondary metabolite (Ahemad and Kibret 2014). IAA is synthesized in two pathways:

1. Conversion of tryptophan to IAA by deamination to indole-3-pyruvic acid following decarboxylation to indole-3-acetaldehyde
2. Decarboxylation of tryptophan to indole-3-acetamide followed by hydrolysis to yield IAA (Arshad and Fankenberger 1991)

Microorganisms like *Erwinia herbicola*, *Azospirillum*, *Klebsiella*, *Bradyrhizobium* and *Agrobacterium* follow the pathway of IAA synthesis via indole-3-pyruvic acid and indole-3-acetaldehyde (Ahemad and Kibret 2014), whereas *Pseudomonas syringae*, *Agrobacterium tumefaciens* and *Pseudomonas* follow the pathway of indole-3-acetamide formation. However, mutants which are completely lacking the property of auxin production (IAA) have been reported to possess multiple alternative pathways (Dimpka et al. 2012).

Isolation is generally done using Luria-Bertani agar medium, King's B agar or nutrient agar. Selective media can be used for the strains such as *Azotobacter* and *Pseudomonas*. Qualitative screening for the IAA-producing activity of these strains is done using Salkowski's method. The method involves treatment of cell-free supernatant with the Salkowski's reagent (35% perchloric acid and 0.5 M $FeCl_3$). Intensity of the colour developed is measured at 530 nm (Ahmad et al. 2004, 2008; Gutierrez et al. 2009). Furthermore, the extract of IAA, cell free supernatant, is mixed with ethyl acetate and 1 N HCL. Solvent systems can be of different types such as ethyl acetate:chlololform:formic acid (55:35:10), benzene:n-butanol:acetic acid (70:25:5) or chloroform:methanol:water (85:14:1) (Ahmad et al. 2004; Ehmann 1977; Bialek et al. 1983).

As discussed earlier, IAA is physiologically the most active auxin, and its application as a pot experiment has very promising effects. Experiment conducted to examine the plant growth-promoting activity of *Pseudomonas putida* showed good results on canola seedlings. IAA produced by the strain had a significant result on the elongation of primary roots which is on average 35% longer than uninoculated seeds (Patten and Glick 2002). In an experiment conducted to study the influence of IAA on root elongation of sugar beet, culture supernatants with different levels of IAA accumulation were quantified by HPLC. Among the 14 isolates, 2 were producing higher amount of IAA. After the seed inoculation, a significant relation was observed between the IAA accumulated in the culture supernatants and the root elongation. In the plants inoculated with the rhizobacterial strains producing higher amount of IAA, the primary root length was decreased and shoot:root ratio was significantly higher compared to the other strains. This concludes that changes in the concentration of the IAA can disrupt the hormonal balance required for growth and development (Loper and Schroth 1986). Tomato seeds inoculated with *T.*

*atroviride* showed positive results with increased root and shoot. But as concentration of tryptophan increased beyond 0.75 mM, the growth was ceased (Gravel et al. 2007). Photostimulatory effect of the strains *Bacillus* and *Pseudomonas* was conducted on *Triticum aestivum* L. under axenic and wire condition using pot experiment. The highest increase in the number of roots was 83.25% with *Pseudomonas* sp. AS-17 and 75% with *Bacillus* sp. NpR-1, EhH-5 and *Micrococcus* sp. AVR-5. *Pseudomonas* also showed highest increase in shoot length over control (Ali et al. 2009). *Paenibacillus polymyxa* RC05, *Bacillus* OSU-142 and *Bacillus megaterium* RC01 had the greatest difference in shoot and total weight, among which *P. polymyxa* RC05 produced the highest root and shoot growth (Cakmakci et al. 2007). Nghia reported the plant growth-promoting activity of *B. megaterium* on rice seedling. The bacteria were isolated from the salt affected soil of rice-shrimp farming systems (Nghia et al. 2017). The results of an experiment obtained after the treatment of rhizobacteria strain with tryptophan and Ag increased the stem length (up to 10%), stem weight (up to 34%), root weight (up to 37%) and also the uptake of macronutrients such as N, P and K. Treatment showed that the usage of both Trp and Ag together causes a significant increase in the parameters under study compared to Trp and Ag using alone and control (Etesami et al. 2009). A comparative study between *Pseudomonas fluorescens* and *Bacillus subtilis* for their growth-promoting activity on onion (*Allium cepa* L.) showed that *Pseudomonas* strain possessed higher potential for IAA production, root and shoot elongation and fresh weight of root and shoot than *Bacillus* (Reetha et al. 2014). Different *Bacillus* strains isolated from soybean rhizosphere have been checked for the plant growth-promoting activity. Aris Tri Wahyudi conducted this experiment and IAA production and germination assay of two *Bacillus* strains, *Bacillus pumilis* strain S2 and *Bacillus shandongensis* SD showed very good results compared to others (Wahyudi et al. 2011). In vitro experiment was designed to study the effect of IAA synthesized by *Pantoea agglomerans* strain PVM on root induction in *Nicotiana tabacum.* The strain recorded more induction of roots compared to leaf explant grown on MS medium supplemented with synthetic IAA. The study confirmed that the biologically produced IAA would be the better option for in vitro root induction in plants (Apine and Jadhav 2011).

### 2.1.2 Gibberellic Acid ($GA_3$)

Gibberellin production promotes the growth of the plants and crop yield (Pandya and Desai 2014). Gibberellins are tetracyclin diterpenoid acids involved in a number of developmental and physiological processes which include seed germination, seedling, stem and leaf growth. They are responsible for the induction of hydrolytic enzyme activity (Rodriguez et al. 2011; Jecobson et al. 1970; Chrispeels and Varner 1967) and also involved in the regulatory aspects of Arabidopsis development such as flowering and hypocotyls elongation (Sivaskthivelan and Stella 2012; Bottini et al. 2004). Chemically $GA_3$ is a tetracyclic dihydroxy γ-lactonic acid containing two ethylene bonds and one free carboxylic acid group ($C_{19}H_{22}O_6$) as given in Fig. 1.2.

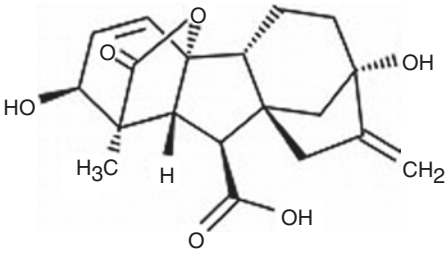


**Fig. 1.2** Chemical structure of gibberellic acid ($GA_3$)

Genes encoding the enzymes responsible for catalysing the $GA_3$ biosynthesis have been reported in *Arabidopsis thaliana*. Cytochrome P450 mono-oxygenase is responsible for the oxidation from *ent*-kaurene to $GA_{12}$. P450 generally catalyses the insection of oxygen into an unactivated substrate with production of water. $GA_3$ gene encodes *ent*-kaurene oxidase which mediates *ent*-kaurene to ent-kaurenoic acid. Bioconversion of *ent*-kaurenoic acid to $GA_{12}$ is catalysed by *ent*-kaurenoic acid oxidase, and the final stages are catalysed by 2-oxoglutarate-dependent dioxygenase (Helliwell et al. 2011; Morrone et al. 2010).

At present, species belonging to fungal genera like *Fusarium*, *Gibberella*, *Sphaceloma*, *Neurospora* and *Phaeosphaeria* have been reported to produce the gibberellic acid. Bacteria that have been reported to produce the gibberellic acid are *Azotobacter*, *Azospirillum*, *Bacillus siamensis* BE76, *Pseudomonas* spp., etc. (Desai 2017; Ambawade and Pathade 2013). Gibberellins are generally produced from fungi *Fusarium* by submerged fermentation. *Methylobacterium oryzae* CBMB20 have been once used for gibberellic acid production. The culture broth of *Methylobacterium* was centrifuged, and pH of the supernatant was adjusted to 2.5 with 2.5 N HCl. $GA_3$ is extracted using liquid-liquid extraction (ethyl acetate/$NaHCO_3$) method. Ethyl acetate layer contains free $GA_3$. This liquid layer is dried and residue is dissolved in absolute methanol. Quantity of dissolved $GA_3$ is determined spectrophotometrically using standard curve (Siddikee et al. 2010; Graham and Henderson 1960). Method for gibberellin detection involved the colorimetric method in a purified preparation. Graham and Thomas developed a new method for gibberellin detection using 2,4-dinitrophenol reagent and KOH (Graham and Thomas 1961). Extraction and purification using chromatographic technique employs water:acetonitrile (76:24) solvent system (Ambavade and Pathade 2013).

Various pot applications have been employed using the $GA_3$ spray to increase the yield of the crop. In 2007, pot application over mustard seed increased the yield and protein content of the crop (Shah 2007). It was also reported that $GA_3$ treatment over salt-stressed mustard plant could stimulate their salt tolerance by increasing photosynthetic rate and nitrogen metabolism (Afroz et al. 2005). Gibberellin application helped the salinity-challenged plants to a different degree in reversal of altered growth and physicochemical processes as demonstrated in an experiment with sugarcane plant (Shomeili et al. 2011). Germination of *Prunus avium* L. seed was increased with the $GA_3$ application and stratification. The effect of thiourea

increased the germination rate of the seeds compared to control. Treatment of $KNO_3$ also had positive effect over the germination (Centibas and Koyuncu 2006).

## 2.2 Nitrogen Fixation

The chemical elements C, H, O, N, P and S are all necessary to sustain life on the earth. Among these elements, nitrogen is the most abundant in the earth's atmosphere (Galloway et al. 2003). Two N molecules bonded by a triple bond forming a dinitrogen molecule comprise about 80% of total earth's atmosphere (Davidson and Seitzinger 2006). Nitrogen is the element that can limit the crop production; hence, biological nitrogen fixation (BNF) becomes a key process in sustainable land management and crop productivity, but it can be limited by stressful conditions such as high temperature, drought and soil acidity (Hungria and Vargas 2000). Conversion of atmospheric nitrogen into ammonia or other nitrogenous compounds available to living organisms is called 'nitrogen fixation' (Postgate 1998). In the biological nitrogen fixation, common soil bacterium *Rhizobium* invades and multiplies within the cortex cell of the roots forming a nodule. This is referred to as the symbiotic relationship between plant and microorganisms (Geddes and Orensik 2016).

Legume rhizosphere soil samples from Karnataka were studied for the nitrogen fixing ability of *Rhizobium*. Qualitative screening was done on nitrogen-free medium. Positive isolates were further studied for antagonistic activity and plant growth-promoting activity (Kallimath and Patil 2018). Tariq S. conducted an experiment to propose the improvement of $N_2$ fixing capacity and yield of mung beans and mash beans by the phosphate management. Total biomass and grain yield was increased with phosphorous application. Along with phosphorous application, nitrogen fixing ability significantly increased with the amount of phosphorous applied. Response to mash beans was found higher as compared to mung beans (Tariq et al. 2007). In 2003–2004, Vijila and Jebaraj proposed an experiment on improving the rhizobium-green gram symbiotic relationship in low nutrient and acid stress soils. *Rhizobium trifolium-Trifolium pratense* symbiosis was found higher with respect to nodule formation, plant height, grain yield, etc. Microbial isolates were specifically isolated from the legumes from acidic soils as acidity factor can result in reduced nodulation (Vijila and Jebaraj 2008).

Rice and Paul measured the nitrogen fixation in water-logged soil, soil-straw and sand-clay-straw mixtures. Acetylene reduction assay method was most prominent. The maximum results were obtained in sand-clay-straw mixture in anaerobic conditions (Rice and Paul 1971). Belimove studied the effect of mixed cultures on growth of two barley cultivars. Inoculation of two mixed cultures *Azospirillum lipoferum* 137 and *A. mysorens* 7 increased grain and straw yield by 23%. However, the addition of low levels of mineral nitrogen resulted in a positive yield response after inoculation with *A. lipoferum* 137 and *A. radiobacter* 10. But, at high levels of nitrogen fertilizers, mixed cultures possessed no advantage over single culture (Belimove et al. 1995). Govindarajan in 2007 evaluated the inoculation effect of

*Burkholderia vietnamiensis* and related endophytic diazotrophic bacteria on yield of rice. Inoculation of the strain *B. vietnamiensis* MGK3 alone produced a significant increase over control. The combined inoculation of this strain with *Glucanacetobacter diazotrophicus* LMG7603, *Herbaspirillum seropedicae* LMG6513, *Azospirillum lipoferum* 4B LMG4348 and *B. vietnamiensis* LMG10929 produced a significant increase in the nitrogen fixation and grain yield of rice (Govindarajan et al. 2008). Field trials conducted to check the potential of different strains to fix nitrogen and growth of different varieties of rice reported that chemical nitrogen fertilizers input lowered the grain yield, whereas rhizobial endophyte strain S11 increased the grain nitrogen content and the grain yield (Yanni et al. 2001). *Klebsiella pneumonia* 342 significantly increased the dry weight of the wheat plant. Percentage increase in total nitrogen for Kp342 inoculated plants was 244 and 498% greater for roots and shoots compared with uninoculated control. Also, the N-concentration in plant tissue also increased with Kp342 inoculation (Iniguez et al. 2004)

## *2.3 Phosphate Solubilization*

Phosphate is one of the macronutrients which is present in both organic and inorganic forms and is essential for biological growth and development (Ingle and Padole 2017). Microorganisms play an important role in the natural phosphorous cycle. Phosphate-solubilizing microorganisms, through various mechanisms, carry out the conversion of inorganic phosphate (insoluble form) to organic phosphate (soluble form) which can be available to plants. This process is known as mineralization. This property of microorganisms has drawn attention to equipage microorganisms to keep up with phosphorous cycling in natural ecosystems (Sharma et al. 2016). The biggest reserves of phosphorous are rocks and other deposits, such as apatite and other primary minerals. Mineral phosphates are associated with the surface of hydrated oxides of Fe, Al and Mn. Most soils contain large reservoirs of phosphorous which may be the accumulation from chemical fertilizers (Rodriguez and Fraga 1999). This may lead to soil health issues and beyond certain limits the yield plateau gets declined. Soil which is rich in phosphorous content constitute about 0.05% phosphorous (W/W) and only one tenth of it is available to plants (Ingle and Padole 2017). These low levels of phosphorous may be caused because of chemical fixation and interaction with other micronutrients such as Zn, Cu and Fe which further cause the imbalance and deficiency of these chelating elements (Krishnaraj and Dahale 2014). Qualitative estimation of phosphate-solubilizing bacteria is done using Pikovaskaya's medium and National Botanical Research Institute's phosphate growth medium (NBRIP) with bromophenol blue (Sujontha and Manawadi 2016; Dhurve et al. 2017). Clear zone around the colony confirms phosphate-solubilizing bacteria. The ability of rhizobacteria to solubilize the insoluble phosphate is described by the term solubilizing index or phosphate solubilization efficacy. It is determined by measuring total halo around the colony and colony diameter (Sunjotha and Manawadi 2016; Schoebitz et al. 2013):

$$\text{Solubilization index} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

Quantification of phosphate-solubilizing ability of rhizobacteria is done using phosphor-molybdate blue colour method (Murphy and Riley 1962; Mihajlovic et al. 2003). Pikovaskaya's broth (adjusted to pH 7) with sucrose and tricalcium phosphate (0.3 g/100 ml) was poured in 250 ml flask. Flasks were autoclaved and loopful cultures of phosphate-solubilizing bacterial strains were inoculated and incubated in shaking condition for 12 days. This suspension was centrifuged and the supernatant was checked for available phosphate using a spectrophotometer at 882 nm and calibrated with standard $KH_2PO_4$ curve. In 2013, Patel and Parmar (Patel and Parmar 2013) collected the soil samples from the rhizosphere of sunflower and isolation was done on Pikovaskaya's agar medium. Quantitative estimation involved the chlorostannous-reduced molybdophosphoric acid blue method. Bacterial cultures were inoculated in Pikovaskaya's broth and incubated for 5 days on shaking conditions. After incubation, broth was centrifuged and the supernatant was tested for soluble phosphorous content. Absorbance of the colour developed after adding the chloromolybdic reagent and chlorostannous acid reagent was measured at 600 nm. Phosphate solubilization activity of six isolates was found to be more than 100 mg/l with highest activity of 181 mg/l. It is also concluded that the decrease in pH and increase in soluble phosphate show the isolates having the ability to produce organic acid at different concentration ranges. Experiment conducted by Perez E. in 2007 aimed at studying the phosphate-solubilizing bacteria colonizing the limonitic crust in the Venezuelan region. The activity of the isolates was found to be high. Screening of phosphate solubilizing in NBRIP medium containing calcium phosphate was found to be biased towards the identification of strains which are Fe-P and Al-P solubilizers, thus reducing the other strains which may exhibit this ability (Perez et al. 2007). Along with *Pseudomonas* and *Bacillus*, other bacteria reported for phosphate solubilization are *Rhodococcus*, *Arthrobacter*, *Chryseobacterium*, *Gordonia*, *Delftia*, *Xanthomonas*, *Vibrio proteolyticus* and *Enterobacter* (Sharma et al. 2013).

Sanjotha and Manawadi conducted an experiment showing the higher phosphate-solubilizing activity of strains such as *Pseudomonas*, *Azotobacter*, *Rhizobium* and *Bacillus*, among which *Pseudomonas* was giving the highest activity of 0.89 mg/l. The method used for quantitative estimation was phosphomolybdate blue colour method (Sanjotha and Manawadi 2016). Hameeda B conducted an experiment on growth promotion on maize by rhizobacteria from compost and macrofauna. Population of *Pseudomonas* sp. CDB 35 was found higher than *Serratia marcescens* EB 67. Plant biomass of the *S. marcescens* EB 67 was better than chemical control (Hameeda et al. 2008). Studies have shown the increase in root and shoot length and phosphorous uptake due to phosphate-solubilizing bacteria. *Azotobacter chroococcum* showed phosphate solubilization activity and increase in grain and straw yield of wheat (Kumar et al. 2001). Eighty percent of the activity encountered with the *Serratia* spp. being the opportunistic pathogen has been reported to enhance the

growth of the plant by inducing the resistance against plant pathogens. Because of the ability to produce the phosphatase enzyme and phosphate solubilization, the isolates can be used for bio-inoculation (Behera et al. 2017).

## 2.4 Zinc and Iron Solubilization

Zinc serves as an important component of the enzymes responsible for the metabolic reactions in plants. Many different processes in plants such as nitrogen fixation, photosynthesis and resistance against stress conditions are mainly influenced by zinc. It is also an important cofactor in activity of more than 300 enzymes. Zinc is critical for the production of different phytohormones described above along with abscisic acid and cytokinins. Thus, the deficiency of zinc on plants can result in the impairment of growth of plant cell, DNA damage, cancer development and other vital processes resulting as a major risk factor for human health globally. Plant growth-promoting bacteria have been proven to be the critical factors in plant establishment in nutrient-stressed conditions (Hussain et al. 2015; Joshi et al. 2013).

Solubilizations of zinc compounds by PGPR have been reported by Fasim et al. 2002 and Naz et al. 2016. Organic acids produced by microbes aid in the solubilization of zinc and phosphorous compounds (Saravanan et al. 2007). Gluconic acid is considered as a major organic acid in the solubilization of minerals. These acids increase the availability of zinc by sequestering cations and subsequently decreasing the pH of the soil (Sunithakumari et al. 2016).

Iron is probably essential for living cells, and it is one of the most common elements on the surface of the earth. However, it lacks its availability that its abundance. Plants obtain the forms of iron from the soil in spite of its insolubility. That's why study of iron solubilization in microorganisms has become an active field recently (Takagi 1976; Neilands 1981). The role of microorganisms in solubilization of insoluble phosphate is becoming important in agricultural field. As discussed earlier, phosphate utilization efficiency in soil is very low because of its fixation with aluminium and iron in acid soil. Laboratory demonstration follows that strains which were tested positive for Pikovskaya's test were then tested for $AlPO_4$ and $FePO_4$ solubilization with Reyes' basal medium containing either BCG and either $AlPO_4$ or $FePO_4$ (Gadagi and Sa 2002).

A study revealed that the mechanism of $AlPO_4$ and $FePO_4$ solubilization mainly involved the secretion of organic acids and pH decrease. Thus, pH becomes an important factor in iron solubilization (Illmer et al. 1995).

A study by Othman et al. 2017 showed that zinc solubilizing bacteria increased the plant height and the plant biomass. Plants also showed a higher chlorophyll content at 0.2 mg/L of $ZnSO_4$. According to Ramesh et al. 2014, inoculation of two ZSB significantly increased the auxin production by 21.9 and 23.1%, respectively, in soybean crop. Biomass of plants, soybean and wheat was also increased by much larger amounts. Sunithakumari et al. (2016) studied the synthesis of gluconic acid produced by ZSB in presence of the substrate ZnO. Similarly, Sushil et al. (2013)

reported the assimilation in soybean seeds by *Bacillus* using different substrates as ZnO, zinc phosphate and zinc carbonate. Study revealed that the isolates found to be more efficient in solubilizing zinc phosphate than the others.

### *2.5 Potash Solubilization*

Potassium (K) is one of the major nutrients limiting plant growth although being in quite abundance in many soils of India. Similar to zinc and iron, it is also present as potassium-bearing minerals and thus unavailable for utilization. Along with other minerals, potassium not only participates in nutrient uptake and its transportation but also have the role in cell synthesis, enzymes, sugars and vitamins. In soil, potassium needs to be replenished continuously with the release of non-exchangeable potassium by either weathering or adding K fertilizers. To overcome the fact of foreign exchange of K fertilizers in India, an alternate source of potassium has been utilized. It is low-grade waste mica containing 8–10% potassium which was generated near mica mines (Verma et al. 2017; Basak and Biswas 2010; Nishanth and Biswas 2008). One of the ways of utilizing mica is through composting which solubilize potassium in more available forms. PGPR present in soil are able to solubilize the K-bearing minerals such as mica, illite and orthoclases (Basak and Biswas 2009; Bennet et al. 1998).

An experiment conducted on the effect of potassium-solubilizing microorganisms on tea plants along with different concentrations of potassium, phosphorous and nitrogen stated that the application of potassium-solubilizing microorganisms with different fertilizers increased the leaf yield and enhanced growth. Productivity index was significantly increased. Organic acids which were produced also improved the yield as well. Thus, a study concluded that the bio-inoculation of fertilizers with reduced potassium level improved the yield of the crop (Bagyalakshmi et al. 2012). Temperature resistance strains have influenced significant effects on the weathering of K-bearing mineral powders, thus improving the harvest and enhancing soil quality (Liu et al. 2016). *Streptomyces* spp. and *Paenibacillus kribbensis* CX-7 were found to be effective strains in solubilizing the potassium and phosphorous and improving the crop yield (Liu et al. 2016; Zhang et al. 2013).

## 3 Antimicrobial Properties Against Phytopathogens

Root pathogens can penetrate the tissues of the roots and can cause damage to the plant. Certain plant growth-promoting bacteria have the antagonistic ability against these pathogens (Haas et al. 2002). Biological control has attention because of the extensive use of pesticide for crop protection and increase in pathogen resistance. It may have an adverse effect on nontarget microorganisms (Singh et al. 2015). Mode of action of control of these bacteria may be through the production of metabolites

contributing to abiosis or production of hydrolytic enzymes such as chitinase and glucanase as the cell wall is mostly composed of chitin and cellulose (Vejan et al. 2016).

## *3.1 Biocontrol Mechanisms*

### 3.1.1 Siderophore Production

One of the factors responsible antagonism is the production of siderophores. Siderophores are low-molecular-weight iron-chelating organic compounds which are produced during iron starvation conditions and have high affinity for Fe (III). These compounds deplete the iron from the environment making it less available to certain microorganisms including plant pathogens. Plants do not absorb the bacterial siderophores but the Fe released by siderophores through a reduction-based mechanisms. Thus, plant growth is stimulated by the excretion of such compounds by improving Fe nutrition or inhibiting the plant pathogens (Loaces et al. 2011; Tian et al. 2009; Saxena et al. 1986; Cesco et al. 2002; Hordt et al. 2000). Siderophore-producing ability of most of the microorganisms makes them the successful competitors in several environments (Loaces et al. 2011). There are more than 500 different siderophores present in the nature, but all have much of the same structure and a functional unit that ligates with iron molecules and peptide backbone interacting with receptors on the membrane of bacteria (Lee et al. 2011).

In an experiment, after TLC of ethyl acetate extract of culture supernatant, the results showed that *Azospirillum lipoferum* D2 produced the different siderophores under iron starvation (Saxena et al. 1986). *Micrococcus luteus* and *Bacillus silvestris*, the two heterotrophic bacteria isolated from southern Baltic Sea, exhibited the ability to produce the siderophores. Specific chemical tests of the siderophore extracts showed that only hydroxamates type of siderophores was excreted (Cabaj and Kosakowka 2009). *Pseudomonas aeruginosa* has been known precisely to produce the siderophores. Various strains of the isolate such as *P. aeruginosa* PSS and *P. aeruginosa* PUPa3 have been checked for their antagonistic activity (Sunish Kumar et al. 2004; De Villegas et al. 2002; Rachid and Ahmed 2005). *Pseudomonas fluorescens* was found efficient in producing siderophores in iron-stressed conditions at pH 7 and 29 °C. Tyrosine was the carbon source which stimulated the bacterial growth and siderophore production (Tailor and Joshi 2011). It is also noted that after supplementing the soil with iron along with the absence of *Pseudomonas* strains (RSP5 and RSP8), siderophore production reduced significantly (Sah et al. 2017). Under Fe-deficient conditions, siderophores produced by *Pseudomonas* (Pyoverdines) show a higher affinity for Fe (III) (Boukhalfa and Crumbliss 2002; Sah et al. 2017). Quantification study carried out by Patel et al. for the production of siderophores reported that *A. faecalis* RZS2 excreted 92.61% of siderophores while *P. aeruginosa* RZS3 produced 43.22% of siderophores (Patel et al. 2016). In one of the studies, Sayyed et al. 2019 evaluated the effects of different physico-chemical

on the production of siderophores by the isolate *Achromobacter* sp. RZS2 isolated from groundnut rhizosphere. Isolate produced siderophores in the presence of urea, which is a chemical nitrogen fertilizer used in agriculture and varying concentrations of metal ions. This ability of isolates can be exploited in the bioremediation of siderophore-mediated contamination and metal contamination of agriculture fields (Sayyed et al. 2019).

### 3.1.2 Cyanide Production

Growth inhibitory bacteria or deleterious rhizobacteria (DRB) are nonparasitic, causing deleterious effects through the production of certain metabolites (Kremer and Souissi 2001). Cyanide is one such metabolite produced by various microorganisms. It is produced directly from glycine and cyanogenic glycosides, which are present in the root exudates (Bakker and Schippers 1987). It is a potential inhibitor of the enzymes responsible for the various metabolic activities such as respiration and assimilation or blocks the transport chain of photosynthesis because of its ability to interfere with cytochrome oxidation (Kremer and Souissi 2001; Kumar et al. 2005). Reports often suggest that hydrogen cyanide (HCN) has certain antimicrobial activity and is effective against the growth of pathogenic fungus. *Pseudomonas* strains have been shown to produce HCN which prevent the growth of root-rot pathogens (Deshwal and Kumar 2013). Synergistic effects of HCN and siderophores for stimulation and suppression of charcoal rot disease have been reported earlier (Khare and Arora 2010). Investigations often suggest the production of HCN in bacteria by iron under the influence of quorum sensing. A study also states that biogenic HCN also contributes in the sequestration of metals and increases the availability of phosphate by indirect means, thus increasing the nutrient availability and plant growth (Sagar et al. 2018). According to Jayaprakashvel et al. 2010, bacterial cyanogenesis is essentially restricted to the proteobacteria such as *Chromobacterium violaceum* and some fluorescent *Pseudomonas*. Certain cyanobacteria such as *Anacystis nidulans* and *Nostoc muscorum* as well as certain strains of rhizobium such as *Rhizobium leguminosarum* have been reported to be the free living HCN-producing bacteria. Overaccumulation of HCN on the phyllosphere region often results in the negative influence on growth and crop yield. A reduction in the yield of wheat due to *Pseudomonas fluorescence* accumulation has been reported (Alimi et al. 2012).

### 3.1.3 Antibiotic Production and Antifungal Activity

Production of antibiotics is one of the aspects of the biocontrol property. Antibiotics produced by PGPR are known to possess various activities, namely, antimicrobial, antiviral, antifungal, cytotoxic, antihelminthic, etc. Numerous antibiotics have been isolated from fungal and bacterial strains. *Gaeumannomyces graminis* var. *tritici*, the causative agent of take-all of wheat, showed variation in the sensitivity to the

antibiotics phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (Phl) produced by *Pseudomonas* spp. (Mazzola et al. 1995; Wani et al. 2016). Antibiotics such as polymyxin, circulin and colistin produced by *Bacillus* spp. are active against many gram-positive and gram-negative bacteria and fungi (Beneduzi et al. 2012). Genetic analysis of several *Pseudomonas* strains has reported a positive association between antibiotic production and disease suppression. Pyoluteorin, an aromatic phenolic polyketide antibiotic, is used in the suppression of seedling pathogens such as *Pythium ultimum.* Vinay et al. isolated from two pyoluteorin-producing fluorescent *Pseudomonas putida* RFP-4 and RFP-19 isolated from the rhizosphere of different legumes (Vinay et al. 2016). As stated earlier, most of the plant pathogens are fungi. Therefore, rhizobacteria are checked for their antifungal activity.

Fungal infections remain the major cause of death. The activity the strains *Alcaligenes faecalis* and *Bacillus cereus* have been checked against *Candida albicans* (CCMB242, CCMB265 and CCMB286). Crude extracts of ethyl acetate were made from rhizobacteria which were found effective against *Candida albicans* CCMB286 (Santos et al. 2011). Several species of *Pseudomonas* sp. have been reported to produce wide range of antifungal antibiotics, viz. cepaciamide A, ecomycins, phenazines, oomycin A, N-butylbenzene sulphonamide, etc. (Hamid et al. 2021). *Pseudomonas* spp. were often checked and found effective as an antifungal agent against *Dematophora* spp., *Fusarium oxysporum* and *Alternaria* spp. Phenazine production was carried out with bacteria *Pseudomonas fluorescence* ESB20 isolated from chili rhizosphere to reduce the in vitro growth of *Pythium* spp. (Soni et al. 2016; Arora et al. 2021). According to Singh et al., *Bacillus* spp. were found potent against *Fusarium* spp. and *Bipolaris* spp. (Singh et al. 2017). *Paenibacillus macerans* MBO2-992 and *Paenibacillus polymyxa* MB02-1007 exhibited significant antagonistic activity against *Ralstonia solanacearum* (Li et al. 2010).

## 4 Conclusion

Geoaccumulation, bioaccumulation and biomagnification have been serious concerns nowadays due to the entry of heavy toxic metals into the ecosystem (Singare et al. 2010). The use of industrial fertilizers and pesticides increases the nitrate content of the soil which then contaminates the groundwater subsequently causing water pollution. Most of the livestock waste accounts for the phosphorous load of the soil. The most damaging activity for soil pollution is deforestation. Burning of land, as a part of deforestation, detriments the quality of the soil (Novotny 1999). Phytopathogens also have antagonistic effect on the useful rhizobacteria. To overcome these problems, rhizobacteria have been suggested as a useful solution. Rhizospheric bacteria such as *Pseudomonas*, *Azotobacter*, *Agrobacterium*, *Bacillus*, etc. have multiple plant growth-promoting traits. Along with that, they show antimicrobial activity against multiple plant pathogens such as *Alternaria*, *Fusarium*, *Penicillium*, *Candida*, etc. To add more, they have been shown to possess pesticide

tolerance, cyanide production and siderophore-producing activities. After successful pot trials of the rhizobacteria as biofertilizers, field trials are suggested for an industrial approach. Another impact of using biofertilizers is as remedial approach regarding soil and water pollution, thus a step forward towards saving the nature and its conservation.

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# Chapter 2
# Metabolomics as a Tool to Study Volatile Organic Compounds Produced by Plant Growth-Promoting Rhizobacteria

**David Guillermo Piedrahita Márquez, Deise Munaro, and Virgilio Gavicho Uarrota**

**Abstract** Plant growth-promoting rhizobacteria-volatile organic compounds (PGPR-VOCs) have a vast number of applications in numerous industries due to their activity against microorganisms, virus and pro-oxidant substances. They could be applied to improve the postharvest quality of numerous crops or as a source of rhizobacteria. Low molecular weight compounds have multiple sources promoting the plant's growth by increasing the amount of nutrients, making synergism and symbiotic processes with plants and enhancing the production of phytohormones and bioactive compounds. In this chapter, the main focus is to show how metabolomics tools are used to unravel secondary metabolites and volatile organic compounds produced by PGPR. It has been discovered that there is a huge chemical diversity and number of PGPR. Among the elucidated bioactive compounds are hydroxycinnamic acids, terpenic compounds, hydrocarbons, carbonyl compounds, alcohols and nitrogen and sulphur compounds. Also, thanks to the metabolomic approach, it has been possible to correlate PGPR with the biological source and correlate the bioactivity with the chemical structure of the metabolites, and this has led to the discovery and use of *Azospirillum brasilense* and *Streptomyces albidoflavus* as plant growth promoters for pharmaceutical and agro-industrial applications, useful for crops like *Zea mays* and *Solanum lycopersicum* which have been the most studied commercial plants in the metabolomic plant-bacteria interaction. Finally, it

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has been concluded that numerous PGPR of the same genus provide similar metabolites in different plant-bacteria interactions. Still there are numerous challenges related to the PGPR study due to their unstable chemical structure and the complexity of their biosynthesis. If those challenges can be solved in a near future, it will be possible to have a PGPR VOC metabolome with a vast amount of information, and the applications of rhizobacteria for the industry will face a substantial increase.

**Keywords** Metabolomic tools · PGPR-secondary metabolites · PGPR-volatile organic compounds

## 1 Overview of the Study of the Plant Growth-Promoting Rhizobacteria (PGPR) Volatile Compounds

Since ancient times, those microorganisms have been useful to stimulate plant growth. But only in recent times, we have discovered the mechanisms and the compounds used in the bacteria involved in the plant metabolism. Among the most well microorganisms involved in this process are the plant growth-promoting rhizobacteria (PGPR), which colonize the rhizosphere/endo-rhizosphere of plants (Backer et al. 2018); recently they have gained a lot of interest in fields such as phytochemistry, biotechnology and agricultural sciences due to their role in the plant development and their contributions to stress resistance (Goswami et al. 2020); their vast spectrum of bioactivity and their numerous applications which go from remediation of contaminated soils to the creation of new products that could replace chemical pesticides and synthetic drugs (Ahemad et al. 2014).

The reason why these bacteria are so useful is due to their role as an economical source of natural compounds, and their activity continues in the plant tissues. An important group of metabolites produced by rhizobacteria are the volatile organic compounds, which are a diverse group characterized by their low molecular weight, inferior to 400 g/mol (Heenan-Daly et al. 2019). These substances are produced according to numerous mechanisms, and depending on the functional groups and the chemical structure of those compounds, we will see changes in the plant-bacteria interaction, and it is necessary to understand the mechanism of action between the compounds and the plants (Pii et al. 2015). It will not only allow the researchers and the industry to understand the process in which the previously mentioned substances can interfere with the plant metabolism, but also it will be a solution to the nutrient loss, the action of radical oxygen species and the decreasing in the chlorophyll content (Batool et al. 2020).

One tool that allows the researchers to understand the production of metabolites and their role in the plant-bacteria symbiosis is the metabolomic analysis; it not only allows the researchers to obtain the chemical profile; but also, it can lead to the discovery of biomarkers with the biggest impact on the plant physiology and to the comprehension of pathways involved in the metabolite synthesis (Iijima 2014).

## 2 Volatile Compound Screening in the Metabolomic Analysis

PGPR-derived compounds can be categorized into enzymes and proteins, volatile organic compounds (VOCs), antibiotics and lipopeptides (Santoro et al. 2015). The second group is by far the biggest of compounds produced by the rhizobacteria, and their primary function consists in the production signals that can be perceived by friendly species or predators; also, they contribute to the shaping ecology at the subcellular, organismal and population levels (Gutierrez-Luna et al. 2010).

The workflow scheme for VOCs is presented in Fig. 2.1. To study these substances, it is necessary to establish extraction, separation, identification and bioanalysis.

protocols that help to establish the volatile chemical profile and their activity. First, to design, the experimental procedures must be known that the volatiles have a low molecular weight (lower than 400 Da), very low boiling points, low vapour pressure and a very easy conversion into gas (Fincheira et al. 2018), which makes challenging the creation of new protocols for studying liquid and gas samples with volatile substances (Monteiro et al. 2017).

First, it is necessary to cultivate various bacteria and make them interact with the plant that the researchers want to study. After the collection of the organism, it is necessary to stop or reduce the cellular metabolism within the biological system in short period of time, especially for unicellular organisms which can suffer mutations in weeks, therefore, the researcher employs a process known as Quenching, which preserves the metabolism integrity; the main objective of this process is to stop the enzyme activity. Methods like the use of liquid nitrogen, methanol at high ($\geq$80 °C) and temperatures ($\leq -40$ °C) and the reduction of the pH environment, are options for the researcher to perform the Quenching, in addition to the quenching method, other variables that the researcher should control to guarantee an efficient inactivation of the system, the size of the microorganism, the number of assays and the development stage of the sample must be carefully control to avoid mistakes in the sampling, usually for volatiles the quenching at low temperatures and the reduction of the pH are more suitable methodologies, considering that volatile compounds are unstable at high temperatures, the most common tool for the quenching of samples with volatiles is the use of liquid nitrogen, which also allows the treatment of biological samples and reducing the risk of the loss of volatiles due to evaporation and adverse reactions (Pinu et al. 2017).

After the quenching process, the researcher proceeds to collect the volatiles of the sample (Audrain et al. 2015) to obtain the volatiles can be used protocols such as solid phase extraction (SPE) hydrodistillation, soxhlet extraction, supercritical fluid extraction, simultaneous distillation-extraction, but the most efficient protocols of all are closed-loop-stripping analysis (CLSA) and headspace-solid phase microextraction (HS-SPME) [10]; those are a faster and cheaper protocols and do not require the heating of the sample, also, both are solvent-less technique which allow the concentration and the extraction of unstable metabolites, reducing the

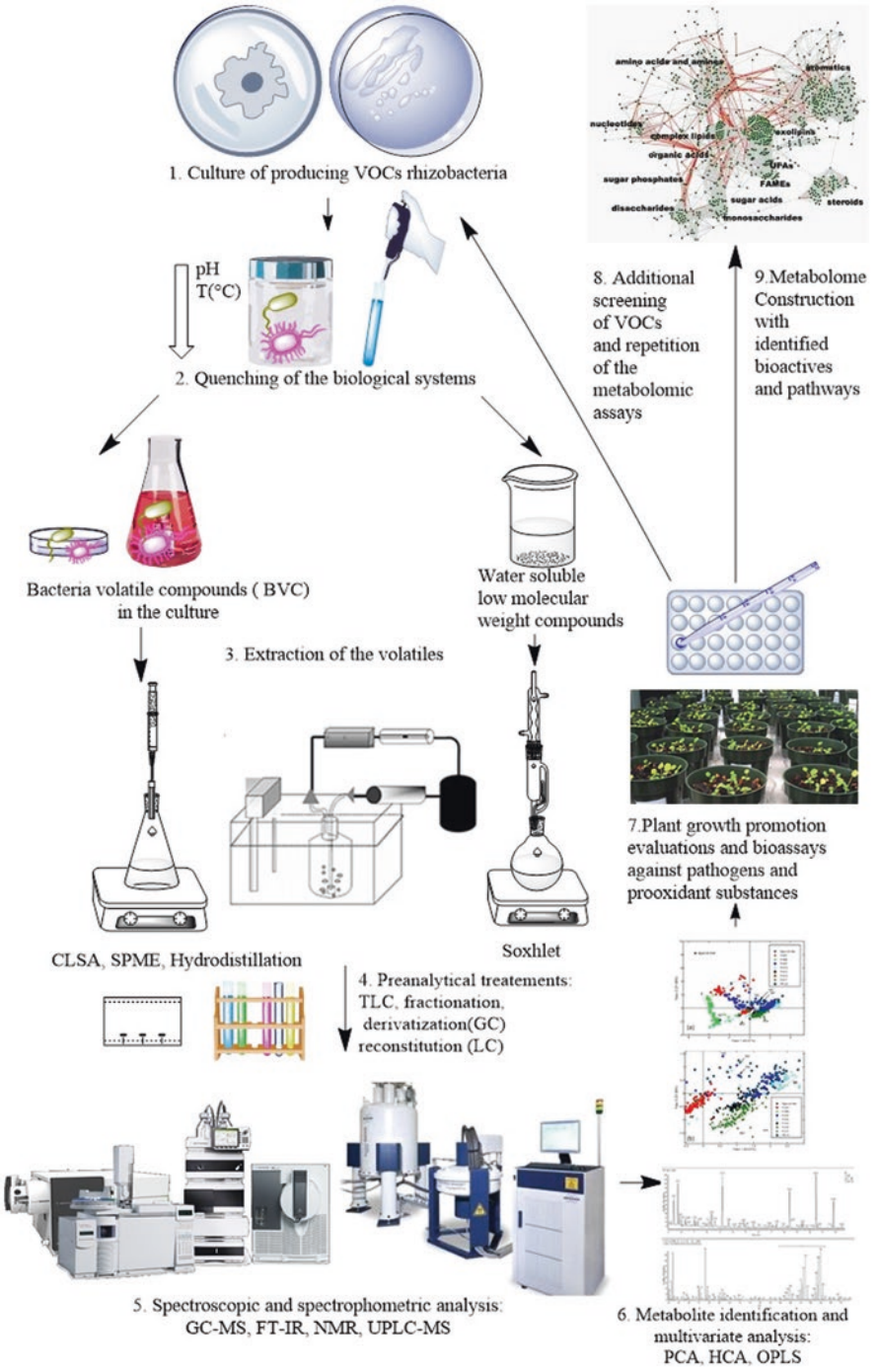


**Fig. 2.1** Workflow of metabolomic projects that study volatile organic compounds (VOCs)

manipulation of the samples and allowing an immediate injection of the sample for the analysis (Bruisson et al. 2019).

After the extraction of the VOCs, some samples may require the respective fractioning to make a preliminary identification of the amount of metabolites contained in the extract or to separate the volatiles of high molecular weight compounds. Techniques such as thin-layer chromatography (TLC) are very helpful for a first approach related to the number of compounds present in the sample and also gave an idea of the purity of the samples. Protocols such as molecular-size exclusion chromatography and ion-exchange chromatography can separate the extracts in fractions for a posterior analysis; however, we have to keep in mind that the volatiles can easily decompose or react, affecting the chemical profile of the sample, making some of these methods challenging to perform. There are solutions if the researcher wants to obtain a fraction from these methodologies like employing cold temperatures or solvents with high affinity to desired volatiles and high boiling point, but considering their cost and their lack of effectiveness compared to the SPME protocols, these methods have been discarded. Also, depending on the metabolites, it is necessary to do the previous steps (Kimball 2016).

The most effective method to know the chemical profile of the low molecular weights compounds contained in a biological system is through the spectrophotometric and spectroscopic analysis. The sample is injected, and it is possible to compare the obtained spectra registered with the information of databases such as NIST, the Golm Metabolome Database (GMD) and the Fiehn Library. The most common analysis for volatiles is the gas chromatography-mass spectrophotometry (GC-MS) and the gas chromatography-flame ionization detector (GC-FID) (Verma et al. 2018; Diez-Simon et al. 2019), but recently the nuclear magnetic resonance (NMR) has been used to study the chemical profile of the rhizobacteria and has been useful to identify new and unknown volatiles (Nemadodzi et al. 2020).

After obtaining the chemical profile, it is necessary to establish the bioactivity of the extracts, and the obtained volatiles, for these bioassays, usually are performed antimicrobial, antifungal and induced systemic resistance (ISR), and the growth of the rhizobacteria and the pathogen are performed by the split-plate method (Ali et al. 2015). Here, the rhizobacteria and the pathogen or the plant are divided by separate compartments, and both have the same culture medium which must allow the growth of both microorganisms. This guarantees the free exchange of gases without the need of physical interaction between the rhizobacteria and the pathogen (Beneduzi et al. 2012; Singh et al. 2021).

If a rhizobacteria inhibits the growth of a pathogen and allows the growth of the plant, it necessary to perform a multivariate analysis which allows the researchers to pick up the possible biomarkers with the biggest impact on the bioactivity. This is the first part of the fifth step in the workflow for metabolomic analysis of volatile organic compounds that consist in an additional screening to identify the usefulness of the bioactive compounds involved in the plant-rhizobacteria interaction. In this case, every step must be repeated, and its mandatory to minimize the variation to avoid the unintentional sampling of irrelevant compounds that introduce noise into the sample and have a negative impact in the quality of the results. The incorrect

interpretation of the date will not allow the identification of some compounds that are released in a specific growth stage or the inability to discover the pathway of the chemical reaction of a metabolite (Olanrewaju et al. 2019).

To avoid those inconvenience, it is necessary to have a more meticulous control of the culture media as well as more control of the extraction process, correcting the media conditions and establishing headspace control treatment without the rhizobacteria, parallel to the microextraction of the plant-PGPR system; also, it is necessary to test various HS-SPME fibres (Lim et al. 2018). The biggest flaw of the SPME method is that the fibres have variations in the affinity, and according to the polarity of the material, it can include or exclude the metabolites depending of their functional groups, limiting the range of compounds that can be detected in one trial, so it is necessary to employ fibres with different composition and absorption which allow to obtain a wider vision of the plant-rhizobacteria volatile compounds (Rath et al. 2018).

Also, in the fifth step, it is recommended to use more advanced separation techniques and spectroscopic methods such as proton transfer reaction time-of-flight mass spectrometer (PTR-TOF-MS) (Mary-Almirall et al. 2017) and 2D NMR (Vlassi et al. 2020) which will reduce the high variability of the volatile chemical profile which depends on the environmental conditions and the interactions of the rhizobacteria with the plant and other microorganisms (Tyc et al. 2015).

Finally, it is necessary to employ multivariate analysis protocols such as principal component analysis (PCA), hierarchical cluster analysis (HCA) and/or partial least square discriminant analysis (PLS) for statistical analysis obtained from the chromatograms and spectrums. Still, to obtain a more precise chemical profile of complex systems that involves symbiosis more rapidly, it is mandatory to employ or create new analytical tools and new software which can provide us more information of new compounds and unknown profiles. Therefore, with this workflow, there have been significant advancement in the profiling of low molecular weight compounds produced by the rhizobacteria that contribute to the plant metabolism (Lubes et al. 2017).

## 3 Bioinformatics Tools for the Interpretation of Metabolomics Data

An analysis of data from high-performance metabolomic experiments using data acquisition platforms for petabyte-scale sizes and complex platforms has been defined as a challenge in bioinformatics (Johnson et al. 2015). Improvements in data analysis software and in silico analysis can provide advances in biological interpretations in identifying metabolites (Meier et al. 2017). Computational tools for analysing a large volume of data can be operated in standardized steps: data processing, chemometric analysis, software for the identification of metabolic structures and pathways and databases (Misra 2018).

## 3.1 Data Processing

During the preprocessing data stage, noise is minimized to improve the quality of the obtained signals, which enhance the detection and quantification of the peaks and facilitating the comparisons that will be performed between the received data (Brown., 2018). Several programs can carry out the step that aims to improve the quality of detection and quantification of the peaks, making the data comparable to each other. In the table below (Table 2.1), we exemplify some programs and their primary purpose.

## 3.2 Chemometrics: Statistical Validation

Statistical analysis tools, both univariate and multivariate, are critical for metabolomic experiment interpretation including the identification of similarities and differences among experimental groups (Shulaev 2006).

Data sets have many variables or hundreds of metabolites, but only a small amount of data is collected for analysis. Statistical analysis of these data is necessary to reduce part of the variables to obtain uncorrelated characteristics. Through methods of significance in ANOVA and t-test, linear combinations of PCA variables or using algorithms were diversely used in studies of compound metabolomics (Gardinassi et al. 2017). Among multivariate analyses, two main methods are frequently used in investigations on metabolomics: supervised and unsupervised analysis. Principal component analyses (PCA), between-class analyses (BCA), hierarchical clusters analyses (HCA) and k-averages are the methods most frequently sought in unsupervised analyses (Costa et al. 2015). The analyses used in

**Table 2.1** Software and packages use for data processing in metabolomics

| Software/package | Support data | Features of program |
|---|---|---|
| *MetaAlign* | LC/MS<br>GC/MS | Preprocessing |
| *MZmine* | LC/MS<br>CE/MS<br>GC/MS | Complete analysis |
| *XCMS* | LC/MS<br>GC/MS | Complete analysis |
| *MeltDB 2.0* | LC/MS<br>GC/MS | Preprocessing |
| *CAMERA* | LC/MS<br>GC/MS | Annotation |
| *AMDIS* | GC/MS | Preprocessing |
| *WMSM* | ESI- LC/MS | Preprocessing |
| *CODA* | LC/MS<br>ESI- LC/MS | Preprocessing |

univariate are student t-test, Mann-Whitney U test and linear regression. The multivariate analysis includes the tests: PCA, PLS-DA, OPLS-DA, BCA and HCA. These tests are found in several software, and these include, among others, SMART, Specmine, eMZed, Metab, Pathomx, SIMCA-P, R Software and MixOmics (Thompson et al. 2020).

### *3.3 Software: Identify a Metabolite and Metabolic Pathway*

Quantifying and identifying metabolites, in short, are the first steps of this type of research. Then, biological information assignments are given in the experimental context (De Carvalho et al. 2019). The investigation of metabolic pathways is involved in the biological process. Some databases and software (Table 2.2) are essential tools that contain metabolic information from different organisms, pathways and specific reactions of several species already studied (Marco-Ramell et al. 2018).

There are two types of analysis that can be executed by software tools: first, those are the analysis of the chemical profile and the prediction of clusters and chemical structures, and second, we have the programs whose main purpose is the analysis of the process suffered by organics compounds like primary and secondary metabolites contained inside a biological system.

First, we are going to focus on the programs in charge of the chemical nature. Most of those programs took a DNA sequence obtained in the analysis of biological samples of the material, and they analyse clusters, predict the presence of certain enzymes linked to the biosynthesis of volatiles, specially various kinds of synthases, and according to these information, predict the functional groups contained in a molecule, the presence of aliphatic, cyclic and aromatic structures as well as their stereochemistry. Those databases cover a vast quantity of compounds from low molecular weight compounds to macromolecules, and those software can be individually or can be integrated for a more efficient prediction of the clusters and the structure of the metabolites (Costa et al. 2015).

The enrichment of pathways through tests makes it possible, for example, to identify metabolic pathways that are overrepresented in the list of p-value calculations using Fisher's test, hypergeometric test or the z-score (Diez-Simon et al. 2019). The use of combinations of libraries to obtain more complex results can provide robustness of the metabolome and better performance of the applied statistical tests, in contrast to using a single database (De Carvalho et al. 2019; Marco-Ramell et al. 2018).

The approach of specific metabolite repositories from different organisms enables precise interactions between metabolic characteristics and biological functions. Also, all data from metabolic studies can be subjected to a global analysis, with platforms that contain filters for specific organisms, technologies and studies (De Carvalho et al. 2019).

**Table 2.2** List of software and packages use for pathway analysis

| Software/package | Weblink | Function of the software |
|---|---|---|
| *RRantiSMASH* | https://antismash.secondarymetabolites.org/#!/start | Prediction and analysis of clusters and chemical structures of volatiles |
| *ClustScan* | http://csdb.bioserv.pbf.hr/csdb/ClustScanWeb.html | Prediction and analysis of clusters and chemical structures of volatiles |
| *NP. Searcher* | https://dna.sherman.lsi.umich.edu | Prediction and analysis of clusters and chemical structures of volatiles |
| *Metscape* | https://metascape.org/ | Prediction and analysis of metabolites and their pathways |
| *metaP-server* | https://mapserver.org/ | Prediction and analysis of metabolites and their pathways |
| *metPA* | http://metap.helmholtz-muenchen.de/ | Prediction and analysis of metabolites and their pathways |
| *MetExplore* | https://metexplore.toulouse.inrae.fr/ | Prediction and analysis of metabolites and their pathways |
| *MSEA* | https://www.metaboanalyst.ca/ | Prediction and analysis of metabolites and their pathways |
| *Mummichog* | https://shuzhao-li.github.io/mummichog.org/ | Prediction and analysis of metabolites and their pathways |
| *Metabonalyst* | https://www.metaboanalyst.ca/ | Prediction and analysis of metabolites and their pathways |
| *YMDB* | http://www.ymdb.ca/ | Prediction and analysis of metabolites and their pathways |
| *MetaBox* | http://kwanjeeraw.github.io/metabox/ | Prediction and analysis of metabolites and their pathways |
| *HMDB* | https://hmdb.ca/ | Prediction and analysis of metabolites and their pathways |
| *KEGG* | https://www.genome.jp/kegg/ | Prediction and analysis of metabolites and their pathways |
| *MetaboLights* | https://www.ebi.ac.uk/metabolights/ | Prediction and analysis of metabolites and their pathways |
| *KeyPathwayMinerWeb* | https://keypathwayminer.compbio.sdu.dk/keypathwayminer/ | Prediction and analysis of metabolites and their pathways |

## 4 Application of the Metabolomic Analysis for the Elucidation of Volatiles Produced by Plant Growth-Promoting Rhizobacteria (PGPR)

Before we continue, it must be clear that the concentration and chemical profile of a PGPR sample depend not only on the bacteria but also from the plants which are interacting with the microorganism. The researchers know it, and during their metabolomics project, they studied both the bacteria and the plant; as a result, it has been possible to elucidate more than 1000 volatile compounds that resulted of the plant-PGPR interaction. Some of these metabolites can act through direct antibiosis and competition with pathogenic microbes or through induced systemic resistance (ISR) and priming of defence responses (Ali et al. 2015; Aloo et al. 2019). Beyond the effect that the volatiles have in the crop yield, the stress tolerance and the plant growth, a lot of those metabolites have not one but more bioactivities that result in an action against pathogens, free radicals and pro-oxidative substances, and their presence could be used as a biomarker of phenomena such as the rise of fresh and dry weight of the plant in harsh conditions or the antimicrobial and antifungal effect of a rhizobacteria in an specific crop.

The diversity of PGPR studied is huge considering that have been studied both Gram-positive and Gram-negative bacteria and that it have been found volatiles with different functional groups, aliphatic structures and cyclic and aromatic compounds (Audrain et al. 2015), as we can see in the (Fig. 2.2).

Still, here we are going to comment about the most representative examples of metabolomic studies in PGPR volatiles, which gave us a bigger amount of information and have potential for the industry. The microorganism which has more information about the volatile compounds in general is *Bacillus subtilis*. There are numerous studies about the interactions of this rhizobacteria with plants such as cannabis, Italian oregano, common grapevine, among others (Aloo et al. 2019). The interaction of the plant with *Bacillus subtilis* increases the microorganisms. It has been studied that numerous rhizobacteria are from the Bacillus genre, and it has been discovered that they are an important source of alcohols such as propanol, methyl-1,2-cyclopentanediol, 3,5,5-trimethylhexanol and various derivatives of butanol such as methylbutanol (Xie et al. 2016).

Another well-studied microorganisms are *Pseudomonas* bacteria, specially *Pseudomonas fluorescens*. It is clear that the increase in the concentration of gibberellins, phytoalexins and membrane-related sterols is due to the action of the Gram-positive microorganisms. It also has been discovered with the metabolomic approach, the effects on to crops like, *Tagetes minuta*, *Origanum majorana* and *Menta piperita* when they have a symbiotic interaction with pseudomonas, present a higher concentration of terpenic compounds such as s-limonene, linalool, pulegone, menthol, terpinen-4-ol, cis-sabinene.

hydrate, trans-sabinene hydrate, a-terpineol and menthone, compared to the control treatment (Cappellari et al. 2020). Besides, the metabolomic approach allowed the researchers to discover that the *Pseudomonas* microorganisms are the source of

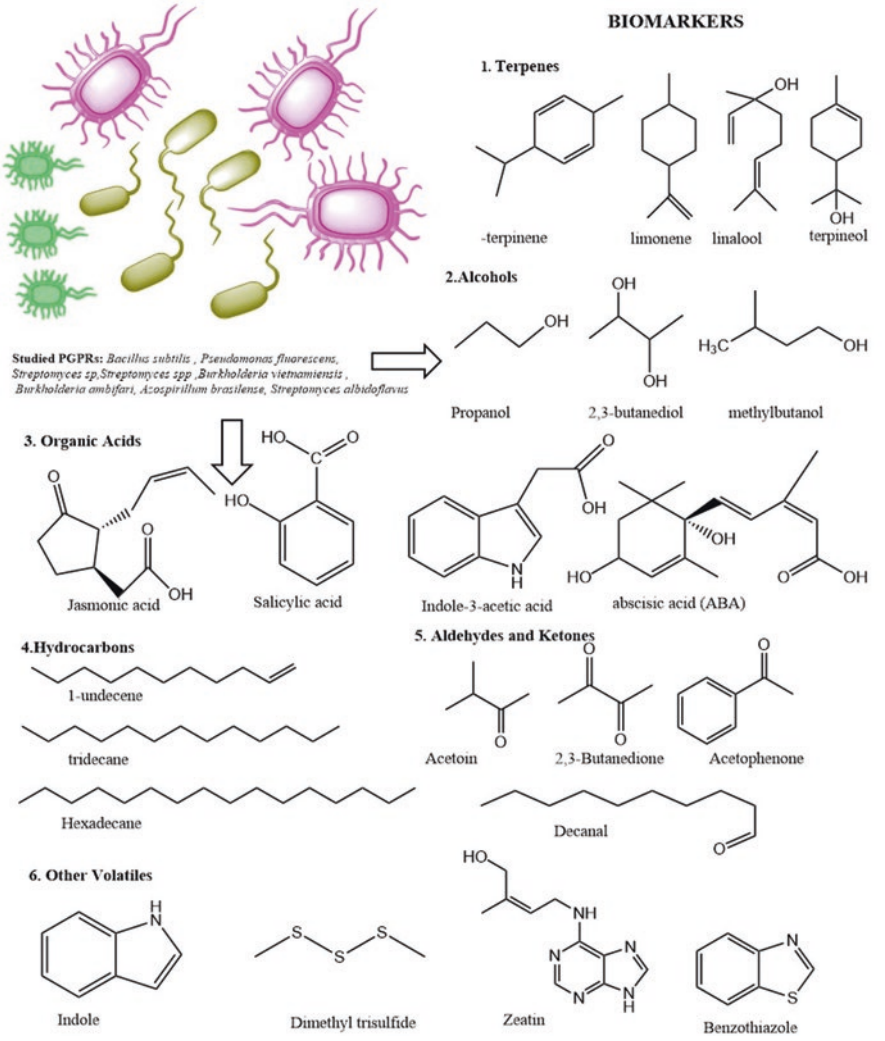


**Fig. 2.2** Examples of volatiles found in PGPR-plant interactions

cytokinins and certain aldehydes and ketones such as decanal, 2-heptanone, 2-nonanone, 2-undecanone, 4-octanone, acetophenone and 1-phenyl-1,2-propanedione, low molecular weight compounds which have simple structures (Maenaka et al. 2020). Production of terpenes, such as γ-terpinene, trans-sabinene hydrate, cis-sabinene hydrate and carvacrol, in addition, metabolomics, allowed the researchers to identify the 2,3-butadienol as the main marker (Yi et al. 2016); the same happens for other *Bacillus*.

The combination of chromatographic techniques with a multivariate analysis has been very useful to know the whole impact of *Streptomyces* microorganisms,

particularly the *Streptomyces* sp. and *Streptomyces* spp. (Oleńska et al. 2020). These microorganisms are the main source of organic acids and auxins. With metabolomics, it was also possible to have a better understanding of the plant-*Streptomyces* interaction. It was discovered how important jasmonates are for the previously mentioned symbiosis in which the correlation with the content of these compounds with the strength of the interaction and the bacteria concentration. Jasmonates basically control a big part of the symbiosis and relieve the biotic and abiotic stress suffered by the plant; this key elements allows an easier interchange of compounds between the microorganism and the plant (Sirhindi et al. 2020).

Numerous metabolomic studies have also studied bacteria from the *Burkholderia* trying to find new biomarkers and how their actions have a positive effect on the crops. Microorganisms like *Burkholderia vietnamiensis CBMB40* have a symbiosis with plants like the *Eucalyptus grandis*. The result showed that the plant-bacteria interaction increased the sesquiterpene concentration and had a positive impact in the biosynthesis of monoterpenes such as cymene and cineol (Kanagendran et al. 2019). Other metabolomic studies showed that *Burkholderia ambifari* have a considerable concentration of ketones such as acetoin, 2,3-butanedionone, acetophenone, 4-methylthio-2-butanone, o-aminoacetophenone, 2-tridecanone and phenylpropan-1-one. The biomarkers are molecules with cyclic structures and complex chains which are different to the carbonyl compounds synthetized by *Pseudomonas* bacteria, helping the researchers to establish differences between the secondary metabolite production of Gram-negative bacteria presents variations according to the genre (Groenhagen et al. 2013).

Another achievement of the metabolomic studies, beyond the in-depth study of the chemical composition of rhizobacteria and the differentiation between the plant and bacteria metabolite as well as the discrimination of biomarkers depending on the microorganism, is the study of synergism between two or more volatile compounds and the bioactivity of an individual and a group of molecules. With numerous extractions and isolations of different metabolites, it has been determined that terpenes have multiple effects in biological system; however, their action is highly dependent of the synergisms between the isoprene derivatives and other low molecular weight compounds, specially polar compounds with oxygenated groups, the synergisms of bacterial terpenic compounds with both alcohols and organic acids such as 2,3-butadienol, indole acetic acid (IAA) and abscisic acid (ABA) (Scalerandi et al. 2018). Numerous bioassays performed by the metabolomic studies showed that the synergism between the oxygenated volatiles and the terpenic compounds leads to mediation of the growth suffered by the plant according to their degree of salinity. Also the previously mentioned synergism can improve the tolerance of the plant to the chill and the drought (Naeem et al. 2018; Khan et al. 2020; Kusale et al. 2021; Najafi et al. 2021).

Also, metabolomic studies helped the researchers know the role of certain compounds. One main example is the auxins which control the production and synergisms of compounds like ethylene and gibberellins (GA), metabolites which have influence in other process like lateral root initiation, floral meristem initiation,

vascular differentiation, apical dominance, embryo development, leaf abscission, parthenocarpy, differentiation of phloem and xylem, floral bud formation and fruit development (Cato et al. 2013).

Besides, the metabolomic approach helped researchers to evaluate the intensity of the bioactivity between two different groups of low molecular weight compounds. One of the most prominent cases is the evaluation of the biological action of hydrocarbons and carbonyl compounds present in rhizobacteria. Numerous studies have established between both organic compounds in their action against phytopathogens such as *Phytophthora infestans*, *Phytophthora cinnamomic* (Méndez-Bravo et al. 2018) and *Rhizoctonia solani* (Huang et al. 2017). Both hydrocarbons and aldehydes and ketones have the same action mechanism against pathogens and pro-oxidant substances, but compounds like tridecane, 1-hexadecane and 1-undecene have a bigger level of specificity than the carbonyl volatiles, showing a stronger action against parasites, bacteria, fungus and virus (Ryu et al. 2015).

One of the main goals of the metabolomics is to find a microorganism whose plant-bacteria interaction leads to the maximum growth and development of the crop who were inoculated with the microorganism and helps the plant to defend itself against pathogens. Two bacteria that may fulfil these requirements are *Azospirillum brasilense* (Jacoby et al. 2017) and *Streptomyces albidoflavus* (Wang et al. 2013): the first one allows a more stable and ideal growth of the plant in various crops, and the second one gives a vast amount of organic acids to the plant that increases their defences against pathogens such as *Alternariasolani*, *A. alternata*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani* and *Botrytis cinerea* (Ryu et al. 2015). With more advancements related to the discovery of new biomarkers, new synergisms and the proper correlation between the composition and the biological action, researchers and industry can create new products based on the bacterial volatile compounds (BVCs) of rhizobacteria that are capable to replace antibiotics, pesticides and fertilizers which are less risky and more effective than the products available at the market.

Other metabolomic researches related to the VOCs in the plant-bacteria interaction have been focused on the communication between the two biological systems. Chaman et al. (2013) used metabolomic profiling and microarray analysis to show which molecules are involved in the defence and signalling process during the symbiosis. Among the volatiles which have influence in the mediation the PGPR-induced priming of the plant against pathogens, they found that salicylic acid has a key role in the process. Metabolomics also showed that there are changes in the metabolomic profile of a plant with rhizobacteria of the same species but of a different strain. It has been shown that the cultivars of rice (*O. sativa*) presented variations according to the PGPR, reflected in the differences of chemical profile and growth (Tenenboim et al. 2016; Sagar et al. 2020).

The study of various PGPR and the volatiles present in the plant-bacteria interaction has led to the discovery of a patron related to the metabolic signature in different plants treated with microorganisms of the *Azospirillum* genus, which signals a coevolution between the host organisms and the PGPR. Investigations like the one performed by Rozier et al. (2016) showcased that the interactions between

*Azospirillum lipoferum* and different maize cultivars share a common route and, therefore, produce the same metabolites. With the use of non-parametric analysis and the use of the MixOmics software, the researchers discovered that the use of rhizobacteria increased the amount of the hydroxycinnamic acids derivates present in the *Zea mays* cultivars.

On the other hand, researchers such as Vinay et al. (2016), Naik et al. (2018) and Valette et al. (2020) discovered that microorganisms like *Paraburkholderia phytofirmans*, *Herbaspirillum seropedicae* and various *Pseudomonas* can increase the accumulation of hydroxycinnamic acid (HCA) derivates, such as feruloylquinic acid, N-p-coumaroylputrescine and N-feruloyl putrescine in the rice crops. Those bioactives are associated with antimicrobial properties and the protection of the plant against biotic and abiotic stress, while the resorcinol derivatives suffered a decrease in their accumulation, which translates in a reduction of their concentration. Without the correlation done by the metabolomic approach using the collected spectrums and analysing them with a PCA in a R software, it would have not been possible to discover a common biosynthetic pathway among bacterial species of the same genus and between plant and bacteria interactions. Also, these analyses conclude that different plant species treated with similar PGPR produce common biomarkers with a positive impact on the plant's health and in their growth. This discovery will make it easier to find multiple bacteria with a similar biological classification which can be an alternative to replace pesticides and agrochemicals due to their action in multiple crops against different pathogens.

Other studies have shown how the untargeted and targeted metabolomic analyses can be used to achieve different goals. Mhlongo et al. (2020) showed that PGPR such as *Pseudomonas fluorescens* N04, *P. koreensis* N19, *Paenibacillus alvei* T19 and *Lysinibacillus sphaericus* T22 induced defence-related metabolic reprogramming in the *Solanum lycopersicum* plants. With the untargeted analysis, the researchers obtained the chemical profile of the benzoic and hydroxycinnamic acids; with the targeted approach, they could study the changes in VOC concentration over a two-day period in response to the four PGPR strains. For this purpose, the researchers employed the CV-ANOVA and OPLS-DA analyses with the SIMCA software.

Plenty of researchers with the aid of software such as SPSS discovered with the help of metabolomic analysis that certain volatiles produced by PGPR described as harmful can have positive effects on the plants. Mellidou et al. (2021) employed the Duncan multiple-range tests to discover that positive impact of MDA in the tomato, soybean and rosemary metabolism under stress conditions. With high MDA, the plants can withstand salt-induced oxidative stress, by activating the xanthophyll cycle-dependent dissipation of excess excitation energy in leaves. Besides, it can increase the alertness of the plant to deal with other situations of stress, increasing the responses against pro-oxidant compounds and improving the growth. The same study with the help of the GC-MS procedures and multivariate analysis showed that rhizobacteria such as *Pseudomonas oryzihabitans* helped the plants to preserve a vast amount of their original volatile composition. The tomato samples inoculated with the rhizobacteria had less metabolomic programming, which can preserve not only their original chemical profile but also their organoleptic properties. Still it was

possible to see that with the metabolomic approach that rhizobacteria changed the concentration of organic acids such as oxalate, malate, galactarate and 2-ketoglutarate.

Finally, the other key objective of the metabolomic studies of rhizobacteria VOCs is the comprehension of the pathways that leads to the production of a metabolite and the changes of the chemical profile. It has established that the PGPR volatiles stimulate the pathways that lead to the increase of defences and the production of key phytohormones. The studies related with *Pseudomonas*, *Burkholderia* and *Bacillus subtilis* interactions with numerous crops showed that BVCs have a direct impact on the auxin, ethylene, salicylic acid and jasmonic acid pathways (Rosier et al. 2018); also with the targeted and untargeted studies, it is been confirmed that most of the low molecular weight compounds produced by rhizobacteria have a dual role: they can improve the nutrition and the growth of the plant, and at the same time, they can confirm resistance to the stress and pathogens. The most prominent volatile with dual role is the acetoin which can be found in both Gram-positive and Gram-negative bacteria. Few volatiles such as salicylic acid or indole acetic acid limit their action to one role, establishing that the volatiles of rhizobacteria in general are versatile substance with multiple mechanisms which need more study (Fincheira et al. 2018). The VOCs not only have an impact on the plant pathways but also those biochemical process alter the pathways of other microorganisms found in the rhizosphere and induce positive changes in the bioavailability of nutrients and the soil chemistry (Saia et al. 2015; Bhaskar et al. 2021). Metabolomics have shown that the diffusion of volatiles through the rhizosphere has a positive impact because the volatiles produced by rhizobacteria enhance the production of numerous bioactive substances which feed other microorganisms which surround the roots of the plant and those bacteria and fungus elicit the induced systemic resistance (ISR) which defends various organisms of phytopathogens and nematodes (Dessaux et al. 2016; Sharma et al. 2020).

Now that the researchers have a more substantial amount of information related to the pathways and the VOCs, there are more ambitious projects like the construction of a soil metabolome, which employs nuclear magnetic resonance (NMR), gas chromatography-mass spectroscopy (GC-MS) and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to obtain a more accurate and complete volatile compound profile of the different PGPR found in an specific environment. Currently, they have the complete mapping of pathways such as monoterpenoid biosynthesis, diterpenoid biosynthesis and polycyclic aromatic hydrocarbon degradation (Honeker et al. 2021).

The construction of data base with the volatile metabolites of PGPR in the soil and the previous research show that the metabolomic studies are useful for the bioprospection of new natural products based on rhizobacteria BVCs and for the understanding of the effect of bacteria in the rhizosphere. Still, those projects, in particular the VOC soil metabolome construction, present many obstacles that the researchers should solve. There are still unknown metabolites, also we must consider that the most common metabolomic analytical techniques are not capable to detect VOCs, and there are still unknown steps and intermediate products metabolic pathway.

Those problems led to incomplete chemical profiles and unclear pathways related to the host-bacteria interactions (Ghirardo et al. 2020; Kalam et al. 2020). The previously mentioned complications and other limitations of the metabolomic analyses of the VOCs are going to be analysed in the next part of the chapter.

## 5 Challenges for the Metabolomic Analysis of Rhizobacteria Volatiles

First, we must understand that is the ultimate level of the postgenomic analysis, and it offers various solutions for the investigations than involve metabolites considering that is not possible to study the effects of volatile compounds just with RNA- or enzyme-based techniques, so it is perhaps the most complicated omic science, and it has some limitations that need to be addressed. First, we should know that the diversity of metabolites is huge; the total of metabolites in plants oscillates between 100,000 and 200,000 compounds, which include both primary and secondary metabolites. This variable increases the technological demands of the metabolomic analysis, specially the untargeted metabolomics; many researchers have proposed to establish a limit related to the number of metabolites that should be studied. In the case of untargeted metabolomics, it is necessary to establish a range of secondary metabolites to be studied and to focus in biomarkers which have been found in similar systems which include bacteria and plants of the same genre than the one that are being studied (Castro-Moretti et al. 2020).

Another challenge of the metabolomics is related to the plant-host studies. Those experiments recently gained a lot of popularity because they can help in studying pathologies with a negative impact on various crops. One of the main challenges comes from the vast number of metabolites that come from the quantity of compounds involved in the symbiosis. For those studies, it is necessary to improve the protocols that allow the fractioning of the sample according to the functional groups and the polarity of the compounds. Besides it is necessary to identify the in vitro activity of individual metabolites against pathogens and them. The researcher must proceed to evaluate the bioactivity of the compounds involved in the synergism (Thompson et al. 2020).

One of the most common problems of the metabolomic approach for the study of plant-bacteria interaction is the fact that the previously mentioned microorganisms can have additional symbiotic interactions with other microorganisms. The origin of one compound or a group of volatiles who share a similar chemical nature (molecular, weight, functional groups, stereochemistry, etc.) can be difficult to trace; they could interact with fungus, bacteria and protists; and at the same time, they have a symbiosis with a plant. The most famous case is related to the origin of sulphide compounds found in plant-bacteria interactions. It is presumed that the sulphide compounds come from mycorrhizae and free-living fungi who interacts with numerous crops like *Phaseolus vulgaris*. This is important since that the sulphide

compounds have been considered by the researchers as bactericidal or fungicidal compounds (Hoyos Carvajal et al. 2015). Sulphide volatiles such as benzothiazole and dimethyl trisulphide are some of the compounds that have been reported as effective against *R. solani*, *Helminthosporium sativum* and *S. sclerotiorum*. It also is known that they have synergisms with terpenic compounds and organic acids, considering the previously mentioned research is perfectly clear that there is a need to consider that the rhizobacteria could have multiple interactions at the same time, and it is necessary that future plant bacteria interaction considers the existence of a simultaneous bacteria fungi or bacteria protozoa symbiosis. This could complicate the experimental design of the metabolomic approach (Wang et al. 2013; Basu et al. 2021). In addition to the synergism of bacteria with multiple organisms and microbes, there are other variables who add difficulty to the experimental design as we can see in Fig. 2.3.

The age of the plant, the action of pathogens, insects and other herbivores which can attack the plant, the humidity, the temperature, the soil structure and nutrition and the water availability have a direct impact in the stability, concentration and bioactivity of volatiles; also if the plant has a disease which the researchers want to treat with *Rhizobacteria*, it is necessary to know the time that has transcurred since the plant show symptoms (Audrain et al. 2015).

Besides, VOCs in rhizobacteria metabolomic studies face more difficulties. The low concentrations, high ranges of polarity and the complexity of rhizobacteria and plant-bacteria interactions are variables that create obstacles for the analysis of low molecular weight compounds in these microorganisms. These factors make that the samples are highly variable, and it complicates the experimental design and the multivariate analysis (Diez-Simon et al. 2019).

To solve most of these problems, researchers use numerous advancements in the software used to process the metabolite structure and pathway data and the newer technologies related to the separation and detection of metabolites. The first advancement was the use of solvent-less techniques to isolate volatiles, considering that this benefits samples with high concentration of labile metabolites.

Nowadays there are new modifications of the gas chromatography such as GC-combustion-isotope-ratio MS (GC/C/IRMS) which when used determines the elemental isotopes of the volatiles found in the rhizosphere. The ratio of isotopes in a compound varies according to its source and forms a distinctive fingerprint which is detected by the equipment and serves to discriminate between samples with a common biomarker. Also it helps to describe the interactions between a rhizobacteria with different plants or the interaction of one common crop with multiple microorganisms. Another advancement is related in the treatment of the samples with high volatile content, treatments during protocols such as the use of direct-injection mass spectrometry (DIMS) and flow injection mass spectrometry (FIMS) which reduce time of the sample injection and give a more defined spectra due to the speed of the process and the fact that those protocols are solvent-free, still those methodologies have an impact in the rate at which the mass spectra is processed. Also a more generalized use of the electrospray ionization (ESI-MS) will improve the quality and the amount of metabolites detected, through the spraying of the sample

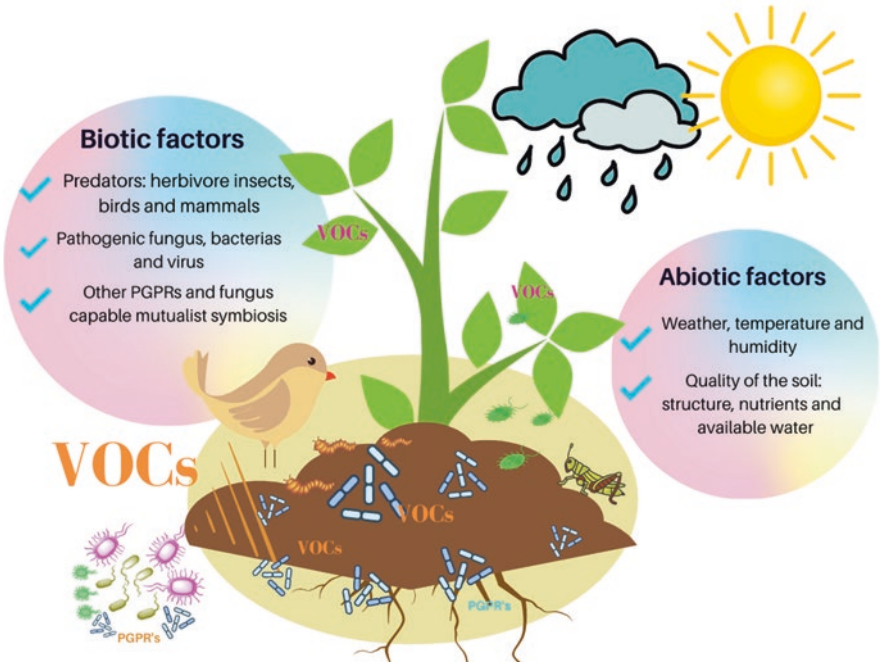


**Fig. 2.3** Factor with impact on the production and pathways of VOCs in the plant-bacteria interaction

in a highly charged environment the TOF analyser makes a better detection of the metabolites, and it is very useful to study the most labile substances and conjugations in the root-microbe interaction which will be difficult to research with conventional GC-MS protocols. This has been useful for the comprehension of the role of volatiles such as phenolic acids and terpenes in the rhizosphere; also it is useful to understand the synergism between non-oxygenated volatiles and compounds like flavonoids (Verma et al. 2018).

## 6 Final Considerations

The study of PGPR compounds have shown the researchers that there is a huge diversity related to the low molecular weight compounds, among the biomarkers there are compounds such as terpenic substances, organic acids, hydrocarbons, gibberellins, aldehydes, ketones, jasmonates and more substances. As a consequence, it is possible to conclude that there are numerous substances of bacterial origin, expected to be found in plant-bacteria interactions, and at the end, the number of volatile substances could be bigger than 100,000–200,000 compounds.

Metabolomic studies of rhizobacteria have shown that microorganisms of the same genre possess substances with similar chemical structure and functional groups which can be useful as biomarkers. Also those studies are effective to correlate the chemical profile or a group of secondary metabolites with the growth and stress tolerance of the plants. In addition, the metabolomic approach gives to the researcher, clues of the antipathogen and antioxidant mechanism of bioactives of bacterial origin in numerous crops. Some of the main objectives of the metabolomic projects have been accomplished, and as result microorganisms such as *Azospirillum brasilense* and *Streptomyces albidoflavus* are being considered for agro-industrial and pharmaceutical applications (Zaman et al. 2021).

Despite of that, the study of volatile metabolites has a vast number of complications due to the complexity of the analytes, the presence of additional symbiotic process with other bacteria and fungus, the low concentrations of certain biomarkers and their instability makes more difficult to trace biomarkers and to comprehend the metabolomic pathways involved in the crop-PGPR interaction. There is a need to improve the technologies, the procedures, the bioinformatic tools and the data treatment used in the metabolomic analysis of volatiles. With more advanced detectors and more precise methodologies, it will be possible to discover more substances and establish their role in phenomena such as the prevention of the pathogen action and the improvement in the plant size and crop yield and health.

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# Chapter 3
# The Role of PGPR-Secondary Metabolites on Plant Photosynthesis


**Virgilio Gavicho Uarrota, R. Z. Sayyed, and Romina Pedreschi**

**Abstract** Light energy is captured and used to convert water, carbon dioxide, and minerals into oxygen and energy-rich organic compounds through photosynthesis, and this process is of high importance in the maintenance of life on Earth. On the other hand, plant growth-promoting rhizobacteria (PGPR) are a diverse group of bacteria that can be found in the rhizosphere, on root surfaces, and in association with roots. PGPR affect the physiology of plants to attenuate to some degree the stressful effects of drought, salt, UV, and a combination of high $CO_2$ content and low atmospheric pressure. PGPR also increase the photosynthetic capacity via photochemical quenching and $CO_2$ assimilation rate. A deep study on how PGPR secondary metabolites modulate the plant photosynthesis is performed. PGPR increase the plant photosynthesis, chlorophyll contents, stomatal conductance, transpiration rate, and photosystem II efficiency of plants even in stressed conditions.

**Keywords** PGPR-secondary metabolites · Photosynthesis · Photosystems I and II · Chlorophyll

## 1 Introduction

Photosynthesis has been defined as the process by which green plants and certain other organisms transform light energy into chemical energy. During photosynthesis in green plants, light energy is captured and used to convert water, carbon dioxide, and minerals into oxygen and energy-rich organic compounds (Bassham and Lambers 2021). It would be impossible to overestimate the importance of photosynthesis in the maintenance of life on Earth. If photosynthesis ceased, there would soon be little food or other organic matter on Earth. Most organisms would

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disappear, and in time, Earth's atmosphere would become nearly devoid of gaseous oxygen. The only organisms able to exist under such conditions would be the chemosynthetic bacteria, which can utilize the chemical energy of certain inorganic compounds and thus are not dependent on the conversion of light energy (Bassham and Lambers 2021).

The increasing demand to reduce the use of chemical fertilizers and pesticides for the development of an agri-food system sustainable for environmental and human health, as well as the current shifting in the agricultural legislation of several countries, has led to an expanded use of bioinoculants. Chemical inputs usually alter the natural physicochemical and biological equilibrium of soil, and microbial consortia used in agricultural management practices could return soil to its natural status. Several plant growth-promoting rhizobacteria (PGPR) have been demonstrated to exert a beneficial effect on plant growth under nutritional and abiotic stress (Romano et al. 2020).

Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. PGPR, also termed plant health-promoting rhizobacteria (PHPR) or nodule-promoting rhizobacteria (NPR), are capable of promoting plant growth by colonizing the plant root. These are associated with the rhizosphere, which is an important soil ecological environment for plant–microbe interactions (Basu et al. 2021; Hayat et al. 2010). PGPR present abundantly in the rhizosphere have beneficial effects on plants. These microorganisms increase stem emergence and stimulate plant growth through several mechanisms. In addition, PGPR can facilitate plant development by improving the availability of certain nutrients, by producing hormones, or by limiting the pathogen growth via direct or indirect mechanisms (Backes et al. 2021; Basharat et al. 2021). Directly, the presence of rhizobacteria can cause modifications to plant metabolism. Examples include N fixation, phosphate solubilization, Fe sequestration, and cytokinin, gibberellin, indoleacetic acid, and ethylene production (Patten and Glick, 1996; Shaikh et al. 2016; Lucas et al. 2014). Indirectly, the presence of rhizobacteria promotes mechanisms that do not involve plant metabolism. Examples include antibiotics (Zakari et al. 2019); lytic enzymes, such as chitinases (Shaikh et al. 2018), cellulases, 1,3-glucanases, proteases (Jadhav et al. 2020), and lipases; siderophore production; competition between pathogens and non-pathogens; induced systemic resistance; and modulation of environmental stress effects (Ahemad and Kibret 2014).

Although the mechanisms used by rhizobacteria are well known, their impact on photosynthetic metabolism remains unclear. Backes et al. (2021) showed that the presence of the beneficial bacteria (*Burkholderia*) reduced the negative impact of the fungus *Drechslera teres*, the causal agent of net blotch in barley, on the photosynthetic performance and modified the net carbon assimilation rate close to the necrotic area. Indeed, the presence of the bacterial strain decreased the quantum yield of regulated non-photochemical energy loss in PSII noted as Y (NPQ) and allowed to maintain the values stable of maximum quantum yield of PSII photochemistry known as Fv/Fm and close to those of the control in the presence of *D. teres.* Sacristán et al. (2020) showed that sugar beet plants inoculated with PGPR strains (i.e., *Pseudomonas fluorescens* and *Pseudomonas chlororaphis*) showed

higher values of maximum quantum yield of PSII and non-photochemical quenching (NPQ). This review aims to investigate and update the current state of art on how PGPR affect the plant photosynthesis and its components.

## 2 The Effect of PGPR on the Net $CO_2$ Photosynthetic Rate

All plants use the photosynthetic carbon reduction or Calvin-Benson cycle for $CO_2$ fixation in which ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the first step producing a three-carbon compound, phosphoglycerate (3-PGA). For this reason, this process is referred as the $C_3$ cycle. A major problem with the $C_3$ cycle is that the enzyme Rubisco catalyzes two competing reactions: carboxylation and oxygenation. The oxygenation reaction directs the flow of carbon through the photorespiratory pathway, and this can result in losses of between 25% and 30% of the carbon fixed. Environmental variables such as high temperature and drought can result in an increase in the oxygenase reaction. Therefore, reducing the Rubisco oxygenase reaction has the potential to increase carbon assimilation significantly and would represent a step change in photosynthesis (Lara and Andreo 2011).

Net photosynthetic assimilation in $C_3$ plants is mostly viewed as a simple balance between $CO_2$ fixation by Rubisco-catalyzed carboxylation and $CO_2$ production by photorespiration. The cornerstone of photosynthesis is the enzyme ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco), that can fix either $CO_2$ (carboxylation) or $O_2$ (oxygenation). Oxygenation is the starting point of photorespiration, in which $CO_2$ is liberated in mitochondrial glycine-to-serine conversion by the glycine decarboxylase–serine hydroxymethyl transferase complex (Tcherkez and Limami 2019). The $C_4$ photosynthesis is an adaptation of the $C_3$ pathway that overcomes the limitation of the photorespiration, improving photosynthetic efficiency and minimizing the water loss in hot, dry environments. $C_4$ plants have greater rates of $CO_2$ assimilation than $C_3$ species. In theory, increases in atmospheric levels of $CO_2$ above current levels can increase photosynthesis by decreasing photorespiration (fixation of $O_2$ rather than $CO_2$ by Rubisco), which increases with temperature and is higher in $C_3$ than $C_4$ and crassulacean acid metabolism (CAM) plants. In addition, rising $CO_2$ generally stimulates $C_3$ photosynthesis more than $C_4$ (Lara and Andreo 2011; Hibberd and Quick 2002).

Results reported by Backes et al. (2021) in barley plants infected with a pathogen and inoculated with beneficial PGPR showed a decrease of net carbon assimilation rate after inoculation with pathogen *D. teres*; contrarily, plants inoculated with PGPR strain showed higher values compared to the control and those inoculated with pathogen, but the values were statistically nonsignificant. Photosynthesis was also reported to increase by 53.22% after tobacco plants being inoculated with PGPR, *Bacillus methylotrophicus* (Begum et al. 2021) in drought stress conditions. Recent work published by Costa-Santos et al. (2021) in tomato using different species of *Bacillus* spp. showed increase in net $CO_2$ photosynthetic rate. Samaniego-Gámez et al. (2016) also reported enhancement of photosynthesis after PGPR

inoculation in peppers, represented as $CO_2$ assimilation rate when compared with non-inoculated plants.

PGPR from rhizosphere synthesize auxins (i.e., indole-3-acetic acid) and this in its turn affect plant cell division, pigment formation and photosynthesis by changing the plant auxin pool (Ahemad and Kibret 2014). Moreover, $CO_2$ resulting from respiration of bacteria have been shown to increase photosynthesis. Hibberd and Quick (2002) reported that $CO_2$ produced in roots can be transported to the shoot. Stem cells in tobacco are supplied with C for photosynthesis from the vascular system and not from stomata. In this way, Rozpadek et al. (2015) suggest that upon endophyte colonization, host plant undergoes changes in its photosynthetic apparatus, leading to increased light harvesting and photosynthesis efficiency.

Photosynthesis converts light energy to chemical energy in the form of energy-rich sugar molecules. The sugars produced not only serve as carbon and energy sources but also as pivotal signaling molecules for plant growth, development, and stress responses (Zhang et al. 2008). In particular, elevated sugar levels induce storage processes and confer feedback inhibition of photosynthesis. Hexokinases (HXK1) are evolutionarily conserved glucose sensors in eukaryotes. To date, *Arabidopsis* hexokinase is the only identified sugar sensor in planta. Hexokinase-dependent glucose signaling requires ABA signal transduction, as the two signaling pathways positively interact with each other. Non-photosynthetic organisms usually obtain sugars either directly or indirectly from photosynthetic organisms (Zhang et al. 2008).

According to Burlak et al. (2013), transcriptome analysis of Arabidopsis plants colonized by a PGPR *Pseudomonas thivervalensis* revealed a low level of transcripts related to photosynthesis, and the real photosynthesis rates were repressed consistently with the reduced growth of plants colonized by the bacterium. Interestingly, the plants treated with *Pseudomonas thivervalensis* were more resistant to subsequent infections by the virulent pathogen *P. syringae* pv. than the control plants. In this case, the downregulation of photosynthesis is thought to be only a transient effect needed for the production of other transcripts necessary for the conditions of colonization and priming plants for fast response against phytopathogen. Zhang et al. (2008) reported that *Arabidopsis* plants exposed to *Bacillus subtilis* colony at a distance, without physical contact with plant roots, presented elevated photosynthesis level through the modulation of endogenous sugar/abscisic acid (ABA) signaling and established its regulatory role in the photosynthetic activity. The studies revealed the elevation of endogenous sugar accumulation in the plant, suppression of classic glucose signaling responses, and overlap in sugar/ABA sensing with suppression of ABA-biosynthetic transcripts. The authors explain such effect by sustained volatile signaling emitted by the PGPR bacterium (Burlak et al. 2013). Recent work of Khangahi and Crecchio (2021) also reported that PGPR inoculations significantly increased the photosynthetic capacity as compared to the non-inoculated durum wheat plants. Efthimiadou et al. (2020) also found a positive effect of PGPR application (foliar and soil) on photosynthetic rate up to 18.4% in maize plants under Mediterranean conditions. Under drought stress, potato plants with PGPR displayed less decrease in net photosynthetic rate than plants without

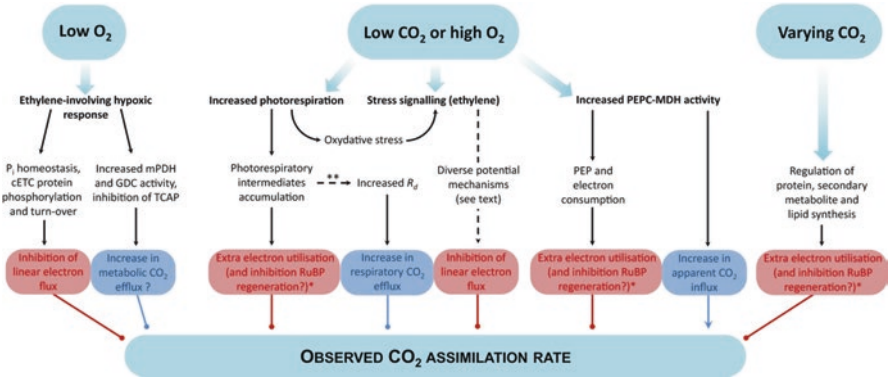


**Fig. 3.1** Summary of metabolic pathways affected by $CO_2$: $O_2$ in the short term and possibly affecting observed $CO_2$ assimilation rate. For simplicity, effects on photosynthesis are shown as falling into two categories: effect on chloroplastic electron transfer chain and/or ribulose 1,5-bisphosphate (RuBP) regeneration (red) and on $CO_2$ production or consumption (blue). See the text for more details on numerical flux values. The asterisk (*) stands for possible variation in the net effect due to possible decrease on redox pressure (e.g., under high light) and reutilization of chloroplastic phosphate to facilitate RuBP regeneration. The double asterisks (**) represent the link between the nitrogen demand by photorespiration and the stimulation of day respiration, further discussed elsewhere. *cETC* chloroplastic electron transport chain, *GDC* glutamate decarboxylase, *mPDH* mitochondrial pyruvate dehydrogenase, *PEP* phospho*enol*pyruvate, *Pi* free phosphate, $R_d$ day respiration, *TCAP* tricarboxylic acid pathway

PGPR application (Batool et al. 2020). Summary of metabolic pathways affected by $CO_2$ is mentioned in Fig. 3.1.

## 3 The Effect of PGPR on the Photosystem II (PSII) Efficiency

Photosynthetic efficiency of the plants can be measured by the maximum quantum yield of PSII ($F_v/F_m$) and is the most common parameter used in fluorescence and is inversely proportional to damage in the PSII reaction center (Gururani et al. 2012). The maximum quantum yield of PSII photochemistry shows the efficiency of light absorbance by the pigment matrix associated with PSII when all PSII centers are in the open state. The Fv/Fm-value may serve as an indicator of plant stress and can be measured quickly on dark-adapted leaves. The parameter ΦPSII is the operating efficiency of PSII when illuminated. At very low irradiance levels, this will be close to Fv/Fm, but as irradiance increases, values of ΦPSII will fall, and energy is dissipated by non-photochemical processes. The photochemical quenching (qP) coefficient reflects the capacity of reaction centers to compete for Chlorophyll (Chl) excited states, and it is related to the redox state of primary quinone acceptor of PSII ($Q_A$). In the case where all reaction centers are open and

capable of photochemistry, qP is maximal (estimated as 1), and the fluorescence yield is low. However, when reaction centers are closed due to reduction of $Q_A$, qP is zero, and fluorescence yields are maximal. Non-photochemical quenching (NPQ) is a measure of heat dissipation and reflects the combination of photo-protective mechanisms. NPQ is affected by non-photochemical quenching that reflects heat dissipation of excitation energy in the antenna system. So, it may be thought of as an indicator of excess excitation energy (Burlak et al. 2013).

In Arabidopsis, it was shown that the maximum and effective quantum yields of PSII (Fv/Fm and ΦPSII, respectively) in PGPR-treated plants were significantly higher than in controls, implying an improvement of energy transfer within PSII (Zhang et al. 2008). Experiments of Gururani et al. (2012) in potato showed that the $F_v/F_m$ values of PGPR-inoculated plants were higher than those of the non-inoculated control plants growing under the same stress conditions. This observation was further confirmed with the estimation of the performance index (PI), essentially an indicator of sample vitality. The PI value is considered an overall expression indicating a type of internal force of the sample to resist external constraints. The PI of PGPR-inoculated plants were significantly higher than that of the non-inoculated control plants under abiotic stress conditions. Datta and co-workers (2011) also showed enhanced growth and yield parameters of chili plants after PGPR inoculation in field experiments. Recent work of Begum et al. (2021) using arbuscular mycorrhizal fungi and PGPR in tobacco (*Nicotiana tabacum*) under drought stress showed a decline in the PSII activity (Fv/Fm) and photochemical quenching coefficient (qP), but an increase in non-photochemical quenching coefficient (NPQ) in leaves of tobacco in all treatments. The (Fv/Fm) was noticeably reduced in non-inoculated plants as compared to control conditions. However, drought stress did not reduce (Fv/Fm) activity in co-inoculated stressed plants by indicating a substantial augmentation of 33.43% compared with uninoculated ones. Similarly, inoculated plants significantly upregulated the qP under both control and stress conditions. However, co-inoculated plants presented approximately 32.69% higher qP under drought stress than uninoculated stressed plants, respectively. NPQ was increased by 51.93% due to drought treatment; however, the drought induced increase in NPQ was also reduced by 25.71%. The presence of PGPR was also reported to decrease the quantum yield of regulated non-photochemical energy loss in PSII noted as Y(NPQ) and allowed to maintain the values stable of maximum quantum yield of PSII photochemistry known as Fv/Fm and close to those of the control in the presence of *Drechslera teres* (*D. teres*), causal agent of net blotch in barley plants (Backes et al. 2021). The inoculation of plants with PGPR significantly reduced Fm values. Considering the Fv/Fm ratio, control plants showed a ratio of 0.82, which was only lower in plants inoculated by PGPR (Costa-Santos et al. 2021) in *Solanum lycopersicum*. The Fv/Fm ratio is an indicator of photosystem II damage in plants. The lower value indicates more damage to the photosystem. Results from the experiment with two genotypes of chickpea plants showed higher damage in sensitive genotype to the photosystem due to drought stress than the tolerant one. Both varieties showed responsiveness to the treatment, but the sensitive genotype was more responsive than the tolerant variety (Khan et al. 2019a,

b). In sugar beet plants, Sacristán-Pérez-Minayo et al. (2020) found significantly higher mean values (0.70) of quantum yield of PSII (ΦPSII) after the PGPR treatment. In relation to maximum quantum yields of photosystem II (PSII) and the non-photochemical quenching (NPQ) parameters, they observed no significant differences between the different treatments used. PGPR inoculation produced significant differences in the quantum yield of PSII (ΦPSII). This parameter indicates the real energy that the plants are using in the photochemical processes, at any given time. The NPQ values for all treatments were very similar, which means that the treatments have, a priori, the same energy loss at the measurement stage. Normally, NPQ reduction is observed in plants subject to different stress conditions (Sacristán-Pérez-Minayo et al. 2020). According to Samaniego-Gamez et al. (2016) in pepper plants, application of PGPR increased the maximum photochemical quantum yield of photosystem II (PSII) (*F*v/*F*m). Inoculated plants exhibited an increase of photochemical quenching (qP) by 27% when compared with non-inoculated plants. The electron transport rate of PSII (ETR) and PSII operating efficiency (*Φ*PSII) was also found to increase in inoculated plants. PGPR markedly augmented effective quantum yield of PSII photochemistry (Y(II)), electron transport rate of PSII (ETR) and photosynthesis capacity in durum wheat (*Triticum durum*) (Khangahi and Crecchio 2021). No statistically significant difference was observed between treatments in terms of *F*v/*F*m in both stress conditions. However, this parameter decreased under stress condition and increased by PGPR inoculation as compared to the control. All the considered chlorophyll fluorescence parameters were influenced by PGPR treatment that also prompted Y(NPQ) and qNto decrease in comparison to the inoculated treatment, in both unstressed and stressed plants. Plants with PGPR treatments were also reported to show higher Fv/Fm and ΦPSII as compared to plants without PGPR under drought conditions in potato plants (Batool et al. 2020).

## 4 PGPR and Their Role on the Chlorophylls *a* and *b*

Chlorophylls are natural green pigments ubiquitously present in plant kingdom, which play an important role in photosynthetic process, a vital function for life on Earth (Singh et al. 2020). Chlorophyll or leaf green is a porphyrin derivative with magnesium as the central atom and is hence a metal complex dye. It is present in the chloroplasts in all green parts of plants as a mixture of blue green chlorophyll *a* and yellow green chlorophyll *b* and constitutes the catalyst for photosynthesis (Puntener and Schlesinger 2000). Chlorophylls are found in virtually all photosynthetic organisms, including green plants, cyanobacteria, and algae. It absorbs energy from light; this energy is then used to convert carbon dioxide to carbohydrates (Britannica 2020).

Co-inoculation with arbuscular mycorrhizal fungi and PGPR considerably increased chlorophyll a, b, total chlorophylls by 96.99%, 76.90%, and 67.96% (Begum et al. 2021) in tobacco plants under drought stress. Using different PGPR strains in *Solanum lycopersicum* plants and compared with the control plants, Chl *a* and Chl *b* were found to increase or decrease depending on the PGPR strain

(Costa-Santos et al. 2021). Khan et al. (2019a, b) while studying metabolic and physiological changes induced by PGPR in two *Cicer arietinum* (chickpea) genotypes reported that the application of PGPR increased chlorophyll content in both genotypes, but the sensitive genotype was more responsive than tolerant genotype (Khan et al. 2019a, b). In tomato plants infested with *Spodoptera litura*, inoculation with PGPR enhanced the contents of chlorophylls *a* and *b* (Kousar et al. 2020). According to Khangahi and Crecchio (2021), PGPR application in durum wheat plants (stressed and non-stressed) improves the concentration chlorophylls when compared to the non-inoculated treatments (Khanghahi et al. 2021). Efthimiadou et al. (2020) also found a positive effect of PGPR application (foliar and soil) on chlorophyll content up to 6.1% in maize plants under Mediterranean conditions. Batool et al. (2020) reported that the drought stress treatments in potato resulted in the reduction of chlorophyll concentration in the leaves while the plants with PGPR treatments showed higher contents of chlorophyll *a* and *b* as compared to plants without PGPR under drought conditions. Mahmood et al. (2016) using two PGPR (*Enterobacter cloacae* and *Bacillus drentensis*) *in mung bean combined with foliar silicon application under saline stress showed* that the salt stress substantially reduced total chlorophyll content, chlorophyll a, and chlorophyll b. The PGPR strains and Si levels independently improved all the aforementioned parameters. Furthermore, the combined application of the *B. drentensis* strain with 2 kg Si $ha^{-1}$ resulted in the greatest enhancement of mung bean physiology, growth, and yield.

## 5 PGPR and Plant Transpiration Rate

Transpiration (E) is the loss of water from plants by evaporation and can be calculated by the kilograms of water lost by E/ kilogram of dry material produced, and this tells us how much water is necessary to produce a certain amount of biomass. The inverse of this parameter is a water use efficiency measure (WUE) or how much biomass can be produced per unit water transpired. It is thought to be a necessary cost or evil to allow the plant to absorb water from the soil and is an inevitable process (Siyavula 2021) as it permits cooling the plant, pull of water and mineral salts upward into leaves, and structural support by maintaining the turgidity in plants (Siyavula 2021).

Plants can open and close the pores by changing the water status of the guard cells. When they take up water from the surrounding epidermal cells, they swell, and their inner surfaces pull away from each other, opening the pore. When they lose water, they come back together, and the pore closes. So, changes in water potential of the guard and epidermal cells are responsible for regulating the size of the stomatal pore. This means, of course, that in order to carry out photosynthesis, plants must open their pores, which means that they will lose large amounts of water. This is the cost of doing photosynthesis on land. In order to take up carbon dioxide, plants have to lose water. But, by regulating the stomatal opening, they can control the amount of water lost and keep it at a reasonable level, while still taking

in adequate amounts of carbon dioxide. There are, however, a number of external factors that affect the rate of transpiration, namely, temperature, light intensity, humidity, and wind and recently observed by PGPR (Siyavula 2021).

The transpiration rate has been claimed to be an important factor controlling the sucrose content of the guard cell apoplast in *Vicia faba* beans. Sucrose has been found more recently to be an important fluctuating osmolyte in the guard cell symplast (Outlaw and De Vlieghere-He 2001) and the guard cell apoplast. The importance of sucrose in stomatal aperture-size regulation lies in the difference between Sucrose concentrations in the guard cell symplast and the guard cell apoplast. Potassium and its counter ions are the well-known fluctuating osmotica that cause stomatal movements through regulation of the aqueous volume of guard cells. Thus, an accumulation of potassium causes stomatal opening, and a dissipation of potassium may cause stomatal closing.

Results from a mechanistic study by Zheng et al. (2018) aiming at better understanding of biophysical changes in the rhizospheric soil due to PGPR point out that the applications of these beneficial bacteria reduce evaporation and increase soil water retention.

All PGPR-treated soils were found to hold more water and had reduced hydraulic conductivity and accumulative evaporation, compared to their corresponding controls. The authors attribute such findings due to production of extracellular polymeric substances (EPS) by bacteria that are potentially responsible for the changes in hydraulic properties and soil evaporation because EPS have a large water holding capacity, alter soil matrix structure and connectivity of pore space, and modify the physicochemical properties of water such as surface tension and viscosity (Zheng et al. 2018).

Efthimiadou et al. (2020) studying the effect of foliar and soil application of PGPR on growth, physiology, yield, and seed quality of maize under Mediterranean conditions found a positive effect of transpiration rate up to 34.3%. Batool et al. (2020) showed that in drought-stressed treatments of potato, the plants with PGPR application showed less decrease in leaf relative water content and the plants with PGPR showed less decrease in membrane stability index. The plants with PGPR treatments maintained higher transpiration rate as compared to plants without PGPR under stressed plant conditions (Batool et al. 2020). Mahmood et al. (2016) using two PGPR (*Enterobacter cloacae* and *Bacillus drentensis*) *in mung bean combined with foliar silicon application under saline stress showed* that the salt stress substantially reduced transpiration rate and relative water content (RWC). The PGPR strains and Si levels independently improved all the aforementioned parameters. Furthermore, the combined application of the *B. drentensis* strain with 2 kg Si $ha^{-1}$ resulted in the greatest enhancement of mung bean physiology, growth, and yield. Co-inoculation with PGPR were also found to increase the transpiration rate in tobacco plants under drought stress (Begum et al. 2021) and in barley plants (Backes et al. 2021). Contrarily, Costa-Santos et al. 2021 observed a reduced transpiration rate in tomato plants after PGPR inoculation.

## 6 PGPR and Their Effect on Stomatal Conductance

Efthimiadou et al. (2020) studying the effect of foliar and soil application of PGPR on growth, physiology, yield, and seed quality of maize under Mediterranean conditions found a positive effect on stomatal conductance. Similar results were also reported by Batool et al. (2020) who reported that due to drought stress stomatal conductance in two potato cultivars was decreased. The authors mention that under well-watered conditions, the stomatal conductance was not significantly different. However, in drought-stressed treatments, the plants with PGPR displayed less decrease in stomatal conductance.

Mahmood et al. (2016) using two PGPR (*Enterobacter cloacae* and *Bacillus drentensis*) *in mung bean combined with foliar silicon application under saline stress showed* that the salt stress substantially reduced stomatal conductance. The PGPR strains and Si levels independently improved the aforementioned parameter. Furthermore, the combined application of the *B. drentensis* strain with 2 kg Si $ha^{-1}$ resulted in the greatest enhancement of mung bean physiology, growth, and yield. According to the model of Llangumaran and Smith (2017), PGPR induce salt tolerance in the following manner: root surfaces are colonized by PGPR, and extracellular polysaccharide matrix acts as a protective barrier against salt stress. Some extracellular molecules function as signaling cues that manipulate phytohormonal status in plants. Enhanced root-to-shoot communication improves water and nutritional balance, source-sink relations, and stomatal conductance. Stimulating osmolyte accumulation, carbohydrate metabolism, and antioxidant activity delay leaf senescence, which in turn contribute to photosynthesis. Backes et al. (2021) showed an increase in stomatal conductance after barley plants being inoculated with PGPR. According to Costa-Santos et al. (2021), the stomatal conductance of tomato plants was highly improved after PGPR application. Figure 3.2 summarizes the main effects prompted by PGPR on the photosynthesis parameters.

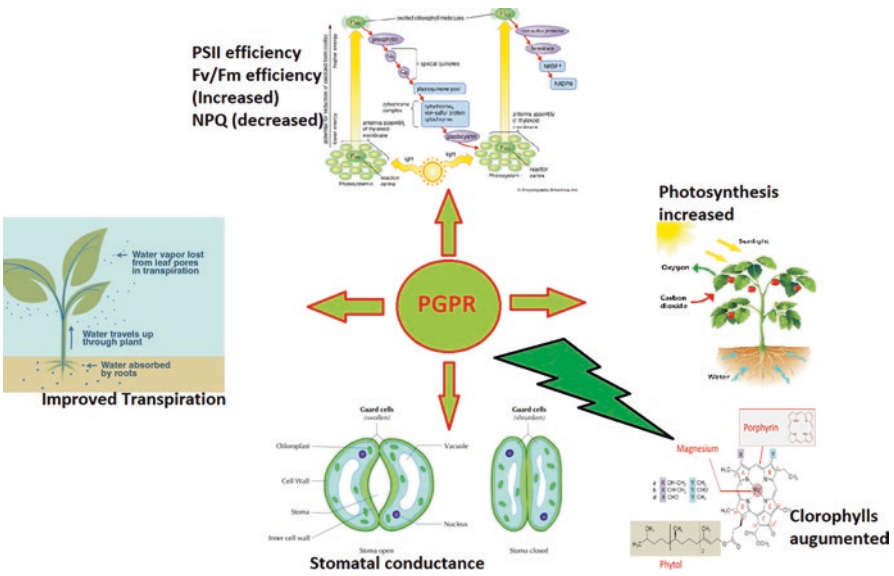


**Fig. 3.2** General overview on the PGPR effects on transpiration rate, photosynthesis, stomatal conductance, chlorophylls, and photosystem II efficiency in plants

## 7 Conclusion

Extensive literature presented in this review on the effect of PGPR in plant photosynthesis, clearly shows that these beneficial bacteria are crucial in the current global scenario of food shortage and global population growth beyond 2050 and 2100. Evidence shows that PGPR can improve the plant photosynthesis even in stressed conditions. With rising emphasis on sustainable agriculture, environmental protection, and food security, exploitation of beneficial soil microbiota is imperative. Abiotic stresses constraint yield and turn agriculture production systems fragile, in addition, persisting climate change intensify the frequency, degree, and resultant damage of stressful conditions. The use of PGPR can be a sustainable approach to deal with such constraints in the soon future. Extensive research must also be performed to better unravel the plant–PGPR communications and signaling toward yield improvement.

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# Chapter 4
# Effect of Volatile Organic Compounds (VOCs) and Secondary Metabolites Produced by Plant Growth-Promoting Rhizobacteria (PGPR) on Seed Quality

**Daniele Nerling, Jaquelini Garcia, Camile Thais Castoldi, Lucieli Santini Leolato, Paloma Guazzelli Della Giustina, Bárbara Bagio, Cristiane Carlesso, and Ânderson Scalvi Sommer**

**Abstract** Plant growth-promoting rhizobacteria (PGPR) in agriculture has become a common practice in recent years. PGPR are beneficial bacteria that inhabit the soil and positively influence plant development and health. Among the many applications studied, seed inoculation with PGPR shows the potential to increase crop productivity. These bacteria have several mechanisms, such as the plant growth stimulation by the production of phytohormones, increased nutrient absorption, biocontrol of pathogens, increased resistance to abiotic stresses, nitrogen fixation, and favor the production of seeds with better physiological quality. It is possible to observe increases in germination percentage, rapid performance and growth, higher seedling length, root length, shoot length, and dry mass. PGPR are responsible for increases in quantities of indole acetic acid and soluble phosphate, increases in enzymatic activity, and energy metabolism in germination and early development of seedlings. In addition, the use of PGPR is currently considered a strategy for

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maintaining agro-ecosystems balance and sustainability in the face of climate change and the prospects for agriculture in the future. Effects of secondary metabolism and volatile compound on the physiological and sanitary quality of seeds have been discussed in this chapter. It is hoped to elucidate the importance of PGPR on quality seeds production and their potential for exploitation for other crops.

**Keywords** Beneficial bacteria · Bioinoculants · Biochemical components · Seedling emergence · Physiological quality

# 1 Introduction

## *1.1 Brief Overview of the Rhizobacteria-Plant Association and Its Benefit*

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria associated with the rhizosphere that can enhance plant growth, development, and health, optimizing resources by directly or indirectly fixing and solubilizing minerals (Raza et al. 2016; Kumar et al. 2019; Patel 2018; Orozco-Mosqueda et al. 2018; Compant et al. 2019). Directly, these microorganisms promote plant growth by acting on pathways involved in chemical transformation (atmospheric nitrogen fixation), nutrient solubilization, and plant tolerance to abiotic stress (Chen et al. 2016; Bharti et al. 2016; Tiwari et al. 2016). PGPR produces antibiotics, siderophores, and phytohormone, such as indole acetic acid (IAA) and gibberellic acid (GA) (Backer et al. 2018; Ijaz et al. 2019; Kafle et al. 2019; Liu et al. 2019a, b; Heydari et al. 2018; Carlson et al. 2020; Nascimento et al. 2020). PGPR affects seed quality indirectly they are antagonists to phytopathogenic organisms such as fungi, viruses, and nematodes, in addition to inducing systemic resistance against diseases (Li et al. 2015; Zebelo et al. 2016; Santoyo et al. 2017; Shafi et al. 2017; Orozco-Mosqueda et al. 2018).

PGPR are associated in different ways with plant cells: extracellular (ePGPR) and intracellularly (iPGPR) (Fig. 4.1) (Raza et al. 2016; Ilangumaran and Smith 2017; Gadhave et al. 2018).

ePGPR are bacteria that develop into plant tissues and non-disciplined nodules but can promote plant growth by substances production, such as nitrogen, phosphorus, iron, and some hormones (Chenniappan et al. 2019). Based on the degree of association with the roots of the plants, ePGPR can be subdivided into three types: those lining near, but not in contact with, the roots; those colonizing the root surface; and those living in the spaces between the cells of the root cortex (Gray and Smith 2005). The main genres belonging to this classification are *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, and *Pseudomonas* (Adesemoye et al. 2017).

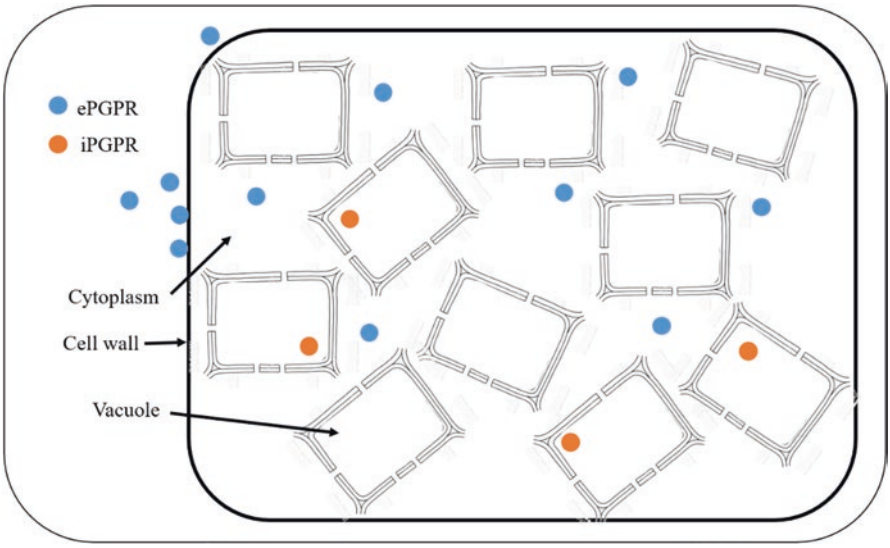


**Fig. 4.1** Schematic representation of intracellular (iPGPR) and extracellular (ePGPR) association plant-bacterium

iPGPR live in plant cells, produce nodules, and live in specialized structures. These symbiotic bacteria are responsible for biological nitrogen fixation (Dinnage et al. 2018). Rhizobacteria inoculation increased *Sorghum bicolor* plant height, stem diameter, aerial biomass, and the root system (Macedo et al. 2019). The seed co-inoculation with two microorganisms improves root system nodulation resulting in a better supply of nitrogen to the seedling (Silva et al. 2017). *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Bacillus*, and *Pseudomonas* are the main kinds of iPGPR (Dinnage et al. 2018; Martins et al. 2019).

PGPR can be used in seed treatment under controlled hydration, which enhances the preparatory processes for germination before root protrusion. This treatment is biopriming, and it can have positive effects on seed quality, such as improving the expression of vigor, germination percentage, germination speed, growth, and development.

## 2 Effects of PGPR on Physiological and Health Seed Quality

Seed quality is a conjunct of characteristics that determine its potential for sowing. Seed quality includes the sum of physical, physiological, genetic, and health attributes responsible for seed performance in the field (Popinigis 1977). Seed physiological and sanitary attributes can be impacted directly by PGPR, such as higher germination percentage and seedlings performance and control of pathogens (Worma et al. 2019; Sufyan et al. 2020; Hyder et al. 2020).

The positive effects of PGPR on seed quality have been verified in some species. Wheat seeds inoculated with *Azospirillum brasiliense* showed higher germination and vigor, seedlings showed a greater length of shoot and root (Brzezinski et al. 2014) and less incidence of pathogens (Munareto et al. 2018). Ilyas et al. (2020 studied the role of exopolysaccharides producing PGPR strains, *B. subtilis* and *A. brasilense*, on germination, physiological and morphological of wheat under drought stress. The authors showed that under osmotic stress, germination percentage, seedling vigor index, and promptness index (PI) values were high in combination-treated wheat seeds as compared to single-strain inoculated seeds. In morphological and physiological parameters, observed an increase in shoot length and root length. Maize seed inoculates with *Azospirillum lipoferum*, *Pseudomonas fluorescens*, and *Pseudomonas putida*, increased the germination percentage, length and weight of root and aerial part of seedlings, and the vigor index (Worma et al. 2019; Amogou et al. 2018; Agbodjado et al. 2016).

The positive effect of PGPR in seed germination and seedling performance has been verified in soybean (De Gregorio et al. 2017), lettuce (Bernardino et al. 2018; Mangmang et al. 2015), tomato (Luna-Martínez et al. 2013), chickpea (Hossain et al. 2016; Sufyan et al. 2020), cocoa (Hardiansyah et al. 2020), pepper (Hyder et al. 2020; Kumari et al. 2019), linen (Bakhit and Moradi 2017), *Cuscuta campestris* (Sarić-Krsmanović et al. 2017), among others.

The application of bacterial biostimulants encourages the healthy growth of crops through the suppression of different plant pathogens and against various types of seed-borne diseases. Some PGPR has a protective action, such as *Pseudomonas fluorescens*, *P. chlororaphis*, *Bacillus thuringiensis*, and *Bacillus amyloliquefaciens* (Hamid et al. 2021). PGPR have antagonistic effects on the development of pathogens in the seed. The use of rhizobacteria inhibited the mycelial growth of *Pyricularia grisea*, *Phoma* sp., *Bipolaris oryzae*, and *Gerlachia oryzae* in rice seed (Moura et al. 2014; Pinho et al. 2019). *Bacillus* strains showed fungal inhibition values that varied between 60% and 80% in bean seed, against *Sclerotium rolfsii*, *Sclerotinia sclerotiurum*, *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina under* in vitro *conditions* (Sabaté et al. 2017). PGPR controlled *Aspergillus* and *Fusarium* in peanut seeds (Syed et al. 2020) and chickpea (Sufyan et al. 2020). The inhibitory effects on the development of pathogens have been observed in chili pepper (Hyder et al. 2020), soybeans (Zilli et al. 2018), beans (Negi et al. 2019), and tomato (Abo-Elyousr et al. 2019).

PGPR increases the content of reserve compounds and enzyme activity in seeds. Storage compounds have been used during the germination process and seedling performance. Bean plants co-inoculated with *Rhizobium* and *Pseudomonas fluorescens* showed seeds with higher protein content (Yadegari 2014). *Azospirillum* and *Azotobacter* increased the quantity and quality of proteins in cottonseed (Nosheen et al. 2016). *Bacillus subtilis* increased $\beta$-amylase activity during wheat seed germination (Li and Hu, 2020). Rapid availability of sugars by enzymatic activity can promote rapid germination, preventing pathogens and favoring the overcoming of stress conditions.

In stressful conditions, soybean seeds inoculated with *Bacillus licheniformis* formed plants more tolerant to water deficit and seed with higher protein and oil content (Mondani et al. 2019). PGPR increased the oil and protein content in bean seeds in salt stress (Khaitov et al. 2020). Increases in soluble protein and germination percentage have been observed in cumin seeds inoculated with PGPR under salt stress conditions (Piri et al. 2020). The use of *Trichoderma* spp. associated with water restriction and pelliculation increased the vigor of maize seedlings (Junges et al. 2014).

PGPR secondary metabolites (SM) can benefit several physiological processes in the plant and seeds (Fig. 4.2). The SM contributes to plant growth and development (Asghari et al. 2020) especially in adverse conditions (Mishra et al. 2018) and can act as signaling compounds within and between plants. Its synthesis varies according to species and the environment (Aftab and Hakeem 2020) because it is regulated by external conditions (Patra et al. 2013) and occurs in specific tissues and organs, according to the role it will have in plant development (Aftab and Hakeem 2020).

VOCs play a significant role in promoting plant growth by regulating the synthesis or metabolism of phytohormones and other compounds. Hydrogen cyanide (HCN) is correlated with the biocontrol of nematodes (Thiyagarajan 2014; Nandi et al. 2015; Kang et al. 2019), diseases suppression (Patel et al. 2020), and at millimolar concentrations, stimulates the germination of seeds of different species. HCN, depending on concentration, inhibits or stimulates seed germination. This volatile secondary metabolite inhibits electron transport and interrupts the cell energy supply leading organisms to the dead (Alemu 2016).

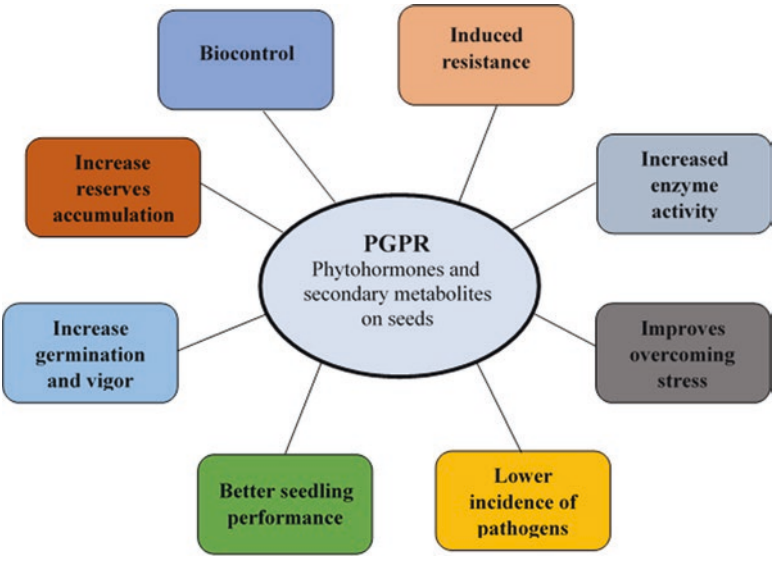


**Fig. 4.2** Main benefits of PGPR in seed performance and plants

Bacterial wilt and canker are serious tomato diseases caused by *Clavibacter michiganensis* subsp. *michiganensis*. This disease is transmitted through the seeds and impacts the world's major tomato growing areas. In a study evaluating fluorescent *Pseudomonads* sp. strains, producers of HCN observed a significant reduction in the disease incidence and its severity, in addition to an increase in growth parameters compared to controls without strains (Banayem et al. 2020). Khan et al. (2012) examined the PGPR inoculation effects for infestations of the nematode *Meloidogyne incognita* in chickpea seeds (*Cicer arietinum*). In the study, *Pseudomonas fluorescens* was the largest HCN producer, able to suppress hatch and kill practically half of the juvenile nematodes, in addition to reducing the egg production in relation to control. Similar effects were observed for *P. fluorescens* in beans (*Vigna radiata*) (Khan et al. 2016). The ammonia produced during nitrification and the toxic cyanide produced by PGPR is responsible for nematode killing (Wilt and Smith 1970). The nematodes infestation may also lead to reduced root hair formation resulting in fewer colonization spots available for root-nodulating bacteria (Khan et al. 2012).

The indole-3-acetic acid (IAA) is a phytohormone produced by several PGPR strains (Goswami et al. 2016). It is responsible for cell stretching and division (Vurukonda et al. 2018). Bacteria affect auxin homeostasis in the plant in a direct way (Tsukanova et al. 2017). There are abundant data indicating that different strains of PGPR synthesize auxin in the crops (Ahmed and Hasnain 2014; Keswani et al. 2020; Chandran et al. 2021). Increased seed germination due to inoculation with PGPR species that produce IAA in different species was reported. A study by Dochhil et al. (2013) reported a higher germination percentage and growth of common bean seedlings (*Phaseolus vulgaris*) due to greater IAA synthesis in situ (71 g/mL and 197 g/mL) (71 g/mL and 197 g/mL) by two *Streptomyces* spp. strains isolated from *Centella asiatica*. Islam et al. (2016) characterized PGPR in cucumber seeds and observed that the 66 selected isolates produced IAA. The PGPR treatments increased the germination rate up to 15.32% and seedling vigor by 148.05%. The effects of IAA-producing bacteria on lettuce plants promoted an increase in the percentage of germination and root growth (Florentino et al. 2017). Even with the positive results, the IAA is not considered a key regulator for the seed germination process (Shuai et al. 2017). Thus, the highest percentage of seed germination due to IAA probably results from other compounds that are also synthesized by these strains, such as gibberellins (Florentino et al. 2017).

In the plants, chitinases act as induced defense responses against biotic and abiotic stresses (Ahmed et al. 2011) or are expressed in organs constitution, as the seeds (Kabir et al. 2016). Enzymes such as the chitinases are responsible for the degradation of cell walls and secreted by PGPR exert a direct inhibitory effect in the growth of pathogenic fungi hyphae by destroying the main component of your cell wall, the chitin, an insoluble linear polymer β-1, 4-N-acetyl-glucosamine (Goswami et al. 2016; Munir et al. 2018; Malik 2019). The tobacco seed's inoculation with *Bacillus amyloliquefaciens* YN201732, promoted seed germination, had protective and therapeutic effects inducing the increase of chitinase and consequently plant resistance to *Erysiphe cichoracearum* (Jiao et al. 2020). Arif et al. (2016, 2017) and

Shahzad (2013) verified in their studies that the associative effect of fertilizers and growth-promoting rhizobacteria even increases the number and quality of sunflower and maize seeds, respectively. The authors state that one of the important factors in the selection of the best strains is the chitinase activity after inoculation. According to Samarah et al. (2020), by inducing increased chitinase through the treatment of pepper seeds, low germination problems due to low humidity and cold were avoided, as well as the occurrence of diseases that caused a reduction in germination and seedling emergence.

Some PGPR strains produce cytokinins and can modify the phytohormones composition in plants (Kumar and Jacob 2019). The expression of gene synthesis and cytokinin content was increased in tomato plants exposed to PGPR *Bacillus subtilis* SYST2 (Tahir et al. 2017). Cytokinins stimulate plant cell division, tissue increase, and expansion and regulate stomatal conductance (Kumar and Jacob 2019). Likewise, studies report that cytokines mitigate oxidative damage due to abiotic stresses like salt, drought, high temperature, and heavy metals as a result of their antioxidant effects (Kataria and Guruprasad 2018). The maize inoculation with the isolated cytokinin producing bacteria *Micrococcus luteus* chp37 stimulated aerial and root biomass by 54% under drought conditions. There was also a germination increase in the maize seeds in one of the soils of this study (Raza and Faisal 2013). Liu et al. (2013) observed that inoculation with *Bacillus subtilis*, a cytokinin producer stimulated *Platycladus orientalis* root biomass by 13.90% and increased its cytokinin concentration by 47.52% in the leaves, about the respective controls under water stress conditions.

Gibberellins are present in plants and some species of fungi and bacteria. Its biosynthesis pathways, enzymes, genes, and regulation is widely known (Hedden and Sponsel 2015). Gibberellin is one of the main germination hormones since acting in dormancy breaking, embryo growth, and seedling emergence (Taiz et al. 2017). PGPR can influence the amount of endogenous gibberellin in plants, similar to other hormones (Tsukanova et al. 2017). Kang et al. (2014) studied the effects of the bacteria of the genus *Leifsonia soli* in cucumber seeds. The bacteria presence increased seed germination compared to the control treatment. The active gibberellic acids identified were GA1, GA4, and GA7. In the same way, Pandya and Desai (2014) selected an isolated from *Pseudomonas monteilii* due to its high production of gibberellic acid and tested its effect in wheat and chickpea crops. The authors report increased germination in both grass and legumes.

The 1-aminocyclopropane-1-carboxylate deaminase, produced by bacteria, reduces ethylene stress in plants, providing resistance and stimulating their growth even in adverse conditions (Bal et al. 2012). This enzyme can hydrolyze 1-aminocyclopropane1-carboxylic acid (ACC), the immediate precursor of ethylene biosynthesis in plants. The products of ACC hydrolysis can supply plants with carbon and nitrogen. The reduction of ACC and ethylene levels prevents the adverse effects of high concentrations of this phytohormone, which can kill the plant (Glick 2014; Amara et al. 2015).

Tiwari et al. (2018) evaluated the effects of rhizobacteria ACC deaminase in *Panicum maximum* exposed to salinity and drought stress. The enzyme presence modified the host plant's response to stress. Significant increases in enhancing growth, water conservation, membrane stability, biocompatible solutes and protein, phenolic contents, and photosynthetic pigments were verified in plants grown under stress conditions. Sagar et al. (2020) evaluated the effect of the inoculation of ACCD and antioxidant positive and halophilic *Enterobacter* sp. on the seed germination and growth of rice and millet seedlings grown in saline and alkaline soil. The combined application of *Enterobacter* sp., ammonium sulfate, and NaCl resulted in a further increase in the seed germination and vigor in rice and millets vis-à-vis control and other treatments. The inoculation of canola seeds by two different rhizobacteria strains *Brevibacterium epidermidis* RS15 (GU968456) and *Bacillus aryabhattai* RS341, both ACC deaminase producing avoided the reduction of seed germination (Siddikee et al. 2015). Like these, many PGPR produce ACC deaminase and thus regulate the presence of ethylene in the plant (Singh et al. 2015, 2019; Saikia et al. 2018). Thus, the use of PGPR is an attractive alternative to bio-inoculants and a tool that, in addition to fertilizers, improves field performance through integrated nutrient management (Maheshwari 2013). In addition to the positive effects mentioned above, PGPR has been used in seed biopriming. The following topic will address aspects related to the association of PGPR with biopriming and its effects on seed quality.

## 3 Effect of PGPR in Biopriming Seed Quality

Biopriming is a seed treatment with beneficial microorganisms and involves the seed soaking in a solution of a specific priming agent. This priming agent restricted water availability under controlled conditions (create ideal conditions for the bacterial inoculation and colonization) followed by drying seeds to their original weight (Sukanya et al. 2018; Mahmood et al. 2016). In this process, seeds are left in the physiological development stage (phase II), ready to germinate quickly. The application of PGPR as a biopriming agent has been tested as co-inoculants with *Azotobacter*, *Azospirillum, Bacillus, Pseudomonas, Serratia*, and *Streptomyces* strains. Seed priming with beneficial microorganisms affects physiological characters, biochemical parameters, and gene expression.

Seeds biopriming agents allowed an improvement in physiological parameters, such as increasing germination and seedling performance. Seedling performance (seedling length, root length, shoot length, dry mass) is a test of vigor based on evaluating the efficiency of the seed's metabolic activity during seed germination and seedling development (Krzyzanowski et al. 2020). Rêgo et al. (2014) reported an increase in root length and diameter and cortex expansion, induced a 2%

expansion of the aerenchymal space, and favored rice seedling root plasticity. Seed biopriming with *Trichoderma* strains reduced the mean germination time, enhanced the seedling vigor and total chlorophyll content in rice (Swain et al. 2021). Sharma et al. (2020) reported higher seed vigor parameters such as root length (11.01 cm), shoot length (7.96 cm), seedling length (18.98 cm), fresh weight (8.00 g), and dry weight (1.31 g) in biopriming soybean seed. Anitha et al. (2015) also reported increased shoot length and significantly higher seedling length in soybean seed biopriming. A better physiological performance observed by the higher germination percentage, rapid performance and growth, and dry matter accumulation have been reported in maize (Rozier et al. 2019), oat (Junges et al. 2019), okra (Roslan et al. 2020), carrots, onion, and kale (Murunde and Wainwright 2018), chickpea, and bens (Kumar et al. 2014).

Biopriming leads to biochemical changes in seeds, such as enhanced production of proteins, hormones, phenol, and flavonoid compounds. These compounds contribute to better plant growth and development performance (Sukanya et al. 2018). Swain et al. (2021) reported increases in enzymes activity like total cellulase, endoglucanase, xylanase, and laccase in biopriming rice seeds. They also produced higher quantities of indole acetic acid, soluble phosphate, and prussic acid, which are responsible for plant growth promotion and the inhibition of rice pathogen populations. In stress conditions, biopriming increases total mineral content in wheat tissues, hydrolytic enzymes (β-glucanase, protease, amylase), EPS, and ACC deaminase activity, stimulating plant growth under stress conditions directly (Brahim et al. 2019).

Biopriming improved seedling development at a molecular level, reflected by the upregulation of specific genes used as molecular indicators of seed quality (Forti et al. 2020). Bioprimed seeds have been efficient mitochondrial development by augmenting energy metabolism, the regulation of respiration, and early reserve mobilization events in crops. Upregulation of the expansin gene is responsible for cell wall loosening and was important for coleoptile elongation in biopriming rice seeds (Sukanya et al. 2018).

PGPR might be used to mitigate the effects of seed storage. During storage occurs seed aging, a gradual, inevitable, and irreversible process. Aging reduces germination and vigor, or seed death, leading to commercial losses and decreased genetic diversity (Liu et al. 2019a, b). Associated PGPR and biopriming techniques can bring results to repair aged seeds. The co-treatment of *Pseudomonas geniculata* with different priming approaches had positive effects on germination and growth of *Bromus catharticus* seedlings after aging. In the study, the co-treatment affected the content and activity of enzymes, such as malondialdehyde, superoxide dismutase, and peroxidase.

Agriculture has been interested in PGPR to promote plant growth and development (Kousar et al. 2020), minimize damage from biotic or abiotic stress (Khademian et al. 2019), and positively interfere with seed quality.

## 4 Conclusion and Future Perspective

Modern agriculture has faced several challenges in food production due to biotic and abiotic stress. Besides, we need sustainable solutions that do not compromise the entire system. Additional studies, together with the exploration of multidisciplinary research, combined with modern tools and techniques, can provide new advances essential for sustainable agriculture.

Just as the use of high-quality seeds is already widespread, the study of their use combined with PGPR has shown encouraging results. PGPR has shown promise in mitigating effects in seed storage. Biopriming is a useful method to increase seed germination and vigor. The focus is to find strategies for use of biopriming seed in biotic and abiotic stress conditions. Several biological agents (fungal or bacterial) have positive effects on seed quality. The information obtained from a detailed analysis of the PGPR-seed relationship (physiological and molecular) will be applicable in understanding mechanisms that result in elevated seed performance and quality. The success of PGPR in seeds has been observed in the laboratory; however, researches under greenhouse and especially in field conditions are necessary. The literature discussed in this research shows that PGPR is important in seed quality and will continue contributing to a better understanding of biological mechanisms that explain seed performance shortly.

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# Chapter 5
# The Role of PGPR-Polar Metabolites, Metal-Chelator Compounds and Antibiotics on Plant Growth

**Daniele Nerling, Camile Thais Castoldi, and Natalia Carolina Moraes Ehrhardt-Brocardo**

**Abstract** The interest in the use of microorganisms in agricultural practices increased significantly in the last years. The use of PGPR in promoting plant growth and health, biological pest control, and plant diseases makes these microorganisms potential substitutes for agrochemicals, thus being able to favor the preservation of the environment. PGPR secretes metal-chelators, such as organic acids and siderophores, making available nutrients for plant uptake. Antibiotic secretion is responsible for plant disease control and has an indirect action in plant growth. PGPR secretes amino acids, particularly in response to osmotic and water stresses, which may act synergistically with other osmolytes produced by plants, promoting plant growth. Among the sugars synthesized and secreted by PGPR, exopolysaccharides (EPS) enable the association of bacteria with plant roots. The contribution to the plant for the production and secretion of EPS by rhizobacteria is evident when the plant is subjected to water and salt stress conditions. EPS allow the maintenance of high moisture content of the soil through the formation of aggregates and improvement of the soil structure, protecting bacteria and the plant from desiccation and increasing the absorption of nutrients by the plant. In this review, we approach the role of PGPR-polar metabolites, metal-chelator compounds, and antibiotics in plant growth and sustainable agriculture.

**Keywords** Antibiotics · EPS · Metal chelators · Rhizobacteria · Siderophore

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## 1 Introduction

PGPR has been shown as effective and inexpensive from the perspective of sustainable agriculture. These microorganisms are involved in an intense network of interactions in the rhizosphere. There are beneficial characteristics in plant growth and development: induce resistance to pathogens, solubilize and make nutrients available, mitigate stresses, and increase crop productivity (Sagar et al. 2020). The association of plant-PGPR also promotes seed germination and root and shoot growth, increases the level of nutrients in soybeans, and improves the biochemical properties of the soil (Saxena et al. 2013; Agboola and Moses 2015; Jabborova et al. 2020). They promote an increase in crop yields, reduce the use of inorganic nitrogen fertilizers (Egamberdieva et al. 2013), and improve nutrient availability for plants (Egamberdieva et al. 2018).

Some PGPR have positive effects on agricultural systems (Table 5.1). This chapter discusses the role of PGPR-polar metabolites, metal-chelator compounds, and antibiotics on plant growth.

**Table 5.1** PGPR activity involving in plant growth

| PGPR microorganism | Activity | References |
|---|---|---|
| *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Bacillus, Pantoea, Arthrobacter, Serratia* | Siderophore production | Ansari et al. (2017), Franco-Sierra et al. (2020), Menéndez et al. (2020), Sheng et al. (2020), Zhang et al. (2020a, b) and Kalam et al. (2020) |
| *Arthrobacter, Burkholderia, Enterobacter, Microbacterium, Pseudomonas, Bacillus, Erwinia, Rhizobium, Mesorhizobium, Flavobacterium, Rhodococcus, Serratia* | Phosphate solubilization | Oteino et al. (2015) and Goswani et al. (2016) |
| *Rhizobium, Bradyrhizobium, Mesorhizobium, Bacillus, Pantoea, Arthrobacter, Pseudomonas, Enterobacter, Burkholderia, Agrobacterium, Xanthomonas, Azospirillum* | Phytohormone production | Egamberdieva et al. (2017), Tsukanova et al. (2017) and Jabborova et al. (2020) |
| *Bacillus species, Pseudomonas species, Burkholderia, Brevibacterium, Streptomyces* | Antibiotic production | Zhou et al. (2019) and Romano-Armada et al. (2020) |
| *Pseudomonas, Bacillus, Burkholderia, Agrobacterium, Paenibacillus polymyxa, Xanthomonas* | Volatile metabolite production | Sharifi et al. (2017) and Buckley et al. (2019) |

## 2 Polar Metabolites

### *2.1 Organic Acids*

PGPR can affect plant growth by a wide range of mechanisms like the production of organic acids and solubilization of inorganic phosphate. Phosphorus (P) is one limiting nutrient for plants; however, in many cases, P is not available in a form suitable for plant uptake. Supplying P by biological means is a realistic alternative to lower the environmental risk and enhance the productivity of ecosystems. Some PGPR can mineralize organic phosphorus in soil by solubilizing complex-structured phosphates. Solubilization of P by organic acid is a direct mechanism of PGPR.

In this context, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Streptomyces*, and another genus can solubilize soil phosphates by the production of organic acids, phosphatases, and phytases. Organic acid, secreted by PRPG, is the primary mechanism of phosphate solubilization. Many phosphate-solubilizing microorganisms secret organic acids such as acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic, glycolic, malonic, carboxylic, formic, fumaric, propionic, and citric acids (Patel et al. 2015; Goswami et al. 2016; Romano-Armada et al. 2020). These acids act as good chelators of divalent Ca2+cations because of lower pH in the rhizosphere, thus causing a release of the bound forms of phosphate (Goswami et al. 2014, 2016; Patel et al. 2015; Kaur et al. 2016).

Some studies have demonstrated the ability of PGPR to produce organic acids (malic, lactic, acetic, citric, and gluconic acids) and their positive effects on phosphorus solubilization. Gluconic acid (GA) is the main responsible for the solubilization of mineral phosphates. Shariati et al. (2017) in a study of comparative genomic analysis of *Pantoea agglomerans*, verified the possession of several genes related to organic acid biosynthesis, especially GA and 2-ketogluconic acid in this microorganism. Li et al. (2018) observed that *Burkholderia multivorans* could promote the root of *Populus* × *euramericana* to secrete organic acid, especially the secretion of GA, which dissolves inorganic phosphorus. The *Klebsiella variicola* strain produced IAA and organic acids that resulted in the acidification and dissolution of rock phosphate in culture conditions of *Helianthus tuberosus* (Nacoon et al. 2020). Sharma et al. (2020) identified nematicidal metabolites produced by the fungus *Purpureocillium lilacinum* cultivated on a Karanja deoiled cake-based liquid medium through bioactivity-guided fractionation against *Meloidogyne incognita*. The organic metabolites isolated and identified from *Purpureocillium lilacinum* was 2-ethyl butyric acid, phenethyl alcohol, benzoic acid, benzene acetic acid, and 3,5-Di-t-butylphenol, which showed promising nematocidal potential.

## 3 Siderophore

Microorganisms have developed efficient iron absorption mechanisms from the environment. Many PGPR have the potential to produce siderophores, such as *Bacillus subtilis*, *Paenibacillus polymyxa* SK1, *Paenibacillus triticisoli* BJ-18, *Mesorhizobium* sp., *Brevibacillus brevis* GZDF3, acidobacteria species (Franco-Sierra et al. 2020; Menéndez et al. 2020; Sheng et al. 2020; Zhang et al. 2020a, b; Kalam et al. 2020). Siderophores act as chelators of iron and other metals, making the mineral available to plant uptake (Hider and Kong 2010). Siderophores also form stable complexes with heavy metals such as $Cu^{2+}$, $Ni^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Hg^{2+}$, and $Ag^{2+}$ (Patel et al. 2018a, b).

Iron is essential for all organisms, including plants, bacteria, animals, and humans. Iron is a transition metal that can exist in two oxidation states: $Fe^{2+}$ and $Fe^{3+}$. Iron variable valence plays a role in redox reactions, trichloroacetic acid cycle, electron transport chain, oxidative phosphorylation, photosynthesis, and respiration. Iron is also responsible for porphyrin, vitamins, antibiotics, toxins, cytochromes, siderophores, pigments, aromatic compounds, nucleic acids, ferroprotein (e.g., ferredoxins and phytoferritins) biosynthesis, involved in biological nitrogen fixation and chlorophyll-protein complexes (Taylor and Konhauser 2011).

In ferrous form ($Fe^{2+}$), the preferred state of absorption by plants, the nutrient is poorly available ($10^{-10}$ to $10^{-9}$ M), and the required level of ferrous iron by living organisms is around $110^{-7}$ to $10^{-5}$ M. Microorganisms produce siderophores capable of forming complexes with iron ions (Saha et al. 2013, 2015; Ahmed and Holmstrom 2014). Organic compounds are secreted near plant roots and join $Fe^{3+}$, forming a ferri-siderophore complex, and binding this complex, to a specific receptor protein present on the microbial cell surface. The ferri-siderophore complex is translocated by active transport and released inside the cell (Fig. 5.1), where it is converted into $Fe^{2+}$ (Khan et al. 2018).

Siderophore biosynthesis initiates from precursors such as citrate, amino acids, dihydroxybenzoate, and N5-acyl-N5-hydroxyornithine. Siderophore biosynthesis in bacteria is accomplished by nonribosomal peptide synthetase (NRPS) enzymes (dependent and independent way), polyketide synthase (PKS) enzymes, and/or by NRPS independent siderophore (NIS) synthetase enzymes (Paul and Dubey 2015; Khan et al. 2018; Ronnebaum and Lamb 2018). Some genus are involved in the production of siderophores. In *Aspergillus fumigatus*, genes *sidA*, *sidD*, *sidG*, *sidF*, *sidC*, and *sidL* were identified. The operon For enterobactin uptake and utilization includes genes like *fepA*, *fepB*, *fepC*, *fepD*, *fepE*, *fepG*, *fes*, and *entS* are involved, while in *Yersinia pestis*, *irp1* and *irp2* genes (Paul and Dubey 2015; Peralta et al. 2016; Khan et al. 2018).

Growth and siderophore production of PGPR are influenced by a variety of physicochemical and environmental factors of the rhizosphere. According to Sayyed et al. (2019), the stress condition of iron might be a decisive factor for siderophore production. Low stress of ferric iron supported the growth yield, while higher level completely repressed siderophores.

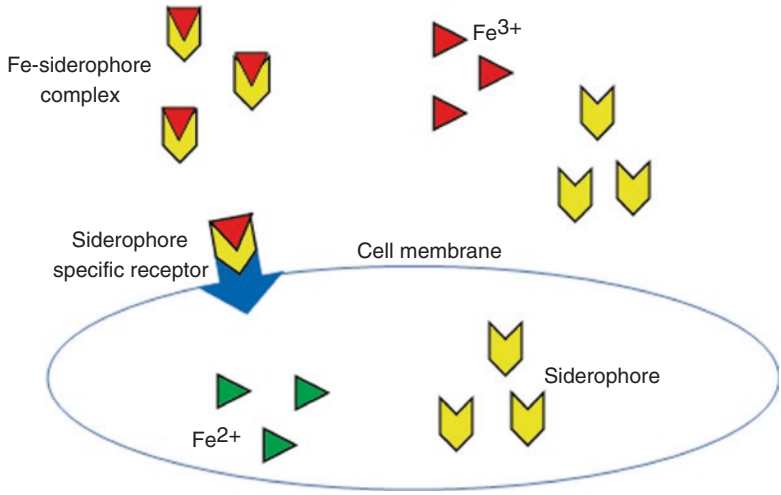


**Fig. 5.1** Schematic representation of iron absorption mediated by siderophores

According to the functional group, siderophores have been classified into three types: (1) hydroxamate-type siderophores, (2) catecholate-type siderophores, and (3) carboxylate-type siderophores. Hydroxamate is produced by fungi, including *Fusarium roseum*, *Aspergillus flavus*, *Rhizopus* sp., *Ustilago sphaerogena*, and *Penicillium* sp., among others. Most of the hydroxamate groups consist of C (=O) N-(OH) R, where R is either an amino acid or a derivative of it. A bidentate ligand forms between two oxygen molecules coming from each hydroxamate group and iron. Each hydroxamate is capable of forming a hexadentate octahedral complex with ferric ion. Catecholate siderophore is produced by bacteria and has a higher Fe-binding affinity than hydroxamate. Each catecholate group supplies two oxygen atoms for chelation with iron and forming a hexadentate octahedral complex. Few bacteria, such as *Rhizobium* and *Staphylococcus* and fungi (*Rhizopus microspores* and *Mucor mucedo*, among others), produce carboxylate siderophore type. These siderophores have carboxyl and hydroxyl groups for iron acquisition (Saha et al. 2015). Different PGPR strains can produce different types of siderophores. *Rhizobium* and *Mesorhizobium* produce catecholate, and *Pseudomonas* spp. produce pyoverdine (hydroxamate siderophore) and pyochelin (catecholate siderophore) (Marathe et al. 2015; Khan et al. 2018; Ringel and Brüser 2018).

Patel et al. (2018a, b) modified the chrome azurol S (CAS) solution method to screen siderophores, resulting in instant color change similar to the traditionally used CAS solution for screening $Fe^{3+}$-specific siderophores. The author found two bacterial cultures isolated from local rhizospheric soil, producing hydroxamate and catecholate siderophores that could remove $Cu^{2+}$, $Ni^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Hg^{2+}$, and $Ag2^{+}$ metal ions from CAS solution resulting in instant color change.

Siderophores can be detected with many techniques, such as spectrophotometry, high-performance liquid chromatography (HPLC), diode array detection (DAD)

analysis, electrospray ionization mass spectrometry (ESI-MS), O-CAS assay, and mass spectrometry, among others (Saha et al. 2015).

Siderophores of rhizobacteria have a wide range of applications, and their production needs to be statistically optimized. Shaikh et al. (2016) indicated siderophore-producing ability of P. *aeruginosa* RZS9. The author obtained statistical-based optimization offered an efficient and feasible approach, with an effective protocol that uses an adequate concentration of succinic acid at a constant temperature. Statistical-based approaches offer ideal ways for process optimization studies in several biochemical and biotechnological processes.

Siderophores have many effects on plant growth, such as they play a significant role in the biological control mechanism against certain phytopathogens. Studies have been illustrated the role of siderophores as a biocontrol agent of *Erwinia carotovora*, *Fusarium oxysporum*, and *Gaeumannomyces graminis* (Saha et al. 2015; Maksimov et al. 2018). *Pantoea dispersa* MPJ9 and *Pseudomonas putida* MPJ6 strains producing siderophores were used in mung bean iron biofortification. Results revealed at harvest time, bio-inoculum-treated plants significantly increased vegetative parameters, iron content (100.3 ppm), protein (0.52 g/g), and carbohydrates (0.67 g/g) as compared to uninoculated plants (Patel et al. 2018a, b). Pii et al. (2015) evaluated the effect of Fe deficiency and *Azospirillum brasilense* inoculation in the growth of cucumber (*Cucumis sativus*) plants. *Azospirillum brasilense* inoculum in soil increases the chlorophyll content, the biomass, and the Fe content of leaves of cucumber plants. PGPR siderophores increased iron content in rice plants, and *Pseudomonas putida* B17 and B19 doubled iron content in rice plants (Sharma et al. 2013).

Bacterial siderophores also exhibit positive effects on the growth and development of *Festuca rubra* L. and *Brassica napus* L. plants, under stress conditions, in soils with a high concentration of heavy metals, and alkaline soil (Grobelak and Hiller 2017). Siderophores produced by *Micrococcus yunnanensis* YIM 65004 (T) and *Stenotrophomonas schelatiphaga* LPM-5 (T) positively influenced the weight gain and the iron (Fe) content of roots and shoots in canola and corn plants under greenhouse conditions (Ghavami et al. 2017). The consortium between *Aneurinibacillus aneurinilyticus*, *Aeromonas* sp., and *Pseudomonas* sp. increased germination, root and shoot length, dry and fresh weight of wheat seedlings compared to single inoculation, and uninoculated plants (Kumar et al. 2018). *Bacillus subtilis* LSBS2 was isolated from the rhizosphere of sesame plants, and the characterization of the siderophore revealed that the isolate produced catecholate siderophore bacillibactin. Nithyapriya et al. (2021) revealed that the multifarious *Bacillus* sp. LSBS2 improved the growth parameters and nutrient content in sesame as well as soil nutrients. These results show the positive effect of siderophores in plant growth and development.

## 4 Antibiotics

The production of antibiotics is the most important mechanism of action of PGPR. The antibiotic compounds produced by PGPR have an indirect action in plant growth by inhibiting the development of invading organisms, suppressing infectious processes, and minimizing the harmful effects caused by phytopathogens (Tariq et al. 2017; Enebe and Babalola 2018; Paliwal et al. 2020). *Agrobacterium*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Lysobacter*, *Pseudomonas*, and *Serratia* are prolific producers of secondary metabolites, which at low concentrations are lethal to the growth or metabolic activities of plant pathogens. Among these unicellular bacteria, *Bacillus* and *Pseudomonas* are prolific producer antibiotics.

*Bacillus* genus produce a wide variety of antibiotics. They are forming by non-ribosomal peptide enzyme (NRPSs) and/or polyketide synthetase (PKS). Examples include Tas A, sublancin, subtilosin, bacilysin, chlorotetain, subtilin, bacillaene, surfactin, iturin, and fengycin. *Bacillus subtilis* control the growth of approximately 23 types of plant pathogens (Meena et al. 2020). Antibiotics produced by *Pseudomonas* sp. include 2,4-diacetyl phloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA), pyoluteorin (Fig. 5.2), pseudomonic acid, pyrrolnitrin, rhamnolipids, oomycin A, cepaciamide A, kanosamine, viscosinamide, butyrolactones, zwittermicin A, aerugine, azomycin, cepafungins, and karalicin (Goswami et al. 2016).

Researchers have studied the effect of some antibiotics on the suppression of pathogens and the development of plants.

The HCN produced by *Bacillus* sp. (strain CtST3.5) and *Pseudomonas* spp. (strain: NBRC 103040, Gamma-81, ATCC 33618, Lzh-T5, and CV25), increased plant growth parameters, such as shoot and root fresh weight and length of the tomato plant. HCN also decreased the population of *Meloidogyne incognita* and the number of nematode galls (El-Rahman et al. 2019).

Phenazine is produced by pseudomonads, derived from the shikimate pathway. Phenazine is a redox-active molecule. This molecule displays an antibiotic activity toward many fungal, bacterial, and oomycete plant pathogens (Bilal et al. 2017; Biessy and Filion 2018). This antibiotic also contributed to plant drought-stress

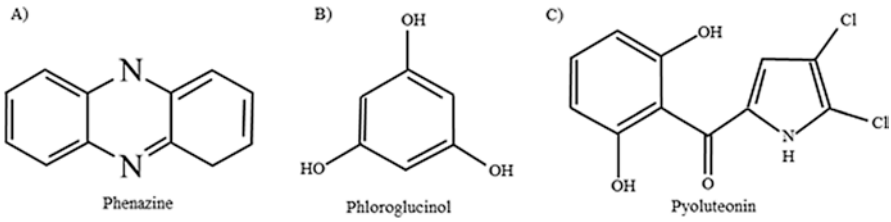


**Fig. 5.2** Chemical structure of phenazine (**a**), phloroglucinol (**b**), and pyoluteonin (**c**) antibiotics produced by PGPR

tolerance. Phenazine-producing *Pseudomonas chlororaphis* reduced ROS accumulation, and catalase activity enhanced in leaves of wheat seedlings grown in saline conditions, reducing osmotic stress (Yuan et al. 2020). Also with wheat seedlings, Mahmoudi et al. (2019) observed that in non-stressed conditions, seedlings colonized by the phenazine (overproducer) differed in dry weight biomass, the root dry weight biomass, and root to shoot investment (root/shoot dry weight ratio, as a percentage) to no-inoculum seedlings. Colonized seedlings translated into greater total root length, root surface area, and number of root tips, compared to the no-inoculum. The greater proliferation of root tips increased water uptake capacity by the seedling root systems.

Phloroglucinol (1,3,5-trihydroxybenzene or phloroglucin) is a product of the degradation of phloridzin and is a precursor of the lignin biosynthesis pathway. This compost increased root formation and leaf number and decreased the time required for rhizogenesis in apple rootstocks (Kim et al. 2020). Phloroglucinol acted synergistically with auxin, which stimulated the rooting of in vitro shoots of sugarcane (Gómez-Kosky et al. 2020), in *Diospyros crassiflora* (Tchouga et al. 2020). Phloroglucinol acted as a hormone using stimulating callus induction and organogenesis in the shoots and roots of *Ornithogalum dubium* (Petti 2020).

Reshma et al. (2018) reported induction of the induced systemic resistance by six strains of rhizosphere fluorescent *Pseudomonas* possessing 2,4-diacetyl phloroglucinol antibiotic genes against rice sheath blight pathogen *Rhizoctonia solani*. Isolate EP5 from *Pseudomonas fluorescens* showed 76.5% inhibition against *R. solani*. EP5-treated rice grains showed the highest germination of 96.6%, mean root length of 15.3 cm, shoot length of 12.6 cm, and vigor index of 2104.9. *P. fluorescens* strain showed higher activity of defense enzymes.

Cycle lipopeptide is another class of antibiotics, with a broad spectrum of activity, and their mode of action involves a cell membrane. These composts inhibit the synthesis of essential cell wall components (Oliveras et al. 2018). The potential use of cycle lipopeptide as biocontrol agents have been demonstrated in many species, such as wheat (Mejri et al. 2017), lettuce (Fujita and Yokota 2018), rice (Omoboye et al. 2019), *Xanthosoma sagittifolium* (Oni et al. 2019, 2020). Lipopeptide produced by *Bacillus* species has controlled potato late blight (*Phytophthora infestans*) and promoted plant growth (Wang et al. 2020).

Pyoluteorin was an aromatic phenolic polyketide antibiotic.Vinay et al. (2016) have detected antibiotic pyoluteorin in isolates of fluorescent pseudomonads strains of different crop rhizospheres. Pyoluteorin antibiotic was most effective against oomycete plant pathogens. In Vinay et al. (2016) study, two strains were proved as pyoluteorin (pltB) positive with different stages of confirmation of pyoluteorin antibiotic production.

## 5 Amino Acids

Studies relate the production and secretion of amino acids by rhizobacteria that affect plant growth. The production of osmolytes by the plant is an indicator of drought tolerance as it performs osmotic adjustment and prevents water loss (Vaishnav and Choudhary 2018) overcome the harmful effects of abiotic stresses.

Paul and Lade (2014) report that rhizobacteria play a fundamental role in plant growth, especially in saline soils, promoting the neutralization of osmotic stress via the release of organic osmolytes. According to the authors, neutralization is possible because the exposure of these organisms to saline environments triggers rapid flows of water along the osmotic gradient out of the cell, causing a reduction in the turgor and dehydration of the cytoplasm. In this way, the cytoplasm is exposed to high ionic strength to achieve osmotic balance, and the accumulation of amino acids allows them to act as osmoprotectants. Kusale et al. (2021) isolated *Klebsiella varicola* SURYA6 from wheat rhizosphere and reported a higher activities of antioxidant enzymes like superoxide dismutase, catalase, and glutathione oxidase in salt stress.

Increases in amino acid concentration and osmolytes (proline, sugar, and protein) contribute to drought tolerance (Askari and Ehsanzadeh 2015; Ilyas et al. 2020; Karimzadeh et al. 2020). Increases in these compounds is one of the defense strategies of bacterial strains to make plants drought tolerant. Increases in osmolyte concentration was significant in inoculated plants and contributed significantly to plant growth promotion under water scarcity by enhancing their defense strategies (Chiappero et al. 2019).

Among the osmoprotectants produced by vegetables under abiotic stress with PGPR inoculation, the accumulation of the proline amino acid and the salt stress condition are the most studied. The works were carried out with maize (Bano and Fatima 2009), wheat (Zarea et al. 2012; Bharti et al. 2016), *Mentha arvensis* (Bharti et al. 2014), *Vicia faba* (Metwali et al. 2015), sunflower (Naz and Bano 2015), and *Sorghum bicolor* (Surender et al. 2015).

## 6 Sugars

The promotion of plant growth by PGPR may occur through the transfer of synthesized molecules by the bacteria to the plant, by increasing the absorption or increasing the availability of nutritional elements, or by the protection of fluctuating environmental conditions (Saghafi et al. 2019). Among the sugars synthesized and secreted by PGPR, the exopolysaccharides (EPS) enable the association of bacteria with plant roots, forming a biofilm.

Biofilm is a complex of bacterial cells that can be linked to different living and nonliving surfaces (Saghafi et al. 2019), providing protection to plant roots against pathogens physically and functionally (Minah et al. 2015). According to Naseem

et al. (2018), EPS are heterogeneous water-soluble mixtures formed by polysaccharides, lipids, nucleic acids, and proteins. In plants, EPS affect plant growth in response to tolerance to different abiotic stresses.

EPS-producing strains have been also reported to produce antioxidant enzymes, i.e., superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which detoxify ROS; therefore, the ability of PGPR to augment the antioxidants can help in imparting drought tolerance. It is the accumulation of organic and inorganic solutes at the cellular level which maintain the cells' turgor properties and also protect proteins, enzymes, membranes, and cellular organelles from oxidative damage (Singh et al. 2016).

The review carried out by Naseem et al. (2018) focuses on the role of EPS-producing rhizobacteria on drought tolerance; it is reported that even in a sandy soil condition and subjected to severe drought; EPS promote the maintenance of high moisture content of the soil, forming a rhizome around the roots and protecting the plant from drying out. EPS create a microenvironment of water maintenance, decreasing dehydration compared to the surrounding environment. Under high osmotic stress, these stains synthesize extra amounts of EPS that alleviate the damage and increase the metabolic process in seeds (Saghafi et al. 2019; Bakhshandeh et al. 2019). Likewise, Ghosh et al. (2019) showed that the presence of EPS in secretions from *Pseudomonas* and *Bacillus* was also able to mitigate the adverse effects of osmotic stress in *Arabidopsis thaliana* plants.

Ilyas et al. (2020) studied the role of EPS-producing PGPR strains, *B. subtilis* and *A. brasilense*, on germination, physiological, morphological, and biochemical parameters of wheat under drought stress. The authors showed that under osmotic stress, germination percentage, seedling vigor index, and promptness index (PI) values were high in combination-treated wheat seeds as compared to single-strain inoculated seeds. Ilyas et al. (2020) also observed an increase in shoot length, root length, leaf area, chlorophyll content, and membrane stability in wheat seed treated with bacterial strains as compared to control in irrigated and drought exposed plants. Bacterial inoculation increased the growth of the plant. It was noted that root/shoot length and leaf area was increased significantly by the inoculation of bacterial strains in corn (Lin et al. 2019; Mishra et al. 2020), quinoa (Aslam et al. 2020), barley (Mahmoud et al. 2020), and mung bean (Kumari et al. 2015).

Other plant physiological responses have been evidenced from the EPS production by PGPR, a mechanism of tolerance to drought and salt stress. Under saline conditions, in soybean, the presence of EPS produced and secreted by *Pseudomonas* allowed an increase in the length of the shoot/root ratio, the number of lateral roots, fresh weight of shoot/root, decreased in Na+/K+ ratio, and binding of free sodium from the soil, making it unavailable for absorption by the plant (Kasotia et al. 2016). Likewise, Choudhary et al. (2015) reported in saline conditions, EPS-producing bacteria reduce the availability of harmful ions, mainly due to the chelation of excessive sodium ions around the roots. Other studies are restricted to the benefits of inoculation with PGPR for the production of soluble sugars by the plant to overcome stress conditions (Chen et al. 2016; Singh and Jha 2016).

Bacterial strains can secrete EPS, they colonize the plant's rhizosphere, adhere to the root surface, and maintain moisture content. They have adhesive properties and make stable aggregates that increase nutrients and water availability, which in turn improves plant development and growth (Asghari et al. 2020; Ilyas et al. 2020; Tewari and Sharma 2020).

## 7 Future Perspectives

PGPR help in boosting plant fitness through several mechanisms of action. The modes of action include direct mechanisms, such as siderophore production, phosphate solubilization, antibiosis, or indirect mechanisms by induction of plant resistance, plant secondary metabolites stimulation, and promotion of plant growth. Molecular and biotechnological tools will help to understand the biology of the rhizosphere, how plants are colonized, and the direct and indirect effect of PGPR on plant growth. The use of multiple strains in a single inoculation might be an efficient approach to reduce the harmful effects of stress on plant growth and improve the cost-benefit of using PGPR. The idea of manipulating genes can help the host plants in developing new traits like phytoremediation and herbicide resistance, among others, which could more suitably regulate metabolism. Although extensive research, none seems to immediately drop artificial chemicals. The current rate of studies and technological advances in nanotechnology and genetic engineering using PGPR will help enhance productivity by reducing our dependence on synthetic fungicides, pesticides, and fertilizers.

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# Chapter 6
# Inhibition of Bacterial and Fungal Phytopathogens Through Volatile Organic Compounds Produced by *Pseudomonas* sp.

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**Abstract** Plant growth-promoting rhizobacteria (PGPR) are being used as an alternative approach to combat plant diseases. About 80–90% of plant diseases are caused by bacterial and fungal pathogens, which remain an inevitable cause for the loss of several crops. Phytopathogenic bacteria and fungi are the major constraints to sustainable agriculture by adversely affecting crop growth and productivity. Owing to the increased pollution and harmful impacts of chemicals to control these pathogens, scientists are now centering on safer biological organisms and their byproducts. Secondary metabolites and volatile organic compounds (VOCs) emitted by various beneficial bacterial strains have a lot of potential for enhancing plant growth and preventing plant diseases. The VOCs produced by the most researched bacterial strains, such as *Pseudomonas* genera, are well recognized for protecting economically imperative plants and inducing resistance against bacterial and fungal phytopathogens. This chapter concentrates on throwing up a better grasp of biological activities of secondary metabolites such as hydrogen cyanide, siderophores, antibiotics, and VOCs produced by *Pseudomonas* spp. Hundreds of various bacterial VOCs, including alcohols, terpenoids, esters, and sulfur compounds, have been discovered. The VOCs emitted by *Pseudomonas* sp., for instance, acetophenone, 1,3-butadiene, 2-undecanone, benzaldehyde, 1,2-benzisothiazol-3(2H)-one, dimethyl trisulfide, dimethyl disulfide, benzothiazole, nonanal, N,N-dimethyldodecylamin, 3,5,5-trimethyl-1-hexanol, isovaleric acid, cyclohexanol, 2-ethyl 1-hexanol, n-decanal, decyl alcohol, etc., are reported for their antagonistic potential, inducing resistance in host plants against several bacterial and fungal

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pathogens. Crop growth enhancement and protection via VOCs is a promising and an ecofriendly method, substituting the harmful impacts of chemicals and ensuring the long-term sustainability in agriculture.

**Keywords** PGPR · VOCs · Induced systemic resistance · Antibiotics · HCN · Siderophore

## 1 Introduction

In the twenty-first century, according to United Nations the global population is increasing continuously which is projected to reach 9.7 billion in 2050,, which may lead to increase in global agricultural production, to fulfill the requirements of rapidly growing population (UNDESAP 2017; Rohr et al. 2019). Our agricultural sector largely depends upon the use of synthetic chemicals in order to revamp the crop production, i.e., synthetic fertilizers, which are used to increase the crop biomass, while synthetic pesticides are used to control pest and diseases in crops to reduce crop loss by 17–30%, particularly for the major staple crops (Naz and Bano 2014; Savary et al. 2019). However, these synthetic pesticides are unendurable due to their harmful residual effects and heavy manufacturing costs (Naz et al. 2014, 2018, 2021a). According to an estimation, around $250 million are required to take single active ingredients in market, having very low success rate about only 1 out of 140,000 synthetic compounds are successful, which is a very unsustainable way to develop synthetic pesticides (Lamberth et al. 2013).

Besides these, the continued use of pesticides makes them less effective because of the production of pesticide-resistant genes in plants (Butt et al. 2019; Ullah et al. 2020; Naz et al. 2021b). Furthermore, the continuous increase in global population has increased the demands for crops and agricultural growth, which has further caused increase in the applications of synthetic compounds. As projected increase in the demand for crops, agricultural growth might result in increased pesticide use of 10-fold and increased fertilizer application of 2.7-fold (Rohr et al. 2019; Jabborova et al. 2020).

Agronomic practices should be taken in consideration to lessen this dependency on synthetic compounds as well as to evolve the viable control measures, and different collaborative efforts should be made, i.e., improving agricultural practices by agronomic practices (Naz and Bano 2015; Ahluwalia et al. 2021). However, the introduction of soil beneficial microorganisms is another effective method to reduce the use of synthetic compounds in agricultural practices, as they have potential to antagonizing soil pathogenic microbes and are capable of increasing plant biomass (Yasmin et al. 2019; Luh Suriani et al. 2020). An extensive range of secondary metabolites is produced by these soil microorganisms which strengthen them to

fight with other pathogenic soil microbes, as they compete with each other for same resources in soil (Naz et al. 2017; Garbeva and Weisskopf 2020; Hamid et al. 2021).

The production of antibiotics, volatile organic compounds (VOCs), and secondary metabolites during microbial lifecycles are some other microbial inhibition tools to cope with pathogenic microbes within soil (Naz et al. 2020; Ye et al. 2020; Khan et al. 2021). The scientific world requires more attention on the production of VOCs due to multiple benefits of their utilization. VOCs are a mixture of volatile metabolites that may be emitted by all living microorganisms and have been shown to be very potent to control the growth of phytopathogenic bacteria and fungus through cross-talk interactions and antibacterial activities. Their antimicrobial effects, along with the reduced hazard for both the environment and human beings and their possible application without the need of a supplemental spray or drench, make the use of VOCs a promising and sustainable approach to replace fungicides of synthetic origin in the control of plant pathogens (Parafati et al. 2017; Tilocca and Migheli 2020; Zhang et al. 2020).

Although numerous modes of action are involved in phytopathogen obliteration, this chapter will dig into novel visions and ideas in biological control of phytopathogens via PGPR by dint of antibiotics and VOCs. Some *Pseudomonas* spp. have been associated with plant growth, suppression of fungal pathogens affecting plants, and detrimental rhizobacteria presenting considerable upsurge in root colonization. These aspects suggest that *Pseudomonas* spp. can serve as excellent biocontrol agents (Gomez-Lama et al. 2018; Reshma et al. 2018).

In this chapter, we focus to explore the role of secondary metabolites, antibiotics, and VOCs produced by the *Pseudomonas* species to sustain plant health by directly suppressing pathogens, inducing plant resistance against phytopathogens, and promoting plant growth, emphasizing their potential as alternatives to synthetic fertilizers and pesticides.

## 2 Microorganisms Emitting Volatile Organic Compounds

The volatile metabolites emitted from both plant and microbial sources are receiving a steady increase in interest. The word "volatilome" has been relatively recently used to describe this diverse and heterogeneous collection of metabolites (Farbo et al. 2018; Tilocca and Migheli 2020). The volatile metabolites of plant and microbial origin are mainly differentiated into organic and inorganic volatile molecules. Among inorganic volatile molecules, most relevant are $CO_2$, $H_2S$, CO, HCN, $SO_3$, $H_2$, $NH_3$, $NO_2^-$, and $SO_2$. The inorganic volatile molecules play an important role in different biological functions, i.e., acting as defense compounds by donating/accepting electrons (Rad et al. 2016; Zhang et al. 2020). These metabolites also have a role in various ecological and biological features along with their antibiotic resistance potential (Avalos et al. 2019; Kenawy et al. 2019).

In agriculture, the application of VOCs with microbial source in the biological control of plants pathogens has been given an unintentional decrease over the last

few years. However, the progress recently made and the general trend to an integrative approach have highlighted the potential advantages of microbiological VOCs in this area. The VOCs are known to be very effective at very low levels (Reshma et al. 2018; Tilocca and Migheli 2020).

In addition to pathogen inhibition and negative impact on fungal spore germination and function of morphogenesis enzymes, VOCs from microbial species have been found to play a role in a variety of biological processes (Deveau et al. 2018; Zhang et al. 2020). VOCs have the capability to kill nematodes which are known to be parasitic for plants (de Freitas Silva et al. 2020; Khoja et al. 2021) to increase plant growth (Hernández-León et al. 2015; Fincheira and Quiroz 2018) and to activate the mechanisms associated with resistance within plants, thus averting the plant from being infected by pathogens (Sharifi and Ryu 2016; Tahir et al. 2017; Zhang et al. 2020).

### *2.1 Production of VOCs by Consortium of Different Microbes*

A single organism can produce a diverse mixture of VOCs when applied, which leads to different outcomes proved by many experiments (Tilocca et al. 2019), whereas in a single ecological niche, there reside many microbial entities through which unexpected achievements can be obtained by consortium application as compared to the application of single microbial strain (Khan et al. 2019). Microbial strains interact with each other irrespective of their genera, phyla, and kingdom (Shaikh et al. 2016; Schulz-Bohm et al. 2017). These interactions lead to the essential biological and ecological outcomes in ensembled role of all the microbiota as a single unique entity. The effectiveness of the interactions occurring between microbiota members *Pseudomonas helmanticensis* Sc-B94 and *Bacillus cereus* Rs-MS53 has been reported to control the pathogenic fungus *R. solani* (Mülner et al. 2019), which was proved to be a strong strain compatibility and cooperative interaction (Asari et al. 2016; Che and Men 2019; Kramer et al. 2020). The production of volatile and nonvolatile compounds by different strains of *Pseudomonas* and *Bacillus* spp. can directly inhibit the growth of pathogen or can help in the acclimatization of the microbial community already residing in the same ecological niche which can also inhibit growth and infection caused by pathogen (Schulz-Bohm et al. 2017; Tilocca et al. 2020; Dimkić et al. 2022).

## 3 Bacterial Volatiles: Tool to Biocontrol of Phytopathogens

Bacterial VOCs play a role in the complex network of interactions that are established between bacteria, bacterial species, and bacteria with other microorganisms as well as with plants. Similarly, these interactions play a variable ecological role including beneficial interaction as well as antagonistic interaction. However,

beneficial cooperation encompasses symbiosis, mutualism, and host resistance interaction, while in antagonistic relationship, one of the interacting species exerts microbicidal activity on other species (Kanchiswamy et al. 2015; Tilocca et al. 2020). The useful bacterial-plant interaction has recently been recognized, which has extend new approaches for the use of bacterial volatilome in promoting plant growth. Furthermore, due to high flexibility of bacterial origin VOCs as well as their efficacy in controlling other pathogens, investigation is made on the utilization of VOCs produced by natural bacteria in defense against plant pathogenic microbes (Reshma et al. 2018; Mulero-Aparicio et al. 2019).

A wide variety of VOCs have been produced by rhizobacteria (*Serratia odorifera*, *S. plymuthica*, *Stenotrophomonas maltophilia*, *P. fluorescens*, *Stenotrophomonas rhizophila*, and *Pseudomonas trivialis*) which are active against an extensive variety of pathogenic microorganisms including bacteria and fungi (Kanchiswamy et al. 2015; Gotor-Vila et al. 2017; Mulero-Aparicio et al. 2019). Bacteria-fungi interaction usually produces some common volatile molecules including 1-octen3-ol, 2-nonanone, 2-undecanone, γ-patchoulene, 3-methylbutanoate, 3-methylbutanal, 2-methylbutan-1-ol, ethanethioic acid, dimethyl trisulfide 2,3,6-trimethylphenol, and 4-methyl-2-heptanone. Among these antifungal activities of some VOCs have already been tested (Tilocca et al. 2020).

## 4 Pseudomonas Volatilome

Various studies revealed that VOCs can inhibit a wide range of plant pathogens, also emphasizing VOCs as possible viable alternatives to pesticides and chemical fertilizers. One of the first examples of VOCs, produced by *Pseudomonas* species from canola and soyabean, exhibits plant growth stimulatory and inhibitory effect in case of plant pathogenic microbes (Agisha et al. 2019). About 23 VOCs are identified, which are produced by *Pseudomonas* species; among these six VOCs inhibited the mycelium growth of *S. sclerotiorum*, a pathogen of more than 400 plant species (Effmert et al. 2012; Thomas et al. 2020). A growth of widespread soil-borne *R. solani* pathogen was inhibited by VOCs from *Pseudomonas* spp. (Elkahoui et al. 2015) and by a variety of other rhizobacterial isolates (Velivelli et al. 2015). However, inhibitory activity against various bacterial pathogens exhibited by many VOCs is reported; for instance, nonanal, benzaldehyde, acetophenone, and benzothiazole are reported to inhibit the proliferation of *Clavibacter michiganensis*, a causative agent of bacterial ring rot disease of potato (Rajer et al. 2017). Similarly, *Xanthomonas oryzae* causing bacterial leaf blight of rice has been reported to be inhibited by 3,5,5-trimethyl-1-hexanol and decyl alcohol (Xie et al. 2018).

Moreover, the bacterial VOCs are also known to inhibit fungal mycelial growth; e.g., isovaleraldehyde, 3-methyl-1-butanol, isovaleric acid, 2-heptanone, and 2-ethylhexanol decrease the mycelium growth of *Phytophthora capsica* (Syed-Ab-Rahman et al. 2019; Freitas et al. 2022). Anti-oomycete activity is displayed by the VOCs of *Nodulisporium* against different *Pythium* species, while VOCs which are

causing inhibition of pathogens were not assayed individually (Sánchez-Fernández et al. 2016). However, these studies spotlight inhibitory activity of VOCs against a wide range of bacterial and pathogens, which could be good alternatives to pesticides.

## *4.1 Role of* Pseudomonas *Volatilome in Biocontrol of Phytopathogens*

*Pseudomonas* is widely recognized for having a diverse storage of plant growth-enhancing and antifungal metabolites, and many of these molecules are volatile compounds (Hernández-León et al. 2015; Yan et al. 2017; Dahiya et al. 2020). For instance, recently, it is reported by Hunziker et al. (2015) that *P. infestans* (a well-known oomycete phytopathogen of potato) can be inhibited by high potential volatiles emitted by *Pseudomonas*. The VOCs produced by *P. fluorescens* and *P. trivialis* are also reported to drastically inhibit the mycelial growth of *R. solani* (Kai et al. 2007). In recent studies, it was revealed that *P. donghuensis* P482 in the rhizosphere of tomato plants emits volatiles that play a significant role in inhibiting the growth of different plant pathogens for instance *Pythium ultimum*, *R. solani*, *Verticillium dahlia*, and *F. culmorum* (Ossowicki et al. 2017).

Evidences related to bacteriostatic were also found in the volatilomes of several strains of *Pseudomonas* spp. particularly in *P. chlororaphis*, which was tested against *Agrobacterium tumefaciens* and fungal, nematode, and insect pathogens for its antagonistic potential (Popova et al. 2014). The VOCs emitted from *P. putida* BP25 including 2-ethyl-5-methyl pyrazine, 2,5-dimethyl pyrazine, 2-ethyl-3,6-dimethyl pyrazine, 2-methyl pyrazine, and dimethyl trisulfide exhibited significant in vitro antimicrobial potential against several pathogens, for instance, *C. gloeosporioides*, *P. capsici*, *G. moniliformis*, *P. myriotylum*, *R. solani*, *R. pseudosolanacearum*, *A. rolfsii*, *R. similis*, and *M. oryzae* (Agisha et al. 2019).

From rhizosphere of soybean, common bean, and canola plants, the *Pseudomonas* strains were isolated and further reported for antagonistic potential against *S. sclerotiorum* owing to their VOCs including dimethyl trisulfide, n-decanal, benzothiazole, nonanal, cyclohexanol, and 2-ethyl 1-hexanol (Fernando et al. 2005; Giorgio et al. 2015). The antagonistic ability of VOCs produced by *P. fluorescens* B-4117 and *P. fluorescens* Q8r1-96 has been reported against plant pathogenic bacterial strains including *A. vitis* and *A. tumefaciens*. Here, it is suggested that *Pseudomonas* species are known to produce VOCs which can be used as a potential tool to control many diseases particularly the crown gall tumors which can be effectively prevented in tomato plants (Dandurishvili et al. 2011). The VOCs produced by *P. fluorescens* WR-1 are also reported to significantly affect and decrease the virulence characteristics of *R. solanacearum in tomato* (Raza et al. 2016). The active VOCs produced by *Pseudomonas* spp. and their biocontrol potential against target phytopathogens have been described in Table 6.1.

**Table 6.1** Active VOCs produced by *Pseudomonas* spp. and their biocontrol potential against target phytopathogens

| VOC-producing *Pseudomonas* sp. | Target pathogen | Active VOCs | References |
|---|---|---|---|
| *P. fluorescens*<br>*P. chlororaphis O6* | *S. sclerotiorum*<br>*E. carotovora/N. benthamiana* | Benzothiazole<br>(2R, 3R)-Butanediol | Fernando et al. (2005)<br>Han et al. (2006) |
| *P. chlororaphis* | *S. sclerotiorum* | Nonanal<br>Cyclohexanol<br>Benzothiazole<br>Dimethyl trisulfide<br>2-Ethyl, 1-hexanol<br>*n*-Decanal | Fernando et al. (2005) |
| *P. donghuensis* | *P. ultimum, R. solani, V. dahlia, and F. culmorum* | Dimethyl sulfide<br>Methyl thiocyanate<br>1-Undecan<br>S-Methyl thioacetate<br>Dimethyl trisulfide | Ossowicki et al. (2017) |
| *Pseudomonas* sp.<br>*P. fluorescens*<br>L13-6-12 | *P. infestans*<br>*R. solani* | 1-Undecene<br>Undecene | Kai et al. (2007) |
| *P. trivialis* 3Re2-7 | *R. solani* | UndecadieneUndeceneBenzyloxybenzonitrile | |
| *P. fluorescens*<br>B-4117 | *A. tumefaciens*<br>*A. vitis* | Hydrocarbon 1-undecene<br>Methanethiol<br>Methyl thiol acetate | Dandurishvili et al. (2011) |
| *P. fluorescens*<br>WR-1 | *R. solanacearum* | Dodecane<br>1-Undecanol<br>1-Nonene<br>Benzothiazole<br>Naphthalene, 1-methyl<br>Ethyl benzene<br>Ethanone 1-(2-furanyl)- | Raza et al. (2016) |

(continued)

**Table 6.1** (continued)

| VOC-producing *Pseudomonas* sp. | Target pathogen | Active VOCs | References |
|---|---|---|---|
| *P. putida* BP25 | *C. gloeosporioides, P. capsici, G. moniliformis, P. myriotylum, R. solani, R. pseudosolanacearum, A. rolfsii, R. similis and M. oryzae* | 2-Ethyl-5-methyl pyrazine<br>2,5-Dimethyl pyrazine<br>2-Ethyl 3,6-dimethyl pyrazine<br>2-Methyl pyrazine Dimethyl trisulphide | Agisha et al. (2019) |
| *P. brassicacearum* | *S. sclerotiorum* | 1-Undecene<br>dl-Limonene<br>m-Cymene<br>2-Undecanone<br>2-Nonanone | Giorgio et al. (2015) |
| *P. aurantiaca* | *S. sclerotiorum* | Nonanal<br>2-Ethyl,1-hexanol<br>*n*-Decanal | Fernando et al. (2005) |
| *P. chlororaphis* | *S. sclerotiorum* | Nonanal<br>Cyclohexanol<br>Benzothiazole<br>Dimethyl trisulfide<br>2-Ethyl,1-hexanol<br>*n*-Decanal | Fernando et al. (2005) |
| *P. fluorescens* | *B. cinerea*<br>*S. sclerotiorum*<br>*C. fimbriata* | Dimethyl disulfide<br>2-Ethyl,1-hexanol<br>Phenylethyl alcohol<br>2-Methyl-1-butanol<br>3-Methyl-1-butanol | Hernández-León et al. (2015)<br>Fernando et al. (2005)<br>Zhang et al. (2019) |
| *P. chlororaphis* subsp. *aureofaciens*<br>*P. stutzeri* | *B. cinerea* | Dimethyl disulfide | Rojas-Solís et al. (2018) |

## 4.2 *Role of Secondary Metabolites Produced by* Pseudomonas *spp. in Plant Disease Control*

Fluorescent pseudomonads are predominant antagonistic bacteria that live in soil. Nowadays, the significance of these bacteria has been acknowledged all over the world, owing to the fact that they are capable of synthesizing a variety of antifungal compounds such as siderophores; fluorescent pigments along with volatile elements, namely, hydrocyanic acid (HCN); lytic enzymes; as well as antibiotics (Ciancio et al. 2016; Jadhav et al. 2017; Yasmin et al. 2020). Some of the noteworthy lytic enzymes produced by *Pseudomonas* spp. are chitinase, protease, and β-1,3-glucanase. These enzymes instigate lysis and hyperparasitism of antagonistic bacteria toward lethal fungal pathogens (Jadhav et al. 2017; Zia et al. 2021).

Various fluorescent pseudomonads are impervious to cyanide due to the existence of a thiosulfate (RhdA): cyanide sulfur transferase that modifies the cyanide to thiocyanate which is less toxic. In many *Pseudomonas* spp., approximately 300 μM cyanide is produced by the oxidative decarboxylation of glycine (Blumer and Haas 2000). Gupta et al. (2002) investigated the *Pseudomonas* to biologically control the charcoal rot instigated by *Macrophomina phaseolina* in peanut.

Sindhu et al. (1997) reported the role of secondary metabolites in the inhibition of phytopathogens and also the inhibiting role of siderophore-producing rhizobacteria and several fluorescent *Pseudomonas* spp. against many bacterial and fungal phytopathogens. Siderophore- producing pseudomonads have been reported in chickpea to markedly reduce the root rot disease (Akhtar and Siddiqui 2009).

*Pseudomonas fluorescens* are known to produce siderophore and control *Pythium ultimum*, and *Pseudomonas stutzeri* produces chitinase which lyse the cell wall of *Fusarium solani*. Antifungal metabolites produced by these *Rhizobacteria* were identified as antibiotics (iturin, surfactins, fengycin, DAPG, phenazine, etc.), cell wall degrading enzymes (proteses, chitinases, cellulases), plant growth-promoting enzymes and hormones (indole-3-acetic acid, ACC-deaminase, phosphatase, nitrogen fixation), N-acyl homoderine lactones, and siderophore (Dahiya et al. 2020).

Another siderophore as pseudobactin produced by *P. putida* was able to suppress the growth of *Fusarium oxysporum* in iron-deficient soil; this suppression/inhibition was abandoned when iron was provided in that soil (de Boer et al. 2003). Several studies have explained the inhibition of fungal pathogens by fluorescent pseudomonads from the excretion of siderophores (iron-chelating), making it inaccessible to other several microorganisms (Shaikh et al. 2014).

The biocontrol potential of siderophore as an antifungal metabolite produced by *Pseudomonas* spp. is shown in Table 6.2.

**Table 6.2** Role of antifungal metabolites of *Pseudomonas* spp. in biocontrol of phytopathogens

| Antifungal metabolites | Producing PGPR | Host | Target pathogen | References |
|---|---|---|---|---|
| **Siderophore** | *P. fluorescence* | Wheat | *G. graminis* | Sayyed et al. (2013) |
| | | Wheat | *F. glycinia* | |
| | | Soybean | *S. oryzae* | |
| | *P. aeruginosa* | Potato | *F. udum*<br>*A. niger* | Sulochana et al. (2014) |
| | *P. fluorescens* | Soybean | *P. ultimum* | León et al. (2009) |
| | *P. putida* | Radish<br>Cucumber | *Fusarium* spp. wilt | Sayyed et al. (2013) |
| | | Beans | *F. solani* | |
| | | Potato | *F. oxysporum* | |
| | *P. cepacia* | Onion | *F. oxysporum* | Sayyed et al. (2013) |
| | *P. aureofaciens* | Wheat | *G. graminis* var. *tritici* | |
| | *P. fluorescence* | beet root | *P. debaryanum* | Dodd and Stewart (1992) |
| | | Cotton | *R. solani* | Hagedorn (1990) |
| | | Tomato | *S. rolfsii* | Thiribhuvanamala et al. (1999) |
| | *P. fluorescence* | In vitro | *P. debaryanum, R. solani*, and *S. rolfsii* | Prasad et al. (2017) |
| **HCN** | *P. fluorescence* | Tobacco, wheat | *T. basicola*<br>*G. graminis* | Voisard et al. (1989)<br>Shaikh and Sayyed (2015) |
| | *Pseudomonas* spp. P76 and P124 | Many crops | *S. rolfsii* | Priyanka et al. (2017) |
| | *Pseudomonas* spp. LBUM300 | Tomato | *C. michiganensis* subspp. *michiganensis* | Lanteigne et al. (2012) |
| | *Pseudomonas* CF1 and CF5 | In vitro | *M. phaseolina* | Reetha et al. (2014) |
| | *P. corrugata* and *P. mediterranea* | In vitro | *B. cinerea* | Strano et al. (2017) |
| | *P. donghuensis* P482 | In vitro | *R. solani* AG2, *F. culmorum* PV and *P. ultimum* P17 | Ossowicki et al. (2017) |
| | *P. fluorescence* | In vitro | *P. debaryanum, R. solani*, and *S. rolfsii* | Prasad et al. (2017) |

## *4.3 Antibiotics Produced by* Pseudomonas *spp.*

According to Haas and Défago (2005), six antibiotic classes are best to perform their biocontrol potential particularly to control root fungal diseases: pyoluteorin, phenazines, pyrrolnitrin, phloroglucinols, hydrogen cyanide (which is volatile), and

cyclic lipopeptides. Most recently, lipopeptide biosurfactants produced by *Pseudomonas* spp. have been implied in biocontrol due to their potential positive impact on the competitive contacts with organisms involving fungi, bacteria, oomycetes, nematodes, protozoa, and plants (Raaijmakers et al. 2010; Shafi et al. 2017; Fira et al. 2018).

Many bacterial species are reported for the isolation of several antibiotics that are known to inhibit cell wall composition of the pathogen, interrupt the cell membrane structures, and impede the synthesis of ribosomal subunits (Maksimov et al. 2011). Fluorescent pseudomonads primarily achieve biocontrol of pathogens by synthesizing specific antibiotics like pyoluteorin, 2,4-diacetylphloroglucinol, pyrrolnitrin, 2-hydroxy phenazines, and phenazine-1-carboxamide and phenazine-1-carboxyclic acid (Mustafa et al. 2019). Antibiotics are not just solely involved in antipathogenic activity; they are also major contributors in instigating ISR in plants as they vigorously suppress disease by offering competitive leverage to biocontrol agents. Host resistance toward plant pathogens is enhanced significantly when ISR and antibiotics act synergistically (Hashem et al. 2019; Ullah et al. 2020).

More than 6000 compounds have been characterized and identified for strong antifungal potential, including phenazine (PHZ) as a key molecule and over 100 more derivatives of PHZ (Mavrodi et al. 2006). Moreover, the products containing PHZ (even more than 180) are known for their strong antifungal, antibiotic, anticancer, insecticidal, anti-protozoan, and antitumor potential (Briard et al. 2015; Guttenberger et al. 2017). Several studies attributed the antimicrobial potential of PHZ produced by *Pseudomonas* strain PCL1391 to the production of ROS (reactive oxygen species) (Laursen and Nielsen 2004) and found very effective against *Botrytis cinerea*, *Gaeumannomyces graminis*, and *F. oxysporum* (Schoonbeek et al. 2002; Chin-A-Woeng et al. 2003). Several PHZ and its derivatives are efficient in controlling numerous fungal diseases (Chincholkar et al. 2013). The *P. chlororaphis* PCL1391 strain has been reported to produce phenazine-1-carboxamide, which can nourish plants with soluble iron at neutral pH (Hernandez et al. 2004; Haas and Défago 2005).

The fluorescent pseudomonads producing DAPG are reported for their strong biocontrol potential (Weller et al. 2007; Troppens et al. 2013); several other research studies have confirmed DAPG as a key antimicrobial metabolite engaged in the biocontrol of fungal phytopathogens (Sonnleitner and Haas 2011; Khare et al. 2018). The DAPG is an efficient and extensively researched antibiotic which is released by pseudomonads to control oomycete and *Pythium* spp. (de Souza et al. 2003).

Pyoluteorin (PLT) is a phenolic polyketide, which has initially been isolated and identified from *P. aeruginosa* and then from fluorescent pseudomonads (Nowak-Thompson et al. 1997). PLT has herbicidal, bactericidal, and fungicidal properties (Takeda 1959). PLT has also been stated to function as an intercellular signal and auto-inducer among distinctive rhizospheric populations of bacterial strains (Brodhagen et al. 2004). It has recently been observed that phloroglucinol in *P.*

*protegens* has a significant impact on PLT gene expression and production (Clifford et al. 2016).

Different metabolites are produced at different concentration of phloroglucinol with distinct phytopathogenic target (Khare et al. 2018). Limited range of gram-negative bacteria are involved in the production of pyrrolnitrin from *Pseudomonas* species (Mujumdar et al. 2014; Weller et al. 2016). Fluorescent pseudomonads produce pyrrolnitrin which has antagonistic nature against fungi, yeast, and Gram-positive bacteria (Jani et al. 2015). *P. fluorescens* BL915 strain secretes pyrrolnitrin which has a property to protect *Rhizoctonia solani* during damping off of cotton (Hill et al. 1994).

Currently, cyclic lipopeptides (CLPs) have been identified as biosurfactant and antimicrobials which is found effective against broad spectrum of phytopathogen involving enveloped viruses, Gram-positive bacteria, and mycoplasmas (Raaijmakers et al. 2006; Tran et al. 2007; Raju et al. 2016). Research has reported that CLPs released by pseudomonads are involved in colonization of seeds and roots. In addition, it also contributes to the formation of biofilm and virulence (Li et al. 2013; Raaijmakers et al. 2010). Fluorescent pseudomonads release different types of CLPs; many of them have not characterized completely. The well-documented and studied groups of CLPs are amphisin, viscosin syringomycin, and tolaasin (Nybroe and Sørensen 2004).

CLPs secreted by *Pseudomonas* are categorized into eight different groups on the basis of variation in length and composition of the oligopeptide and fatty acid tails (Olorunleke et al. 2017). The ability to agitate biological membranes are associated with the antimicrobial properties (Raaijmakers et al. 2006; Dumée et al. 2015). *P. protegens* produce orfamide which is a type of potential CLPs having insecticidal property (Nandi et al. 2015). Fluorescent pseudomonads releasing several metabolites having broad-spectrum phytopathogenic activities are preferred in the field of agriculture. Currently, Izzah-Shahid et al. (2017) reported that application of PCA, CLP, and lahorenoic acid A substantially enhanced growth of wheat by producing *P. chlororaphis* and *P. aurantiaca* during development. Sharifazizi et al. (2017) also found that fluorescent pseudomonad strain Ps170 has the capability to control blight-causing pathogen in pear by releasing DAPG, PLT, PRN, and PCA. Metabolites of fluorescent pseudomonads are currently being used as biological controls to secure the plant from causative agents such as causing protozoa and nematodes (Meyer et al. 2009; Jousset et al. 2010; Clifford et al. 2016). Antibiotics produced by *Pseudomonas* spp. and their biocontrol potential against phytopathogens have been described in Table 6.3 and Fig. 6.1.

**Table 6.3** Antibiotics produced by *Pseudomonas* spp. and their biocontrol potential against fungal pathogens

| Antibiotics | *Pseudomonas* spp. | Host/disease | Targeted fungal phytopathogen | References |
|---|---|---|---|---|
| 2,4-DAPG | *P. fluorescens* | Wheat | *G. graminis tritici* | Weller et al. (2007) |
| | | Tobacco | *T. basicola* | Keel et al. (1992) |
| | | Sugar beet | *P. ultimum* | Nielsen et al. (1998) |
| | *Pseudomonas* spp. | Sugar beet | *P. ultimum* | Shanahan et al. (1992) |
| | *P. fluorescens* (CHAO) | Tobacco | *T. basicola* | Keel et al. (1992) |
| | *P. fluorescens* CHAO | All diseases | *G. graminis* tritici | Fenton et al. (1992) |
| | *P. fluorescens* Q2-87 P. ßuorescens F | Sugar beet | *P. ultimum* | Rosales et al. (1995) |
| | *P. fluorescens* Pf | Sheath blight | *R. solani* | Rosales et al. (1995) |
| | *P. aurantiaca* | Wheat | *F. oxysporum* | Garagulia et al. (1974) |
| | *P. fluorescens* VUPf5 | Wheat | *G. graminis* var. tritici | Lagzian et al. (2013) |
| | *P. fluorescens* | Rice | *X. oryzae* pv. oryzae (Xoo) | Velusamy and Gnanamanickam (2003) |
| | *P. aeruginosa* | Banana | *F. oxysporum* f. spp. cubense FOC | Ayyadurai et al. (2006) |
| | *P. fluorescens* | Groundnut | *A. niger, A. flavus*, *S. rolfsii* | Sherathia et al. (2016) |
| | *P. brassicacearum* | In vitro | *R. solanacearum* | Zhou et al. (2012) |
| | *Pseudomonas* spp. | Tomato | *C. michiganensis* subspp. *michiganensis* | Lanteigne et al. (2012) |
| Aerugine | *P. fluorescens* | Pepper<br>Cucumber | *Phytophthora*<br>*C. orbiculare* | Lee et al. (2003) |
| Pyrrolnitrin | *P. fluorescens* | Grass<br>Cucumber<br>Soybean | *S. homoeocarpa*<br>*Pythium* spp.<br>*P. ultimum* | León et al. (2009) |
| | *P. cepacian* | Maize<br>Sugar beet<br>In vitro only | *B. maydis*<br>*A. cochliodes*<br>*C. truncatum* and *F. sambucinum* | Homma (1994)<br>Burkhead et al. (1994) |
| | *P. chlororaphis* O6 | *Tomato* | *F. graminearum* and *R. solani* | Park et al. (2011) |
| | *P. fluorescens* | Cotton and cucumber | *R. solani* | Hammer et al. (1997) |
| | *P. fluorescens* | Cotton<br>Cotton | *V. dahliae*<br>*T. basicola* | Howell and Stipanovic (1979) |

(continued)

**Table 6.3** (continued)

| Antibiotics | *Pseudomonas* spp. | Host/disease | Targeted fungal phytopathogen | References |
|---|---|---|---|---|
| | *P. fluorescens* Pf-5 | Spring and fall disease of Kentucky bluegrass | *D. poae* | Rodriguez and Pfender (1997) |
| | *P. cepacia* | Potato | *F. sambucinum* | Burkhead et al. (1994) |
| | *P. cepacia* | Sunflower | *Sclerotinia sclerotiorum* | McLoughlin et al. (1992) |
| Viscosinam ide | *P. fluorescens* | Sugar beet | *R. solani*<br>*P. ultimum* | Nielsen et al. (1998) |
| Pyoluteorin | *P. fluorescens* | Cotton<br>Sugar beet | *Pythium* spp.<br>*Pythium* spp. | Howell and Stipanovic (1980) |
| | P. fluorescens Pf-5 | Damping off | Members of oomycetes spp. Pythium | Kraus and Loper (1995) |
| Phenazines | *P. fluorescens* | *Wheat* | *G. graminis* var. tritici. | Thomashow and Weller (1988) and Thomashow et al. (1990) |
| | P. aeruginosa | Pigeon pea and chickpea | *F. oxysporum* f. spp. ciceris and *F. udum* | Anjaiah et al. (2003) |
| | *Pseudomonas* spp. MCC 3145 | In vitro | *C. circinans*, *C. dematium, F. oxysporum* | Patil et al. (2017) |
| | *Pseudomonas* spp. | *Wheat* | *R. solani* | Jaaffar et al. (2017) |
| | *Pseudomonas* spp. | *Tomato* | *F. oxysporum* | Chin-A-Woeng et al. (1998) |
| | *P. fluorescens* | Wheat | *G. g.* Var. *tritici* | Thomashow and Weller (1988) |
| Oomycin A | *Pseudomonas* spp. | Damping-off (cotton) | Pythium spp. | Gutterson et al. (1988) |
| 3-de-epoxy-2,3-didehydro-rhizoxin | *Pseudomonas* spp. | Net blotch Wheat bunt | *Pyrenophora teres* Drechs *Tilletia caries* Tull | Wright et al. (1999) |
| Agrocin 84 | *Pseudomonas* spp. | Crown gall (fruit trees) | *A. tumefaciens* | Kerr et al. (1984) |
| Pseudobactin B10 | *Pseudomonas* spp. | Flax wilt | *F. oxysporum* | Kloepper et al. (1980) |
| Cyclic lipopeptides | *P. fluorescens* | Sugar beet | *R. solani* and *P. ultimum* | Nielsen et al. (2000, 2002) |
| | *P. fluorescens* | Tomato | *P. infestans* | Tran et al. (2007) |
| | *Pseudomonas* SH-C52 | Groundnut | *S. rolfsii* | Le et al. (2012) |

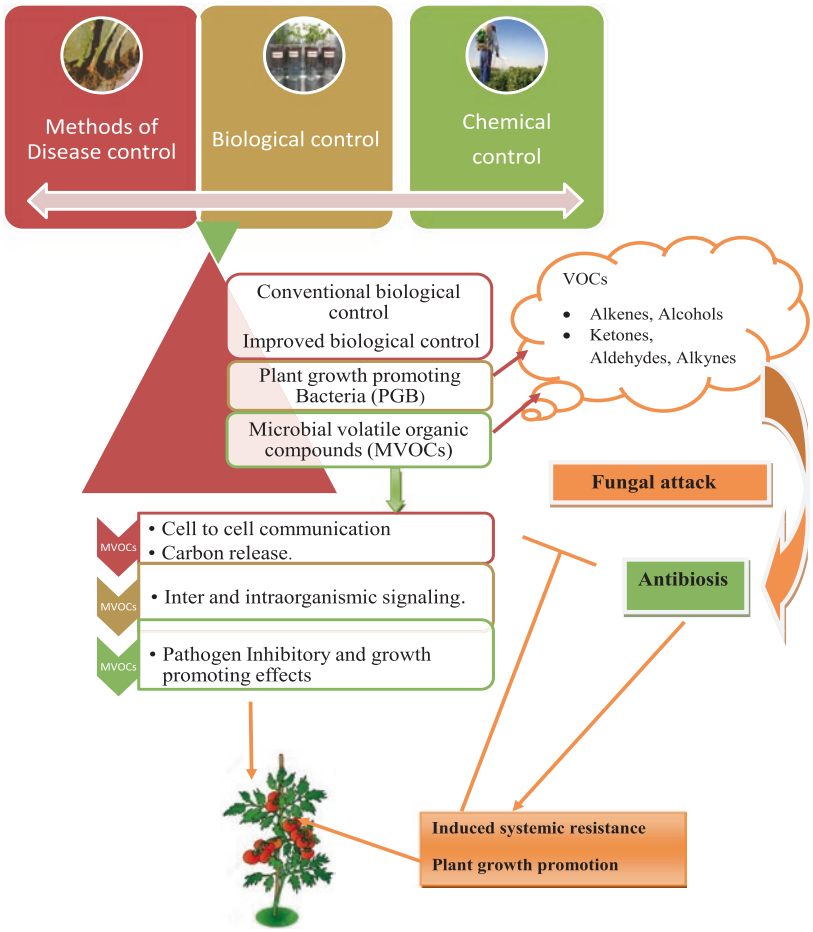


**Fig. 6.1** Role of VOCs and antibiotics in plant growth promotion and induced systemic resistance

## 5 Conclusion

*Pseudomonas* spp. are **plant growth-stimulating** bacteria that are often observed with diverse phyto-beneficial characteristics. The biological activities of hydrogen cyanide, siderophore, antibiotics, and VOCs **produced by these species** highlight their potential to act as alternatives to unsustainable agricultural chemical inputs and to feed a continuously growing population. In this chapter, we have investigated the biocontrol potential of secondary metabolites and VOCs produced by *Pseudomonas* species (Tables 6.1, 6.2, and 6.3), which have more and diverse abilities to fight phytopathogens. Therefore, future research should focus on the growth-stimulating effects of antibiotics and VOCs on various crop and vegetable species. This chapter represented here focuses on the antibiotics and particularly VOCs

emitted by *Pseudomonas* spp. in axenic culture conditions, whereas growing evidence suggests that interaction between different microorganisms could boost the production of VOCs which have been shown to have inhibition against pathogens. This will allow to identify the biologically relevant VOCs that are effectively involved in the inhibition of microbial pathogens. While a number of studies have also investigated the impact of VOCs in one biological function, there are likely to be similarities in the functions of these VOCs. For instance, nonadecane and heptadecane exhibited their role in pathogen suppression, plant growth promotion, and induced resistance, which suggests that the biological activities are not the isolated entity. Studies have shown the pathogenic suppression in the presence of the VOCs, but it is also important to know the involvement of these inhibitory VOCs on plant growth. Further investigation on the efficacy of VOC under field conditions can be a promising approach. There is a dire need for further exploration for the testing of a wider range of VOCs for field applications.

In conclusion, studies reviewed here demonstrate antibiotics, siderophore, hydrogen cyanide, and VOCs can be manipulated to serve as sustainable alternatives to agricultural chemical inputs, which can potentially reduce our overreliance on the current unsustainable methods at a time when population growth, and food demand, is likely to substantially increase.

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# Chapter 7
# How Phytohormones Synthesized by PGPR Affect Plant Growth?


**Deivid Luis Vieira Stefen, Francielle Regina Nunes, Giselle Regina Rodolfo, Cristiane Segatto, Thalita Cardoso Anastácio, and Cristiano Reschke Lajus**

**Abstract** Plant growth-promoting rhizobacteria (PGPR) are important microorganisms that can induce the secretion of phytohormones, like auxins, gibberellic acid, abscisic acid, cytokinins, and ethylene, and play an important role in plant growth. Plant growth benefits due to the addition of PGPR include increases in germination rate, root growth, yield, crop quality, leaf area, chlorophyll, nitrogen, protein content, tolerance to stresses, shoot and root weight, delayed leaf senescence, and tolerance to pests and diseases. PGPR confers tolerance to plants under stressful environments promoting their growth. Then, the possible explanation for the mechanism of biotic and abiotic stress tolerance includes the production of phytohormones. In this chapter, we present the benefits that phytohormones produced by PGPR bring to plants, indicating that the use of PGPR becomes an appropriate strategy and a trend in the sustainable development of plants under environmental stresses.

**Keywords** Plant growth-promoting rhizobacteria · Stress tolerance · Sustainable agriculture

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## 1 Introduction

Producing enough food for a growing population is a challenge for food security. The damaging effects of chemical fertilizers and pesticides have led to increasing interest in improving agricultural practices (Tsukanova et al. 2017). As an alternative to mitigate these difficulties, beneficial soil microorganisms have been used (Odoh 2017). Plant growth-promoting rhizobacteria (PGPR) contribute to increasing productivity and sustainability in agriculture. Then, these bacteria associated with the plant rhizosphere can be beneficial for plant growth, yield, and crop quality (Sureshbabu et al. 2016). In addition, decreases the dependence on agricultural chemicals which unbalance the agroecosystem (Kumar et al. 2020).

The rhizosphere, the layer of soil influenced by plant roots, is a complex ecosystem colonized by diverse organisms where microbial activities occur (Verma et al. 2019; Bukhat et al. 2020). There is a great diversity of PGPR in the rhizosphere that comprise microorganisms of the genera such as *Acetobacter*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Lactobacillus*, *Micrococcus*, *Pseudomonas*, *Serratia*, *Cellulomonas flavigena* (Disi et al. 2019; Hassan et al. 2019; Duy et al. 2016; Hossain et al. 2015), *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* (Kumawat et al. 2019; Harman and Upho 2019; Etesami and Maheshwari 2018; Lamont et al. 2017).

PGPR have also recently been known to produce phytohormones like secondary metabolites such as auxin, cytokinin, gibberellins, abscisic acid (ABA), and ethylene, which are synthesized through plant-secreted precursors and are called plant growth regulators (Jeyanthi and Kanimozhi 2018). These phytohormones may play a regulatory role in plant growth and development at extremely low concentrations (Fig. 7.1).

Many rhizosphere bacteria synthesize and secrete hormones allowing plants to absorb these products through their roots and thus increasing plant growth under stress conditions (Backer et al. 2018; Etesami and Maheshwari 2018; Parray et al. 2016). The production of such plant growth regulators by PGPR can give additional support to the growth of host plants (Ahemad and Kibret, 2014), improvement of mineral nutrition, plant resistance to abiotic stresses, and water relations. Then, this chapter discusses how phytohormones produced by PGPR affect plant growth is discussed.

## 2 Indole-3-Acetic Acid (IAA)

Auxins are molecules naturally produced by plants and involved in almost all aspects of plant physiology, controlling cell division, expansion, differentiation, and abiotic stress relief (Paque and Weijers 2016).

Although auxins are the main regulators of the developing plant, IAA and its biosynthesis-determining genes are also found in a wide range of different bacteria

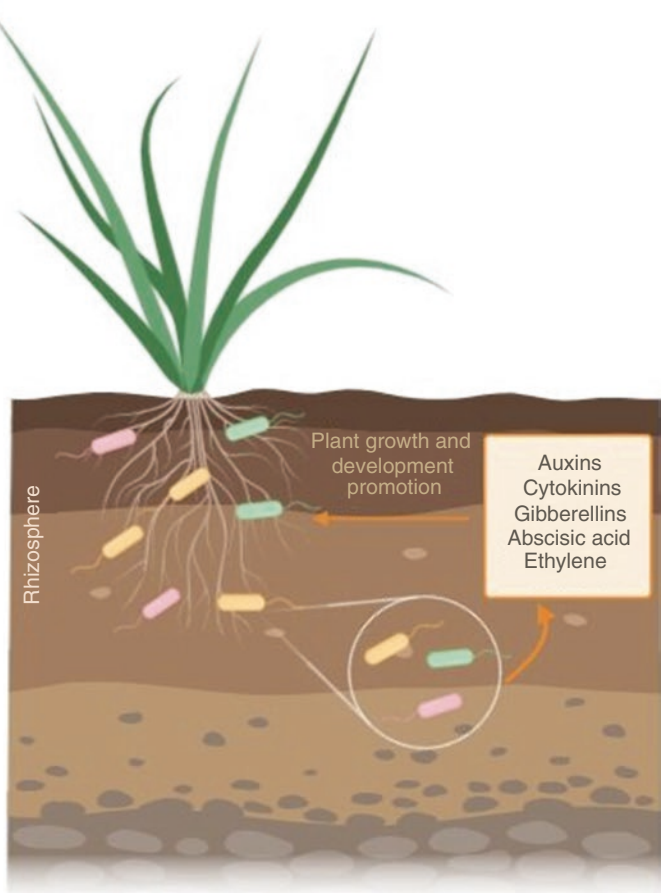


**Fig. 7.1** Phytohormones secreted by PGPR have an effect on plant growth and development

or fungi (Matsuda et al. 2018). Even though IAA may impact gene expression in some bacteria, it does not function as a bacterial growth factor, but as a signal to communicate with plants in an ecological context to gain profits from improving plant growth (Olenska et al. 2020).

Auxins are produced and excreted by more than 80% of the rhizosphere bacteria, such as A*zospirillum* spp., *Azotobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., and S*taphylococcus* spp. (Park et al. 2017). The amounts of auxins produced vary among bacterial strains. In bacteria, auxin synthesis was found from only one precursor, tryptophan (Park et al. 2021). Beneficial rhizospheric bacteria predominantly use the indole-3-pyruvate pathway for the production of auxins, while plant-associated pathogenic bacteria use indole-3-acetamide (AMI) more often (Li et al. 2018). In the presence of *Azospirillum* spp., there is a positive correlation between stimulation of plant root cell membrane activity and increased levels of IAA and indole-3-butyric acid (IBA). Bacteria also provide other plant

growth-regulation compounds for their host plant, such as indole-3-acetaldehyde, indole-3-lactic acid, indole-3-ethanol, indole-3-acetamide (AMI) (Patten et al. 2013).

However, at low concentrations, bacterial auxins stimulate the stretching of the primary roots of plants, but at higher doses, auxins promote the formation of lateral and adventitious roots, which can increase the absorption of minerals and increase the production of root exudates that increase bacterial proliferation (Verbon and Liberman 2016). Through the production of IAA phytohormones, induce significant changes in root system architecture, thus increasing the lateral branching of the root and root hair formation, thereby there is an increase in nutrient absorption by root systems that promote plant growth (Grover et al. 2021).

The soil properties can positively influence the amount of IAA received by the plant, thus altering the lifetime of the IAA and its diffusion speed. In soil poor in organic matter and clay, IAA may be less adsorbed than clay soil, spreading faster (Suarez et al. 2014). The plant species are also important to explain the direction and extent of the effect of rhizobacteria on plant growth, demonstrating sensitivity to a certain amount of IAA, which depends on genetic and physiological factors, and are likely to be indicative of the net effect of rhizobacteria on plant growth. However, a plant can actively alter the amount of IAA production by bacteria (Suarez et al. 2014). In addition, bacteria-derived auxins can prevent the effects of various environmental stresses, such as drought, salinity, or soil pollution (Kudoyarova et al. 2019).

An example that can be cited is a *B. licheniformis* strain HSW-16 mitigated saline stress and stimulated the *T. aestivum* to grow thin correlation with high concentrations of IAA (Singh and Jha, 2016). Similarly, *Enterobacter* spp. NIASMVII strain produced amounts of IAA that correlated with increased *T. aestivum* seed germination (Sorty et al. 2016). Was observed a correlation between the increase in *Serratia* synthesis to IAA (Zaheer et al. 2016). The inoculation of corn plants with the bacterial strains *P. fluorescens* S3X and *C. necator* 1C2 obtained positive effects on corn tolerance to moderate water stress, helping to maintain corn yield. Bacteria significantly promoted shoot biomass and P and N use efficiency by maize plants helping to maintain maize productivity with a less water supply (Pereira et al. 2020). Recent results show that *Bacillus thuringiensis* KVS25 alone or in conjunction with silicon can be employed to mitigate the of silver nanoparticles (AgNPs) in *Brassica juncea* seedlings; this mitigation can be attributed to the activities of improved antioxidant enzymes that help in detoxification of reactive free radicals, in reducing the harmful effects to *B. juncea* seedlings under the stress of AgNPs, thus reducing the negative effects of AgNPs by PGPR *B. thuringiensis* KVS25 may be associated with the release of IAA (Vishwakarma et al. 2020).

## 3 Gibberellic Acid (Gibberellin)

Gibberellins (GA) amount to more than 100 compounds, constituting the largest class of phytohormones, which are found in both plants and microorganisms. Phytohormone acts on the growth of the stem and leaves of vegetables by regulating

height; it also acts on fruit development, flowering, and retarding the aging of plant tissues (Tsukanova et al. 2017). Rhizobacteria have the potential to increase plant growth as it is capable of producing this hormone, as observed in maize roots with inoculation of different strains of *Azospirillum* (Bottini et al. 2004). Gibberellin phytohormones GA1 and GA3 and indole-3-acetic acid were detected in *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*. These bacteria are associated with *Gramineae* species and promote plant growth and yield (Kang et al. 2014a, b). Bacteria capable of producing gibberellins are *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, and *Bacillus* (Sharma and Kaur 2017).

Gibberellin production by PGPR promotes the growth and yield of many crop plants. An example is gibberellin-producing bacteria used in plants with dwarfism. The reversal of dwarfism was demonstrated in both rice and maize seedlings inoculated with *Azospirillum* sp. (Bottini et al. 2004). *Leifsonia soli* SE134 suspensions significantly promoted the growth of cucumbers, tomatoes, and young radishes due to gibberellins production capacity (Kang et al. 2014a, b). Cucumber plants in symbiotic association with PGPR showed had higher shoot length, plant biomass, chlorophyll contents, and increased amino acids and crude protein contents (Kang et al. 2012). The bacteria *Bacillus cereus*, *B. macroides*, and *B. pumilus* in addition to promoting growth in red pepper also increased the level of endogenous gibberellin (Joo et al. 2005).

Recent work has suggested an important role in plant survival under drought stress conditions. The inoculation by rhizobacteria that promote plant growth provides an increase in the root system, constituting a strategy of plants adaptation in water deficit conditions, which may reflect intolerance and higher crop productivity. Mutualistic symbiosis of maize and *Pseudomonas fluorescent* enhanced stress maize drought tolerance. The PGPR application can increase the level of proline, abscisic acid, auxin, gibberellin, and cytokinin in maize leaves under water deficit conditions (Kang et al. 2014b). The symbiosis between PGPR and plants improves plant development, nutrient uptake, and $N_2$ fixation. Nodules of different *Leguminosae* species contain more gibberellin than adjacent roots, suggesting bacteria modify hormonal levels in the nodules, favoring the biological nitrogen fixation (Bottini et al. 2004).

## 4 Cytokinin

Cytokinins (CK) are an important plant hormones group. Are obligatory for cell cycle progression. Microorganisms, which belong to diverse genera such as *Pseudomonas*, *Azospirillum*, and *Bacillus*, produce cytokinins and use them as a chemical signal to communicate with plants and also as a strategy to invade the plant host (Goswami et al. 2016). The cytokinins produced by PGPR are present in small amounts in roots, but it is sufficient to increase cell division leading to root hair formation and root development, favoring water and nutrient absorption. Microorganisms contain more than 30 growth-promoting compounds from the

cytokinins group, and about 90% of microorganisms found in the rhizosphere are capable of releasing cytokinins increasing the cytokinins content in soil solution (Amara et al. 2015). When the rhizosphere is inoculated with cytokinin-producing bacteria it can stimulate plant growth (Kudoyarova et al. 2019).

While cytokinins have direct impacts on various plant processes, generally the balance between auxin and cytokinin levels determines meristem functioning, root system architecture, lateral organs formation, premature leaf senescence, and generative organs development. Cytokinins regulate chlorophyll biosynthesis and chloroplast biogenesis and are involved in plant resistance to biotic and abiotic stresses (Tsukanova et al. 2017). Since some bacteria are able of producing both of these hormones, tissue auxin to cytokinin ratio can be important in determining plant response to rhizobacterial inoculation (Kudoyarova et al. 2019). The introduction of bacteria capable of synthesizing cytokinin in the rhizosphere of wheat and lettuce promoted an increase in leaf area, as cytokinins stimulate cell division and elongation (Grover et al. 2021). At the same time, CK promotes the growth of the aerial part of plants, it can inhibit root elongation and branching. *Bacillus amyloliquefaciens* UCMB5113 inhibited primary root growth of *Arabidopsis*, due to bacterial CK production and increased root CK levels. The reduction of the CK status in the plant causes larger root system formation. However, CK is required for shoot growth, and a CK status systemic reduction reduces sink strength in the young shoot tissues and thus inhibits their growth (Albrecht and Argueso 2017). The cytokinin-producing bacteria inoculation into the rhizosphere may not inhibit root growth if they are transported to shoots. The production of cytokinins by bacteria allows them to be transported to shoots, reducing the accumulation of cytokinin in root and, therefore, not interfering with development. The inoculation of a bacterial suspension of *B. subtilis* IB 22, producing ribosylated CK, in wheat rhizosphere did not reduce root biomass accumulation (Kudoyarova et al. 2014).

## 5 Ethylene

Ethylene is an important natural plant hormone that is involved in several physiological processes; leaf abscission, floral senescence, fruit ripening, root growth, seed germination, regulation of release of dormancy, root nodulation, and injuries in various tissues. The regulatory role of ethylene depends on its concentrations that are produced in plant tissues in response to metabolic and environmental stress. Low levels of ethylene are known to stimulate plant growth while its higher levels inhibit normal plant growth (Iqbal et al. 2017).

The plants synthesize 1-aminocyclopropane-1-carboxylate (ACC), which is the precursor for ethylene, in response to exposure to various types of environmental stress, such as cold, drought, flooding, infections with pathogens, and the presence of heavy metals. The magnitude of ethylene production by a plant tissue is regulated by the availability of the substrate ACC, high levels of ethylene, produced under stress conditions, can halt certain processes such as root elongation or nitrogen fixation in legumes, and cause premature senescence (Tsukanova et al. 2017).

PGPR can express ACC-deaminase and lower the amount of ethylene in the plant by degrading ACC (Singh et al. 2015). ACC-deaminase gene which regulates endogenous ethylene levels in plant roots is present in some strains of *Azospirillum*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Burkholderia*, *Ralstonia*, *Pseudomonas*, and *Enterobacter* (Gamalero and Glick 2015). The plant ACC role during PGPR inoculation was evident when, in studies with *Medicago truncatula,* the absence of ethylene production by plants caused over nodulation in their roots (Penmetsa and Cook 1997). When the ACC-deaminase gene together with its regulatory region was transferred into a root colonizing bacteria, deficient in ACC-deaminase activity, it increased the root length of canola plants (Bechtold and Field 2018; Wang et al. 2000).

Salinity stress boosts ethylene production; however, studies have shown the capability of plants inoculated with rhizobacteria containing ACC deaminase to sustain the salinity by demonstrating a normal growth pattern (Gupta and Pandey 2019). Tomato seedlings grown in the presence of NaCl salt increased the fresh and dry weights due to the ACC-deaminase activity produced by *Achromobacter piechaudii* (Hashem et al. 2016; Sagar et al. 2020). Drought, like salinity, also induces accelerated ethylene production in plant tissues which leads to abnormal growth of a plant (Ma et al. 2020). However, Mayak et al. (2004) observed an interesting phenomenon that PGPR *Achromobacter piechaudii* significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress. Inoculation with ACC deaminase bacteria partially eliminated the effects of water stress in *Pisum sativum* (Zahir et al. 2008).

Some PGPR also controls plant protection reactions to pathogenic bacterial infection. It results from the inhibition of ethylene production by plants, induced by the bacterial synthesis of ACC-deaminase. The symbiosis of such microorganisms with plants exposed to abiotic stress conditions, such as soil with high salinity and flooding, mitigates physiological stress reactions, masking their symptoms (Etesami et al. 2015; Jha and Saraf 2015). These microorganisms not only derive the action of ethylene from the plant but are also capable of producing ethylene, which when at low levels can provide plant growth. However, it is not known what the dose response is for this hormone to change from an inhibitor to a plant growth promoter. A wide range of factors are believed to be involved, such as the environment, symbiotic interactions, plant species habitats, and metabolic reactions induced by plant genetics (Souza et al. 2015).

## 6 Abscisic Acid

Abiotic stresses such as salt and drought are important factor that leads to losses in crop yields around the world (Curá et al. 2017). Among phytohormones, abscisic acid (ABA) is synthesized in response to abiotic stresses previously mentioned. It inhibits seed germination, induces plant senescence and abscission of leaves and fruits, promotes stomatal closure, and affects the root system architecture (Munemasa

et al. 2015; Sah et al. 2016). Bacteria may synthesize ABA under stress conditions or metabolize ABA from soil solution, decreasing ABA plant concentrations (Belimov et al. 2014) (Fig. 7.2), and it depends on plant growth conditions.

The scheme above demonstrates that production or consumption has different results, but PGPR can affect the plant ABA level and activate stomatal conductance control mechanisms, in this way influencing its growth and abiotic stress resistance (Numan et al. 2018; Ilangumaran and Smith 2017). Previous studies showed that PGPR alleviate abiotic stresses in crop plants. Shahzad et al. (2017) reported an increase in ABA in PGPR-inoculated plants under abiotic stress conditions.

*Arabidopsis thaliana* at drought stress when inoculated with *Azospirillum brasilense* sp. 245 strain had changes in root architecture, stimulated photosynthesis, photoprotective pigments, and retarded water loss, with enhanced ABA levels. Thus, PGPR contributed to mitigating drought stress effects on plants via rhizobacterial-induced drought endurance (Cohen et al. 2015). *Vitis vinifera* inoculated with ABA-producing strains such as *Bacillus licheniformis* Rt4M10 strain and *Pseudomonas fluorescens* Rt6M10 strain had an ABA content increases, and the plant becomes more resistant to drought (Salomon et al. 2014). *Bacillus aryabhattai* strain SRB02 enhanced ABA levels in soybean under heat stress and were observed stomatal closure during heat stress (Park et al. 2017). In wheat, the PGPR *Arthrobacter protophormiae* SA3 strain and *Dietzianatrono limnaea* STR1 strain help to tolerate salt stress conditions. In the same study, *Bacillus subtilis* LDR2 strain provided tolerance to crop against drought stress. Abscisic acid levels showed an increase under salt and drought stress conditions for SA3 and LDR2 inoculations. On the other hand, STR1 did not result in a significant impact on the ABA content (Barnawal et al. 2017).

In addition, some studies show an ABA levels reduction. The inoculation with *Azospirillum brasiliense* SP-7 strain and *Herbaspirillum seropedicae* Z-152 strain in maize promoted an increase of tolerance to drought stress. Compared to control non-inoculated plants, the inoculated plants showed higher biomass production;

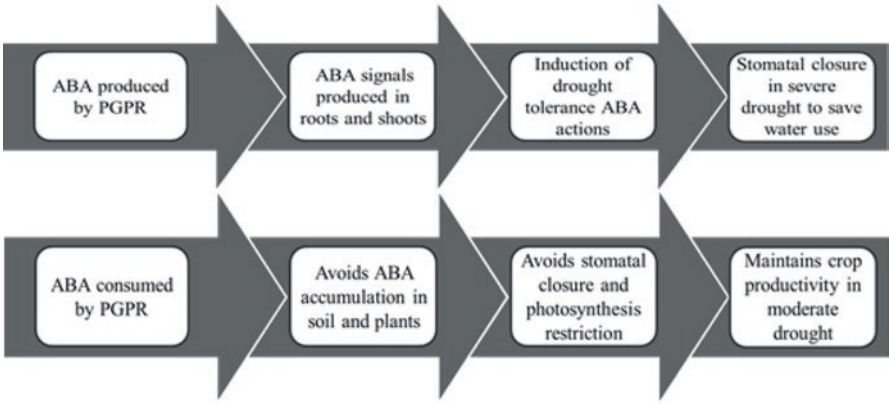


**Fig. 7.2** ABA production (**a**) or consumption (**b**) by PGPR

higher carbon, nitrogen, and chlorophyll levels; and lower levels of abscisic acid and ethylene (Curá et al. 2017). Vives-Peris et al. (2018) working with a species of citrus inoculated *Pseudomonas putida* KT2440 strain or *Novosphingobium* sp. HR1a strain rhizobacteria, subjected to salt stress for 30 days, observed the ABA levels were lower in inoculated plants. In addition, the maximum efficiency of photosystem II decreased to a lower extent in inoculated plants. This last result confirms ABA consumption by PGPR performance, in order to prevent photosynthesis inhibition.

It is worth mentioning that the effects each strain produces on plants are variable and this may depend on both plant species and environmental factors (Tsukanova et al. 2017).

## 7 Concluding Remarks

Climate change has increasingly affected agriculture in recent years. Conditions of water stress, high temperatures, and salinity, among others, are situations that cause a reduction in the productivity of agricultural crops. Considering that PGPR excrete phytohormones and these exert a positive influence on the development of crops under stress, incentives should be given to their implementation in agriculture. PGPR use will surely become a reality and will be a crucial tool for sustainability and maintaining long-term productivity without the use of agrochemicals.

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# Chapter 8
# The Role of PGPR Secondary Metabolites in Alleviating Allelopathic Effects (Biotic Stress) and Induced Tolerance in Plants


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**Abstract** Plant growth-promoting rhizobacteria (PGPR) exert numerous benefits to the plants with which they are associated, including improved tolerance to environmental stress sources. That is possible due to the mechanisms of tolerance induced by bacteria in the plant, such as promoting plant cell osmotic balance to improve water relations, stimulation of carbohydrate metabolism and photosynthesis, and accumulation of secondary osmoprotective metabolites, in addition to the production and regulation of phytohormones. During exposure to stressors including high temperatures, water deficit, low availability of nutrients, and the presence of heavy metals, one of the defense mechanisms of PGPR is the production of secondary metabolites. Besides, PGPR also produces siderophores, which improve plant iron acquisition. In situations of contaminated soils, metabolites produced or induced by PGPR can also reduce the bioavailability of heavy metals for plants. On the other hand, plants also produce secondary metabolites, and these metabolic pathways involve primarily processes that synthesize organic compounds, which directly function in their growth and development, making them essential to their survival and reproduction. The mechanisms by which plants protect themselves from herbivory, infection by pathogenic microorganisms, growth of other plants, and enact defense responses are mostly linked to secondary metabolism. Plants can release them into the environment in various forms, such as leaching, volatilization, waste decomposition, and root exudation, performing diverse ecological functions or reducing other plants' growth and establishment.

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## 1 Introduction

Historically, secondary compounds were considered final products of metabolism with no apparent function. However, from the nineteenth century and the beginning of the twentieth century, there was a deepening of studies involving these compounds by organic chemists interested in their use in phytomedicines, poisons, and flavorings. Also, many products of secondary or specialized metabolism have important ecological functions in vegetables and are therefore of great relevance in agriculture (Taiz and Zeiger 2009).

The secondary metabolites produced by plant growth-promoting rhizobacteria (PGPR) in the soil can favor the growth and development of plant species, such as bacteria that promote plant growth. The range of bacteria reported being able to benefit plant growth and control plant pathogens includes several species belonging to the genera such as *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Streptomyces*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Flavobacterium*, *Burkholderia*, *Bradyrhizobium*, *Mesor*, *Rhodococcus*, and *Serratia* (Berg et al. 2002; Sobral et al. 2004; Sessitsch et al. 2005; Chen et al. 2006; Fernandez et al. 2007; Ahmad et al. 2008; Soltani et al. 2010; Sayyed et al. 2015; Nithyapriya et al. 2021). The number of bacterial species that demonstrate plant growth promotion has increased substantially in recent decades. This results from numerous studies with a wide range of plants in search of sustainable agriculture tools, as well as advances in molecular biology techniques that have allowed advances in bacterial taxonomy.

In addition to the secondary metabolites produced by PGPR, the mechanisms by which plants protect themselves from herbivory, infection by pathogenic microorganisms, competition from other plants, and other forms of defense may be linked to secondary metabolism, and the metabolites produced may be responsible for transmitting information from plants to their surroundings (Oliveros-Bastidas 2008). The purpose of this chapter is to present information about the secondary metabolites produced by PGPR and plants in the soil and how these metabolites produce allelopathic effects and induce plant growth.

## 2 Concepts About Plant Growth-Promoting Rhizobacteria (PGPR)

The PGPR represent a wide variety of soil bacteria that, when grown in association with a host plant, result in the stimulation of the growth of their host (Vessey 2003; Shaikh et al. 2016). This group of microorganisms is of particular interest in the

rhizosphere, as they may represent 2–5% of the total population of soil bacteria (Antoun and Prevost 2005). The predominant bacterial species in the PGPR community that have emerged as the most widely studied candidates and with the potential to improve plant growth and health are *Pseudomonas* and *Bacillus*. The contribution of these two PGPR to plant growth includes solubilization of phosphates, production, and release of phytohormones, such as indoleacetic acid and gibberellins; biocontrol of soil phytopathogens; siderophore production; antibiosis, i.e., production of antibiotics; and inhibition of plant ethylene synthesis (Bottini et al. 2004; Cawoy et al. 2011; Chen et al. 2006; Jangu and Sindhu 2011; Velineni and Brahmaprakash 2011; Basu et al. 2021), as illustrated by Kumar et al. (2011).

Many PGPR act antagonistically against phytopathogens, producing antimicrobials or interfering with virulence factors (Rezzonico et al. 2005). Actinobacteria are one of the most abundant classes of PGPR in the rhizosphere, capable of producing a wide range of secondary compounds with antibacterial, antifungal, antiviral, nematicidal, and insecticidal properties (Turner et al. 2013). Other bacteria also act as antagonists of plant diseases, including *Pseudomonas fluorescens*, capable of producing the antifungal compound diacetylphloroglucinol (DAPG). The production of DAPG by *Pseudomonas* spp. also demonstrated the ability to modulate transcription in another PGPR (*Azospirillum brasilense*), increasing the expression of genes involved in the colonization of grassroots and in promoting plant growth (Combes-Meynet et al. 2011).

## *2.1 PGPR and Production of Secondary Metabolites*

Secondary microbial metabolites include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, inhibitory enzymes, immunomodulatory agents, antagonist and agonist receptors, pesticides, and growth promoters of animals and plants (Demain 1998). The synthesis of these compounds is extremely dependent on environmental and growth conditions (Madigan et al. 2008; Basu et al. 2021), and, generally, they are encoded by genes grouped in chromosomal DNA or, less frequently, in plasmid DNA (Demain 1998).

In general, secondary metabolites are produced by specific species or genera, for physiological, social, or predatory reasons, being closely linked to the ecology of organisms (O'Brien and Wright 2011). Generally, secondary metabolic pathways are activated in situations of the nutritional deficit, biosynthesis, or presence of an inducer (usually of low molecular weight), and/or by decreasing the rate of microbial growth. Such events generate signals that drive a series of regulatory events, resulting in chemical differentiation, which activates the secondary biosynthetic pathways (Demain 1998). The main secondary metabolites produced by rhizospheric microorganisms are antimicrobials, exopolysaccharides, lipoquitooligosaccharides, and phytohormones.

The production of antimicrobial molecules (antibiotics) derived from secondary metabolism is widely distributed in microorganisms. It is estimated that 40% of

filamentous fungi and actinobacteria produce antibiotics when isolated from nature (Demain and Fang 2000). Also, 77% of soil myxobacteria have antibiotic activity against *Micrococcus luteus*, in addition to having antifungal activity and, in some species, antibiotics against Gram-negative bacteria (Foster et al. 2001). The production of antibiotics by microorganisms is closely associated with the fact that these molecules increase the selective advantage of the producer in environments that are poor in nutrients because they can decrease or eliminate neighboring competitors (O'Brien and Wright 2011). For this reason, bacteria isolated from natural environments tend to be highly resistant to antibiotics (Dantas et al. 2008).

Antimicrobial agents produced by bacteria include the so-called bacteriocins (Mourad et al. 2009), which are secondary protein compounds, active against bacteria closely related to the producing bacteria (Riley and Werts 2002). Several types of bacteriocins produced by rhizobia have already been described (Schwinghamer 1975; Schripsema et al. 1996), being called rhizobiocins (Sridevi and Mallaiah 2008). Bacteriocin production and interspecific competition appear to be closely related (Mourad et al. 2009).

In addition to antimicrobial substances, certain bacteria can produce polysaccharide chemicals, which are released into the extracellular medium. A feature that has drawn attention to rhizobia is its ability to synthesize large quantities of these substances, called exopolysaccharides (EPS), both in vivo and in vitro. EPS production by rhizobia can reach 70% of cellular energy expenditure under certain environmental conditions (Castellane and Lemos 2007), even though it is very rare for a bacterium to use it as an energy source (González et al. 1996).

Studies with several *Rhizobium* species have shown that EPS play an important role in the interaction between the symbiotic legume and the bacterium, acting on the cell signaling of both organisms (Kirichenko et al. 2004; Becker et al. 2005), functioning as a receptor molecule micro-symbiont, promoting cell-cell interaction, and triggering the nodulation process (Kirichenko et al. 2004). EPS act in the formation of nodules and microcolonies (Nicolás 1996), increasing the adhesion of the bacteria and promoting its growth in the intercellular spaces necessary for both nodulation and the root organogenesis, and acting as molecular signals during the development of the nodule (Kosenko et al. 2001). It is also noteworthy that EPS actively participates in protecting the bacterium from environmental stresses, such as desiccation, osmotic and pH fluctuations, and the presence of toxic metallic elements in the soil (Castellane and Lemos 2007), as well as predation and attacks by antimicrobial molecules (Staudt et al. 2012).

In addition to the variation between species and genera, the composition of EPS can vary according to changes in the environmental conditions that surround the cell at any given time. Environmental factors or specific culture conditions can impact the chemical composition of the polysaccharide to be formed. Among the critical factors for the production of EPS, the following stand out: bacterial growth phase, available carbon and nitrogen sources, oxygenation rate, temperature, and pH (Staudt et al. 2012).

PGPR are capable of synthesizing different types of phytohormones under different environmental conditions such as cell age and the presence or absence of

nutrients that influence the activities of secondary bacterial metabolism (Cacciari et al. 1989). The main phytohormones produced by PGPR are in Table 8.1.

The production of auxins, gibberellins, and cytokinins by bacteria of the *Azospirillum* genus has already been identified in several studies (Barbieri et al. 1986; Pati et al. 1995; Bashan and de-Bashan 2010). There is evidence that plants inoculated with phytohormone-producing strains of *Azospirillum* respond positively to inoculation (Arshad and Frankenberger 1998). Some studies have shown that rhizobia also can produce phytohormones, such as IAA and other indole compounds (Wang et al. 1982; Sekine et al. 1988) and several types of cytokinins (Sturtevant and Taller 1989).

**Table 8.1** Origin and function of phytohormones produced by PGPR

| Phytohormone | Origin | Function | References |
|---|---|---|---|
| Auxins | Found in the form of AIA and its halogenated derivatives; indole-3-butyric acid (IBA), with tryptophan being its main precursor | Division, extension, and differentiation of plant cells and tissues, with importance in the production of secondary roots | Arshad and Frankenberger (1998) and Bashan and de-Bashan (2010) |
| Gibberellins | Although the most well-known gibberellin (GA) is gibberellic acid (GA3), derived from a fungus, the most active GA in vegetables is GA1, responsible for stem elongation | Promote cell division and elongation, without the characteristic inhibitory effects of auxins; in addition, involved in seed dormancy breaking processes | Davies (1995) and Bashan and de-Bashan (2010) |
| Cytokinins | Aminopurines with N6 are replaced by ribosides, ribotides, and glucosides. The most common cytokinin is zeatin, which can be converted to other cytokinins | Responsible for inducing cell division in plant tissues, the main regulator of cytokinin biosynthesis; acting in the morphogenesis of stems and roots, maturation of chloroplasts, increase in cell volume, germination of shoots, and senescence | Bashan and de-Bashan (2010) and McGaw and Burch (1995) |
| Ethylene | Hydrocarbon ($C_2H_4$) is synthesized from the amino acid methionine, usually in response to stresses. It is also known as the ripening hormone | Acts from the germination of seeds to the senescence of several organs and the ripening of fruits | Arshad and Frankenberger (1998) |
| Abscisic acid | Sesquiterpene derived from mevalonic acid | Promoting and inhibiting plant growth; senescence and abscission of fruits and leaves and in plant responses to biotic and abiotic stresses, acting on osmotic control and the stomata opening/closing process | Walton and Li (1995) and Bashan and de-Bashan (2010) |

## 2.2 How PGPR Induce Tolerance in Plants Under Environmental Stress

The main environmental stresses that significantly reduce the production of biomass and grains from crops of economic interest are related to water deficit, cold, heat, and salinity (Kaur et al. 2008; Thakur et al. 2010; Ahmad and Prasad 2012). In the Caatinga biome, in Brazil, functional analyzes identified genes related to the response to osmotic stress (synthesis of osmoprotective compounds and accumulation of potassium ions) and preferential use of carbon and nitrogen, when comparing the microbiome of soils preserved under seasonal changes (Lacerda-Junior et al. 2019). This reflects differences in the genetic potential for nutrient cycling and carbon acquisition in the environment. According to Lacerda-Junior et al. (2019), the functions within the carbohydrate group, in with water deficit, are related to the metabolism of labile carbon sources, such as monosaccharides (L-rhamnose, D-ribose, L-arabinose) and oligosaccharides (maltose/maltodextrin) or osmoprotective sugars (mannitol and inositol), which also appear to play a role in tolerating water deficit.

To try to understand the relationship between the application of PGPR, the soil, and the tolerance of plants to water deficit, Zheng et al. (2018) incubated soil samples from different textural groups with *Bacillus subtilis* strain UD1022 and demonstrated that EPS modulate changes in soil water retention capacity and evaporation characteristics through three potential mechanisms: (a) EPS is hygroscopic and it can retain large amounts of water; (b) EPS can modify the distribution of soil pores; and (c) EPS can decrease the surface tension of the water and increase the viscosity. These results show that the use of PGPR increases the availability of water for the plants, decreases the drying processes, and relieves the stress experienced by the roots during the water deficit. Other studies have also shown that the use of PGPR can increase resistance to water stress in tomato and pepper plants (Mayak et al. 2004), common beans (Figueiredo et al. 2008), wheat (Timmusk et al. 2015), and corn (Naseem and Bano 2014).

EPS are produced by a wide variety of microorganisms (Souza and Garcia-Cruz 2004), accumulating on the surface of cells (Coronado et al. 1996), and their use has been associated with a mechanism for adapting rhizobia to a wide variety of environmental stressful conditions such as saline soils, temperature variations, and water stress. Also, EPS participates in the degradation of compounds harmful to plant growth and also helps in the control of phytopathogens by inhibiting colonization, virulence, and survival of the pathogen in the host plant (Roper et al. 2007) and protection from environmental stresses (Coronado et al. 1996).

Soil salinity significantly affects the productivity and yield of crops worldwide. The increase in the tolerance of plants to salt stress can be stimulated by the association with PGPR that is tolerant to salt through a process called induced systemic tolerance (IST) (Yang et al. 2009). This is based on the ability of the plant's defense system to respond more quickly in situations of stress induced by the association with beneficial bacteria. The main effects of IST include improvement in water

relations, osmotic adjustment of plant cells, detoxification, regulation of phytohormones, improvement in the acquisition of nutrients by the roots, and photosynthetic efficiency (Arora et al. 2018; Ilangumaran and Smith 2017; Vaishnav et al. 2019; Verma et al. 2019).

Plants are more sensitive to the effect of salt stress when compared to microorganisms, which have several mechanisms of osmotolerance. These mechanisms include increased $K^+$ uptake, in addition to the accumulation of osmoprotectors in the cytosol, and the production of EPS involving the bacterial envelope. These compounds help to alleviate the effect of stress on bacterial cells, keeping their metabolism unchanged and allowing growth promotion and stress relief in associated plants (Paul and Lade 2014; Vaishnav et al. 2019; Verma et al. 2019).

Osmoprotectors (such as proline and trehalose) produced by PGPR are absorbed by the roots of plants and assist in maintaining the osmotic balance and preventing oxidative damage in saline conditions (Zarea et al. 2012). Some PGPR are also capable of limiting the uptake of salt ions in the root, by capturing cations in their EPS matrix or changing the root structure with the formation of rhizosheath. Ion homeostasis occurs by increasing the exclusion of $Na^+$ ions by the roots and increasing $K^+$ uptake, or by modifying ion transporters (Ilangumaran and Smith 2017). Activation of the antioxidant machinery induced by PGPR allows the reduction of oxidative damage caused by saline stress, by increasing the activity of enzymes for the conversion of reactive oxygen species (ROS) into nonreactive species (Chen et al. 2016).

The production and regulation of endogenous phytohormones is another mechanism that can induce stress tolerance, such as the production of auxins, gibberellins, and cytokinins by PGPR. These substances are absorbed by plant roots and stimulate their endogenous production, increasing the root surface and, consequently, in better absorption of water and nutrients (Ilangumaran and Smith 2017). PGPR also have mechanisms for regulating stress-related hormones, such as ethylene. Ethylene levels increase under stress conditions, and auxin response factors are inhibited in such conditions, impairing plant growth. The production of ACC deaminase by PGPR restricts ethylene biosynthesis, resulting in its decrease and allowing better response to the vegetal stimulus by auxins (Yan et al. 2014).

The release of extracellular molecules by PGPR, including EPS, lipochitooligosaccharides (LCOs), polyamines, and volatile organic compounds (VOCs), among others, acts on signaling pathways that stimulate the defense mechanism against diseases and plant development, besides, to regulate functions that increase tolerance to sources of stress (Smith et al. 2015). EPS are among the most studied extracellular molecules, indispensable for the formation of biofilms and functional nodules during symbiosis with plants, acting mainly on soil particles and root surfaces, stabilizing the soil structure, and increasing the water and nutrient retention capacity (Upadhyay et al. 2011). LCOs are secreted by rhizobia as nodulation factors in response to flavonoids present in root exudates. In addition to initiating the formation of nodules, studies report that these *nod* factors act as signs of stress response in legumes. Inoculation of soybeans with *Bradyrhizobium*

*japonicum*, for example, provided improvements in nodulation and plant growth in saline environments (Miransari and Smith 2009).

Polyamines are low-molecular-weight molecules secreted by some PGPR with antioxidant activity that modulate ROS homeostasis, eliminating free radicals and stimulating antioxidant enzymes. The most studied polyamines are spermidine, spermine, and putrescine, which are involved in various stress response processes in plants (Gupta et al. 2013). The activation of these metabolic compounds contributes to the control of osmotic stress in plants (Zhou et al. 2016). VOCs released by PGPR are low-molecular-weight compounds, such as ketones, aldehydes, and hydrocarbons, known for their role in stimulating plant growth in stressful situations, by stimulating the production of phytohormones. These can also induce improved plant tolerance to abiotic stress, as in *Arabidopsis thaliana* plants inoculated with *Bacillus subtilis* in which VOCs mediated ion homeostasis under saline stress (Numan et al. 2018; Shao et al. 2016).

## 3 Allelopathic Compounds Produced by Plants

Allelopathy is defined as a direct or indirect effect, harmful or beneficial, that compounds produced and released into the environment by one plant exert on the other. These effects occur from the production and release of some substances from secondary metabolic routes, also called allelochemicals. These chemical or secondary compounds can be released into the environment in various forms, such as root exudation, leaching, volatilization, and decomposition of residues (Rice 1984).

Plants invest a large number of resources to synthesize, accumulate, and release metabolites. The production of these is linked to photosynthetic processes, in which part of the carbon skeletons deviates from the primary metabolism to the synthesis of secondary compounds (Dewick 2002; Lewinsohn and Gijzen 2009; Taiz and Zeiger 2009). Secondary plant metabolites can be classified into three chemically distinct groups. The first includes terpenes, the largest class of secondary metabolites, insoluble in water and biosynthesized from the routes of mevalonic acid and methylerythritol phosphate. The second comprises phenolic compounds, which constitute a very heterogeneous group, with approximately 10,000 compounds, biosynthesized from the routes of malonic acid and shikimic acid. The third group includes nitrogenous compounds, synthesized from common amino acids. Despite this classification, secondary metabolites have their interconnections with primary metabolism with specific distribution and are restricted to a group of species or plant species (Taiz and Zeiger 2009).

Secondary metabolites have been identified as largely responsible for allelopathic effects on the environment, including compounds from the group of phenolics, terpenoids, alkaloids, coumarins, tannins, xanthones, flavonoids, sterols, and quinines, among others (Trezzi 2002; Patil 2007; Labbafy et al. 2009). Phenolics, for example, can reduce the growth of spontaneous plants in the field, as they are

directly related to the absorption of nutrients, complexing the chemical elements present in the soil (Bertin et al. 2003).

There are several examples of plants with allelopathic effects on the environment (Labbafy et al. 2009; Hagemann et al. 2010). However, there is a great difficulty in identifying the chemical substances present in plants and in determining the action of these substances in the environment, mainly about the responses of plants to the application of these compounds (Belz 2007). One of the oldest examples of allelopathy involves root exudation and the inhibition of plant growth, caused by the presence of the black walnut (*Juglans nigra* L.). Used as a shade tree and with highly valued wood, it has been reported for years as an arboreal species that interferes with the growth of neighboring plants (Rice 1984). This is due to root exudates that contain specific metabolites, often released in large quantities in the rhizosphere, affecting the soil macro- and microbiota and its surroundings (Vidal and Bauman 1997; Bertin et al. 2003).

The toxicity of black walnut is associated with the presence of the secondary metabolite juglone (5-hydroxy-1,4-naphthoquinone), which its living tissues are usually found in a reduced nontoxic form, but when exposed to air, it can oxidize and be toxic to the plants. The degree of toxicity depends on the age and diameter of the plant's trunk, as well as environmental conditions. Juglone is very important in protecting plants against pathogenic organisms, and, because it is not easily leached into the soil profile, it can persist under the treetops, where the roots are located. Both photosynthesis and respiration of plants exposed to the compound are affected (Jose and Gillespie 1998).

Other examples of plants that produce root exudates with known allelopathic activities are wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*). Wheat allelopathy is associated with the presence of phenolic compounds, such as p-hydroxybenzoic, vanillic, p-cumaric, syringic, ferulic, and hydroxamic acids. These are classified as cyclic carbamates, and synthesized in the metabolic route of shikimic acid, more precisely in the production of the amino acid tryptophan (Niemeyer 1988). Sorghum has proven allelopathy, producing a complex of lipid substances and proteins generically called sorgoleone, having as its main compound 2-hydroxy-5-methoxy-3-[(Z, Z)-8′,11′,14′-pentadecatriene]-p benzoquinone, which is naturally released to the soil from the trichomes of its roots (Santos et al. 2012). Sorgoleone has been characterized as a bioherbicide that inhibits the growth of spontaneous plants in concentrations of 10 μmol (Bertin et al. 2003). This chemical compound has different modes of action in plant systems, such as, electron transport inhibitors in photosynthesis and respiration.

Several abiotic factors induce the gene or enzyme activity in the biosynthesis of allelochemicals, their accumulation, or release (Belz 2007). According to Pavarini et al. (2012), plant secondary metabolites are synthesized by different biochemical pathways, and their contents are regulated and susceptible to environmental variations. As this information is still limited, it is a challenge for many researchers, both during the collection of samples in the field and the detection and quantification of compounds and the development of accurate analytical techniques.

### *3.1 Cover Plants with Allelopathic Potential*

The identification and understanding of the action of compounds with allelopathic potential produced by cover plants can assist in the selection of species for management purposes in the emergency control and development of spontaneous plants. The chemical effect of cover crops involves the production of compounds, especially those of phenolic nature, biosynthesized from the malonic and shikimic acids routes (Taiz and Zeiger 2009), which perform several functions, such as defense against herbivores and pathogens (Zasada et al. 2005; Meyer et al. 2009) or, also, the reduction of growth and establishment of other plants (Taiz and Zeiger 2009; Hagemann et al. 2010; Inderjit et al. 2011). These interactions can occur between microorganisms, between microorganisms and plants, between cultivated plants, between spontaneous plants, and between spontaneous plants and cultivated plants.

The rye (*Secale cereale* L.) and turnip (*Raphanus sativus* L.) have been used as cover crops in no-till systems to control the emergence of spontaneous plants, exercising physical, chemical, and/or biological effects (Vilanova et al. 2014; Comin et al. 2018; Souza et al. 2019, 2020). Rye exudes various compounds from the roots or releases them by decomposing its biomass. Among them, benzoxazolinone constitutes the most important group of secondary metabolites already known in this species. These metabolites are prominent in higher plants, mainly in cereals such as wheat, rye, and corn (*Zea mays*) (Friebe 2001). They exert effects such as inhibiting the growth and germination of spontaneous or cultivated plants and protecting against bacteria, fungi, and insects (Zanatta et al. 2006). Benzoxazolinones are cyclic hydroxamic acids, also called cyclic carbamates, synthesized in the metabolic route of shikimic acid, which is responsible for the production of aromatic amino acids in plants, such as phenylalanine, tyrosine, and tryptophan (Taiz and Zeiger 2009).

Hydroxamic acids produce compounds such as DIBOA (2,4-dihydroxy-1,4-benzoxazolinone-3), DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazolinone-3), and products of their degradation, decarboxylated to the form BOA (2-benzoxazolinone) and MBOA (6-methoxy-2,3-benzoxazolinone). Works carried out by Copaja et al. (2006) demonstrate that DIBOA is the main hydroxamic acid found in the leaves of rye and is in small concentrations in the roots. These are directly linked to plant defense, in addition to being involved in inhibiting the growth of spontaneous plants and resistance to insects and nematodes, such as *Meloidogyne incognita* (Zasada et al. 2005).

Benzoxazolinones play a fundamental role in the metabolism of phenolic compounds, interfering in the pathway of phenylpropanoids, mainly in the activity of their main enzymes, such as phenylalanine ammonia lyase (PAL) and peroxidase (POD), which are involved in the biosynthesis of most phenolics (Parizotto et al. 2011). The active substance benzoxazolinone, present in the species *Secale cereale* L., when released in the soil has a herbicidal action on neighboring plants, reducing the number of competing plants and causing rye to increase its access to light, water, and nutrients, favoring its adaptation in the culture system (Alves et al. 2001).

Similar reactions occur with glyphosate, a broad-spectrum herbicide, which acts on plants, blocking a secondary metabolism stage of plants and causing their death.

Rye plants, in addition to having herbicidal action and interfering with the metabolism of phenolic compounds by the presence of benzoxazolinones, also produce phenolic acids, which are synthesized from the amino acid phenylalanine, such as ferulic acid, synaptic, vanillic, caffeine, and p-cumárico, found mainly in caryopsis of the plant (Taiz and Zeiger 2009; Weidner et al. 2000). These phenolic acids act as precursors to a series of natural polymers, which protect against ultraviolet light, defense against herbivores and pathogens. They also interfere with the hormonal balance and levels of indoleacetic acid (IAA), which is involved in plant growth. This change can come either suppressively, through chlorogenic, caffeic, and ferulic acids, or stimulating, with p-cumáric and vanillic acids (Oliveira et al. 2009).

Other bioactive compounds are present in the rye bran and, mainly, in the cuticular layer (cutin and waxes) of the leaves of the plant, as is the case of alkylresorcinols (ARs). ARs belong to a special class of phenols, non-isoprenoid phenolic lipids, characterized by the presence of the phenyl group attached to a side alkyl chain with an odd number of carbons, and synthesized from the aromatic polyketide route, in which the subunit aromatic is formed by one molecule of acetyl-CoA and three molecules of malonyl-CoA (Correia et al. 2006; Gonzaga 2008). Resorcinol derivatives, such as ARs, have bactericidal, herbicidal, fungicidal, and antitumor activities (Kozubeck and Tyman 1999). They are also largely responsible for the movement of water from the aerial part of plants, resistance to the entry of pathogens, and signaling and activation of other mechanisms, such as induction of proteins related to plant pathogenesis (Ji and Jetter 2008).

Another form of defense, protection, and important evolutionary adaptation present in rye plants is the mechanical support for the production of different complex phenolic macromolecules, called lignins. Lignins are polymers of lignans (dimers or trimers of C6–C3 units) that perform primary and secondary functions in plant metabolism, and among the main lignans identified in rye are syringaresinol, pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, and medioresinol (Bondia-Pons et al. 2009). They are key components of water transport tissues, in addition to having protective and physical resistance functions, strengthening stems and vascular tissues (Taiz and Zeiger 2009).

Black oats (*Avena strigosa*), a food source for humans and animals and a ground cover plant, are also a source of phytochemicals, especially phenolic compounds, which have antioxidant and allelopathic activity (Jacobi and Fleck 2000; Hagemann et al. 2010). The first study with oats was carried out by Fay and Duke (1977) when looking for genotypes with greater allelopathic potential and greater production of the scopoletin compound, which is known to inhibit plant growth. The main phenolic acids found in oats are caffeic, syringic, ferulic, and synaptic acids. Besides, oats are also known to produce hydroxamic acid, such as BOA. These compounds have an allelopathic effect on other plants and can be found in oats and in oat roots (Sicker and Schulz 2002). Flavonoids, especially anthocyanins, are also present in black oats, giving the plant pigmentation and defense (Souza et al. 2019). The plant

also produces triterpenoids, which in the roots are the avenacines and in the aerial part the avenacosides, important compounds that give resistance to the plant against pathogens (Luis Junior 2011).

Another species also widely used as a cover plant in single cultivation or intercropped with other plants and with allelopathic potential is the turnip (*Raphanus sativus*) (Rehman et al. 2013; Papetti et al. 2014; Souza et al. 2019). In general, it is known that species of the genus *Brassica*, such as turnip, have the potential to control the emergence of plants (Boydston and Hang 1995). Among the compounds with allelopathic potential already found in turnip are glucosinolates and phenolic acids caffeic, p-coumaric, syringic, ferulic, and synaptic (Rehman et al. 2013; Papetti et al. 2014; Souza et al. 2019), in addition to flavonoids, especially quercetin, which protects against UV rays, pigmentation, resistance to diseases, and inhibition of germination and plant growth (Parvez et al. 2004; Pereira et al. 2009; Papetti et al. 2014).

This species is also known to synthesize large amounts of glucosinolates (Maldini et al. 2017), which are nitrogenous compounds belonging to the group of glycosides, being stored in the cellular vacuoles of vegetables (Silva 2014). When the plant cells are disrupted, the glucosinolates present are hydrolyzed by the enzyme myrosinase, giving rise to compounds such as isothiocyanates, thiocyanates, nitriles, and indoles, which are known for their allelopathic and natural biocidal effects (Neves 2005; Ediage et al. 2011). Glucosinolates can be found in the roots, seeds, leaves, and stems of turnip plants, being in higher concentrations when the plant is younger. These compounds act in defense of the plant against fungal diseases and infestation of diseases and insects, in the metabolism of nitrogen, and in the regulation of plant growth (Blazevic and Mastelic 2009). Studies by Norsworthy et al. (2007) with extracts from different species of *Brassicas* spp., glucosinolate producers, grown as cover crops in an organic pepper system, showed inhibition of germination and reduced seedling emergence and the size of spontaneous plant species *Digitaria sanguinalis* and *Amaranthus palmeri*.

## *3.2 Effects of Cultivation Systems and Plant Phenological Stages on Secondary Metabolite Production*

The concentration of allelochemicals in the soil solution is regulated by several factors that establish their effects on plants and organisms, as illustrated by Moreira and Siqueira (2006). Depending on the species, both at low concentrations of allelochemicals and as concentrations increase, positive effects for the crop of interest appear, due to the negative effect that the allelochemical has on spontaneous plants, but can also reach lethal conditions for the plant of interest. Therefore, plant management and ecosystem dynamics influence the concentration and effects of bioactive substances. By identifying the compounds produced by cover crops, inferences

can be made about the characteristics and mode of action of these compounds (inhibition, stimulus, or no effect) on other plants, revealing a strategy for the control of spontaneous plants in the system cultivation (Zanatta et al. 2006; Patil 2007).

Allelochemicals can be easily degraded and released into the soil during the decomposition of plant residues (Moreira and Siqueira 2006; Souza et al. 2019), and this process can vary according to the species, the type of soil, and the phenological stage of the plants (Meyer et al. 2009; Rueda-Ayala et al. 2015; Sangeetha and Baskar 2015; Sampaio et al. 2016; Tanwir et al. 2017; Souza et al. 2019). Weidner et al. (2000) found that the content of total phenolic compounds varies according to the phenological stage of rye, with the highest content (44.24 μg $g^{-1}$ dry matter) at 22 days after flowering, decreasing at the end of the maturation stage of the grains (6.5 μg $g^{-1}$ dry matter), at 57 days after flowering. The production of secondary metabolites can also vary according to the development of the plant, including leaf development, emergence of new organs, biochemical, physiological, ecological, and evolutionary processes (Czelusniak et al. 2012).

When studying the presence of phenolic compounds with allelopathic potential in rye grown in an agroecological no-till system, Souza et al. (2019) found higher contents of BOA and MBOA in the elongation stage (60 days after sowing (DAS)) in single (2.74 and 2.53 mg $g^{-1}$ dry matter) and intercropped (2.58 and 247 mg $g^{-1}$ dry matter), when compared to the earing (80 DAS) and flowering (100 DAS) stages. In the same study, the authors observed that after the lodging of the plants, only trans-cinnamic acid, a precursor to lignins, was detected in methanolic extracts, and its content increased, mainly, at 15 days after lodging of the species. The highest levels of cinnamic acid in rye are probably associated with the lignification process that occurs after the lodging of the plant. Regarding BOA, Tanwir et al. (2017) also observed that the levels of expression of the gene linked to this compound were higher during the germination of rye, as well as the levels (8.5 μmol $g^{-1}$ dry matter) when compared to the period of seedling development (4.5 μmol $g^{-1}$ dry matter).

In studies with turnip grown in an agroecological no-tillage system, it was observed that, about phenological stages, the highest phenolic content was found at 100 DAS, when the plant was in the grain filling stage, especially when intercropped with *Secale cereale* (3.24 mg $g^{-1}$ dry matter) and *Avena strigosa* (3.83 mg $g^{-1}$ dry matter) (Souza et al. 2019). Turnip, due to its lower C/N ratio and less fibrous material due to the lower lignin content, when compared to rye, has a faster release of nutrients and, probably, of the compounds contained in plant tissue. When working only with the dry extract of the leaves of the species *Raphanus sativus* L., Silva (2014) found levels of up to 47.02 mg GAE $g^{-1}$ DM (gallic acid equivalents per gram of dry matter) of total phenolics. Beegamashi (2010), on the other hand, when quantifying the total phenolic content of the aqueous extract of the residues of leaves and forage turnip stems, found, on average, 17.7 mg GAE $mL^{-1}$ and, for the hydroethanolic extract, 8.68 mg $mL^{-1}$ GAE.

## 4 Final Considerations

The presence of pathogens and herbivores, as well as abiotic stresses, may lead to the production of secondary metabolites, but organisms that help plant performance, such as PGPR, are also involved in the process. Those processes present a great complexity, changing with plant species and variety, environmental conditions, and the use of microbial inoculants. PGPR has been studied, and some of them are presently used in crops and vegetables, and there are open avenues for further research and product development. Plants, in their evolution, have developed several mechanisms to overcome the diverse challenges and threats they face in the environment. Such challenges include competition with other plants, attacks by pathogens and herbivores, and physical stress factors, such as drought. Plant secondary metabolites may be produced constantly, helping in competition with other plants, or their production is triggered as a response to environmental conditions.

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# Chapter 9
# Role of Actinomycetes in Mitigating the Impact of Climate Change: Mechanisms of Action and Perspectives


**Marika Pellegrini, Rihab Djebaili, Giancarlo Pagnani, and Maddalena del Gallo**

**Abstract** Climate change induces several abiotic stresses that negatively influence growth and yield of crops. Higher $CO_2$ levels, temperature increase, and change in intensity and frequency of precipitations have a significant impact on productive landscapes, both quantitatively and qualitatively. One of the sustainable approaches to mitigate abiotic stresses on crops is the use of plant growth-promoting bacteria (PGPB). These rhizobacteria through direct and indirect mechanisms provide plants with defensive capabilities against various abiotic stresses. Among PGPB, actinomycetes have recently gained increasing attention for their ability to mitigate stresses and improve agricultural productivity. These rhizosphere-inhabiting class of bacteria often form a close association with plants and have been shown to improve plant growth particularly), and even greater growth is expected in the coming years in the presence of drought, temperature, salinity, and alkalinity stresses. This chapter focuses on the potential of actinomycetes in mitigating the impact of climate change and on the prospects for using their formulations in sustainable agriculture.

**Keywords** Actinomycetes · Climate change stress · Crop plants · Halophiles · Salt tolerance

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## 1 Introduction

In recent decades, the increase in food demand due to the growth of the world population has led to an increase in production beyond expectation (Blattner 2020), and even greater growth is expected in the coming years. To meet this demand, agriculture has responded by almost completely depleting natural resources (loss of 70% of global freshwater and loss of 40% of arable lands) (Poore and Nemecek 2018). Beyond this unsustainable exploitation, agriculture is responsible for producing 14% of the world's greenhouse gases (methane, nitrous oxide, and carbon dioxide) (Poore and Nemecek 2018), inducers of climate change. Climate change in return negatively affects agriculture at different levels. The major endangerment for agriculture is climate variability, with an increase in extreme weather severity (e.g., droughts) and natural events (e.g., heavy rains, floods, pests) (Zhang et al. 2006). Farmers often face erratic rainfall, pests, natural disasters (Ullah et al. 2016), and a decrease in agricultural lands (Mahato 2014). The modification of these environmental factors disrupts soil quality and biodiversity, inducing salinization and decreases of water and nitrogen content (Mall et al. 2017). These changes negatively affect agricultural production by limiting plant growth rate, transpiration, respiration, and photosynthesis processes (Mall et al. 2017; Morison 1987; Peng et al. 2004; Rezaei et al. 2015; Wang et al. 2011). Several studies have investigated the climate change impact on agriculture (Dalezios et al. 2016; De Silva et al. 2007; Dehghan et al. 2019; Grover et al. 2016; Iglesias et al. 2012; Porter et al. 2013; Thomson et al. 2006; Vaze et al. 2011). The effects on climate changes, and in particular on temperature shifts, are different according on the climate zone (Karki and Gurung 2012). Countries in the Nordic temperate zone will be able to grow new crops, increasing arable land and crop yields (Ewert et al. 2005; Iglesias et al. 2012; Olesen and Bindi 2002). Conversely, Southern temperate zone countries will be affected by a decrease of arable area and agricultural yield (Olesen and Bindi 2002; Reidsma et al. 2010). In these countries, climate warming also shortens the crops cycle, lowering the productions (Olesen and Bindi 2002). The rational management of the natural resources through a conservation agriculture – minimal tillage, crop rotation, and carbon sequestration – could represent a valid tool to mitigate climate change effects (Serpantié 2009; Verhulst et al. 2012). However, this type of agriculture is accompanied by a reduction of yields and productivity and an increased need for labor (Giller et al. 2009). The use of beneficial microorganisms in agriculture is an alternative approach that improves crop productivity and soil health (Kalam et al. 2020; Basu et al. 2021; Etesami and Beattie 2017; Etesami and Maheshwari 2018; Hamid et al. 2021; Kour et al. 2021; Lugtenberg et al. 2002).

## 2 Plant Growth-Promoting Bacteria and Induced Systemic Resistance

Photosynthesis and root activity depletion are the main mechanism by which climate change limits agricultural productivity (Bhattacharyya et al. 2016). Beyond the impairment of these activities important for plants to thrive, climate change destroys crops by compromising plant-microbe interactions. Plant growth and development are strictly related to the microorganisms associated with its rhizosphere (Gouda et al. 2018). Among these microorganisms, plant growth-promoting bacteria (PGPB) allow plants to overcome climate change effects (Gouda et al. 2018; Sureshbabu et al. 2016) by limiting several biotic and abiotic stresses (Dastagir 2019; de-Bashan et al. 2012; Egamberdieva and Kucharova 2009; Lugtenberg and Kamilova 2009). The application of PGPB has been demonstrated to be an effective strategy to cope with the deleterious effects of various environmental stresses (Bacilio et al. 2004; Dastagir 2019; de-Bashan et al. 2012; Etesami and Beattie 2017; Etesami and Maheshwari 2018; Grover et al. 2011; Kim et al. 2012; Qin et al. 2016). This ability occurs thanks to the production of several molecules (e.g., elicitors, antibiotics) and the induction of an induced systemic resistance (ISR) (Compant et al. 2005; Dastagir 2019; Etesami and Maheshwari 2018; Glick 2004, 2010, 2014; Pagnani et al. 2018, 2020). Plants can respond to certain stress by activating resistance mechanisms limited to the damaged organ or systemically spread throughout the plant (Romera et al. 2019). The latter include the systemic acquired resistance (SAR) and ISR. SAR is induced by pathogens and pests, while ISR is mediated by beneficial microbes living in the rhizosphere (Choudhary et al. 2007; Meena et al. 2017a). PGPR-induced ISR has been reported in several plant species (e.g., cress, bean, carnation, cucumber, radish, tobacco, and tomato). ISR induced by PGPR has been demonstrated to be effective in reducing pathogenic attacks by fungi, bacteria, and viruses (van Loon et al. 1998). Some elicitors produced by PGPR also have a role in the ISR (Bakker et al. 2003; van Loon et al. 1998). These ISR elicitors can be components of cell walls (e.g., lipopolysaccharides and flagella) or metabolites (siderophores and antibiotics) (Bakker et al. 2003; Iavicoli et al. 2003).

## 3 Actinomycetes as Mitigators of Climate Change

Among PGPB, actinomycetes are a class of gram-positive filamentous bacteria widely distributed in soil with interesting plant growth-promoting traits (Demain and Sanchez 2009; Djebaili et al. 2020; Erikson 1949; Gayathri and Muralikrishnan 2013; Grover et al. 2016; Subramanian et al. 2016). Plant growth enhancement by actinomycetes strains has been widely reported (Cruz et al. 2014; Djebaili et al. 2020; Jog et al. 2012). These bacteria can produce various metabolites (e.g., hydrolytic enzymes), degrade soil organic matter (e.g., lignocelluloses, chitin, and pectin) (Gasmi et al. 2019; Strap 2011), and resist unfavorable environmental conditions,

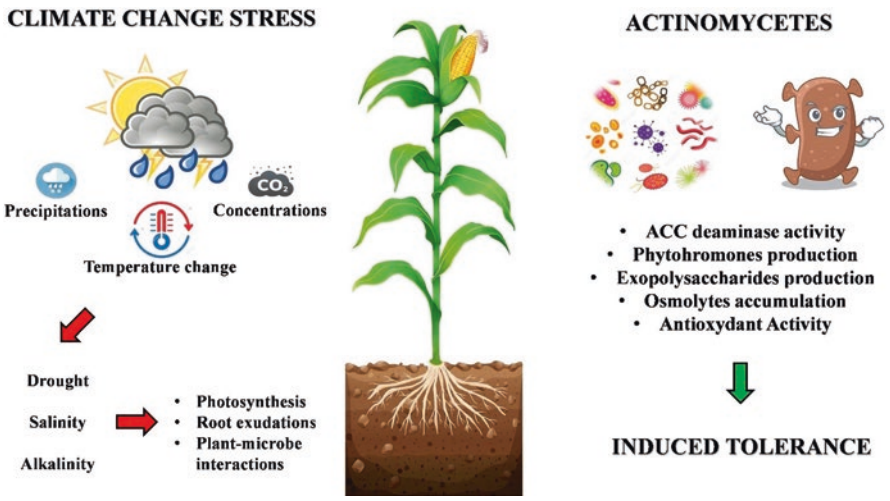


**Fig. 9.1** Climate change and abiotic stress reduction with actinomycetes strains

thanks to sporulation (Alexander 1977). They have several traits that can be related to the promotion of plant growth and suppress plant diseases (Fig. 9.1) (Golinska et al. 2015). Their plant growth stimulation includes hormones and growth regulators production such as indole acetic acid (IAA) and cytokinins which participate in root development, exudation, and plant growth (Boukaew et al. 2013; Subramanian et al. 2016). They participate in the decomposition of crop residues to make nutrients available for plants and produce metabolites important for soil fertility (e.g., geosmin) and humus formation (Abdulla 2007; Subramanian et al. 2016). Furthermore, they limit stresses through the activity of the enzyme 1-aminocyclopr opane-1-carboxylate (ACC) deaminase, which converts ACC to ammonia and α-ketobutyrate, subsequently reducing ethylene levels in plants (Glick 2005). Based on these capabilities, actinomycetes strains can reduce the deleterious effects of various abiotic stresses, such as drought, salinity, temperature fluctuations, and alkalinity (Grover et al. 2016; Sakure et al. 2015; Yandigeri et al. 2012).

## *3.1 Drought Stress*

Drought stress is a growing danger for plants that, in a few decades, is expected to cause 50% of agricultural productivity losses (Kim et al. 2012; Tripathi et al. 2016; Ilyas et al. 2020; Khan et al. 2020a). Drought stress is a result of the alteration of rainfall throughout the year – in terms of quantity and distribution – with a consequent decrease in soil water content (Jaleel et al. 2009). Drought stress severity depends on the type of precipitations, evaporation, and the ability of soil to retain water (Farooq et al. 2009; Wery et al. 1994). Drought limits the absorption of water

and nutrient in plants (Prathyusha and Bramhachari 2018) because it causes plants stomata to close and limitation of $CO_2$ absorption, gas exchange, and turgescence. These effects alter the photosynthesis system, causing leaf senescence and plant death (Anjum et al. 2011; Cornic and Massacci 1996; Jaleel et al. 2009; Smirnoff 1993; Taiz and Zeiger 2006; Wahid et al. 2005).

Usually, rhizospheric microorganisms are adapted to environmental changes (Prathyusha and Bramhachari 2018) and can help plants to counteract them. Actinomycetes, in particular the genus *Streptomyces*, can grow under drought stress and other environmental constraints (Abbasi et al. 2020; Goudjal et al. 2013). These microorganisms can stimulate plant growth through many different processes (e.g., phosphate solubilization, atmospheric nitrogen fixation, nutrients availability, siderophores, and indole acetic acid production) (Abbasi et al. 2020; Djebaili et al. 2020; Passari et al. 2016; Taj and Rajkumar 2016) and produce a wide range of secondary metabolites (Doumbou et al. 2001; Erikson 1949; Taj and Rajkumar 2016). The actinomycetes' role in drought stress mitigation has been described in several plants such as *Zea mays* L. (Chukwuneme et al. 2020a, b; Selim et al. 2019; Warrad et al. 2020), *Triticum aestivum* L. (Li et al. 2020), *Solanum lycopersicum* (Abbasi et al. 2020), *Kalmia latifolia* L. (Hasegawa et al. 2004), and *Mentha* × *piperita* (Zade et al. 2019) and has been mainly ascribed to the following (Hasanuzzaman et al. 2013b; Naseem et al. 2018; Solans et al. 2011; Warrad et al. 2020):

- Increase of water, minerals, antioxidant activity, and nutrient availability
- Production of hormones
- Osmoprotection

The increase in nutrient availability is achieved, for example, by an increase in sugar content (i.e., sucrose, fructose, and glucose) during water stress. This increase allows carbon storage, cell homeostasis regulation, free radicals elimination, and protection against oxidative stress and improves plant tolerance (Abbasi et al. 2020; Gagné-Bourque et al. 2016; Gontia-Mishra et al. 2020; Sami et al. 2016). Among hormones, IAA regulates the microbial physiology, microbe-microbe interaction, and tolerance to environmental stress (Duca and Glick 2020). The importance of IAA production in improving plant growth under normal and under stressed conditions has been widely reported (Abbasi et al. 2020; Glick 2012; Jog et al. 2012; Palaniyandi et al. 2014; Yandigeri et al. 2012; Zade et al. 2019). Microorganisms producing exopolysaccharides (EPS) can improve plant osmoregulation: Naseem et al. (2018) and Yang et al. (2009) showed that plants treated with these microorganisms were able to resist to water deficit and accumulate more compatible solutes compared to those untreated (Sandhya et al. 2009). Selim et al. (2019) reported the role of the actinomycete strain Ac5 in the mitigation of the adverse effects of drought in *Z. mays* L. This mitigation has been related to the reduction in hydrogen peroxide ($H_2O_2$) levels, an increase in antioxidant activity, and the accumulation of compatible solutes such as sucrose, proline, and glycine betaine (Selim et al. 2019). The alleviation of drought stress in plants is also reflected in the activity of ACC deaminase enzyme, which lowers ethylene levels and improves plant growth under stress

and, through the hormonal regulation, improves the antioxidant activity and the nutrient availability (Glick 2005; Meena et al. 2017b; Taj and Rajkumar 2016). Plants can counteract water deficit by lignifying the cell walls and building up the callose (Prathyusha and Bramhachari 2018), and their biosynthesis is influenced by bacterial inoculation (Hasegawa et al. 2004). Inoculation of bay laurel (*Kalmia latifolia* L.) with *Streptomyces padanus* strain AOK-30 stimulated callose accumulation compared to untreated plants (Hasegawa et al. 2004).

## *3.2 Salinity Stress*

One of the major environmental stressors hindering globally growth and yield of crops is salinity (Kamran et al. 2019). Soil salinization induces various biochemical and physiological changes within plants (Arora et al. 2018; Kusale et al. 2021a, b; Nabati et al. 2011). The excess of sodium causes the following (Kamran et al. 2019):

- Cellular homeostasis imbalance
- Nutrient deficiency
- Oxidative stress with reactive oxygen species (ROS) production
- Growth inhibition and cell death

Beyond the negative consequences on crops, salinity negatively affects soil composition, organic matter content, and soil microbial biomass (Zhang et al. 2019). Salinity of soils endangers crop yield and production capacity (Arora et al. 2018; Sagar et al. 2020). Saline soils can be restored by chemical remediation (e.g., leaching, flushing, treatment with gypsum, and lime). However, these treatments take a long time and cause a decrease in plant and microbial biodiversity (Egamberdieva et al. 2019). Recently, the use of halotolerant PGPB and their secondary metabolites has been proposed as a valid economic and eco-friendly tool to restore saline-degraded soils and induce halotolerance to plants (Sunita et al. 2020). Among the actinomycetes, the genera *Aeromicrobium*, *Actinomadura*, *Actinopolyspora*, *Gordonia*, *Marinactinospora*, *Marinophilus*, *Microbacterium*, *Micromonospora*, *Nocardiopsis*, *Nonomuraea*, *Prauserella*, *Pseudonocardia*, *Rhodococcus*, *Saccharopolyspora*, *Salinactinospora*, *Salinibacterium*, *Salinispora*, *Streptosporangium*, *Streptomonospora*, *Streptomyces*, and *Verrucosispora* have been recovered from hypersaline regions (Valan Arasu et al. 2016). These halophilic actinomycetes have the capability to grow at moderate and extreme concentrations of NaCl (15% and 30%, respectively), thanks to the presence of mechanisms that blocks the entrance of NaCl and maintain the cell structure (Quillaguamán et al. 2010; Vargas et al. 2008). Halophilic strains induce salinity stress tolerance in plants by the following (Dodd and Perez-Alfocea 2012):

- Hydraulic conductance
- Osmotic accumulation
- Toxic $Na^+$ ions sequestration

- Higher maintenance of osmotic conductance
- Maintenance of photosynthetic activities

Inoculation with halophilic actinomycetes has been shown to improve plant salinity stress tolerance and promote crop growth by inducing ISR and promoting plant growth/health (e.g., IAA production, ACC deaminase). Qin et al. (2014) reported that ACC-producing strains belonging to the genera *Arthrobacter*, *Streptomyces*, and *Isoptericola*, isolated from *Limonium sinense* and reinoculated on it, improved seed germination, seedlings growth, and flavonoid production under salt stress. A *Streptomyces* strains was also isolated and effectively reinoculated in *Solanum lycopersicum* under salt stress also by Damodharan et al. (2018), who reported an increase in plant biomass. Sadeghi et al. (2012) demonstrated that an isolate of IAA-producing *Streptomyces* promoted growth and development of wheat under high salt stress conditions. Positive results have also been underlined for *Serratia* by Nadeem et al. (2013), who reported that *S. ficaria* improved germination and yield of wheat in a saline field.

## *3.3 Alkalinity Stress*

Soil alkalinization is mainly due to the presence of high concentrations of carbonates ($CO_3^{2-}$) and bicarbonates ($HCO^{3-}$), which reduce the mobilization and availability of nutrients, causing imbalances (Chen et al. 2011). This causes desertification in many soils, particularly those along the coasts and in warmer countries due to the strong evaporation of irrigation water (Rashid et al. 2019), causing various negative effects on crops, in some respects similar but, to a greater extent, to those produced and described in the previous paragraph for salinity stress (Shi and Sheng 2005). Alkaliphilic bacteria are able to grow within an extracellular pH up to 11 and can be used to mitigate alkalinity stresses (Siddikee et al. 2011). They can be classified into three major groups (Jiang and Xu 1993):

- Alkaliphilic (pH 10–11)
- Moderately alkaliphilic (pH 7–10)
- Alkalitolerant actinobacteria (pH 6–11)

These microorganisms occur in various extreme environments (Li et al. 2006; Yu et al. 2013) with high salinity (i.e., haloalkaliphiles or haloalkalitolerants) or in thermobiotic conditions (i.e., alkalithermophile or alkalithermotolerants) (Shivlata and Satyanarayana 2015). The genus *Nocardiopsis* is the most present in alkaline environments (Ara et al. 2013). The other alkaliphilic actinomycetes belong to the genera *Arthrobacter*, *Cellulomonas*, *Corynebacterium*, *Georgenia*, *Isoptericola*, *Microcella*, *Micromonospora*, *Nesterenkonia*, *Nocardioides*, *Saccharomonospora*, *Saccharothrix*, *Streptomyces*, and *Streptosporangium* (Shivlata and Satyanarayana 2015). Despite extensive works on the identification and characterization of actinomycetes and other bacteria from alkaline environments, few have evaluated the

ability of these bacteria to induce alkalinity tolerance in crops. Induced tolerance is related to the possibility for these microorganisms to be used as biofertilizers and biocontrol agents (Shivlata and Satyanarayana 2015). In particular, in alkaline environments, they can increase the availability of assimilable iron by reduction of $Fe^{3+}$ (Valencia-Cantero et al. 2007; Wu et al. 2014) and increase the availability of phosphorus by solubilization (Palaniyandi et al. 2013). Furthermore, important ecological roles have been ascribed to these bacteria:

- Decomposition of recalcitrant biopolymers in haloalkaline sites (Kaur et al. 2014; Sorokin et al. 2012; Tseng et al. 2011)
- Complete degradation of nitriles (Sorokin et al. 2007)
- Rock weathering process enhancement (Cockell et al. 2013)
- Recycling of humic acids (Wu et al. 2011)
- Bioremediation of hydrocarbon-contaminated sites (Shivlata and Satyanarayana 2015)

### *3.4 Temperature Changes*

Temperature changes and extreme temperatures are abiotic factors that limit plant development and growth, affecting the geographic distribution of plant species (Krasensky and Jonak 2012). High temperature affects plant germination and the reproductive system (Hasanuzzaman et al. 2013b) and alters the water level in the leaves, electron flow, $O_2$ emission, and $CO_2$ concentrations, which cause the closure of stomata (Allakhverdiev et al. 2008; Szymańska et al. 2017). At the molecular level, changes in temperature alter the cytoskeleton and the functioning of proteins, enzymes, and RNA and disrupt the gene expression involved in stress protection (Hasanuzzaman et al. 2013b; Semenov and Halford 2009).

Low temperatures induce osmotic stress – which disrupts cell turgor, membrane permeability, and protein activity and generate ROS – disturbing the antioxidant system and leading to lipid peroxidation (Khan et al. 2015; Maeda et al. 2005; Szymańska et al. 2017; Zhang and Tian 2009; Zhang et al. 2013). These damages have a direct effect on photosynthesis and cell metabolism and induce early senescence in plants (Allen and Ort 2001; Krasensky and Jonak 2012). Both cold (0–10 °C) and freezing (<4 °C) affect the physiology, biochemistry, and distribution of plants (Megha et al. 2018; Sanghera et al. 2011). The plant's response to these changes depends on the temperature level, the duration of exposure, and the plant species (Szymańska et al. 2017). Plant response to low temperature stress includes regulation of the membrane system, production of compatible solutes, and regulation of cellular redox balance (Janská et al. 2010; Krasensky and Jonak 2012; Szymańska et al. 2017), and then acclimatization and recovery (Zhang et al. 2013). At the molecular level, plants activate genes linked to the stress response, such as osmoprotectants, detoxifying enzymes, transcription factors, protein kinases, and

phosphatases, and the regulation of the gene expression related to stress tolerance (Krasensky and Jonak 2012; Megha et al. 2018).

High temperatures limit the water level in plants, affecting crop productivity, due to dehydration (Eid et al. 2019; Wipf et al. 2020). Plant responses to high temperatures change according to the degree and duration of temperature and type of plant; at extremely high temperatures, damage or death of cells occur, with the collapse of the cellular organization (Hasanuzzaman et al. 2013a). Heat stress negatively affects the stability of proteins, RNA, and cytoskeletal structures and creates metabolic imbalance by altering the efficiency of cellular processes (Ruelland and Zachowski 2010).

Some PGPB tolerate thermal stresses by means as follows:

- Accumulation of cellular metabolites, such as carbohydrates, amino acids, and proteins (Ali et al. 2011)
- Activation of hormonal metabolism (Khan et al. 2020b)
- Modification of lipid metabolism, for the protection of the membrane against electrolyte leakage (Ali et al. 2011)

These PGPB can mitigate the negative effects of cold in plants (Barka et al. 2006) by regulating gene expression, enhancing antioxidant activity, and producing compatible solutes (Ali et al. 2011, 2009; Mukhtar et al. 2020; Sarkar et al. 2018; Srivastava et al. 2008). Treatment with PGPB also increases proline accumulation, which protects against osmotic stress and regulates membrane permeability (Ali et al. 2011). Furthermore, temperature resistance can be induced by the production of EPS and the accumulation of heat shock proteins (HSPs), stabilizing the membranes against high temperature (Ali et al. 2009).

Few reports are available on the ability of actinomycetes to protect plants from the adverse effects of temperature changes (Grover et al. 2016). However, these strains are more resistant to heat and thermal stress than other groups of bacteria (Kumar et al. 2013), and their filamentous morphology offers good nutrition under environmental constraints (Grover et al. 2016). The actinomycetes' mechanisms to stabilize cell membranes remain poorly understood (Wang et al. 2020). However, plant growth-promoting traits of actinobacteria and their ability to produce phytohormones improve plant resistance, resilience, and survival under stressful conditions (Grover et al. 2016; Yandigeri et al. 2012). In particular, actinomycetes improve plant resistance to temperature variations by stimulating cell damage recovery, photosynthesis, and the accumulation of compatible solutes such as proline (Eid et al. 2019). The stimulus to the production of compatible solutes by actinomycetes has been reported by Hamedi et al. (2013) as an important protection strategy against extreme temperatures and freezing. The ability of some actinomycetes to produce gibberellins (GA) is another key element in the mitigation of temperature stress. Kang et al. (2015) reported that *Serratia nematophila* PEJ1011 relieved low temperature stress in *Capsicum annuum* L. and improved plant growth under cold conditions by increasing the level of gibberellins. The ability to produce EPS and the activation of the antioxidant system are other mechanisms that help the plant to decrease ROS levels and protect cell membranes against oxidative stress

induced by thermal stress (Wang et al. 2020; Xiong et al. 2020). The EPS production by *Glutamicibacter halophytocol*a KLBMP 5180 has a role in ROS elimination and helps in plant tolerance under stress conditions (Xiong et al. 2020). Furthermore, the inoculation of *Streptomyces* spp. has been shown to be effective in protecting celeriac plants (*Apium graveolens* L.) under freezing stress in the presence of fungal pathogens, mitigating freezing-induced cell membrane injury (Wang et al. 2020).

## 4 Conclusions and Future Perspectives

Microbiological approaches can be one of the emerging tools to increase the productivity of agricultural soils subjected to environmental stresses triggered by climate change. Among these tools, the use of actinomycetes as plant inoculants and biostimulants represents a valid strategy: these bacteria have many characteristics that make them suitable for this purpose. This chapter has shown that these microorganisms can be applied to mitigate the negative effects of climate change, namely, drought, salinity, alkalinity, and temperature stresses. Their abundance and metabolic versatility offer a robust new tool for mitigating the negative effects of climate change. The genus *Streptomyces* appears to be the most responsive, with an interesting pool of plant growth-promoting characteristics and biomolecule assets. The growing demand for sustainable tools and worsening of climate change requires a great deal of effort to address the current environmental challenges. Given all the potentialities described for actinomycetes, so interesting and peculiar, it is important to deepen the research carried out so far, in particular the isolation of new species, their characterization, and the experiments on crops in various agricultural environments more subject to climate change.

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# Chapter 10
# Metabolites of *Bacillus* spp. to Control Fungal Phytopathogens


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**Abstract** The health of crop plants is at a stake majorly due to the fungal phytopathogens. About 60 fungal pathogens are responsible for causing a major threat to plants and causing 30% drop in crop production worldwide. One of the greatest challenges is to manage the plant diseases caused by pathogenic fungi owing to the complex nature of the soil's environment. Various options such as chemical, biological control, agricultural practices, and varietal resistance are available to date to reduce the growth of fungal phytopathogens. Promising prospects have been shown by using either plant growth-promoting rhizobacteria (PGPR) or their metabolites as a biological control. The PGPR produce an extensive array of metabolites that contribute to the sustainable agricultural industry as they exert mechanisms like induced systemic resistance (ISR), growth promotion, and antibiosis. The most broadly characterized and studied PGPR include the *Bacillus* genera, as their metabolites are extensively known for protection against plant pathogenic fungi. The present chapter focusses on bringing up a better understanding of *Bacillus* spp. Antifungal metabolites such as siderophores, hydrogen cyanide, and antibiotics. Their antibiotic metabolites, for instance, phenazines, 2,4-diacetyl phloroglucinol, pyoluteorin, pyrrolnitrin, viscosinamide, oomycin, pantocin, tensin, kanosamine, zwittermicin A, and iturins, are having potent antagonist effects against various pathogenic fungi. All these points validate that the metabolites of *Bacillus* spp. play a crucial role for efficient control of diseases triggered by phytopathogenic fungi. Taken together the metabolites of *Bacillus* spp. serve as a sustainable and

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environment-friendly substitute to chemical fertilizers with many other advantages of targeted delivery and easy formulation strategies.

**Keywords** PGPR · Induced systemic resistance · Secondary metabolites · Antibiotics

## 1 Introduction

In the most recent many years, crop efficiency has been fundamentally based on the utilization of high-yielding varieties and in the application of high measures of manures and pesticides. Regardless of crop protection measures, present losses are assessed at 20–40% for the significant food crops around the world (Savary et al. 2012). Thus, novel systems for increasing crop yield, with less dependence on synthetic items, should be created. Comparable to plant mineral nutrition, two techniques that can add to this objective are the development of more efficient crop varieties which have robust nutrient sequestration along with improved rhizosphere management (Shen et al. 2013).

Presently the entire globe is bearing two intense problems: first is overpopulation and the next one is global heating. According to Jiménez-Mejía et al. (2022), the human populace is consistently growing, and there is prediction of it reaching ~8 billion by 2020 and ~9 billion in 2020, which puts more demand on space, production of food, and surroundings in order to upgrade the standards of life. At the same time, restricted accessibility of lands and draining natural supplies negatively impact aptitude for expanding agricultural outcome (Ahluwalia et al. 2021). Feeding this massive number of people is a true obstacle, a venture that wants rise in agricultural productivity. The agriculturists along with the scientists are facing the crucial challenge of obtaining the demands of expanding populace in finite supplies without badly impacting the habitat (Agaras et al. 2015). Moreover, in the present decade, manufacturers and purchasers are progressively concentrating on health and food's variety and sensorial and nutritional qualities.

Currently, it has been reported that there is massive waste of food grains which is caused by pest insects during the production of food and food materials. There is a huge challenge of managing diseases such that previous techniques are not useful enough and should be made better. To guarantee food safety and social stability, management of plant diseases needs to strike by exaggerating agricultural production, decreasing food impurity by microbial toxin, and safeguarding the flow of mixed and affordable priced foods (Ahmad et al. 2016; Kumar et al. 2016). In the given theme, plant growth-promoting bacteria are being used by safe environment approach as a more effectual and environment-friendly alternative for the betterment of crop growth, production, and disease control (Kumar et al. 2015).

## 2 Phytomicrobiome: The Beneficial Interaction

A plant developing under natural conditions is certainly not an entity; it is an intricate network (Luh Suriani et al. 2020) with its partner relationships that are subtle and relatively steady. An organized and controlled network of microorganisms is constantly connected with the plant (Chaparro et al. 2014; Naz and Bano 2015). This community is termed as phytomicrobiome which in addition to the plant is called as the holobiont (Berg et al. 2016; Smith et al. 2017; Backer et al. 2018). All multicellular organisms, particularly all eukaryotes, have microbiome relationships. As a matter of fact, these likely originate before the establishment of plants on the land (Berg et al. 2014). Since their earliest evolution, the microbial community has been linked to land plants, to help early terrestrial plants combat with challenges of nutritional access, stressful environmental conditions, and pathogenic encounter (Smith et al. 2015). Phytomicrobiome has elements such as bacteria and fungi that are linked with all the vital parts of plant (roots, leaves, flowers, stems, and fruits) (Berg et al. 2016). Even though the conditions among these structures change considerably, paving way to microbial populations inhabiting the others. The microbial communities associated with the plant roots which are known as the rhizomicrobiome are the most densely inhabited and intricate among all those accompanying higher plants.

Zhang et al. (2017) reported that the plant has a considerable hold on the formulation of rhizomicrobiom. It produces various compositions of root exudates (Backer et al. 2018), which might be more favorable as a supplier of reduced carbon for some microorganism compared to other microbes. The plant itself also has capability to produce some compounds as signals that are known to bring about certain species and regulate their functions pursuits (Nelson and Sadowsky 2015; Naz and Bano 2014; Smith et al. 2017). Moreover, the microbial community of soil tackles several facets for their own regulation and functions (Leach et al. 2017).

Plant-related bacteria could be ordered into beneficial, deleterious, and neutral groups owing to their effects on plant growth and are referred to as plant growth-promoting rhizobacteria (PGPR) usually (Yasmin et al. 2017; Sayyed et al. 2019). The rhizospheric colonization of PGPR, the rhizoplane, or within the root is independent of the mechanisms of vegetal growth promotion (Basu et al. 2021). Only 1 to 2% of bacteria enhance plant growth in the rhizosphere which is a well-stated fact (Sayyed et al. 2019). A wide genus of bacterial populations has been classified as PGPR, of which *Bacillus* spp. Are the most prevalent (Shaikh et al. 2016a; Manasa et al. 2021).

## 3 Fungal Phytopathogens

At various stages of the development, many crop plants have been adversely influenced by phytopathogens since the establishment of agriculture procedures. According to history crop losses have occurred majorly due to fungal phytopathogens. Phytopathogens are the main culprits causing epidemics which are the reason of great loss to the human life. Jadhav et al. (2017) reported demise of 750,000 people and migration of about 2 million people to the United States because of the potato blight pandemic instigated by *Phytophthora infestans*. Phytopathogens are significantly depriving about 800 million people to get sufficient food by extensively affecting the agricultural production worldwide that was estimated as 10–20% (Naz et al. 2017; El Enshasy et al. 2020). Plant diseases caused by fungi result in billions of dollars of financial loss each year (Khare et al. 2018; Naz et al. 2021a).

According to the estimation of FAO, nearly 25% of the loss of crop across the world is because of the pests and the different diseases of crops (Europe and Unece 2015). The productivity of the developing countries is more affected by it because of the agriculture as it performs a major role in the economy and its growth for the company (Dubey et al. 2010). Pathogenic microbes pose harmful impact on the environmental ecosystem as well as on the agriculture worldwide. Fungal pathogens among all the other plant pathogens like bacteria and viruses are causing two third of the plant diseases and their yield reduction. Some fungal pathogens have drastic effect on the human health and the agriculture of the country (Naz et al. 2014; Gul et al. 2016; Jadhav et al. 2017). Different inhibitory proteins and enzymes are involved in the toxin production which triggers the virulence of fungus (Ghazaei 2017). Fungus has two modes of reproduction – sexual and asexual – varying from simple to complex cycles of life. The spores that are produced during the life cycle of fungus have different ways of transmission like air, wind, water, and animals from one place to another. Environmental factors such as moisture, wind, and temperature majorly affect the growth and reproduction of fungus (Kazemian et al. 2019). Most of the plants that are economically important are threatened by the different fungal species. It has been studied that one plant species is susceptible to more than one type of fungus. The yield of staple crops is also threatened by fungus attack not only in Pakistan but worldwide also (Naz et al. 2017; Naz et al. 2018; Butt et al. 2019).

In managing the plant fungal pathogens, the plant pathologists face a lot of problems like ecological contamination and obstruction among microorganisms. The ID of suppressive soils to different soil-borne plant microorganisms, for example, *Gaeumannomyces graminis* var. *tritici*, *Fusarium oxysporum*, *F. solani*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, and *Sclerotium cepivorum*, have restricted the illness advancement regardless of the good climate (Kenawy et al. 2019; Yasmin et al. 2020; Ullah et al. 2020).

Since the initiation of horticulture rehearses, misuse of crops by phytopathogens has been common at different phases of their turn of events. Nonindustrial nations are more defenseless against misfortune in the profitability since agribusiness

assumes a leading part in the monetary development of these countries (Dubey et al. 2010). Various chemical, physical, and biological methods are being used for the prevention of diseases in plants. However, chemical applications may cause hazards to human health and increase environmental pollution. Therefore, it is important to find alternative, eco-friendly approaches to control bacterial/fungal diseases of crop plants (Ram et al. 2018; Naz et al. 2020).

## 4 Biocontrol of Fungal Phytopathogens

In the previous years, various methods have been put into use to curb phytopathogens; these methods display variation in terms of their efficiency against pathogens. Frequently employed method is chemical control that involves the use of synthetic products for the purpose of defense against pathogens. Owing to their quick action and practicality, manufactured pesticides have become preferable mode of limiting surfeit of pests. Conversely, unrestricted use of these chemicals wreaks havoc on ecosystems and well-being of humans (Akhtar 2015; Naz et al. 2021b). Therefore, modern agronomic practices focus more on eco-friendly means for efficient management and extermination of noxious phytopathogens.

Soil is a living body which is home to a wide range of microbes; these microbes develop intricate association with each other for the purpose of survival. Rhizosphere is the region that surrounds plant roots; it has huge impact on microbial associations taking place in the soil (Basu et al. 2021). Rhizosphere serves as habitat for numerous useful rhizobacteria that identify as PGPR. Plant roots are heavily colonized by such bacteria, and they massively influence the growth preferment of plant (Sayyed et al. 2019; Yasmin et al. 2019). Apart from extensive genetic diversity of prokaryotes, PGPR are also immensely involved in managing plant disease via different approaches in order to augment crop productivity. Biocontrol of diseases affecting plants by means of antagonistic PGPR is an immensely efficient, cost-effective, and eco-friendly approach that serves as a substitute to the usage of artificial pesticides (Jadhav et al. 2017; Riaz et al. 2021a). A preeminent approach that can cut chemical usage in agricultural setting is the use of soil-inhabiting microorganisms for combating plant diseases (Shaikh et al. 2016a, b). Rhizobacteria are capable of accumulating and thriving on roots of valuable agricultural plants, and they actively compete with other microorganisms present in rhizosphere.

Antagonistic organisms that are effective against a plethora of soil-inhabiting fungal phytopathogens exhibit multiple modes of action, namely, synthesis of antibiotics, hydrolytic enzymes along with enzymes responsible for initiating cell lysis (Vinay et al. 2016), secretion of siderophore, engaging in active competition to bind with target substrates, and developing association with specific sites on the roots, along with induced systemic resistance (ISR) (Fig. 10.1) (Mukherjee et al. 2018). Triumphant antagonistic bacteria frequently demonstrate synergistic amalgamation of different approaches for initiating an effective antifungal response.

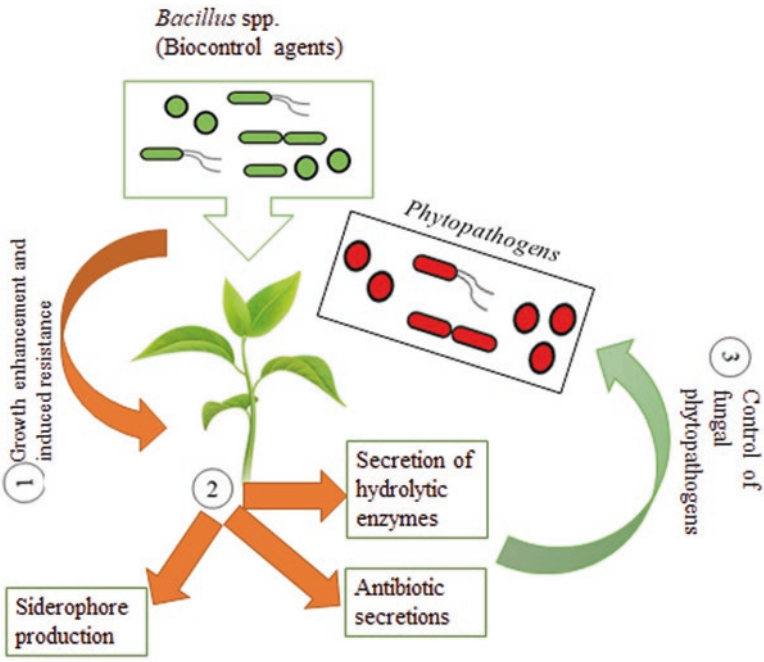


**Fig. 10.1** Potential of *Bacillus* spp. to control fungal phytopathogens

## *4.1 Role of* Bacillus *spp. in Fungal Disease Control*

The mycelial growth of fungi is inhibited and controlled by the *Bacillus* spp. antagonistic activity, thus limiting the plant's damage by a fungal disease (Chowdhury et al. 2015; Abdallah et al. 2016), enhancing the plant productivity and growth (Narasimhan and Shivakumar 2015). *Bacillus* spp. can be established efficiently in the rhizospheres with no any prolonged impacts on the populations of other bacterial species (Chowdhury et al. 2015). *Bacillus* spp. are known to affix to the cell walls of fungal pathogens, and they deform and crack the fungal hyphae while secreting cellulase, chitosanase, glucanase, protease, HCN, and siderophores, which cause distorted functions and their cell structure owing to leakage of the protoplast and vacuolation (Khedher et al. 2015; Babu et al. 2015; Backer et al. 2018). Antifungal peptides that are synthesized using bacteria perform a significant role in destroying the fungi which lead to plant pathogenesis (Riaz et al. 2021a). Antifungal peptides, for instance, surfactin, iturin, mixirin, fengycin, pumilacidin, and the cyclic peptides, also play their role in the destruction of the fungi inhabiting the rhizosphere (Yamamoto et al. 2015; Zihalirwa Kulimushi et al. 2017).

## 5 Secondary Metabolites from *Bacillus* spp. Involved in the Biocontrol of Fungal Pathogens

The characteristic feature of potential biocontrol agents (BCAs) is that they should be able to prepare secondary metabolites exhibiting antimicrobial properties against a wide range of phytopathogens. The naturally originating secondary metabolites are known to produce from primary metabolism as by-products that do not have a pivotal role neither as an energy source nor as a reserve substance (Baba et al. 2021). Regardless of the fact that they don't assume a conspicuous part in the inner financial system of the living being, their function in subsistence capacities is viewed as critical (Sharma et al. 2020). In homology to the other potential biocontrol PGPR, *Bacillus* spp. contains the secondary metabolites which are being used by them to antagonize the harmful effects of phytopathogens. Antagonism of these *Bacillus* spp., against phytopathogens is propelled by the secondary metabolites, for instance, HCN, siderophores, and antibiotics.

### *5.1 Hydrogen Cyanide (HCN)*

Hydrogen cyanide (HCN) is a volatile secondary metabolite; it is produced by many rhizobacteria and effects different organisms. HCN can be a cause of death to many organisms as it disrupts the energy supply to the cell by inhibiting electron transport. Diverse groups of bacterial species produce various HCN including the species of *Aeromonas*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Ahemad and Kibret 2014; Alemu 2016; Abd El-Rahman et al. 2019).

Nandi et al. (2017) reported glycine as the instantaneous cyanide's metabolic precursor, where HCN synthase enzyme catalyzes the decarboxylation of glycine into $CO_2$ and HCN. The HCN synthase is a product of the hcnABC synthase gene cluster and functions as an oxygen-sensitive and membrane-associated enzyme that shares its role in cyanogenesis process (Rijavec and Lapanje 2016). Different HCN exhibit toxicity against phytopathogens and impede the cytochrome c oxidase, mainly the ones produced by *Bacillus* spp. (Goswami et al. 2014). Maximum cyanide is described to be generated under microaerophilic conditions between 34 °C and 37 °C (Short et al. 2018). *Bacillus* spp. is isolated from soybean on the basis of having powerful plant growth-promoting traits and biocontrol features like production of HCN, siderophore, hydrolytic enzymes, and different antibiotics (Goswami et al. 2014). Comparing the nonproducing strains to HCN-producing *Bacillus* spp. and siderophore, the former could serve as effective biocontrol agents against plant pathogens (Raza et al. 2016). HCN-producing *Bacillus* spp. are described in Table 10.1.

**Table 10.1** Role of antifungal metabolites of *Bacillus* spp. in the biocontrol of phytopathogens

| Antifungal metabolites | Producing PGPR | Host | Target pathogen | Reference |
|---|---|---|---|---|
| Siderophore | *Bacillus* spp. | In vitro | *P. debaryanum*, *R. solani*, and *S. rolfsii* | Prasad et al. (2017) |
| | *B. pumilus* | Wheat | *G. graminis* var. *tritici* | Sayyed et al. (2013) |
| | *B. subtilis* | Pepper | *Fusarium* spp. *wilt* | Yu et al. (2011) |
| | *B. halotolerans* | Date palm | *F. oxysporum* f.sp. *albedinis* | Slama et al. (2019a, b) |
| | *B. amyloliquefaciens* | In vitro | *V. dahliae kleb*, *F. oxysporum*, *F. solani*, *S. sclerotiorum*, *R. solani*, *P. parasitica* | Li et al. (2014) |
| | *Bacillus* spp. | In vitro | *Fusarium oxysporum* f.sp. *cubense* | Goswami et al. (2014) |
| | *Bacillus* spp. | In vitro | *M. phaseolina*, *F. oxysporum*, *F. solani*, *S. sclerotiorum*, *R. solani*, *Colletotrichum* sp. | Kumar et al. (2012) |
| | *B. subtilis* | In vitro | *F. oxysporum* f.sp. *melonis*, *R. solani*, *S. rolfsii*, *F. solani* | Caulier et al. (2019), Singh et al. (2017) and Kushwaha et al. (2020) |
| | *B. subtilis* | Wheat | *F. oxysporum* | Mardanova et al. (2016) |
| | *B. subtilis* | In vitro | *Doratomyces* spp. 14raKKLD, *Fusarium* spp., *F. avenaceum*, *F. redolens*, *C. coccodes* 14raKK6 | Mardanova et al. (2016) |
| HCN | *Bacillus* spp. | In vitro | *M. phaseolina*, *F. oxysporum*, *F. solani*, *S. sclerotiorum*, *R. solani*, *Colletotrichum* sp. | Kumar et al. (2012) |
| | *Bacillus* spp., *Bacillus* spp. | In vitro | *P. debaryanum*, *R. solani*, and *S. rolfsii* | Prasad et al. (2017) |
| | | In vitro | *F. oxysporum* f.sp. *cubense* | Goswami et al. (2014) |

## *5.2 Siderophore Production*

A siderophore is secreted by organisms and is a high-affinity, low-molecular-weight, ferric-chelating compound. Siderophores are minute peptidic molecules that have functional groups and side chains; both of these deliver a high-affinity set of ligands to synchronize the ferric ions (Shaikh et al. 2016b). Based on their structural features, iron-coordinating functional groups, and ligand types, the bacterial siderophores are classified into four main classes (hydroxamates, carboxylate, pyoverdines, and phenol catecholates) (Pahari et al. 2017). Various siderophores are reported for

cultivable microorganisms; among them few are particular to species, while other few are commonly recognized and employed by various microbes (Patel et al. 2018).

Some microorganisms give rise to siderophores that chelate to iron already present in soil and avert the iron sustenance of their phytopathogen, and as a result they limit the expansion and root colonization by the phytopathogen; this happens when there is an imbalance in the amount of iron (Patel et al. 2018; Sayyed et al. 2019). Rhizobacterial-produced siderophores are also known to initiate ISR in the host plants (Nithyapriya et al. 2021) and the soil repressiveness and incriminated to control various diseases of plants biologically (Shaikh et al. 2016a, b).

Siderophore that produces PGPR acts as a biological agent to control the pathogen while depriving it from iron sustenance (Riaz et al. 2021b). Siderophore-producing rhizobacterial species are known to quickly inhabit the plant roots of various important agricultural crops and secrete a range of antifungal metabolites/compounds (Adeleke and Babalola 2020). Bacterial-produced siderophores are reported to inhibit the growth of fungal pathogen of rice. Siderophores producing PGPR act as biocontrol agents, depleting the nutrients from the soil, showing positive antagonism against numerous pathogenic fungi, thus producing greater and expanded crop yields (Sayyed et al. 2019). The rhizobacterial produced siderophores are reported to inhibit the mycelial growth of *Colletotrichum gossypii* (Nawaz et al. 2018). Microorganisms have the aptitude to produce potent siderophores that turn out to be ecologically competent biological control agents (BCAs); these BCAs are strong in root-colonizing (Patel et al. 2018).

Siderophores produced by *Bacillus* spp. exhibit strong affinity toward the ferric ion. Different potent siderophore, like pyoverdin, can hinder the growth of pathogenic fungi (Saha et al. 2016). Siderophore-producing *Bacillus firmus* D 4.1 showed a powerful antagonistic potential against the *Sclerotium* spp. *Alternaria*, *Pyricularia oryzae*, and *Fusarium oxysporum* (Sayyed et al. 2019). *Bacillus cereus*, *B. amyloliquefaciens*, and *Bacillus subtilis* revealed strong siderophore production and antagonistic potential against *Fusarium solani*, *Sclerotium rolfsii*, and *Rhizoctonia solani* (Kushwaha et al. 2020). The biocontrol potential of siderophore as an antifungal metabolite produced by *Bacillus* spp. is shown in Table 10.1.

## 6 Antibiotics Produced by PGPR

HCN and siderophore are produced by *Bacillus* spp. Moreover, the biocontrol capabilities of these strains effectively depend on the production of antifungal antibiotics, induction of systemic resistance, and aggressive root colonization in the host plant (Haas and Keel 2003; Fira et al. 2018). The use of antagonists produced by microbial strains in agricultural crops against phytopathogens has been suggested as a substitute to chemical pesticides. The antibiotic production is more commonly linked with the capability of plant growth-promoting bacteria which act as antagonistic agents against plant pathogens (Glick et al. 2007; Shafi et al. 2017). The biocontrol activity of antibiotics depends on the secretion of molecules that kill or

reduce the growth of the target pathogen, which now has become current research trend (Whipps 2001; Lugtenberg and Kamilova 2009; Fira et al. 2018). Antibiotics are heterogeneous group of organic and low-molecular-weight compounds that are ruinous to the metabolic activities of other microorganisms (Duffy et al. 2003).

Antibiotics produced by PGPR comprise of phenazine-1-carboxamide, 2,4-diacetyl phloroglucinol, phenazine-1-carboxyclic acid, pyrrolnitrin, pyoluteorin, omycin-A, viscosinamide, kanosamine, butyrolactones, zwittermicin A, ecomycins, aerugine, rhamnolipids, cepaciamide A, pseudomonic acid, antitumor antibiotics, azomycin, and antivirals karalicin and cepafungins. These antibiotics are reported to hold antifungal, antiviral, antibacterial, insect and mammalian antifeedant, antioxidant, anti-helminthic, phytotoxic, cytotoxic, antitumors, and plant growth-improving activities.

## *6.1 Antibiotics Produced by* Bacillus *spp.*

Most of the antibiotics of *Bacillus* spp. are potent against Gram-negative and Gram-positive bacteria and several disease-causing fungi, for instance, polymyxin, colistin, and circulin (Maksimov et al. 2011). Zwittermicin A (aminopolyol) and kanosamine (aminoglycoside) are produced by *B. cereus* UW85 strain which suppresses oomycete pathogens that augments the biocontrol potential of alfalfa (He et al. 1994).

In order to use bacteria as biocontrol agent to resolve biological problems, many researchers have recommended the application of Gram-positive species (sporulating) such as *Bacillus* and *Paenibacillus* spp. that are known to confer more stability to population during the storage of bacterial inoculums (Emmert and Handelsman 1999; Kokalis-Burelle et al. 2006).

The biological process of microorganisms to antagonize the other disease-causing organisms while secreting antimicrobial compounds is known as the antibiosis which has capability to even kill the pathogenic organisms. As already mentioned, *Bacillus* strains are remarkable producers of the various antimicrobial substances (Stein 2005; Hamdache et al. 2013; Cochrane and Vederas 2016). Several studies and many critical reviews have contributed a considerable information to the elucidation of significant influence that *Bacillus* lipopeptide*s* or antimicrobial compounds have on phytopathogens (Romero et al. 2007; Ongena and Jacques 2008; Roongsawang et al. 2011; Béchet et al. 2012; Dimkić et al. 2013, 2015). *Bacillus* species such as *B. subtilis*, *B. cereus*, *B. amyloliquefaciens*, *B. megaterium*, *B. licheniformis*, *B. mycoides*, *B. mojavensis*, *B. sphaericus*, *B. pumilus*, and *B. pasteurii* are highly effective producers of antibiotic compounds and their inhibiting effect against phytopathogens through antibiosis (Cawoy et al. 2011). The cyclic lipopeptides (CLPs) have three major families, viz., iturin, fengycin, and surfactin. Members from another recently reported family kurstakin are well-established compounds with strong biocontrol potential (Ongena et al. 2005; Béchet et al. 2012; Fira et al. 2018). These are very highly significant antimicrobial compounds as they

might be produced in innately pertinent quantities throughout the growth process (Debois et al. 2014; Cawoy et al. 2015).

Several studies have been dedicated to interpreting a great variety of lipopeptides from *Bacillus* genera with high efficacy and the direct antagonistic effect on the plant pathogens (Table 10.2). The majority of studies have shown a direct inhibitory effect of the fengycins producing *B. subtilis* against pathogenic fungi, especially from the *Fusarium* genus (Cao et al. 2012). *B. subtilis* SQR 9 exhibited in vitro inhibition potential against *Fusarium oxysporum* in cucumber (Falardeau et al. 2013). Different strains of *B. subtilis* have been reported to suppress the ear rot of maize and head blight of barley and wheat caused by *Fusarium graminearum*. Fengycin has been reported by several researchers for their strong antifungal potential (Liu et al. 2005; Chan et al. 2009), particularly against *Fusarium moniliforme* and *Fusarium culmorum* (Hu et al. 2007; Rebib et al. 2012). Several other strains of *Bacillus subtilis* (EA-CB0015; GA1 and CPA-8 strains) have been described as the potential fengycin C producer, a very strong agent against *Mycosphaerella fijiensis*, *Monilinia laxa*, and *R. solani*, respectively (Toure et al. 2004; Yánez-Mendizábal et al. 2012; Falardeau et al. 2013; Villegas-Escobar et al. 2013; Mnif et al. 2015). Several other studies have demonstrated a good antifungal potential of iturin A in protecting crops (Kita et al. 2005). *B. subtilis* strains producing iturin A were found very effective against *Rosellinia necatrix*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, and *Gloeosporium gloeosporioides* (Cho et al. 2003; Cazorla et al. 2007). Moyne et al. (2001) showed that *B. subtilis* (AU195), a producer of two bacillomycin D, was very suppressive against *Aspergillus flavus*. Mohammadipour et al. (2009) and Mnif et al. (2015) reported a strong antifungal potential of surfactin against *C. gloeosporioides* and *A. flavus*.

Antibiotics produced by *Bacillus* spp. and their biocontrol potential against fungal phytopathogens have been described in Table 10.2 and Fig. 10.2.

## 7 Role of *Bacillus* Volatilome to Control Phytopathogens

*Bacillus* species are known to produce volatile organic or inorganic metabolites just like their typical metabolism. The VOCs produced by bacterial species are involved in the multifaceted network of interactions which has been established among several bacterial species, with several other microorganisms, and plants as well. Such interconnections have a versatile ecological role, while having the beneficial or antagonistic interactions (Kanchiswamy et al. 2015; Ullah et al. 2020; Yasmin et al. 2020).

The recent recognition of the favorable effects of plant-*Bacillus* interactions has opened up new possibilities for using bacterial volatilome to boost plant growth development. Furthermore, due to the diversity of VOCs being derived from *Bacillus* species and their efficacy in suppressing other microorganisms, research is concentrating on harnessing natural production of bacterial VOC as a technique for plant disease biocontrol. According to this viewpoint, only a few research have been

Table 10.2 Antibiotics produced by *Bacillus* spp. and their biocontrol potential against fungal pathogens

| *Bacillus* spp. | Active antibiotic compounds | Targeted fungal phytopathogens | Reference |
|---|---|---|---|
| *B. subtilis* | Iturin/fengycin | *P. ultimum* | Ongena et al. (2005) |
| | | *B. cinerea* | Meena and Kanwar (2015) |
| | Iturin/fengycin | *P. fusca* | Romero et al. (2007) and Meena and Kanwar (2015) |
| | Iturin A/fengycin | *P. digitatum* | Waewthongrak et al. (2015) |
| | Bacillomycin Ls/fengycin | F. *oxysporum* | Luo et al. (2015) |
| | Iturin A/surfactin | *P. fugeniae* | Lin et al. (2011) |
| | Iturin/surfactin | *P. phaseoli*; *B. cinerea* and *B. lactucae* | Etchegaray et al. (2008) and Hinarejos et al. (2016) |
| | Iturin A/subtulene A | *C. gloeosporioides* and *S. rolfsii* | Thasana et al. (2010) |
| | Fengycin | F. *oxysporum* | Cao et al. (2012) and Falardeau et al. (2013) |
| | Fengycin | F. *graminearum* | Liu et al. (2005), Chan et al. (2009) and Meena and Kanwar (2015) |
| | Fengycin | F. *culmorum* | Rebib et al. (2012) and Falardeau et al. (2013) |
| | Fengycin | F. *moniliforme* | Hu et al. (2007) |
| | Fengycin | *M. laxa* and *M. fructicola* | Yánez-Mendizábal et al. (2012) and Falardeau et al. (2013) |
| | Fengycin | R. *solani* | Guo et al. (2014), Toure et al. (2004) and Ongena et al. (2005) |
| | Fengycin C | *B. cinerea* | Villegas-Escobar et al. (2013) |
| | Iturin A | M. *fijiensis* | Cazorla et al. (2007) |
| | Iturin A | F. *oxysporum* f.sp. *Radicis-lycopersici*, *R. necatrix* | Cho et al. (2003) and Falardeau et al. (2013) |
| | Iturin A | *G. gloeosporioides*, *R. solani*, and *Phomopsis* spp. | Kita et al. (2005) and Mnif and Ghribi (2015) |
| | Bacillomycin D/surfactin, Mycosubtilin | *A. flavus* and *C. gloeosporioides* | Moyne et al. (2001), Mohammadipour et al. (2009) and Mnif and Ghribi (2015) |

| *Bacillus* spp. | Active antibiotic compounds | Targeted fungal phytopathogens | Reference |
|---|---|---|---|
| *B. subtilis* NCIB 8872 | Antibiotics plipastatins A and B | *Fusarium oxysporium*, *Aspergillus flavus* | Volpon et al. (2000) |
| *B. subtilis* SSE4, RB14 | Subtulene A, iturin A | *C. gloeosporioides* and *S. rolfsii* | Thasana et al. (2010) and Ohno et al. (1995) |
| *B. subtilis* B47 | Iturin $A_2$ | Southern corn leaf blight | Ye et al. (2012) |
| *B. subtilis* fmbJ | Bacillomycin D | *Aspergillus flavus* | Gong et al. (2014) |
| *B. subtilis* SQR 9 | Fengycin and bacillomycin | *F. oxysporum* f.sp. *cucumerinum* | Cao et al. (2012) |
| *B. subtilis* EU07 | YrvN protein-based subunit of protease, Acyl-homoserine lactonase | *F. oxysporum* f.sp. *radicis-lycopersici* | Baysal et al. (2008) |
| *B. subtilis* JA | Fengycin, mycosubtilin, subtulene | *F. graminearum*, *R. solani*, *Pythium irregulare*, and *Cladosporium fulvum* | Chen et al. (2008) |
| *B. subtilis* B-916 | Bacisubin | *R. solani*, *Alternaria oleracea*, *A. brassicae*, *Magnaporthe grisea*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea* | Liu et al. (2007) |
| *B. subtilis* CMB32 | Iturin A, fengycin, and surfactin A | *Colletotrichum gloeosporioides* | Kim et al. (2010) |
| *B. subtilis* 14B | Bac 14B | *Alternaria solani* | Hammami et al. (2012) |
| *B. subtilis* F-29-3 | Fengycin | Effective against filamentous fungi | Vanittanakom et al. (1986) |
| *Bacillus* spp. | Iturin A | *P. aphanidermatum*; *B. cinerea*, F. *oxysporum*, *F. graminearum*, *R. solani*, F. *oxysporum*, *P. irregulare*, *B. cinerea* | Leclere et al. (2005), Béchet et al. (2013) and Zhao et al. (2014) |
| *B. circulans* | Iturin A | *F. oxysporum* f.sp. *lycopersici* | Hsieh et al. (2008) |
| *B. thuringiensis* | Kurstakin | *Colletotrichum gloeosporioides* | Kim et al. (2004) and Mnif and Ghribi (2015) |
| B. *pumilus* | Pumilacidin | *R. solani*, *P. aphanidermatum*, and *S. rolfsii* | Melo et al. (2009) |
| *B. vallismortis* | Bacillomycin D | *F. graminearum*, *A. alternata*, *R. solani*, *C. parasitica*, *P. capsici* | Zhao et al. (2010) |
| *B. vallismortis* ZZ185 | Bacillomycin D (*n*-C14) and Bacillomycin D (iso-C15) | *F. graminearum*, *A. alternata*, *R. solani*, *C. parasitica*, and *P. capsici* | Zhao et al. (2010) |

(continued)

Table 10.2 (continued)

| *Bacillus* spp. | Active antibiotic compounds | Targeted fungal phytopathogens | Reference |
|---|---|---|---|
| *B. vallismortis* BS07 | Surfactin and fengycin | *Phytophthora capsici* and *Colletotrichum acutatum* | Park et al. (2013) |
| B. *licheniformis* | Surfactin | *M. grisea* | Tendulkar et al. (2007) |
| *B. mojavensis* | Surfactin | F. *verticillioides* | Snook et al. (2009) |
| | Mojavensin A | F. *oxysporum* f.sp. *cucumerinum* and F. *verticillioides* | Ma et al. (2011) |
| *B. mojavensis* A21 | Fengycin, surfactin, and pumilacidin | Many fungal | Ayed et al. (2014) |
| *B. amyloliouefaciens* | Iturin/fengycin | *A. phoenicis*, *B. sorokiniana*, F. *oxysporum* f. sp. *licopersici* | Hsieh et al. (2008) and Benitez et al. (2010) |
| | Iturin/surfactin | *A. alternata*, *A. flavus*, *A. niger*, *A. ochraceus*, *B. obtuse*, *C. acutatum*, *C. gloeosporioides*, F. *oxysporum*, *F. solani*, *F. semitectum*, *F. proliferatum*, *F. nygamai*, *F. polyfialidicum*, and *P. expansum* | Dimkić et al. (2013, 2015) |
| | Iturin/surfactin | *R. solani* | Souto et al. (2004) |
| | Bacillomycin D/fengycin | F. *oxysporum* f.sp. *spinaciae* | Zhao et al. (2014) |
| | Surfactin/fengycin | S. *sclerotiorum* | Alvarez et al. (2012) and Meena and Kanwar (2015) |
| | Surfactin/fengycin | *P. italicum*, F. *culmorum*, *B. cinerea*, *M. grisea*, and *E. graminis hordei* | Sun et al. (2006) and Mnif and Ghribi (2015) |
| | Iturin A | A. *citri*, *C. gloeosporioides*, and *P. crustosum* | Arrebola et al. (2010) |
| | Bacillomycin D | F. *oxysporum* f.sp. *cucumerinum* | Koumoutsi et al. (2004), Xu et al. (2013) and Li et al. (2014) |
| | Bacillomycin L/D | *R. solani* | Zhang et al. (2013a) and Chowdhury et al. (2015) |
| | Surfactin | S. *sclerotiorum*, *R. solani*, and *F. solani* | Li et al. (2014) |
| | Surfactin | *V. dahlia*, *F. oxysporum*, *F. solani*, and *P. parasitica* var. *nicotianae* | Li et al. (2014) |

| *Bacillus* spp. | Active antibiotic compounds | Targeted fungal phytopathogens | Reference |
|---|---|---|---|
| *B. amyloliquefaciens* AS 43.3 | Surfactin, iturin, fengycin, a bacillibactin, bacilysin, bacillaene, difficidin, and macrolactin | *Fusarium* head blight in wheat | Dunlap et al. (2013) |
| *B. amyloliquefaciens* WH1 | WH1fungin | *R. solani* | Qi et al. (2010) |
| *B. amyloliquefaciens* LBM 5006 | Iturin-like and fengycin-like peptides | *Aspergillus* spp., *Fusarium* spp., and *B. sorokiniana* | Benitez et al. (2010) |
| *B. amyloliquefaciens* FZB42 | Plantazolicin | Fungal plant pathogens | Scholz et al. (2011) |
| *Bacillus amyloliquefaciens* SQR9 | Fengycin | *V. dahliae kleb*, *F. oxysporum*, *F. solani*, and *P. parasitica* | Li et al. (2014) |
| | Bacillibactin | *V. dahlia*, *F. oxysporum*, *F. solani*, *S. sclerotiorum*, *R. solani*, and *P. parasitica* | Li et al. (2014) |

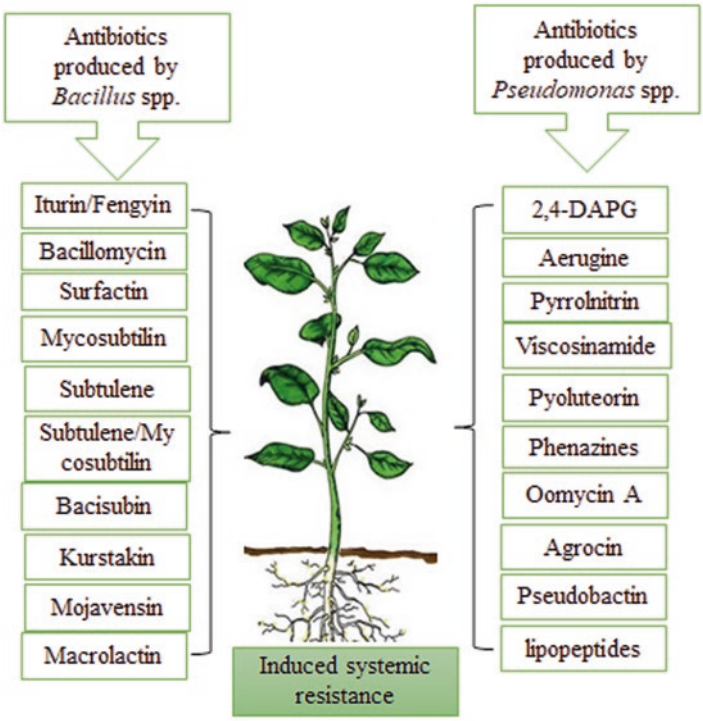


**Fig. 10.2** Role of antibiotics produced by *Bacillus* spp. in inducing systemic resistance in plants

conducted to understand the metabolic efficacy of the volatilome produced by bacterial species on the target organism, despite the fact that VOCs derived from bacterial species are widely recognized to play a critical role in activating or inhibiting several other microorganisms (Kanchiswamy et al. 2015).

The VOCs produced by *Bacillus amyloliquefaciens* SQR-9 have been reported very effective against *R. solanacearum* to control wilt pathogen of tomato (Raza et al. 2016). Volatilome produced by *B. amyloliquefaciens* NJN-6 effectively controlled the banana fusarium wilt disease and inhibited the spore germination and growth of F. *oxysporum* f.sp. *cubense* causing fusarium wilt on banana (Yuan et al. 2012). *Clavibacter michiganensis*, the causative agent of ring rot disease of potato, was significantly inhibited by VOCs emitted from *B. subtilis*, particularly with acetophenone, nonanal, benzaldehyde, and benzothiazole (Rajer et al. 2017). A mixture of VOCs emitted by *Bacillus atrophaeus* CAB-1 is predominately composed of o-anisaldehyde, 2,3-dimethoxybenzamide, and hexadecane which have effectively controlled the growth of gray mold pathogen *B. cinerea* in tomato (Zhang et al. 2013b).

*Bacillus*-released VOCs, for instance, 3,5,5-trimethyl hexanol and decyl alcohol, also suppressed the proliferation of *Xanthomonas oryzae*, the causative cause of leaf blight in rice (Xie et al. 2018). *Bacillus* VOCs also found effective to inhibit the

growth of fungal pathogen other than inhibiting the growth of bacterial pathogens. The VOCs, e.g., 2-heptanone, 3-methyl-1-butanol, 2-ethylhexanol, isovaleric acid, and isovaler aldehyde, emitted from *Acinetobacter* and *Bacillus* significantly inhibited the mycelial growth of *Phytophthora capsica* (Syed-Ab-Rahman et al. 2019). Similarly, VOCs emitted from *Bacillus* endophytes decreased the vegetative sclerotia structures of *S. sclerotiorum* (Massawe et al. 2018). Specifically, the *Bacillus*-generated VOCs like, 3-butadiene, 3-butadiene, and benzaldehyde are involved in modifying the gene expression of virulence factor of *Ralstonia solanacearum* and *Xanthomonas oryzae* (Tahir et al. 2017; Xie et al. 2018).

## 8 Conclusion

The inhibitory activity of hydrogen cyanide, siderophores, and antibiotics may bring revolution in the agricultural sector in the upcoming future as they are safer, sustainable, and profitable to use as compare to synthetic pesticides. These bacteria produce antifungal antibiotics, induced systematic resistance in host plant, and interfere in the interaction between phytopathogen and biocontrol agent during root colonization and throughout plant development. Despite having extensive researched and well-documented data regarding biocontrol agents of microbes, the synthetic pesticides have very huge share in the market. There are lesser number of biopesticides having biological controls and metabolites in the commercial sector. Production of bio-fungicides from secondary metabolites of *Bacillus* spp. is now in initial stages. *Bacillus* spp. have amazing antagonistic inhibitory properties against fungal phytopathogens which can effectively use in preparation of bio-products. Hence its time to pay special attention toward these microorganisms so that their wonderful capabilities can be used for the betterment of mankind by manufacturing bio-products, especially bio-fungicides and bio-pesticides.

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# Chapter 11
# Antifungal Antibiotics Biosynthesized by Major PGPR


**Paul A. Correa, Asia Nosheen, Humaira Yasmin, and Mohammad Javed Ansari**

**Abstract** Plant growth-promoting rhizobacteria (PGPR) play an essential role in the protection, crop growth, and health promotion, improving soil biophysiological and biochemical properties and thus promoting soil health. Extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR) such as *Pseudomonas*, *Bacillus*, *Glomus*, *Azospirillum*, *Rhizobium*, *Serratia*, *Arthrobacter*, *Streptomyces*, *Stenotrophomonas*, *Sinorhizobium fredii KCC5*, *Klebsiella*, *Burkholderia*, *Staphylococcus*, *Herbaspirillum*, *Agrobacterium*, *Azotobacter*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus* and *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, etc., respectively, are well documented for the ability to biosynthesize antifungal, antibiotic, anthelminthic, antioomycete, and antiviral substances as their underlying mechanism for biocontrol. The antagonistic potential of PGPR is ascribed to their ability to produce siderophore, surfactants such as viscosin and viscosamide; antimicrobial compounds such as phenazine-1-carboxylic acid, 2,4-diacetyl phloroglucinol, pyoluteorin, pyrrolnitrin, kanosamine, zwittermicin A, pantocin, and type three secretion systems (T3SS), known to inhibit oomycin; etc. Two highly conserved genes N-acyl homoserine lactose and sigma factors form a cascade of endogenous signals that regulate the biosynthesis of antifungal antibiotics in PGPR. These genes modulating the synthesis of these antimicrobial substances have highly conserved sequences in these PGPR and can be harnessed for green agriculture.

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## 1 Introduction

The United Nations (UN) projects that by the year 2100, the global population will increase exponentially by about 4.3 billion. This increment of the world population implies that there will be an absolute need to produce more than double or even triple food to provide for the over 11 billion citizens' food needs globally (United Nations 2017; Rohr et al. 2019). Agricultural systems and crop production practices have evolved from the hunter-gatherer systems to modernized commercial agricultural farming systems that depend on agricultural inputs such as systemic chemical inputs, i.e., pesticides to curb crop damage in the field by pests, herbicides to reduced competition for soil minerals and sunlight, fungicides to inhibit fungal growth or attack on farm produce in stores, and inorganic fertilizers to increase crop biomass and provide major and minor soil minerals. Put together, all these uses of systemic chemical inputs reduce crop yield loss in the field and the store. The hindrances to crop can induce annual losses up to 17%–30% for the five major crops of global importance like wheat, rice, corn, potatoes, and soybean (Savary et al. 2019).

Despite the development, production, and proliferation of the use of inorganic agrochemicals, they were soon proven to be unsustainable due to high cost of production, impact on the environment, impact on human and animal health, impact on soil, impact on soil microflora, impact on agriculture and aquatic ecosystems, etc., thus continue to be a grave concern (Dhananjayan et al. 2020; Parameswari et al. 2020).

An alternative solution is through the use of antagonistic and beneficial microorganisms (De Silva et al. 2019; Morales-Cedeño et al. 2020; Thomas et al. 2020). These microbes are known for their ability to antagonize pathogen soil microbes and improve plant growth and biomass (Rai et al. 2020). Such microbes are referred to as plant growth-promoting rhizobacteria (PGPR) and produce a wide variety of secondary metabolites enabling them to compete with other microbes in the soil, as they have evolved to compete for the same resources with soil (Bruisson et al. 2020; Vafa et al. 2021). Many compelling reports have revealed that bacteria from many genera of soil-inhabiting bacteria such as *Streptomyces* spp. biosynthesize diverse range of essential secondary metabolites, such as antibiotics, antifungal, antioxidants, etc. (Bruisson et al. 2020; Kusale et al. 2021). These secondary metabolites have structural diversity reflecting their varied spectra of activities such as mediation of intra- and interspecies communication, defense against competing species, nutrient acquisition, symbiotic interactions, etc. (Macheleidt et al. 2016; Spiteller 2015). Research on secondary microbial metabolites have been concentrated on nonvolatile compounds. However, attention is being focused more on microbial volatile compounds (mVOCs). Microbial VOCs are a class of secondary metabolites with low molecular weight (<300 Da), high vapor pressure, and low boiling

point and are generally lipophilic (Schulz-Bohm et al. 2017). Their ability to diffuse through gas- and water-filled pores within the heterogeneous soil matrix makes them suitable for both short- and long-distance signaling (Kanchiswamy et al. 2015; Maffei et al. 2011; Schulz-Bohm et al. 2017). Volatile organic compounds are essential for antibiosis and signaling symbiotic interactions under competitive soil conditions for other competitive organisms (Effmert et al. 2012). Microbial volatile compounds (mVOCs) can antagonize pathogens in their surroundings, and reports have demonstrated their potential use as an alternative to chemical fertilizer and pesticides and could serve as a lasting alternative solution for soil fertility improvement and pest control with negligible adverse effects on animal life and the environment (Thomas et al. 2020).

## 2 Inorganic Agrochemicals

It is estimated that inorganic agrochemicals cost approximately $250 million to isolate and produce a single active substance for the market, with a success rate of 1:140,000 synthesized compounds (Lamberth et al. 2013). Inorganic nitrogen fertilizer is produced by the Haber-Bosch process in which hydrogen (H) and nitrogen (N) are converted to $NH_3$. This is a high-energy-demanding process as it occurs at high temperature and pressure and generates a carbon footprint and approximately 1.2% of anthropogenically produced $CO_2$ emissions (Nørskov et al. 2016). Inorganic nitrogen fertilizer application can induce crop stress in fields and makes crop production most considerable anthropogenic alteration of the global nitrogen cycle (Smil 1999). Furthermore, overuse of agrochemicals such as pesticides, fungicides, and herbicides can induce pesticide resistance development, making these chemicals less effective against their target pest, fungi or herb, etc. Application of inorganic nitrogen to the soil improves soil microbial ability to produce $NO_2$ (Ahirwar et al. 2020). The nitrous oxide ($N_2O$) produced by microbial action such as nitrification and denitrification on inorganic fertilizers in soil and the $NO_2$ released into the atmosphere causes depletion of the stratospheric ozone layer, which serve as a shield against harmful UV rays emanating from the sun, leading to increased UV-B radiation stress. Scientific evidence revealed that this has led to increased $NO_2$ in the atmosphere since the 1960s due to fertilizer applications (Davidson 2009). As crop production is projected to triple by 2100, agricultural expansion could lead to an excessive increase in the use of pesticides, fertilizers, fungicides, herbicides, etc., and it is estimated that this could induce up to 10-fold increases in pesticide use and up to 2.7-fold increases in fertilizer application (Rohr et al. 2019). In addition, the excessive and indiscriminate long-term application or use of pesticides, fungicides, and other agrochemicals affect soil ecology and macro- and microsoil environment which may lead to the alterations in or removal of soil beneficial or probiotic soil organisms or microflora (Kalia and Gosal 2011; Singh and Wright 2002; Subhani et al. 2000). Studies have shown that loss of soil microflora can adversely affect the crop production in many ways including lack of inducement of plant defense; loss

of soil organic matter due to non-decomposition; noninfluence of plant growth; inadequate stress response by plant; lack of some major soil mineral such as N, P, and K since nitrogen mineralization process such ammonification, nitrification, and phosphate solubilization are processes conducted by some soil microbes such as *Nitrobacter*, *Nitrosomonas*, *Acetobacter*, etc.; loss of induced systemic resistance (ISR) by microbes in plants; reduction in soil respiration; etc. (Kour and Sayyed 2019; Achari 2020); meaning this can induce poor crop performance in the field, low yield, poor nutrition, hunger, and starvation.

During these recent years, there is a frantic search and research to develop a sustainable agricultural pest and disease control methods to cut down on overuse of agrochemicals such as fertilizers, pesticides, fungicides, herbicides, etc. by encouraging and supporting a change in farming practices (Brzozowski and Mazourek 2018). There are currently various methods of improving crop tolerance to pest, disease, and abiotic and biotic stresses. These methods include genetic engineering to enhance crop resistance to pest, disease, and disease stress. This method reveals a practical exhibition of improved disease, pest, stress resistance, and ability to reduce pathogen damage and possibly reduce the overuse, reliance, dependency, and requirement agrochemical inputs. Despite its promising nature of genetic engineering products, genetically modified (GM) crops' regulatory networks and transactions needed to legalize and commercialize these crops take a very long time (Kanchiswamy et al. 2015). The GM crops have not been proven safe for human consumption through chemical trials; changing the genetic makeup of crops may result to changes in the food supply that introduce toxins or allergens that trigger allergic recreations; some GM crops may harm the environment through increased use of toxic herbicides and pesticides, and genetic modifications can lead to a mass loss of a particular crop due to disease, and modernization of agriculture leads to genetic changes which can lead to genetic erosion (Van de Wouw et al. 2010).

Agrochemicals can have a severe impact on the ecosystem and environment including effects such as environmental threat, numbers of crop pollinator decline (honeybee, butterflies, etc.), food poisoning and contamination of food chains of domestic and wild cause death, pesticide resistance, bioaccumulation, loss of natural antagonistic to pest, losses to neighboring crops, fishery and bird losses, and groundwater contamination. For these reasons, conventional use of agrochemicals and pest use have come under severe pressures and challenged by the legislature in various countries. Many countries have adopted risk reduction policies; farmers, especially in European countries, are looking for support for integrated pest management (IPM). Keeping along with crop yield and quality while reducing the reliance on pesticides or agrochemicals is a big challenge for the farming communities around the world currently (Lamichhane et al. 2016).

## 3 Biocontrol Approach

Biocontrol approach to pest and disease control is the use of beneficial microorganisms with an antagonistic, inhibitory, suppressive, or biological potential against crop pest or pathogen and have neutral, mutual, harmless, or beneficial effects on crop plants (Zakaria et al. 2019). Currently, due to population explosion and increased demand to feed the ever-increasing number of mouths, the agricultural sector is facing many challenges of food production which include but not limited to climate change, the induction of increased heat and temperature; abiotic stresses such as salinity, drought, flooding, etc.; and biotic stress factors such as increased pest and pathogen attack on crops (Gopalakrishnan et al. 2015). To mitigate and alleviate the pest and disease problems, increase crop survival under stress conditions, and improve yield and global food security, eco- and environmentally friendly crop protection approaches are a promising approach for the future. The world is experiencing a global cry on the use of agrochemical such as fertilizers, herbicides or weedicides, fungicides, pesticides, etc., due to hard lessons learnt such as the *Silent Spring 1962* which triggered global environmental concerns and movement to conserve the environment, natural resources, and nature. The world became more concerned and increased the call for the use of eco-friendly, stable, and sustainable methods of crop production and protection in the field. Some of these environmentally and ecologically friendly crop production and protection approaches are embedded in organic agriculture or farming and promote the use of biofertilizers, biopesticides, and crop residue return. Organic farming employs plant growth-promoting rhizobacteria (PGPR) for crop protection, growth improvement, improvement to abiotic and biotic stress tolerance (Zope et al. 2019), change or improve soil biophysiological and biochemical properties and thereby improve overall crop productivity and yield and ensure global food security (Hamid et al. 2021; Fazeli-Nasab and Sayyed 2019). Over the past decades, a good number of PGPR have been reported to antagonize fungal and bacterial plant diseases; some of the PGPR with this potential to inhibit and kill plant pathogens include *Pseudomonas*, *Bacillus*, *Rhizobium*, and *Serratia*. Their ability confers this potential of PGPR to inhibit or kill plant pathogen to biosynthesis antibiotic or concoction of various antibiotics. The employment of plant-pathogen microbial antagonist has been postulated and proven as an alternative to the use of agrichemicals to conserve nature and the ecosystem. Several compelling scientific reports have revealed that PGPR alleviate many plant diseases caused by various plant pathogens such as fungi, bacteria, viruses, and nematode and herbivore irritation. By all scientific experimental and filed report indication, PGPR are a promising alternative to the best environmental and eco-friendly plant pest and disease control method (Singh et al. 2019).

The PGPR can be employed as biofertilizers and biocontrol method of plant pest and diseases that edge out conventional methods of use of agrochemicals in many ways including their non-toxicity, natural occurrence and co-evolution with plants for millions of years, feasible application, ability to stimulate plant growth and development, ability to improve soil structure and texture, improve soil health,

confer plants tolerance to abiotic and biotic stresses, etc. These merits of PGPR come from their different potential modes of action ranging from their ability to produce antioxidants, antibiotics, volatile organic compounds, and biosurfactants and capacity to solubilize and fix various minerals such as nitrogen (N), phosphorus (P), and potassium (K); produce phytohormones (Khan et al. 2020) such as auxins, cytokinin, ethylene, gibberellins, etc.; produce vitamins and enzymes; and mobilize complexed soil minerals. In addition, PGPR have the ability to synthesize substances that can advance plant growth and development, have beneficial plant health substances, antifungal and bacterial substances, and secondary metabolites that can kill or inhibit phytopathogen, etc. This potential of PGPR is conferred by their ability to release antibiotics into the rhizospheric root zone of plants and plant surfaces or immediate environment and thus making PGPR a prospective alternative to agrochemicals fertilizers, pesticides, fungicides, and bactericides.

Antibiotics are a group of the heterogeneous low-molecular-weight organic complex, such as circulin, mycosubtilin, subtilosin, subtilancin, fengycin, surfactin, difficidin, 2,4-DAPG, pholuteorin, HCN, PCN, PCA, 1-OH-PHZ, etc., can inhibit the growth, development, and metabolic processes in different microorganisms (Kumar et al. 2015).

Over the past decades, in vitro and in situ experiments have revealed that antibiotics are more effective in inhibiting the development of target pathogens. The ability of rhizobacteria to produce and exude at least one antibiotic plays an essential role in plant development by inhibiting many phytopathogens (Glick 2014; Glick et al. 2007). Antibiotics are classed into two major groups: volatile antibiotics and nonvolatiles antibiotics (Table 11.1). Antibiotics have been widely reported over the years to possess antimicrobial properties such as antiviral, antibacterial, antifungal, insecticidal, anthelminthic, anti-nematode, antioxidant, phytotoxic, and cytotoxic effects and influence the promotion of plant growth (Ulloa-Ogaz et al. 2015; Ulloa-Benítez et al. 2016).

Microbial volatile organic compounds (mVOCs) are considered natural products that have a wide range of actions and can increase the inhibition rates of target microorganisms due to their cell membrane permeability and high efficiency of diffusion into the voids of air and soil (Toffano et al. 2017). At present, the MVOCs that have been validated for use are for their antifungal capabilities, antibacterial activities, promotion of plant growth, induction of plant disease resistance, transduction signals, and their use in microorganism identification (Albayrak 2019). However, there is only a few reported uses of mVOCs for the preservation of fruits and vegetables with most studies focusing on fungi or yeast VOCs and the preharvest activity of VOCs in controlling plant disease. Studies on the control of postharvest diseases using bacterial VOCs and some MVOCs with antifungal activity in vitro have been reported. However, still, these studies often focus on single pathogens and screened antifungal strains, and screening in vivo is lacking (Kanchiswamy et al. 2015). In addition, even though the good antifungal activity of mVOCs against pathogens in vitro has been shown, in vivo, the antifungal activity was found to be significantly reduced or nonexistent (Gotor-Vila et al. 2017). In addition, (i) at present, plant diseases are often thought to be caused by one species or specific strain;

**Table 11.1** Summary of antifungal antibiotics produced by PGPR, the biosynthesis, genes, type of molecule elicited, pathogen and disease antagonized, and mode of action from some PGPR

| Producing PGPR & strains | | | | | Microbial activity | | | | Pathogen | Disease | Crop | Elicited substance(s) | Mode of Action | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PGPR** | **Biocontrol Strain, specie** | **Active Biocontrol molecule** | **Gene (s)** | **Type of compound** | **Antifungal** | **Antibacterial** | **Antiomycin** | **Antiviral** | | | | | | |
| **Bacillus** | *Bacillus sp* | Circulin, Colistin | ? | Macrocyclic cysteine knot-peptide | √ | √ | | | *Magnaporthe grisea* | Rice blast, | rice | Metabolite | Disrupt microbial cell membrane | Beneuzi et al., 2012; |
| | *B. subtilis*, | Iturins | ituABC | Lipopeptide | √ & Biopesticide | | | | *Alternaria solani, F.graminnearum,* | Foliar disease, CMV, blight disease, Fusarum wilt | Tomato, pepper, Lettuce (*Lactuca sativa*) | Metabolite | Disrupt microbial cell membrane | Rojas-solis et al., 2013, Kim et al.,2017; Ntushelo et al., 2019 |
| | | Mycosubtilin | MycABC | Lipopeptide | √ | √ | | | *Fusarium graminaerum, saccharomyces cerevisiae* | Fusarium head blight, | Cereal crops | Metabolite | Acts on the cytoplasmic membrane | Leclere et al., 2004;(Ongena et al., 2007) |
| | | Subtilin | SpaS (spaBCD) | Single-peptide | | √ | | | *Fusarium graminaerum, fusarium oxysporum* | Fusarium head blight, Fusarium witl, | Melon, Tomato | Metabolite | Disrupting cell membrane | Chung et al., 1992; (Abdallah et al., 2018) |
| | *Bacillus, B. amylolique facticns* | subtilosin | sboA | Modified peptide | | √ | | | *Broad-spectrum action against bacteria Ralstonia,* | Tomato wilt | Tomato | Metabolite | Disrupting cell membrane by perturbing the lipid bilayer | (Abdallah et al., 2018) (Velho, Basso, Segalin, Costa-Medina, & Brandelli, 2013) |
| | *B. velezensis CC09, B. amylolique faciens* | Amyloly sin | Aml (aml MTK REF) | peptide | | √ | | | *Listeria monocytogenes* | Listeriosis | | Metabolite | Inhibits cell wall biosynthesis by inteeracting with lipid II, protein modifcations | (Arias et al., 2013) |
| | | Amylocyclicin | acnABCDEF | Peptide | √ | √ | | | *Gaeumannomyces graminis var. tritici & Bipolaris sorokiniaana* | Take-all | Wheat | metabolite | Disrupting cell membrane | Bithell et al., 2016; MacDonal et al., 2018 |
| | *B. amylolique faciens* | Amysin | ? | Peptide | √ | | | √ | *CMV* | CMV, tomato mottle virus | tomato | Metabolite | Disrupting cell membrane | (Kaewklom, Lumlert, Kraikul, & Aunpad, 2013) |

(continued)

**Table 11.1** (continued)

| Producing PGPR & strains | | | | | Microbial activity | | | | Pathogen | Disease | Crop | Elicited substance(s) | Mode of Action | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PGPR | Biocontrol Strain, specie | Active Biocontrol molecule | Gene (s) | Type of compound | Antifungal | Antibacterial | Antiomycin | Antiviral | | | | | | |
| | | Thuricin 17 | secE, nusG, thn(1, 2,3) albA | Peptide | √ | √ | | | *Pantoea* | | Corn leaf bligt | Metabolite | Cidal or static by removing key substances from niche | (Nazari & Smith, 2020); |
| | | Thuricin H, Z | Thn(PEDRA1 2A3BT1) | peptide | | √ | | | | | | | | |
| | | Sublancin | Sunl (sunA, sunT, bdbA, sunS, bdbB) | Single-peptide (glycol-peptide) | √ | √ | | | *Trichoderma sp* | Post-harvest diseases & disease of roots and shoot; green mould disease | Pleurotus ostus (commercial mushroom) | Metabolite | Cidal or static by removing key substances from niche | Ji et al., 2015;(Akond et al., 2016); |
| | | TasA | TasA, yqxM, sipW; & spo0A & spo0H; abrB | Protein (cpnt of Eps) | √ | √ | | | | | pepper | Metabolite | Modifcation of other proteins | Axel G. Stover and Adam Driks., 1999;(I. Singh, 2018) |
| | | Bacilysin | bacA BCD E | dipepetide | | √ +yeast | | | *R. solani, Xanthomonas oryzae* | Fire blight disease, baccterial blight, bacterial leaf streak | Apples, pears, family Rosaceae | Metabolite | Induce changes in cell structure | Steinborn et al.,2005; (X.-H. Chen et al., 2009); (Wu et al., 2015) |
| | | Ericin | eriA and eriS | Single-peptide | | √ mild /subtilin | | | *Clavibacter michiganensis* | Bacterial C anker | tomato | Metabolite | | Stein rt al., 2002a |
| | | Mersacidin | Mrsa ADE KFG KMR 1R2T | Peptide(Protein), Lantiotic | | √ | | | *Gram-positive phytopathogens, actinobacteria,, Clavibacter michiganence* | Bacterial wilt and canker of tomato | tomato | Metabolitei | Inhibits cell wall peptidoglycan biosynthesis; inhibition antibiotic resistance | (Brötz, Bierbaum, Markus, Molitor, & Sahl, 1995); (Altena, Guder, Cramer, & Bierbaum, 2000) |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *B. subtilis* | Bacilysocin | ytpA | peptide | √ | √ | √ | √ | *Bacteria, fungi, protozoans, viruses* | Broad-spectrum antimicrobial activity | Various crop specie | Metabolite | Cell wall disruption | (Tamehiro et al., 2002) |
| | *B. subtilis* | chlorotetain | ? | dipeptide | √ | | | | *Listeria monocytogenes* | | | Metabolite | Cell wall disruption | (T. Wang, Wu, Chen, Lin, & Yang, 2016) |
| | *B. subtilis* | mycobacillin | ? | Cyclic peptide | √ | | | | | | | | Inhibit phytopathogens resistant genes | (Sajitha, Dev, & Maria Florence, 2016); |
| | *B. subtilis* | rhizocticins | RhiA-rhiM | Phosphor-oligopeptide | | √ | | | | Tubercrop | | | Transport system antagonism | (Petronikolou, Ortega, Borisova, Nair, & Metcalf, 2019) |
| | *B. amylolique faciens* | bacillaene | baeBCDEKGHIJLMNRS | polyketide | √ | √ | | | *Burhholderia* | Bacterial blight, bacteerial panicle blight | Millet,Rice | Metabolite | Competing for resources | Arguelles et al., 2009, (Caulier et al., 2019a) |
| | *B. amylolique faciens* | difficidin | dfnLMKJIHGFEDBCYXA | Lipopeptide(polyketides) | | √ | | | *E. amylovora* | Fire blight disease | Apple, pear | Metabolite | Disrupting cell membrane | Arguelles et al., 2009,(X.-H. Chen et al., 2009) |
| | *B. amylolique faciens* | Macrolactin | YkyA, mlnABCDEFGH, pdhA | Lipopeptide | √ | | | | *Fusarum oxysporum Fusarum graminearium* | Fusarum wilt of banana; fusarum head blight | Banana, wheat | Metabolite | Disrupting cell membrane | Arguelles et al., 2009, (Yuan et al., 2016) |
| | B. *amylolique faciens; B. subtilis* | Bacilysin | YwfH, ywfG, bacEDCBA, ywfA | Lipopeptide | √ | √ + Algal species | | | *Xanthomonas oryzae* | Bacterial blight | Rice | Metabolite | Disrupting cell membrane | Arguelles et al., 2009,(Knight et al., 2018) |

(continued)

**Table 11.1** (continued)

| Producing PGPR & strains | | | | | Microbial activity | | | | Pathogen | Disease | Crop | Elicited substance(s) | Mode of Action | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PGPR | Biocontrol Strain, specie | Active Biocontrol molecule | Gene (s) | Type of compound | Antifungal | Antibacterial | Antiomycin | Antiviral | | | | | | |
| | *B. amyloliquefaciens, B. subtilis; B. anthracis, B. cereus, B. thurigiensis* | Bacilliba ctin | dhbF BEC A | Lnon-ribosomal peptide (siderophore) | √ | √ | | | *Aspergillus flavus, Fusarium graminearium* | Bacterial blight | Rice, | metabolite | Disrupting cell membrane, siderophore | Arguelles et al., 2009, (Knight et al., 2018); (Hertlein et al., 2014) |
| | *B. megatorium* | Megacin s | MegA, MegC and MegA im | Large protein, Lipopetide, | | √ | | | *Rhizoctonia solani* | | soybean | Metabolite | Disrupting cell membrane | Zheng et al., 2000 |
| | *Bacillus megateriu m* | Schizoki nen | Stb1 geno me | Organic iron transporters/ dihroxamate | √ | | | | *Iron-oxidizing bacteria* | | | Secondary metabolite | Chelating iron and disrupt iron transport | (Budzikiewicz, Münzinger, Taraz, & Meyer, 1997) |
| | *Several bacillus strains* | Baccillo mycin D | bmyA BC/( BYA; BY;B YC) | Mixture of two lipopetide | √ | | | | *Candida, species, Aspergillus flavus, Sclerotinia sclerotiorum* | Ear and kernel rot , white mold, cotton rot, drop | Cotton, maize, | Metabolite | | Gu et al., 2017, |
| | *B. subtilis, B. amyloliquefaciens* | Surfactin | SrfA A,srf AB, srfAC & srfAD | Lipopeptide | √ | √ | | | | | | Secondary metabolite | Disrupting cell membrane | Meena and Kanwar., 2015; Kim et al.,2017 |
| | *B. subtilis, B. amyloliquefaciens* | Fengycin (fengycin A, fengycin B & pliplastin A | FenC DEA B | Lipopeptide | √ | v | | | *P. fusca* | | melon | Metabolite | Disrupting cell membrane | Meena and Kanwar., 2015; Wu et al., 2007; Romero et al., 2007 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *B. cereus UW85* | Zwittermicin A (aminopolyols), Kanosamine (aminoglycoside) | NRPS & PKS (ZmaABCFKOQ) ZmaDERGHIJLMNPSTUV | Non-ribosomal peptide | √ | | √ + antiprotists, insects | | *Phytophthora medicaginis* | | alfalfa | Metabolite | | Matilla et al., 2017, Kevany et al., 2008 |
| | *B. ploymyxa* | Polymixin | | Lipopeptide | √ | √ | | | | | | metabolite | Disrupting cell membrane | Velkov et al., 2010; Zhai et al., 2010 |
| ***Pseudomonas*** | *P. protegens Sp. Nov* | 2,4-DAPG/Phl; pyrolnitrin(PRN); Pyouteorin (PLT) | phlACBDEFO; prnABCD pltLABCDEFG | phenolic polyketide | √ | √ +antihelmintic | | | *Clavibacter michigens subsp michigensis* | Bacterial Canker | Tomato | Phytoalexins | Supression | Lanteigne et al. 2012). Dwivedi and Johri 2003). Bangera and Thomashaw 1996; NNowak-thompson et al., 1999; Delaney et al. 2001). Weller et al., 2007 |
| | *P.fluorescens* | Ferribactin, C, H, HCN, 2,4-DAPG, PCA, | GrrA/GrrS hcnABC PhlEDBAF, phnAB | Siderophore Metabolite, Polyketide, | √ | √ | | | *R. solani, Macrophomina phaseolina, S.sclerotiorum, S. rolfsii, Pythium aphabidermatum, sarocladium oryzae,* | Sheath blight disease, rice sheath rot, BBTV | Rice, Banana | Phytoalexins | Inhibit mycelial growth | Jay Shankar singh., 2013; M. Bruto 2014., Reddy et al., 2008,M. Gita Bangera and L. S thomashow., 1999, |
| | *P. fluorescens pf5* | Pholuteorin | 10 ORF pltLABCDEFG MR | phenolic polyketide | √ | | √ | | *Pythium ultimum, Glomerella tucumanensis,* | *Pythium* damping-off, red root rot | sugar cane | | Reduce symptoms | Matilla and Krell., 2017 Fernando et al. 2005; Hassan et al. 2011 |

(continued)

**Table 11.1** (continued)

| Producing PGPR & strains | | | | | Microbial activity | | | | Pathogen | Disease | Crop | Elicited substance(s) | Mode of Action | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PGPR** | **Biocontrol Strain, specie** | **Active Biocontrol molecule** | **Gene (s)** | **Type of compound** | **Antifungal** | **Antibacterial** | **Antiomycin** | **Antiviral** | | | | | | |
| | *Pseudomonas protegens, Dryoteris fragans* | Phlorogl ucinols (2,4-DAPG), Pseudoas pidinol | PhlA CBD E | Polyketide | √ | | | | *Clavibacter michigens subsp michigensis* | Bacterial Canker | Tomato | Phytoalexins | Supression | Abdel-Ghany et al., 2016, |
| | *P.chlororaphis PCL1391* | Phenazines;HCN & derivatives like PCA, PCN, 1-OH-PHZ | phzMSH hcnABC, phnAB | Hetrocyclic Nitrogenous Metabolite | √ | √ | | | Anti-neamtodes, antitumor off *G. graminis* var. *tritici, rhizotonia solani Kuhn & broad spectrum fungal acctivity* | take-all disease, root rot, | Wheat, cocoyam, Rice | | Not clear yet implicated to act through a redox electron exchange. | (Blumer & Haas, 2000) Chen et al. 2014; Dasgupta et al. 2015 Guttenberger et al. 2017), Mavrodi et al. 2001, 2006; |
| | *P.aureofaciens ,* | 2,4-DAPG, Phenazine, | phlA, phzMSH | Hetrocyclic Nitrogenous Metabolite | √ | √ | | | *G. graminis* var. *tritici* | Take-all disease | wheat | Phytoalexins | inhibition | Paulitz et al.,2000;Pascale et al., 1997; |
| | | furanone, butyrolactones | | | | | | | | | | | | |
| | *P.aerogino sa,* | HCN | hcnABC, | Secondary metabolite | | | Nematicidal | | *Meliodogyne hapla; F. solani;* | | Alfalfa | Secondary metabolite | | Lee et al., 2011; kang et al., 2018 |
| | *P.fluorescens FD6* | 2,4-DAPG, Phenazine, pyrrolnitrin, pyoluteorin, cyclic lipopeptide, furanomycin | phlA prnA, $Gm^r$ pltA GacS/GacA | Hetrocyclic Nitrogenous Metabolite | √ | √ | | | *Clavibacter michigens subsp michigensis* | Bacterial Canker | Tomato | Phytoalexins | Supression | Trippe eta al., 2013, Raaijmakers et al., 2006 |
| | *Pseudomonas pyrrocinia , P. cepacia* | Pyrrolnitrin, | prnA | Nitrogenous compound | √ | | | | *F.sambucinum* | | potato | Secondary metabolite | Iinhibits electron transport chain | (Tripathi & Gottlieb, 1969) |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P. chlororaphis* PA23 | Pyrrolnitrin, HCN | PrnABCD hcnABC | Halogenated compound | √ | √ | *Nematicidal properties | | Prn has a broad spectrum phytopathogens; *Meliodogyne hapla; F. solani;* | | | Secondary halometabolite, | Iinhibits electron transport chain | Lee et al., 2011; kang et al., 2018 |
| | *Pseudomonas fluorescens* | Oomycin A, Pyloluteorin, 2,4-DAPG | pltA, phlA | Peptide | √ | √ | | | *Antioomycetes, Pythium Ssp, Pythium ultinium,* | Pre-emergent damping-off | Cooton, sugarbeat | Metabolite | | (Immanuel, Hemamalini, & Gnanamanickam, 2012) |
| | *Pseudomonas spp* | Viscosin, | ViscABC; | Cyclic lipopeptide (CLP) | √ | √ | | | | | | | Disrupting cell membrane | |
| | *P. syringae* | Amphisin, syringomysin; Tolaasin' | gacS syrB1,B2; sryC; sryD and syrP; syrE1 | CLP | √ | √ | | | *Streptomyces* | | | | Inhibits biosynthesis of membrane bound peptidoglycan | Bendeer et al., 1999; Helman et al., 2019 |
| | | | -8; salA | | | | | | | | | | | |
| | *Pseudomonas putida* | Pseudobactin | | Organic iron transporters | √ | | | | bateria | Bacterial wilt | Tomato, cucumber | | Chelating iron atoms | (Burr, Caesar, & Schrolh, 1984) |
| | *Pseudomona sp.* | Massetlolide 1A | Mass ABC | Cyclic lipopeptide (CLP) | √ | √ | | | | | | | Disrupting cell membrane | De Bruijn et al., 2008 |
| Serratia | *S. phymathica A153* | Andrimid | Adm X and Hfg | Hybrid polyketides/ Non-ribosomal peptide | √ | √ | | | *Agrobacterium* | Crown gall disease | Broad spectrum | | Blocking the carboxyl transfer reactin | Maltilla et al., 2016,(Matilla, Nogellova, Morel, Krell, & Salmond, 2016) |
| | *S. marcescens B2* | Marcescins, prodigosin | rphD-rphZ | metabolite | √ | √ | | | *Pyriculari oryzae* | rice blast | Rice, cyclamen | | Chitinase action and induced systemic resistance | Kawasaki et al., 2009;(Someya & Akutsu, 2005) |
| | *S. hygroscopicus* | Phosphinothricin, bialaphos | prpA (orfM & trs) | Amino acid | | | (Herbicide) | | | | | | Inhibits glutamine synthetase | (Dragićević et al., 2013), (Schwartz et al., 2004) |
| | *Serratia spp.* | Pyrrolnitrin | prnABCD, | Halometabolite | √ | √ | | | | | | Halometabolite | | |

(continued)

**Table 11.1** (continued)

| Producing PGPR & strains | | | | | Microbial activity | | | | Pathogen | Disease | Crop | Elicited substance(s) | Mode of Action | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PGPR** | **Biocontrol Strain, specie** | **Active Biocontrol molecule** | **Gene (s)** | **Type of compound** | **Antifungal** | **Antibacterial** | **Antiomycin** | **Antiviral** | | | | | | |
| | *S. plymuthica*, | Pyrrolnitrin Andrimid Haterumalid | prnABCD, PKS | Phytohormones/ siderophores | √ | √ | | | *Dickey solani, Yersinia enterolitica* | Blackleg/ soft rot | Potato | Secondary halometabolite | Inhibit bacterial synthesis | Awodi et al., 2017 Matilla et al., 2016b Cleto et al., 2014 Petersen & Tisa, 2013; Matilla & Salmond, 2014; Czajkowski & Wolf, 2012 |
| | *S. ruhidaea* | prodigiosin | | Alkaloid | √ | | | | *Fusarium oxysporum* | Foot and root rot disease | tomato | Metabolite | | Kamou, N.N., Dubey, M., Tzeleppis, G et al 2016 |
| Enterobacter | *Enterobacter cloacae* | Cloasins Hydrogen cyanide (HCN)/ Prussic acid, Indole acetic acid (IAA), Bacteriocin | hcnABC | circular DNA molecule | | √ | | | *Enterobacter* and *Klebsiella* | defoliation | Maize, papaya, chilli. Etc. | Secondary metabolite | Inhibition β-lactamases | Deepa et al. 2010; Tieze et al. 1969 |
| | *Pantoea agglomerans(Enterobacter agglomerans)* | Herbicolin, microcin, andrimid pantocin, agglomerin | mcbABCDEFG | Lipopolysaccharides, phytohormones | √ | √ | | | Moulds, | Tunerogenic diseases, fire blight | Wheat, rice, citrus fruits | Metabolite | Competition mechansm or induction of plant resistance | Dutkiewicz, J., Mackiewicz, B., Lemieszek, M. K., Golec, M., Milanowski, J. (2016). |
| *Burkholderia* | *Burkholderia cepacia,* | Siderophore Enacyloxin IIa, Bactolactin, Quinolines, afcA lipopeptide | sidABCDEF Enacyloxin IIa afcA | polyketide | √ | √ | | | *Phytophthora capsici, Fusarium oxysporum*, and *Rhizoctonia solani* | Blight and fruit rot, wilt, root & foot rot | Onion& Agronomical important crops | Secondary metabolite | Inhibition of protein synthesis | (Jung, Hong, Park, Kim, & Shin, 2018), (de Lamo & Takken, 2020) |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Azospirillum* | *Azospirillum spp* | Siderophores, HCN, lytic enzymes, Poly-β-hydroybutyrate (PHB), | SREA<br><br>hcnABC<br><br>phaABC &phaE | polyester | √ | √ | | | Iron-oxidizing Phytopathogen | | | Secondary metabolite | | (Sant'Anna et al., 2011),(Dobrogojski, Spychalski, Luciński, & Borek, 2018) |
| | *A. lipoferum* | Siderophores | sidABCDEF | Organic iron transporters | √ | | | | | | | Low-molecular iron chelating compound | Chelating iron me | (Sayyed, Chincholkar, Reddy, Gangurde, & Patel, 2013) |
| | *A.basilense* | Siderophores, | SREA | Organic iron transporters | √ | | | | *Colletotrichum acutatum* | anathracnose disease/foliar disease | Ceareal crops, legumes, tree crops | Low-molecular iron chelating compound | Chelating iron me | (Tortora, Díaz-Ricci, & Pedraza, 2011),(Řezanka, Palyzová, Faltýsková, & Sigler, 2019) |
| | *A. fluorescens* | | | | | | | | | | | | | |
| | *A.niger* | Ochratoxins | | mycotoxins | | | | | *Stachybotrys chartarum* | Black mold | Fruits & vegetavles, peanuts | | | |
| Agrobacter | *Agrobacter vinelandii* | Azotobactin | sidABCDEF | Siderophore | √ | | | | Iron-oxidizing phytopathogens (*Thiobacillus ferrooxidans*) | | | | Chelating iron me | (Sayyed et al., 2013) |
| Agrobacterium | *Agrobacterium tumefaciens* | agrobactin | sidABCDEF | Siderophore | √ | | | | Iron-oxidizing Phytopathogens (*Thiobacillus ferrooxidans*) | | | | Chelating iron me | (Sayyed et al., 2013) |
| *Azotobacter* | *Azotobacter chrococum* | Catechol & hydroxamate | SidABCDEF | siderophore | √ | | | | Iron-oxidizing phytopathogens (*Thiobacillus ferrooxidans*) | | | | Chelating iron me | (Sayyed et al., 2013) |

(continued)

**Table 11.1** (continued)

| Producing PGPR & strains | | | | | Microbial activity | | | | Pathogen | Disease | Crop | Elicited substance(s) | Mode of Action | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PGPR | Biocontrol Strain, specie | Active Biocontrol molecule | Gene (s) | Type of compound | Antifungal | Antibacterial | Antiomycin | Antiviral | | | | | | |
| *Arthrobacter* | | Arthrobactin | sidABCDEF | Siderophore | √ | | | | Iron-oxidizing phytopathogens (*Thiobacillus ferrooxidans*) | | | | Chelating iron me | (Sayyed et al., 2013) |
| *Erwina* | *Erwinia herbicola* | Ferrioxamine E | SidABCDEF | Siderophore | √ | √ | | | Iron-oxidizing phytopathogens (*Thiobacillus ferrooxidans*), *Erwina amylovora* | Fire blight, apple blossom | Apple, Pear | | Chelating iron me | (Sayyed et al., 2013) |
| *Flavobacterium* | *Flavobacterium anhuiense* | | SidABCDEF | | | | | | Oomycetes | | Pepper, phytophthora capsici | | | |
| | *TRM1* | | | | | √ | | | *R. solanacearum* | *R. solanacearum*-disease | Tomato moneymaker | | | Kwak et al., 2018 |
| *Rhizobium* | *Rhizobium sp* | hydroxamte | sidABCDEF | Cyclic hydroxyic acid | √ | | | | *F. oxysporum f.sp.ciceris, Fusarum spp, F.solani, R. solani, pythium sp* | | Chickpea, banana , tomato | Secondary metabolite | Inhbits matrix metalloproteinases, | (Datta & Chakrabartty, 2014; Ranjbar Sistani, Kaul, Desalegn, & Wienkoop, 2017) (Laronha et al., 2020) |

however, pathogens in pre- and postharvest crops generally occur as a part of complex communities (Lucas et al. 2015). Interestingly, most laboratory studies focus on single-pathogen strains grown in pure cultures (Lucas et al. 2015). (ii) Postharvest diseases of fruits and vegetables are complicated and different from those of pre-harvest. (iii) The storage and shelf conditions of fruits and vegetables are different from those found in plate experiments (Lucas et al. 2015).

This review chapter presents antifungal antibiotics produced by PGPR that are essential for plant health, the induction of plant resistance to fungal pathogens, and promotion of plant growth, highlighting the capacities for use as an alternative solution to synthetic fertilizers and pesticides.

## 4 Microbial Volatile Compound (mVOC) Antagonism of Phytopathogens

The earliest evidence of the antagonistic role of volatile microbial compounds (VOCs) against phytopathogens was demonstrated in a study by Ferdinand et al. (2005); since then many compelling scientific reports have elucidated volatile microbial compounds' (mVOCs) potential to inhibit a variety of phytopathogens, ubiquitous plant pathogens such as bacteria, fungi, oomycetes, etc. that are hampered by mVOCs. This spotlights the potential of mVOCs and their suitability for use as an alternative to agrochemicals such as pesticides, bactericides, and fungicides.

### *4.1 mVOC Antagonism of Fungi*

The earliest study evidence elucidating the antagonistic role of mVOCs against phytopathogens is reported in 2005. In this study, *Pseudomonas* sp. isolated from soybean and canola was reported to antagonize *Sclerotinia sclerotium*, a fungal phytopathogen with a wide range of more than 400 plant species, and causes white mold commonly known as cottony rot, watery soft rot, drop, stem rot, crown rot, and blossom blight (Fernando et al. 2005). Recently, *Pseudomonas* species as a PGPR is reported to produce over 23 VOCs, and out of these, 6 are shown to reduce fungal mycelial growth of *S. sclerotiorum* significantly. In a similar report, VOCs produced by two strains of the *Bacillus* endophytic species is shown to impact negatively on the weight and number of the vegetative, long-term survival structures, the sclerotia of the *sclerotiorum* (Massawe et al. 2018).

### 4.2 mVOC Antagonism of Bacteria

Volatile organic compounds from a range of PGPR are reported to inhibit the growth of ubiquitous soil-borne bacterial plant pathogens. The VOCs from *Burkholderia ambifaria* and a variety of other rhizobacteria isolates (Groenhagen et al. 2013; Vetivelli et al. 2015) are capable of antagonizing soil-borne phytopathogens such as *R. solani*. Microbial volatile compounds are demonstrated to antagonize bacterial phytopathogen. *Bacillus*-produced VOCs such as benzaldehyde, benzothiazole, nonanal, acetophenone, etc., are shown to significantly inhibit *Clavibacter michiganensis*, potato root rot, the causative agent for bacterial ring rot disease of potato (Rajer et al. 2017). These VOCs from the *Bacillus* are further revealed to antagonize the growth of *Xanthomonas oryzae* that causes the bacterial disease blight of *Oryza sativa* (rice) by producing VOCs such as decyl alcohol and 3,5,5-trimethylhexanol which VOCs inhibit the pathogen growth (Xie et al. 2018).

### 4.3 mVOC Antagonism of Oomycetes

Further, the mVOCs also possessed a wide range of action against pathogenic oomycetes. This is demonstrated by the ability of VOCs from the *Bacillus* and actinobacter to diminish the mycelial growth of the oomycetes *Phytophthora capsici*. The *Bacillus* and actinobacter PGPR produce 3-methyl-1-butanol, isovaleraldehyde, isovaleric acid, 2-methyl-1-ethyl hexanol, and 2-hepta hexanone that specifically antagonizes *Phytophthora capsici* (Syed-Ab-Rahman et al. 2019). In another report, it is also demonstrated that *Nodulisporium* exudes VOCs that antagonize oomycete action on several *Pythium* species. However, the mechanism of inhibition is still unclear as the VOCs involved in the inhibition of oomycetes are not individually assayed and studied (Ulloa-Benítez et al. 2016).

### 4.4 mVOC Antagonism of Virus

Recently, there are compelling reports revealing PGPR antagonistic potential against plant viruses. A virus that attacks cucumber (*Cucumis sativus* L.) known as cucumber mosaic virus (CMV) is reported to be antagonized by *B. velezensis* strain PEA1 (Abdelkhalek et al. 2020). Cucumber mosaic virus has many types of host ranging from monocots to dicots and infects over 1200 species of 100 plant families all over the world (Mochizuki and Ohki 2012). In the study by Abdelkhalek et al. (2020), samples are collected from opened fields at Alexandria governorate in Egypt with severe leaf mosaic symptoms and chlorosis characteristic CMV-like symptoms. Cucumber mosaic infection (CMV, category, *Cucumovirus*, family *Bromoviridae*) is one in every foremost dangerous and financially crucial plant

infections, causing extreme loss to trim quality and surrender round the world (Scholthof et al. 2011); PEA1 was proven to inactivate CMV replication and induce systemic resistance against CMV infection in *D. stramonium* leaves. In similar studies, PGPR such as *B. amyloliquefaciens* and *Pseudomonas* sp. strain have also been shown to be effective against tobacco streak virus and *Babuvirus* of cowpea and banana, respectively, by producing antiviral peptides, PR proteins, chitinase, phenolics, enzymes, etc.

Put together, these reports elucidate the role of mVOCs that antagonize various ubiquitous soil-, air-, and waterborne phytopathogens such as fungi, bacterial, oomycetes, and nematodes, spotlighting the potential of these PGPR VOCs as possible alternatives to agrochemical such as fungicides, pesticides, and bactericides (Abdelkhalek et al. 2020).

## 5 Structural Differences of mVOC That Influence Their Inhibitory Function

Optical enantiomer studies reveal that the presence of a chiral center in the structure of these mVOCs influences their antagonistic function against target phytopathogens. A recent study revealed that the presence of chiral centers in 1-octen-3-ol that have two optic isomers; (R)-(-)-1-octen-3-ol and (S)-(+)-1-octen-3-ol influence the antagonistic potential and should verify the target infective agent. Intriguingly, in an associate degree experiment, wherever these two optical isomers studied for their aggressive potential against the fruit spoilage antibiotic drug chrysogenum, (R)-(-)-1-octen-3-ol repressed reproductive structure germination of five out of seven isolates, whereas (S)-(+)-1-octen-3-ol repressed reproductive structure germination of merely two isolates, spotlighting that completely different enantiomers exhibit variations in their antagonistic potential (Yin et al. 2019). Besides, (R)-(-)-octen-3-ol modulated the transcription of a more significant number of penicillin chrysogenum genes. This pinpoints a vital point of consideration in the determination of the specificity of mVOCs for target pathogens, ushering a novel area for future research in the search for bioactive chiral mVOCs and revealing chemical structural information that can lead to the artificial synthesis of these VOCs to replace agrochemicals such as pesticides and fungicides.

## 6 Mechanisms of Volatile Microbial Compounds Against Plant Pathogens

Plant growth-promoting rhizobacteria (PGPR) are broadly reported to possess a broad range of antagonistic activities that interferes with phytopathogens' growth and development (Berg 2009; Mrabet et al. 2013; Soylu et al. 2005). The

biosynthesis of siderophores, antibiotics, bacteriocins, and lytic enzymes is extensively studied among harmful bacteria (Mrabet et al. 2013; Jabborova et al. 2020). The mode of action of some antifungal antibiotic substances produced by PGPR still remains unclear; however, compelling reports reveal that PGPR' antifungal antibiotic substances antagonize phytopathogens by interfering with the pathogens' critical structural components (Olanrewaju et al. 2017). These pathogen inferences include various mechanisms such as the disruption of cell membranes of phytopathogens, termination or interference with the electron transport system, immediate inhibition of endogenous and exogenous respiratory systems, inhibition of growth and development parameters such phytopathogen metabolism, etc. (Jadhav et al. 2017). In another mechanism, the PGPR produce substances that can prime plants without actually any direct plant-pathogen interaction of the target pathogen and induce plant resistance to disease caused by phytopathogens. The antifungal antibiotic compounds produced by PGPR can act against pathogens by regulating and modulating their growth conditions adversely through the production of substances such antibiotic and antifungal compounds, combinations which can interfere with the virulence factor of these phytopathogens and enhance the plant's capacity to develop resistance or heal (Tariq et al. 2017). Apart from their antagonistic role, the antifungal antibiotic compounds are also involved in mutualism, intra- and interspecies regulation of plant cell development process, and modification of plant surrounding – rhizosphere (Bitas et al. 2013). Compelling scientific reports have also shown to regulate root exudation process; transcription of iron transporters such as SbIRT1, SbIRT1, SbYS1, and SbYS2; and defense signaling pathways such as COI1 and PR-1 (Hernández-Calderón et al. 2018). Antagonistic isolates of those genera vary in the host, and individual strains principally have a variety of plant morbific hosts. They manufacture structures for attachment and infection and kill their hosts by cell wall degrading enzymes (CWDEs), usually together with antimicrobial secondary metabolites (Karlsson et al. 2017; Köhl et al. 2019; Nygren et al. 2018). These lytic enzymes aren't essential; however, their production is triggered by a complicated signal when recognizing the host. Surface compounds like lectins from the host cell membrane, surface properties, and diffusible host-released secondary metabolites play essential roles within the recognition and signal pathways like MAPK cascades, cAMP pathway, and G-protein signal (Karlsson et al. 2017; Köhl et al. 2019). Recognition of the flora host then ends up in transcriptional reprogramming and expression of the "molecular weapons" concerned in host attack and lysis, as well as bound CWDEs. Mycoparasitism-related sequence families in *Trichoderma* like ech42 and prb1 are upregulated throughout mycoparasitism as results of the first activities of CWDEs, oligosaccharides and oligopeptides, are discharged by the host that is then perceived by *Trichoderma* receptors and thereby act as inducers (Karlsson et al. 2017). This attack by a necrotrophic mycoparasite leads to an additional increase of porousness, degradation of host cell walls, and death of the host. A synergistic transcription of varied genes concerned in cell membrane degradation was additionally according to *Trichoderma atroviride* in association with *B. cinerea* and *Phytophthora capsici* (Reithner et al. 2011), the final results of the complete cascade of events. Enzymes like CWDEs are

complicated proteins consisting of many one hundred or a thousand amino acids with the performance to change state the conversion of specific substrates into specific merchandise. Functioning of enzymes depends not solely on organic compound sequences, however, additionally, on their complicated tertiary structures (Iyer and Ananthanarayan 2008). Flowering of those structure or disordered polypeptides results in catalyst denaturation and irreversible loss of the protein activity. Enzymes are sensitive to physical denaturation, e.g., by heat or cold temperatures; chemical denaturation by numerous factors from acids to chelating agents; and microorganism denaturation, e.g., by proteases. High sensitivity of catalysts to denaturation could be the main obstacle in technological processes so that enzyme stabilization throughout production and application is typical in specialized applications. Proteases, cellulases, lipases, amylases, and different enzymes are created at industrial scales by microorganisms. They are ordinarily utilized in the paper manufacturing process, food manufacturing, medical device cleaning, plant product manufacturing, and in addition, as several standard family cleansing processes like laundry (Aberer et al. 2002). Enzymes used for such technical applications are tested for a few years, and it has been established that enzymes have a safe toxicologic profile with a decent record of activity health and safety for the patron. Studies unconcealed that enzymes appear unlikely to be dangerous to the aquatic setting thanks to their ready biodegradability and, therefore, the discovered expected effects on marine organisms (Aberer et al. 2002). Cell wall-degrading enzymes are ordinarily created within the setting by microorganisms through degradation of organic matter from dead plant tissues and dead microorganisms as well as dead flora hyphae and unendingly play an essential role in nutrient athletics in all told ecosystems. Given this background activity of protein CWDEs in natural ecosystems, application of hyperparasites in biological management won't considerably increase cell membrane-degrading activities within the setting. Hyperparasites manufacture low amounts of flora CWDEs throughout short time periods domestically in microniches once they act with their hosts (Karlsson et al. 2017).

Microbial ordering analysis unconcealed huge numbers of cryptic antibiotic cistron clusters coding still new antibiotics. Antimicrobial metabolites are the most potent mode of action of microorganisms against competitors permitting antibiotic-manufacturing microorganisms competitive benefits in resource-limited environments (Raaijmakers and Mazzola 2012). Production of antimicrobial metabolites, principally with broad-spectrum activity, has been according to the biocontrol bacterium happiness to *Agrobacterium*, *Bacillus*, *Pantoea*, genus *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Actinomycete*, and plenty of alternative genera. *Bacillus* lipopeptides such as iturin, surfactin, and fengycin are confirmed in *Bacillus* (Ongena and Jacques 2008). In the genus *Pseudomonas*, several antibiotic metabolites like DAPG, pyrrolnitrin, and phenazine are studied (Raaijmakers and Mazzola 2012). Several antibiotics are created only if a microbic population reaches bound thresholds. This quorum-sensing development is ubiquitous in phenazine-producing genus *Pseudomonas*. Genomic info reveals that conjointly these genera have the potential to supply several still unknown secondary metabolites with attainable antimicrobial activity. Conjointly fungous antagonists will turn out

antimicrobial compounds. For *Trichoderma* and closely connected *Clonostachys* (former *Gliocladium*), 6-PAP, gliovirin, gliotoxin, viridin, and plenty of additional compounds with antimicrobial activity are investigated (Ghorbanpour et al. 2018). Microorganisms synthesizing antimicrobial metabolites with the potential to interfere with antibiotics in phytopathogens mostly should be excluded from use as microbial biocontrol agents (MBCAs).

## 7 Intrinsic Antibiotic Resistance

Intrinsic resistance conferred to the plant and microbes by their outer membrane and multidrug resistance possessed by Gram-negative bacteria. The outer membrane is impermeable to many molecules of the Gram-negative outer membrane, while the multidrug-resistant is insensitive to a number of classes of Gram-positive antibiotics. Phytopathogens are resistant to several classes of antifungal antibiotic substances, and rhizospheric bacteria exhibit this resistance by possessing intrinsic resistome which predates inherent resistance (Fig. 11.1).

## 8 PGPR as Biocontrol Agents Induce Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR)

The PGPR plant interaction and phytopathogen plant interaction are reported to induce ISR and SAR, respectively. Rhizobacteria plant interaction causes jasmonic acid and ethylene signaling pathways, through *jar1* and *etr1*, in which the signal is generated by nonpathogenic-related protein 1 (npr1) and thus enhance the defense capacity of the plant and trigger activation of induced systemic resistance. On the other hand, phytopathogen plant interaction induces salicylic acid response signaling pathway through *NahG*, which induces *npr1*, which activates pathogen-related proteins and induced systemic acquired resistance (Kamle et al. 2020).

## 9 Major Antifungal Antibiotics of PGPR

The antifungal antibiotics in Table 11.1 can be broadly classified into various groups such as ribosomal peptides, nonribosomal peptides, bacteriocins, AMP enzymes, polykedties, lipopeptides (thiotemplate NRPs), siderophores (thiotemplate NRPs), non-thiotemplate NRPs, volatiles, volatile inorganic compounds, volatile compounds, and alcohols (Fig. 11.2). These classes of antifungal antibiotic in Fig. 11.2 have various applications ranging from medicine, agriculture, biotechnology, and industry.

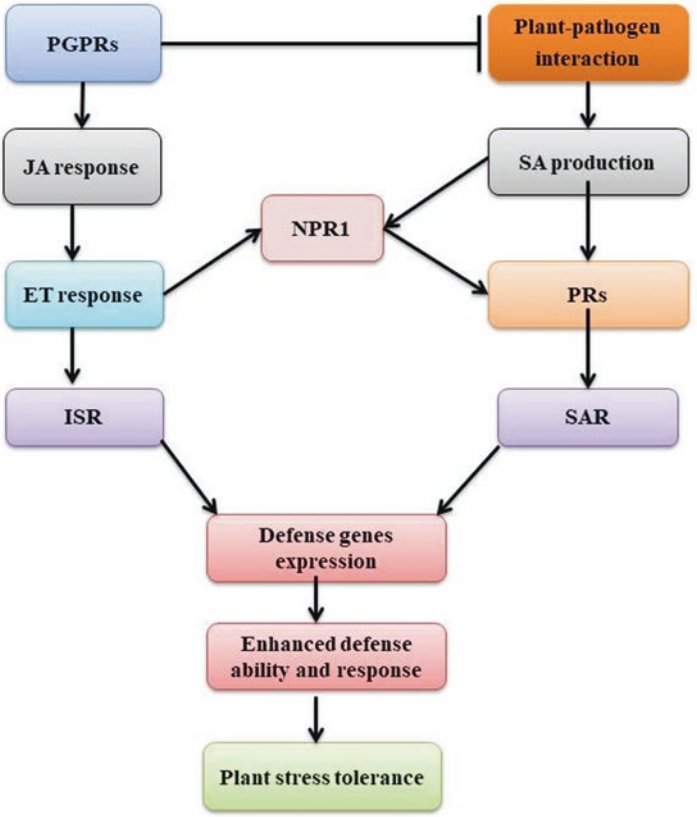


**Fig. 11.1** PGPR-induced SAR and ISR

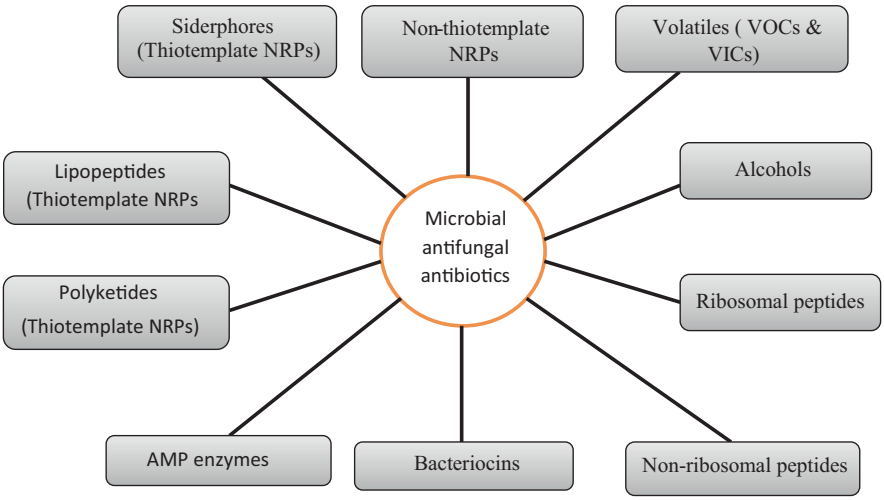


**Fig. 11.2** Major classes of antifungal antibiotics

## *9.1 Antifungal Antagonist Antibiotics Against Plant Pathogens*

The conventional use of chemical fungicides is a common practice in modern farms and stores, and reports show that native antagonist against plant pathogens, use of chemical fungicides for control of *Fusarium* wilt, is a general practice in many of the cultivated plants (Arunodhayam et al. 2014). Such measures are mostly ineffective. Moreover, chemical measures might establish imbalances within the microbiological community, unfavorable for the activity of helpful organisms that otherwise improve crop health. The demand for different chemical management of plant pathogens has become stronger because of the issues regarding the protection and environmental aspects of chemicals. Indiscriminate use of pesticides leads to the development of resistance in pathogens, and bioaccumulation of these chemical pesticides in plant tissues may cause potential health hazards to humans. A strategy to overcome these problems is the use of biocontrol agents. However, biological management offers the potential to enhance crop production inside the present resources, besides avoiding the matter of chemical resistance (Dekker 1976; Khan et al. 2014). Biological control is the potential tool for the management the *Fusarium* wilt disease. A variety of soil microorganisms have demonstrated antagonistic activity in the control of various soil-borne plant pathogens, including *Fusarium* wilt pathogen. *Fusarium* wilt suppressive soils are known to occur in many regions of the world, and suppression has generally been shown to be biological in origin. The potential for controlling *Fusarium* wilt, a significant threat in tomato in many parts of the country, was evaluated by using *Trichoderma harzianum*, Pseudomonads (microorganisms of saprophytic nature), and *Glomus intraradices*, an arbuscular mycorrhizal fungus. Secondly, the process of using these three microorganisms (*Trichoderma harzianum*, *fluorescent Pseudomonas*, and *Glomus intraradices*) was adjusted in a manner that the optimum population of bioagents remains in the rhizosphere to combat the disease. Antagonists recovered from *Fusarium* suppressive soils have been used to reduce *Fusarium* wilt diseases of several different crops (Paulitz et al. 1987; Postma and Rattink 1992; Alabouvette et al. 1993; Minuto et al. 1995; Larkin et al. 1996). It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms initially isolated from other plant species (Lucy et al. 2004).

It is also reported that fungal antagonists like bacteria can produce antimicrobial compounds (Table 11.1). Reports revealed that the soil fungal genus *Trichoderma* of the family of hypocreaceae produce volatile antifungal compounds such as 6-PAP, gliovirin, gliotoxin, viridin, and many more compounds with antimicrobial activity (Ghorbanpour et al. 2018; Köhl et al. 2019). Fungal organisms producing antifungal and antimicrobial metabolites with the potential to interfere neural system and with antibiotics of human and animals must be excluded from use as biocontrol agents for plants and animals (Köhl et al. 2019). The inhibitory effect of fungal and microbial secondary metabolites on fungal spore germination or hyphal growth of pathogens makes fungal and microbial exudate compounds as an alternative to chemical control.

# 10 Regulation of Antibiotic Synthesis

## *10.1 GacS/GacA System*

GacS/GacA is a well-conserved two-constituent signal transduction system, and it's reported as a master regulator of bacterial pathogenicity and virulence factor in many Gram-negative bacterial phytopathogens such as *Pseudomonas* spp. It comprises of a hybrid sensor kinase GacS and a cognate response regulator or coordinator GacA (Heeb and Haas 2001). This system is capable of detecting and responding coordinately to external stimuli, including microenvironment physiological state. The GacS/GacA system is reported to positively modulate polyketide antibiotic 2,4-DAPG produced by *P. fluorescens* 2P24 biosynthesis (Zhang et al. 2020). GacS/GacA is also revealed to regulate the type III secretion system (T3SS) of effector proteins into host cells and play a critical function in disease induction, while in the opportunistic plant pathogen *Pseudomonas aeruginosa*, GacS/GacA is shown to regulate the expression of T3SS encoding genes negatively. Experiments have shown that the GacS/GacA system regulates motility, siderophores, pigment production, and phaseolotoxin biosynthesis. It is also demonstrated to modulate the output of two antifungal metabolites nunamycin and nunapeptin which are cyclic lipopeptides produced by *P. fluorescens*. The synthesis of syringomycin is activated by GacS/GacA and three transcription factors salA, syrF, and syrG following plant signaling molecules (Christiansen et al. 2020). However, a study by Wang et al. (2020) revealed that mucins and especially their affiliated glycans have efficacy and induce the sensor kinase RetS through its Dismed2 domain in *P. aeruginosa*. This report shows that RetS-dependent signaling directly inhibits GacS/GacA two-constituent signal transduction system whose activity is linked to a chronic infection state. RetS signaling leads to the downregulation of type VI secretion system (T6SS) and inhibits T6SS-dependent bacterial killing by *P. aeruginosa* (Wang et al. 2020) (Fig. 11.3).

## *10.2 N-acyl Homoserine Lactone (NHL) and Sigma Factors in Antibiotic Biosynthesis*

### 10.2.1 Quorum Sensing

Bacterial phenotypic characteristic expression such as bioluminescence, biofilm formation, motility, virulence factor production, exoenzyme, antibiotics and antifungal substance production, etc. is a cell density-dependent process that is regulated by the perception of cell-to-cell signaling in a process called quorum sensing (QS). In Gram-negative and positive bacteria, LuxIR is the QS system which functions through the production of N-acylated homoserine lactone (AHL) signaling (Waters and Bassler 2005). The QS is reported to modulate the biosynthesis of

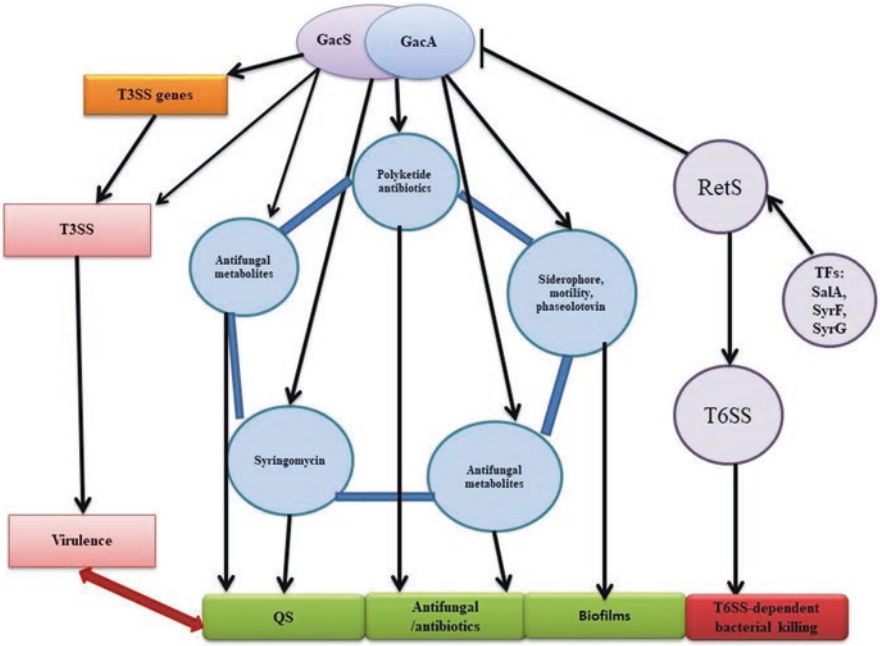


**Fig. 11.3** Regulation of antibiotic synthesis

phenazines and pyrrolnitrin in *Pseudomonas chlororaphis* and *Pseudomonas aeruginosa* (Selin et al. 2012). In *P. aeruginosa*, there is a three-interlinked QS system; las, rhl, and pqs are involved in the regulation synthesis of pyocyanin, a phenazine virulence factor an phenazine which is used a phenotypic marker for analyzing QS. Production of pyocyanin is a sophisticated process that involves near identical operons named $PhzA_1B_1C_1D_1E_1F_1G_1$(phz1) and $phzA_2B_2C_2D_2E_2F_2G_2$(phz2) that regulates the biosynthesis of phenazine-1-carboxylic acid (PCA); PhzM- and PhzS-modifying enzymes convert PCA to pyocyanin (Higgins et al. 2018).

The QS modulate the biosynthesis of polyketide antibiotics in *Burkholderia*. The QS in *Burkholderia* triggers BtaR2-BtaI2 (luxRI) genes that activates acyl-HSL (AHL) synthase that induces polyketide antibiotic biosynthesis (Mohan and Sahu 2018). In *Serratia* and *Erwinia carotovora*, a cluster of nine-gene complex (carRA-BCDEFGH) that functions in assembling antibiotics where N-(3-oxohexanoyl)-L-homoserine (OHHL) is produced as a by-product of the independent carI gene activates which carR transcription factors. The OHHL-dependent transcriptional activation permits the cells to synchronize expression of carbapenem with cell density. This is referred to as orphan quorum sensing and identified in the soil bacteria *Burkholderia thailandensis* and differs from the classical QS as its receptors do not respond to the characteristics of QS signaling parties. The orphan receptors respond to antibiotics like trimethoprim and sulfamethoxazole and as a result, elicit the expression of the malA-M involved in the biosynthesis of the cytotoxic antibiotics

malleilactone. This path may be a survival-of-the-fittest strategy for competing in matrix of soil bacterial niches (Mohan and Sahu 2018).

The QS is demonstrated to be targeted by quorum quenching (QQ); at molecular level, QS gene expression can be inhibited in the emitting cell; the chemical messenger itself may be degraded; and finally, the detection of the signal molecule by the cognate receptor and/or by the regulatory protein(s) may be blocked. The QQ can be an alternative strategy of biocontrol methods (Rosier et al. 2016). It is reported that some rhizobacteria naturally produce enzymes that degrade AHLs, AHL lactonases, or AHL acylases. These hydrolytic enzymes cleave the AHL molecule, for instance, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* (Fetzner 2015). In a further development in this light, mVOCs of PGPR are also shown to quench the QS signal molecules of phytopathogens. It is reported that *P. fluorescence* and *S. plymuthica* VOCs quench signal molecules for cell-to-cell communication in the pathogens of the genera *Agrobacterium*, *Chromobacterium*, *Pectobacterium*, and *Pseudomonas* (Chernin et al. 2011). Exposure to these molecules reduces the amount of AHL biosynthesis by these phytopathogens, negatively influencing the expression of QS expression genes. The QQ is a future alternative to displace bacterial competitors and thus protect plants from phytopathogens (Hartmann et al. 2014). Phytopathogens are shown to respond to both AHL produced and released by pathogenic bacteria like *P. aeruginosa* and *Serratia liquefaciens* MGI and *P. putida* of tomato plant increased systemic-induced resistance (SIR) against fungal foliar pathogen *A. alternaria*. This helps plants prepare for a future invasion of phytopathogens by activity plant defense mechanism. This AHL from either phytopathogens or symbiotic bacteria may induce plant defense gene expression involve in defense (Chernin et al. 2011).

## *10.3 Virulent Factor Regulation*

The presence of virulence factor molecules influence the amount of antibiotic molecules produce by pathogens in a niche matrix (Kreikemeyer et al. 2003). The virulence factor concentration is directly proportional to the amount of antibacterial substances produce. The virulent factor regulation is intricately linked to the QS regulator system and modulated by AHL synthase, synergistically or reciprocally to produce antibiotics. The *Agr* regulates the transcription of RNAIII, which is an RNA molecule that regulates the virulent factor expression both at transcriptional and translational levels. The *Agr* downregulates gene products such as protein A (spa) and upregulates fibronectin-binding protein (*fnb*), enterotoxins, exfoliatins, and phenol-soluble modulin virulent factors (Azimi et al. 2020).

## *10.4 Type VI Secretion Systems (T6SS)*

Type (VI) secretion system (T6SS) is a molecular-level toxic effector protein weapon that the microbes like bacteria injects into neighboring closely related strain of bacteria to lyse kill them and out compete them in their niche matrix (Allsopp et al. 2017; Ho et al. 2014; Hood et al. 2010). T6SS is typically composed of 15–23 different protein, and among them 13 proteins (TssA-M) are highly conserved in many bacteria and encode structural component of the T6SS (Mougous et al. 2006; Pukatzki et al. 2006). T6SS effector protein has a VgrG protein trimmer and a PAAR-domain containing protein. It is recently reported that a metallopeptidases $VgrG2b_{C\text{-}ter}$ can elicit toxicity in bacterial periplasm and can be neutralize by a cognate immunity protein. The toxicity of $VgrG2b_{C\text{-}ter}$ induces serious morphological anomalies characterized by blebbing of the bacterial membrane at the site of septation, reflecting an inhibition of cell division by β-lactam antibiotics (Wood et al. 2019).

## *10.5 Type III Secretion Systems (T3SS)*

The T3SS is a system in Gram-negative bacteria species of essential phytopathogens. The T3SS is composed of a complex multiprotein assembly that transverses the inner and the outer membranes and host cellular membranes to secret effector toxins and translocate them directly into the host cells. The T3SS like T6SS is virulence factor that plays an essential role in the establishment and spreading of *P. aeruginosa* infections (Williams et al. 2015). A recent study discovered and characterized a number of inhibitors of *P. aeruginosa* T3SS based on phenoxyacetamide scaffold. The T3SS effector toxins of *P. aeruginosa* (ExoS and ExoU) and various Gram-negative bacteria impede quick innate immune response to the invading and colonizing bacteria by quenching the phytopathogen antibiotic substances and out competing it (Williams et al. 2015).

## *10.6 Outer Membrane Protein F Gene (OprF)*

The outer membrane protein F is important with crucial function in bacteria. It functions in the antibacterial role of host neutrophil elastase both in vitro and in bacteria. The *P. fluorescens* 2P24 produces 2,4-DAPG, a major antibiotic substance that protects plant from soil-borne disease. The release of 2,4-DAPG biosynthesis enzymes encoded by the gene phlACBD locus is regulated by a sensitive network. A very recent study revealed that oprF negatively regulates 2,4-DAPG biosynthesis through random mini-Tn5 mutagenesis, while sigma factor X (SigX) upstream of the oprF gene positively modulates its biosynthesis in *P. fluorescens* (Li et al. 2018). The

SigX is also reported to be involved in the regulation of many factors including virulent factors (Bouffartigues et al. 2014; Gicquel et al. 2013).

### *10.7 The Ecological Role of PGPR Antifungal Antibiotics*

The PGPR antifungal antibiotics play a crucial function in the ecosystem in many ways including eradication of soil-borne fungal and biotic diseases naturally; enhancing sustainably agricultural practices (green agriculture); improving crop resistance to various pest, diseases (fungal, bacterial, viral, and oomycetes), pathogens, and adverse soil conditions; reducing the use of environmentally unfriendly agrochemical; and reducing wanton killing of beneficial or symbiotic microbes to crops. This invariably reduces agrochemical accumulation in animal food chain and reduces the chance of toxicity. PGPR antifungal antibiotics promote rhizobacteria interactions which could lead to the production of other plant growth-promoting substances or molecules in the natural environment.

## 11 Role of PGPR Antifungal Antibiotics in ISR

Induce systemic resistance (ISR) is the plant resistance to disease and pathogens triggered by biological or chemical substances produced by pathogens. The ISR protects nonexposed plant host or plant parts against possible attack by pathogenic microbes, herbivores, and/or insects. The ISR is reported to work with quorum sensing system and other regulatory systems in providing protection against pathogens and disease. A recent report shows that the NHL-deficient rhizobium radiobacter mutant Rrf4NM13 demonstrated a reduced plant growth-promoting and resistance-inducing activities in mono- and dicotyledonous plants (Alabid et al. 2020). These point at synergistic role of NHL and QS regulatory systems in modulating antibiotic biosynthesis and induction of induced systemic resistance (Coquant et al. 2020).

### *11.1 Ribosomal Peptides (rps)*

These are ribosomally synthesized peptides from short precursors and are modified into matured form by posttranslational modifications (Oman and Van Der Donk 2010). They are called bacteriocins and have a low molecular weight. The RPs can antagonize the growth activities of various bacteria of closely related strains (Chopra et al. 2015). Different enzymes regulate modifications and generate antifungal antibiotics of various chemical structures, thus, conferring these antimicrobials with additional inhibitory potential and specificity (Chopra et al. 2015). The RPs as

bacteriocins show different metabolic activities relating to quorum sensing regulation and induction of genetic competence or cell lysis (Schmidt 2010; Shafi et al. 2017)

## *11.2 Nonribosomal Peptides (nrps)*

The NRPs are derived from a family of secondary metabolites with broad structural differences and functionality as antibiotics, siderophores, surfactant pigments, immunosuppressors, and antitumor substances (Wang et al. 2014).

## *11.3 Bacteriocins*

Bacteriocins are bacterial proteins produced by one strain of bacteria and biologically active against other bacteria of a closely related strain or specie. Almost all bacteria produced at least one bacteriocin (Table 11.1). During their synthesis, the active peptides of bacteriocins undergo posttranslational modifications, conferring them with different potency of the antagonism (McIntosh et al. 2009). Bacteriocins are classed based on their biosynthesis pathways into classes I, II, and III (Abriouel et al. 2011; Nes et al. 2007). The class I bacteriocins are all the bacteriocins that have posttranslationally modified peptides, for instance, antibiotics; while classes II and III are translationally non-modified peptides and larger than 10 kDa in size (Abriouel et al. 2011).

Class I bacteriocins encompasses small AMPs with a size range of 19 to 38 amino acids with lots of posttranslationally modified peptides. Class I bacteriocins is further classed into subclasses such as subclass I.1, I.2, and I.3; these have a typical lantibiotic structure (an inner-residual thioester bond consisting of modified amino acids). Lantibiotics consist of 2,3-dehydroalanine (Dha) and (Z)-2,3-didehydrobutyrine (Dhb). The intramolecular addition of Dha and Dhb to the cysteine residue gives rise to lanthionine and methyl methionine bridge formation (Willey and van der Donk 2007). The similarity in the structure is evident in the similarity between the structure of subtilin (subclass I.1) and nisin A lantibiotics (Abriouel et al. 2011). Bacteriocins from the subclass I.4 go through several modifications; in the case of subtilosin A, it is a head-to-tail cyclic peptide with a peculiar interresidue linkage (Cys-Phe bond) (Kawulka et al. 2004; Marx et al. 2001). Class II bacteriocins are linear, non-modified peptides of less than 10 kDa in size that are resistant to heat and acid-base treatment. This class also has subgroups such as based on their conserved AA in their N-terminus. The subclass II.1 has YGNGVXC (where X is any AA) motif which is associated with pediocin-like peptide, whereas subclass II.2 has DWTXWSXL motif which is specific to thuricin-like peptide, and subclass III is made up of small non-modified AMPs with no unique motif in their

AA sequence. Lastly, class III bacteriocins of large heat-susceptible molecules are characterized by phospholipase activity (Cleveland et al. 2001).

Bacteriocins display varied mode of action such as protoplasm vesicularization, pore formation, or cell disintegration; this is due to the modifications and structural differences they exhibit. They antagonize bacteria as phytopathogens (Gautam and Sharma 2009). The class I and II bacteriocins owing to their amphiphilic or hydrophobic properties oppress phytobacteria by attacking their bacterial envelope; lantibiotic class I.1 has a dual mode of action; they can antagonize bacteria by inhibiting their cell wall biosynthesis through binding to lipid II – a principal transporter peptidoglycan subunits through the inner cell membrane. The bacteriocins can use lipid II and can also antagonize bacteria by using it as docking molecule to insert lantibiotics to the cell membrane, inducing perforations or pore formation and consequentially leading to cell death (Chatterjee et al. 2005; Cotter et al. 2005). More recent papers also reported a similar dual mode of antagonistic action of bacteriocins (Parisot et al. 2008). Numerous modulatory systems regulate the production, secretion, and immunity of bacteriocin like any other antifungal antibiotic compounds. Bacteriocins production traced to particular cellular processes and events stress. A case in point, subtilin production is linked to and depends on cell density, and its production is increased during nutrient deprivation (Abriouel et al. 2011). It can function as its production inducer (Kleerebezem 2004).

## *11.4 AMP Enzymes*

The AMP enzymes include lytic such as chitinases, cellulases, proteases, and glucanases. The lytic enzyme is generally referred to as cell wall-degrading enzymes (CWDEs) (Alamri 2015; Caulier et al. 2018). The CWDEs are active against fungal phytopathogens, whose cell walls are composed of chitin and glucans, where different glycoproteins are embedded (Geraldine et al. 2013; Gomaa 2012).

## *11.5 Polykedties (pks)*

Polyketides are bioactive compounds that are produced by microorganisms and biosynthesized from acyl-CoA precursors such as malonate and methyl malonate. Their interest as biocontrol agents is a result of their potential as antibacterial, antifungal, antitumor, immunosuppressors, and numerous other antagonistic potential. Their biosynthesis is dependent on diverse multifunctional polyketide synthases (PKSs), and their structure is derived from fatty acid synthases (FACs) with similar chain elongation, precursor, and the network (Smith and Tsai 2007). The PKS has a sequential elongation module flanked by initiation and termination modules. Their mode of action is by their three unique domains; the initiation module is composed of acyltransferase (AT), acyl carrier protein (ACP), and a β-ketoacyl synthase (KS).

The AT domain recruits and catalyzes the binding of a monomer carrier substrate to the ACP domain. The ACP domain possesses the second catalytic domain (KS) on the next elongate module and catalyzes the chain elongation reaction that occurs through decarboxylative Claisen thioester condensation (Hertweck 2009). Aside from these core domains, the elongation domain also has auxiliary domains that modulate ketoreduction, dehydration, and enoyl-acyl reduction that takes place before chain elongation. These modifications enhance the diversity and complexity of mature polyketides and confer differences in their mode of action and reactivity. Lastly, the termination module possesses an additional thioesterase that catalyzes the macrolactonization and release of matured polyketides (Cane and Walsh 1999).

Type I PKs include large multifunctional enzymes containing several domains bonded covalently and arranged linearly; the type II PKs are multienzyme complexes constituting a number of the monofunctional enzyme in composite forms during the biosynthesis of PKs; and finally, the type III PKs are chalcone synthetase-like PKs that regulate the acid CoA thioesters directly without needing any ACP domain (Chen and Du 2016). In prokaryotes, type I PKs are more reported (Challis 2008).

The PKs are very diverse, and intermediate metabolites between the three forms exist such as bacillaene, compactin, fusarin C, and salinosporamide A (Fisch 2013; Hertweck 2009). Generally, seven PK family are reported, and *Bacillus*, a widely studied PGPR, produce only three including bacillaene, difficidin, and macrolactin, and these antagonize phytopathogens by selectively inhibiting protein synthesis. Bacillaene antagonizes many bacteria and fungi such as *Myxococcus Xanthus* or *Staphylococcus aureus* and *Trichoderma* spp. or *Fusarium* spp. (Müller et al. 2014; Um et al. 2013). Difficidin and oxydifficidin (difficidin-oxidized form) are type I PKs encoded in the dif. Operon inhibits bacterial pathogens including clostridium perfringens, *Erwin amylovora*, *E. coli*, or *Xanthomonas oryzae* (Aleti et al. 2015; Chen et al. 2009; Wu et al. 2015).

## *11.6 Lipopeptides (Thiotemplate NRPS)*

Lipopeptides are thiotemplate-based nonribosomal peptides (NRPs) biosynthesized through NRPS sequential elongation of amino acids as iterative or non-iterative paths (Caulier et al. 2019). Like PKs, NRPs have a modular organization with an initiation, elongation, and termination modulation, and each module is subdivided into core domains with catalytic domains differing from that of PKs (Süssmuth and Mainz 2017). The NRP biosynthesis is described previously by Weinig et al. (2003). It starts with an adenylation A domain that functions in recruiting and phosphorylating amino acid monomer into aminoacyl adenylate intermediate. The intermediate is implicated in the peptidyl carrier protein (PCP) also called T-domain through a thioester bond. The PCP serves as a bridge linking the condensation (C domain) that forms the C–N bond between the recruited aminoacyl and the peptide acyl chain in formation. The termination module has a thioesterase domain (TE) that catalyzes

the release of the final peptide acyl chain. The elongation module can be supplemented with accessory domains cyclization domain (Cy), epimerization domain (E), and methylation domain (M). The modifications of the nascent peptide chain lead to a different mature structure (Pistorius 2011).

These four families of LPs (kurstakins, surfactins, iturins, and fengycins) are produced by *Bacillus* (Jacques 2011) due to the flexibility of their biosynthesis and, thus, very heterogeneous. These families share the same structural features characterized by the nature and organization of the peptide moiety or fatty acid tail. The LP biosynthesis is regulated by environmental factors like carbon sources, oxygen availability, pH, and temperature. Warm temperatures (≥37 °C) and anaerobic conditions increase the production of surfactins, while lower temperatures (25–37 °C) and aerated bioreactors favor fengycin and iturin family metabolites. Surfactin production is also regulated by quorum sensing (QS) (Caulier et al. 2019). Fengycins and iturins have potent antifungal activity against several phytopathogenic fungi, while iturin-like mycosubtilin R, subtulene A, and eumycin have antibacterial properties. Iturins, fengycins, and surfactins predominantly show antiviral and antibacterial microbial properties. They antagonize *R. solanacearum* or *X. oryzae* and *Listeria monocytogenes*. Surfactins can antagonize fungal phytopathogens such as *F. oxysporum* and *R. solani* (Fira et al. 2018).

Lipopeptides have a peptide moiety bound to a lipid tail, a structure which confers them with an amphiphilic property. This characteristic confers them an excellent surfactant and executes an essential role in their biological functions and antimicrobial properties. Lipopeptides antagonize phytopathogens by disrupting their plasma membrane by pore-forming activity leading to the cells of target microbes to die, and their antiviral mode of action is similar. They disintegrate the lipid bilayer of virions, thus rendering answers to the weak LP activity against plant viruses as very few of them are enveloped (Luo et al. 2014; Malviya et al. 2020). Lipopeptides also influence other metabolic processes like biofilm formation. Motility, virulence, plant root colonization, and defenses implicated in the degradation of hydrophobic substrates could be used for polluted soils bioremediation (Arora et al. 2013).

## *11.7 Siderophores (Thiotemplate NRPS)*

Siderophores chelate iron, reducing its bioavailability; this antagonizes the growth of iron-requiring phytopathogens such as *F. oxysporum* f. sp. *capsici*; *ferric* itoic acid and bacillibactin are examples of such ferric iron-chelating siderophores. They are composed of a 2,3-dihydroxybenzoate (DHB) molecule bound to glycine, and it is used as a precursor by trimodular NRPS machinery to produce bacillibactin which is obtained after condensation of three units of DHB-glycine-threonine (Sundaram and Hertweck 2016; Nithyapriya et al. 2021). The final synthesis is catalyzed by a terminal thioesterase domain leading to the production of a methylated trilactone ring link to three catecholate moieties. This cyclic structure enables the sequestration of the metal atoms (Evans et al. 2011).

### *11.8 Non-thiotemplate NRPS*

These are antimicrobial NRPs biosynthesized through the non-template mechanism, and an excellent example of these are rhizocticins di- and tri-phosphopeptides. They have an L-2-amino-5-phosphono-3-cis pentanoic acid (APPA) connected to an arginine (rhizocticine A) (Hamano et al. 2013). The NRPs can also be substituted with an additional valine, isoleucine, and leucine to give rise to rhizocticine B, rhizocticine C, and rhizocticine D, respectively. The NRPs work through the integration into the target microbe, and their cleavage by the host cell peptides releases the fungitoxic L-APPA moiety that interferes with threonine metabolism in fungi cells. Strikingly, rhizocticine A can antagonize nematodes such as *C. elegans* (Hamano et al. 2013).

Rhizocticine compounds and two other peptides such as bacilysin and chlorotetain possess anticapsin and display a robust antibacterial property regulated by anticapsin moiety that inhibits the glucosamine-6-phosphate synthase (Borisova et al. 2010). This inhibition suppresses peptidoglycan, which is the main constituent of the bacterial cell wall (Boes et al. 2019). Anticapsin also inhibits the production of chitin and the fungal membrane's main proteins; bacilysin and chlorotetain exhibit antifungal activity against *A. fumigatus* or *C. albicans*. Bacitracin and mycobacillin are cyclic polypeptides, and bacitracin is dodecapeptides that have cyclic heptapeptides linked to a thiazoline ring and are active against Gram-positive bacteria in which they inhibit the bacterial cell wall biosynthesis by preventing the lipid carrier from re-entering the reaction cycle of peptidoglycan synthesis (Boes et al. 2019). Bacitracin antagonistic mechanism can also affect membrane functions, hydrolytic enzymes, or biosynthesis of ubiquinone precursors. Mycobacillin is an antifungal cyclic tridecapeptide altering the membrane of fungi like *A. niger* (Caulier et al. 2019)

## 12 Volatile Compounds

The PGPR produce a concoction of different volatile carbon-based solids and liquids of low-molecular-weight (<300 $gmol^{-1}$) signaling molecules with a low boiling point and high vapor pressure of 0.01 Kpa at 20 °C that readily sublimes into the gas-phase via vaporization at pressure making them useful signaling molecules for short and long distances (Fincheira and Quiroz 2018; Kanchiswamy et al. 2015; Pagans et al. 2006). The PGPR produce these volatile compounds via their direct and indirect pathways; the natural ways involve the release of phytohormones, and the indirect methods involve the release of volatiles that prevent pathogen attack by producing compounds such as alcohols, aldehydes, ketones, sulphides, HCN, siderophores, antibiotics, hydrolytic enzymes, and antioxidants (Goswami et al. 2016; Sagar et al. 2020). Microbial volatile compounds are a new and emerging field with application in medicine, biotechnology, and agriculture and basic science. The

recently developed mVOC database (v1) (http://bioinformatics.charite.de/mvoc.) has increased with microbes and in targeted microbial species information with data on emitted volatiles. In 2017, mVOC 2.0 database was presented with close to 2000 volatile organic compounds (VOCs) from close to 1000 microbial species (Lemfack et al. 2018). Volatile organic compounds such as nonal, benzothiazole, and 2-ethyl-1-hexanol are reported to 100% inhibit mycelial and sclerotial germination of *S. sclerotiorum* (Fernando et al. 2005).

## *12.1 Volatile Inorganic Compounds*

Volatile inorganic compounds synthesized by microbes such as carbon (IV) oxide, carbon monoxide, hydrogen gas, hydrogen cyanide, hydrogen sulphide, nitrogen gas, ammonia, and nitric oxide are mainly by-products of primary metabolism. Nitrogen-containing compounds are mostly produced by denitrifying bacteria; during the denitrifying process, NO is produced by nitric-oxide reductase or nitric-oxide synthase and has a wide range of antimicrobial activity. The NO is known to induce systemic-acquired resistance (SAR) in plants against phytopathogen such as *R. solanacearum* and *A. contario*; ammonia, a secondary metabolite from the catabolism of the amino acids L-aspartate, is reported to antagonize soil-borne oomycetes such as *Pythium* spp. Hydrogen cyanide from glycine metabolism directly poisons aerobic microbes by inhibiting metal-containing enzymes such as the cytochrome c oxidase activity in the respiration chain (Cherif-Silini et al. 2016).

Bacteria in anorexic soil (deep soils) produce VICs as hydrogen and hydrogen sulphide, and these VICs serve as electron acceptors, amino acid precursors, and antimicrobial metabolites. Hydrogen sulphide is produced by microbes from sulfate reduction or as a by-product of L-methionine and L-cysteine catabolism through direct cleavage of L-methionine or a transamination followed by reductive demethylations (Even et al. 2006; Schulz and Dickschat 2007). Hydrogen sulfide inhibits phytopathogens like *A. niger* and *Penicillium italicum* (Fu et al. 2014), and it is also shown to act as a bacterial defense mechanism against antibiotics (Shatalin et al. 2011). Strikingly, ammonia increases the resistance of Gram-positive bacteria to antibiotics, too (Bernier et al. 2011).

## *12.2 Nonvolatile Microbial Compounds*

Nonvolatile microbial compounds of antifungal antibiotics are compound of sizeable molecular weight such as phenazine, phenazine-1-carboxylic acid (PCA), 2-hydroxyphenacine, and pyrrolnitrin (Zhang et al. 2006) including several metabolites such as protease, lipase, HCN, and siderophores (Poritsanos et al. 2006)

### *12.3 Alcohols*

The PGPR produce alcohol-based volatiles such as nonanal, 2-ethyl-1-hexanol, which showed 100% inhibition of mycelial and sclerotial germination of *S. sclerotiorum* (Fernando et al. 2005). The success of strain PA23 as a biocontrol agent (BCA) against *Sclerotinia* on canola suggested that it might serve as a potential BCA against *Sclerotinia* infection of sunflower.

## 13 Conclusion

Plant growth-promoting rhizobacteria (PGPR) have multiple activities in terms of biofertilization, biocontrol, and bioremediation, all of which exert a positive influence on crop productivity and ecosystem functioning; encouragement should be given to its implementation in agriculture. The stable formulations of PGPR should be implemented in agriculture by replacing the use of chemical fertilizers, pesticides, and artificial growth regulators which have numerous side effects to sustainable agriculture. The PGPR promote plant growth not only by supplying nutrients to the plant but also by producing phytohormones, inducing stress resistance, or preventing pathogen-induced plant diseases. Thus, the development of the biofertilizer market and the promotion of bacterial inoculations in the field are an environment-friendly way to meet the worldwide need to raise crop yields. A piece of broad knowledge on the regulation of antifungal antibiotics can help in the development and employment of PGPR with improved efficiency and reliability. Besides, the molecular regulation of antifungal antibiotic and communication between the different species of PGPR and crop plants helps much when it comes to the selection of the compatible strains that can be released under some field conditions. Research about the communication between different types of antibiotic and its interaction with the abiotic environment, plant pathogens, and the plants is still at its beginning stage. However, the intensification of the research in the field can help in a better understanding level about the interaction of PGPR, pathogens, plants, and the abiotic environment around the rhizosphere. The most effective biocontrol agents which overcome the negative crosstalk in the environment around the rhizosphere are crucial in this development. Moreover, the knowledge on the antifungal antibiotic regulatory genes and the ecology of these PGPR their natural environment can help to introduce the non-indigenous strains. In addition to that, it also helps to select the biocontrol strains which can be suitable for different ecological conditions and for various species of the crops in other parts of the world.

## 14 Future Perspective

Studies revealing the antagonistic capacity of antifungal antibiotics substances such as subtilin, subtilosin, ericin, TasA, iturin, fengycin, surfactin, zwittermicin, 2,4DAPG, HCN, PCA, PCN, pholuteorin, etc. of these PGPR have been performed in vitro, and just a few of them have been evaluated in association with plants in the field and several inhibitory substances involved in pathogen antagonism have not been elucidated yet. Furthermore, all reports put together, we know only 1% of the bacterial diversity in a natural environment, leading us to assume that many other inhibitory substances remain unexplored (Cesa-Luna et al. 2020). It will be excellent to carry out more evaluation and assessment of the efficacy of antifungal antibiotic substances of PGPR in association with plant in the fields and do more isolation and characterization of PGPR antifungal antibiotic potential to discover more for a broader range of application. More molecular work is needed to be carried out to reveal specific genes and regulatory proteins or factors, biosynthesis pathways, responsible for the synthesis of these antifungal antibiotic substances and under which ecological conditions, associations, and interactions in the rhizosphere of plants.

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# Chapter 12
# Extreme Environments as Potential Sources for PGPR


**Meriam Bouri, Samina Mehnaz, and Fikrettin Şahin**

**Abstract** Extreme environments represent unique ecosystems with conditions inhospitable for life, on the edge of temperature, hypersalinity, pH extremes, pressure, dryness, etc. Organisms able to thrive in such hostile habitats are called extremophiles. Due to biodiversity and adaptations of extremophiles to different stresses, consideration for their potential in several industrial processes including biotechnology, food production, and medical and pharmaceutical sectors has increased. Recently, extreme environments gained an importance as potential sources of plant growth-promoting agents for the enhancement of crop health and growth in sustainable agriculture. The main purpose of this chapter is to point out how microorganisms living under extreme conditions could be applied in agriculture for plant growth enhancement. Therefore, an overview of extreme environments and extremophiles is devoted essentially to biodiversity and successful stories of extremophile applications. Then, approaches regarding the use of microorganisms from extreme environments for agriculture purposes are analyzed, before going to overreported mechanisms and aspects of extremophiles in plant growth amelioration, especially under abiotic stresses. Although plant-beneficial values of microorganisms from extreme environments are recognized in this chapter, challenges and perspectives of their application in agro-ecosystems are also discussed.

**Keywords** Abiotic stresses · Extreme environments · Extremophiles · Plant growth promotion · Sustainable agriculture

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## 1 Introduction

The role of the telluric microbial communities in the plant growth, nutrient management, and biocontrol activity is well accepted. Plant-associated beneficial bacteria colonizing the rhizosphere were first defined by Kloepper and Schroth (1978) as plant growth-promoting rhizobacteria (PGPR). Afterward, the term has been modified to plant growth-promoting bacteria (PGPB) to include other strains that are non-rhizospheric in origin (Andrews and Harris 2000). Some rhizospheric fungi were also reported to have plant growth-promoting (PGP) attributes. However, the mechanisms of association with plant and growth enhancement features are completely different than bacterial ones. Despite the fact that most studies on plant growth biostimulation have been devoted to bacteria and/or rhizobacteria, the whole plant microbiome with complex multi-interactions is involved in the plant fate. PGP microbes deplore different mechanisms that may improve plant growth including nutrient flux management, soil fertility improvement, and extracellular molecules modulating such as hormones, secondary metabolites, antibiotics, and various signal compounds. Therefore, many researchers have been interested in the use of these microorganisms and their compounds as biostimulants in sustainable agriculture to enhance the crop production. As selection and recruitment of the plant microbiome are mediated by the specific profile of the plant rhizobial disposition, agro-ecosystems have been the main source of PGPR/PGPB isolation for many studies. Based on the specificity of the consortium between plants and their microbiome, rhizosphere of various crops was so far mined for plant-beneficial bacteria. Recently, particular attention was accorded to the exploration of extreme environments for PGP microorganisms.

Microbes inhabiting biotopes under high salinity, cold temperatures, dry conditions, etc. are endowed with specific adaptive features which allow them to thrive under harsh conditions. Using microbes from extreme habitats/niches for abiotic stress alleviations in agriculture was the key strategy of this new PGP perspective. There has been significant interest in finding life in extreme environments. The adaptive mechanisms developed by these organisms, called also extremophiles, in order to survive under extreme conditions, were found to be of great importance for multidisciplinary studies. Interestingly, extremophiles showed specific molecular strategies and versatile metabolic diversity beyond the paradigms of modern biology. The stability and peculiar activity of some extremophilic biomolecules such as enzymes made them useful alternatives to labile mesophilic molecules. Although extremophiles were investigated mainly for biotechnological purposes, over the last few decades, these specific biotopes have been the pivot of different branches of life sciences including phylogeny, microbial ecology, astrobiology, and agronomy.

In this chapter, characteristics of extreme environments, their microbial diversity, their pertinent applications, and their importance as sources of plant-beneficial microorganisms will be briefly reviewed. Constrains related to PGP extremophile application will be also discussed to draw up future strategies for the optimization of their efficacy in the enhancement of sustainable crop production.

## 2 What Is an Extreme Environment?

In ecology, the term "extreme" commonly refers to unfavorable environmental factors that depress the ability of organisms to function (Li et al. 2014). An extreme environment is defined as a habitat under extreme conditions that are considered inhospitable for life, e.g., temperature, pressure, radiation, and accessibility to different energy sources are beyond the optimal range. Ecosystems such as volcanoes, deep-see vents, very arid deserts, geographical poles, upper atmosphere, and outer space are considered as extreme environments. The organisms surviving under these circumstances are known as extremophiles and are often considered as the result of long-term evolution. In fact, life in extreme environments was thought to be impossible, until not very long ago. Thus, physiologists theorize that extremophiles have experienced a longtime natural selection to get adapted to their living conditions (Garland and Carter 1994). Furthermore, a variety of extremophiles have shown that they not only can tolerate hostile conditions but that they may also require those conditions for survival.

Extreme environments can be classified depending on the extreme physicochemical conditions (Table 12.1).

According to their extreme environments, organisms can be called thermophiles (hot environments), hyperthermophiles (very hot environments), psychrophiles (cold environments), acidophiles (acidic environments), alkaliphiles (alkaline

**Table 12.1** Different types of extreme environments (Gómez 2011)

| Type of extreme environments | | Conditions | Examples |
|---|---|---|---|
| Extreme temperature | Cold | Below −5 °C | Lake Vostok, geographical poles |
| | Hot | Higher than 45 °C | Geothermal areas of Yellowstone (USA), some regions of Iceland, and Kamchatka (Russia) |
| Extreme pH | Acidic | Below 5 | Rio Tinto (Iberian Pyrite Belt, SW Spain) and Iron Mountain in California (USA) |
| | Alkaline | Above 9 | Soda lake of Magadi (Kenya), Mono Lake in California (USA), and Saltpans (e.g., Cappadocia, Turkey) |
| Extreme ionic strength | | Ionic concentration higher than seawater, >3.5% | The Dead Sea (Israel), the Great Salt Lake (USA), and the Santa Pola saltern (Spain) |
| Extreme pressure environments | | Extreme hydrostatic or lithostatic pressure | Aquatic habitats at depths of 2000 m or more and deep-subsurface ecosystems |
| High-radiation environments | | Abnormally high radiation doses | Deserts, the top of high mountains, and in the surface of ISS (International Space Station) |
| Xeric environments | | Dry habitats with extremely limited water | Cold and hot deserts |
| Oligotrophic environments | | Low levels of nutrients to sustain life | Deep oceanic sediments, polar ice, and the deep subsurface |

environments), barophiles (extreme pressure environments), and halophiles (saline environments).

Extremophiles may be divided into two main categories: extremophilic organisms which require extreme condition(s) to grow and extremotolerant organisms which can tolerate extreme conditions and keep surviving under critical physicochemical values, despite the absence of their "normally" required growth conditions. Some organisms can develop in habitats under different extreme physicochemical conditions; they are called polyextremophiles. For example, organisms living in deep oceans are generally exposed to cold, high pressure, and low nutrient content as well.

Extreme environments can be habitats for all three domains of life, i.e., bacteria, archaea, and eukarya. Nevertheless, most extremophiles are microorganisms including some eukaryotes such as algae, fungi, and protozoa (Rampelotto 2013).

## 3 Microbial Diversity in Extreme Environments

Microbial diversity can be approached from very different perspectives, including not only the phylogenetic diversity but also molecular, functional, and dynamic points of view. Phylogenetically, extremophiles show a very high diversity which makes studies very complex. Some extremophiles belong to well-distinguished orders or genera, whereas others can be classified as non-extremophiles in same orders or genera. This is the case of some psychrophiles or barophiles, for which members may be found broadly dispersed in the phylogenetic tree of life (Rampelotto 2013).

Generally, populations of extreme environments are dominated by archaea. In fact, this third domain of life was in some part discovered due to the first studies on extremophiles, with deep consequences for evolutionary biology. Archaea are considered to be the best organisms qualified in adapting to the most extreme conditions. For example, the most acidophilic microorganisms belong to the archaea genus *Picrophilus* (e.g., *Picrophilus torridus*) with the ability to develop at 0.06 pH, while the archaeal *Methanopyrus kandleri* strain 116 can grow at 122 °C, considered as the highest extreme temperature for life (Rampelotto 2013).

Extremophile bacteria are dominated by cyanobacteria which often form microbial mats with other bacteria, from Antarctic ice to continental hot springs. In fact, living in aggregate seems to help bacteria to increase their resistance toward harsh conditions by aiding the community as a whole, to face the environment. This aggregation is expressed mainly in biofilm formation defined as the coexistence of multiple species of bacteria that assembled together as a whole community (Donlan and Costerton 2002) by the secretion of extracellular polymeric substances (EPS). This population tends to act as a harmonious bacterial community despite being crammed into a tiny limited space. Among this community, resistant cells within the

biofilm would protect the sensitive ones and thereby enhance the resistance level of the population. In this regard, biofilm is highly considered as a bio-architectural mechanism displayed by bacteria in general, and extremophiles in particular, to overcome environmental harsh conditions (López et al. 2010; Msarah et al. 2018).

For a long time, it was thought that life forms in extreme environments were restricted to prokaryotes, until a wide variety of extremophile fungi has been discovered, recently, across a broad range of severe conditions, especially in hypersaline and extremely cold environments (Selbmann et al. 2013; Gunde-Cimerman and Zalar 2014). The most acidophilic of all microorganisms are filamentous fungi (*Cephalosporium* sp., *Aconitum cylatium*, *Trichosporon cerebriae*) which have all been reported to grow at pH 0 (Schleper et al. 1995). Alone or in symbiosis with cyanobacteria or algae, fungi can survive in acidic and alkaline conditions, hot and cold deserts, snow and ice, dry rock surfaces, deep oceans, and hypersaline and metal-rich water in mining regions, but not in hyperthermophile environments.

Although extremophile fungi are the most frequently phylogenetic lineage of eukaryotes encountered in extreme environments, some microorganisms like yeasts, algae, Rotifera, several species of Nematoda, and some arthropods and Tardigrada (water bears) have been also isolated from extreme environments (Erdmann and Kaczmarek 2017). Yeasts like *Rhodotorula* spp. and some *Candida*, *Cryptococcus*, and *Purpureocillium* strains can develop in acid mine drainage waters (Gross and Robbins 2000; Oggerin et al. 2013). Red unicellular algae of the order *Cyanidiales* have been also isolated from hot acidic aquatic systems (Brock 1973). Tardigrades are the most impressive polyextremophilic eukaryotes. Also known as water bears, Tardigrades are microscopic invertebrates which have been found everywhere, from the top of mountains to the depth of sea, from tropical rainforests to the Antarctic, and volcanoes and muds. They undergo hibernation to survive extreme temperatures ranging from −272 to 151 °C, extreme pressure of 6000 atm, radiation exposures (X-rays and gamma rays), and extreme dehydration (vacuum-like conditions) (Rampelotto 2013). Tardigrades have even survived exposure to outer space and have become a perfect model organism for space research (Erdmann and Kaczmarek 2017).

It has been widely admitted that the more extreme the environmental conditions of a niche, the lower the diversity of organisms. However, with the development of molecular biology techniques, particularly new high-throughput DNA sequencing technologies, extreme environments revealed unexpected microbial ecosystems with very high level of genetic diversity. In addition to profound achievements of sequencing techniques in phylogenetic and evolution perspectives, molecular strategies and transcriptomic studies highlighted some specific biochemical pathways and peculiar biomolecules of extremophiles with different potential uses in many fields.

## 4 Relevant Applications of Extremophiles

Since the discovery of life in extreme environments, extremophiles have become a pivotal issue of research for many areas (Fig. 12.1). Understanding adaptive mechanisms of microbes to extreme environments is of prime importance from both evolutionary and ecological perspectives. Advances in DNA sequencing have extended our insights into molecular evolution and adaptive landscapes that occur in microbiome under successive selections by harsh condition(s) (Blum et al. 2016). Molecular biological evidences about prokaryotes have strongly suggest that hyperthermophiles lie close to the last common ancestor of all terrestrial life (Rampelotto 2013). Metagenomics and comparative genomics have underlined the importance of genome plasticity, including codon bias, nucleotide skew, and horizontal gene transfers (HGTs), in the evolutionary adaptation of microorganisms to extreme conditions, through gain and loss of functions (Zeldovich et al. 2007; Hemme et al. 2010; Mehta and Satyanarayana 2017). Adaptive abilities of extremophiles allow them to survive under hostile environmental conditions. Exploring ecological systems in extreme regions is very important, as they can be models to explore relationships between diversity and environmental factors variations and enhance our knowledge about ecological problems related to some particular natural systems like the change of green cover and glacial retreat in some mountains, drying lagoons, heavy-metal-contaminated sites, etc., especially in facing the global warming scenario. For example, microorganisms of cold regions show a broad-spectrum degradation activity of complex hydrocarbons (such as petroleum products) under low-temperature environments, and they were identified as potential agents in biodegradation (Dhakar and Pandey 2020). Moreover, survival mechanisms of hyperalkaline bacteria opened new perspectives for bioremediation of hyperalkaline pollutions as well

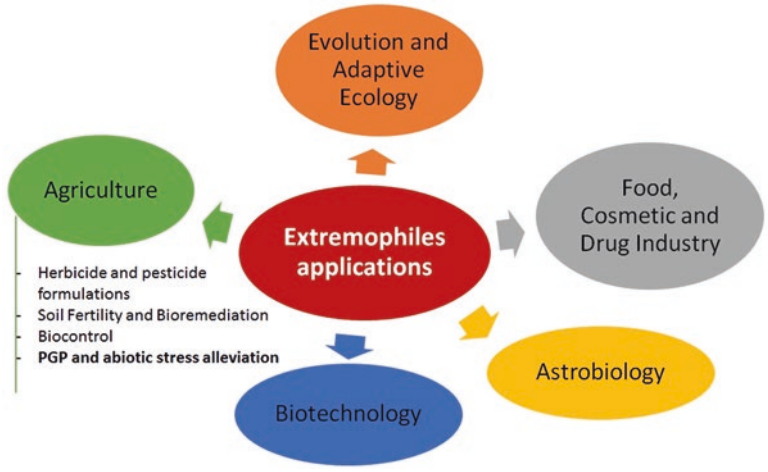


**Fig. 12.1** Main areas of extremophile applications

as the detection of microbial life in serpentinization-like conditions on Earth and other planets (Ohlsson et al. 2019). Microbial ecosystems of extreme environments like hot springs, hydrothermal vents, and other sites under heats of volcanic activities in terrestrial or marine zones may be analogous to potential life forms adapted to extraterrestrial environments. Studying the organisms from Earth's upper atmosphere provides important clues in Astrobiology. In this regard, some extremophiles can be considered as model organisms to explore the existence of life in outer space, besides that they are best candidates for exposure to outer space conditions (Thombre et al. 2020).

From biotechnological perspectives, adaptation mechanisms of extremophiles turned them into a top spot for the mining of relevant bioactivities as extraordinary alternatives for standard biological processes and metabolites. Metabolisms of extremophiles can be based upon methane, sulfur, and even iron pathways besides the conventional photosynthesis. The resulted molecules are of high stable activity at extreme conditions that makes them good alternatives to standard mesophilic molecules. Some extremophile enzymes (extremozymes) remain catalytically active under nonstandard conditions of temperature, salinity, pH, and/or solvent conditions. Thus, they have been widely used in industrial biotechnology. This is particularly the case of the DNA polymerases isolated from the thermophiles *Thermus aquaticus*, *Pyrococcus furiosus*, and *Thermococcus litoralis*, otherwise known as Taq (Tindall and Kunkel 1988), Pfu (Lundberg et al. 1991), and Vent (Mattila et al. 1991), respectively. Other success stories of biotechnological applications of extremophiles and their molecules include various extremozymes used in the process of making biofuels (Barnard et al. 2010), carotenoids used in the food and cosmetic industries (Oren 2010), cold-active beta-galactosidase to make lactose-free milk (Coker and Brenchley 2006), the production of new drugs (Herbert 1992), organisms used in the mining process (Johnson 2014), or even in the production of electricity (Dopson et al. 2016). Applications of extremozymes in agriculture and pharmaceutical, textile, and food and beverage industries are potentially based on economic and environment-friendly advantages. For example, in agriculture, biosurfactants from extremophile microorganisms are used as adjuvants in herbicide and pesticide formulations. Also, they can be applied to improve the soil quality in arid zones by enhancing the soil structure and wettability, in addition to the bioremediation of the soil and to the biocontrol of phytopathogens (Sachdev and Cameotra 2013).

Microbe-based biotechnology in agriculture has also opened new possibilities concerning the application of some extremophiles to the soil for the enhancement of crop production through the plant growth-promoting (PGP) and/or the biological control of plant pathogens (Orellana et al. 2018). Over the last two decades, particular attention was attributed to the relation between some extremophile microorganisms and plants. Besides their potential biocontrol activities, extremophile bacteria are likely to play important roles in water management and nutrient flux between the soil and plants, especially under deficiency stress. However, their exploitation as plant growth-promoting bacteria (PGPB) or plant growth-promoting rhizobacteria (PGPR) may face ecological compatibility issues.

## 5 Approaches of Extremophile Use in Plant Growth Promotion

It has long been known that plants can shape the microbiome in their rhizospheres and within their roots. Furthermore, under stress conditions, plants can acquire specific microbial species to tolerate stress and therefore grow and prosper in a given ecosystem. Advances in plant-bacterial interactions showed that inoculating plants with plant growth-promoting rhizobacteria (PGPR) can be an effective strategy to enhance the crop growth and improve culture tolerance to different stresses (e.g., drought, heat, and salinity).

Nevertheless, using autochthonous or allochthonous PGP agents in agriculture is still a controversial issue. A problem of cross-compatibility was recorded in some rhizobacteria which exhibit variable PGP activity limited in a range of niche application (crops, soil types, and environmental conditions). Therefore, the application of extremophiles for PGP purposes may require additional considerations regarding some differences in ecological contexts.

### *5.1 Biotopes and Ecological Niches of Isolation*

According to several ecological studies, stress tolerance enhancement effect of plant-associated microbiomes is the result of a coevolution under habitat-specific harsh conditions (Riva et al. 2019). For example, drought stress was reported to shape the structure of plant-associated microbial communities (Koberl et al. 2011; Kavamura et al. 2013). Therefore, biotopes under specific environmental stress may hold beneficial symbiont candidates that could help plants to resist the same stress conditions (Patel et al. 2016). Based on this strategy, several studies have been turned on the investigation of extremophiles from soils under major stresses related to agricultural problems such as drought, salinity, cold stresses, and heavy metal contamination. Cold regions, arid and semiarid areas, alkaline/acidic environments, and industrial soils were extensively mined during the last few years in the research of microbes with plant growth-promoting attributes (Yadav 2017). Based on the same principle, heavy-metal-contaminated regions were investigated for potent PGPR that can be used in soil phytoremediation (Hao et al. 2014; Rangel et al. 2017; Singh et al. 2019). With recent advances in sequencing technologies, the diversity of extremophiles in soil was found to be high enough to exceed expectations. Nevertheless, many of them are from Bacteria Domain and may belong to both specific and ubiquitous genera. In Antarctic soils, psychrotroph species of *Psychrobacter* and *Planococcus* coexist with other widely dispersed genera like *Chryseobacterium*, *Brevundimonas*, and *Paenibacillus* that could tolerate low temperatures (Wery et al. 2003). Some of these Antarctic microorganisms have also been reported for their capacities to improve the physiological performance of some plant species other than their native host plants (Fardella et al. 2014). Surprisingly,

soils of arid zones, like Northern Arizona (Dunbar et al. 1999), Arizona Sonoran Desert (Nagy et al. 2005), and Rocky Mountains (Nemergut et al. 2005), were specifically predominated by members of *Acidobacteria*. On the other hand, most of the rhizospheric bacteria from plants under desert farming conditions exhibit stress resistance and PGP features that may confer a certain level of tolerance to their host plants (Marasco et al. 2012). Moreover, bacteria associated with *Salicornia* plants grown under hypersaline biotopes in Tunisia showed potential PGP activity and showed multiresistance capacity to high-temperature, osmotic, and saline stress (Mapelli et al. 2013). Therefore, plants under extreme conditions are considered as ideal habitats for potential PGP candidates that can be applied in sustainable agriculture to overcome water shortage, salinity, cold, and other problems in arid lands.

## *5.2 Plant-Associated Extremophiles*

Microbes associated with crops are named plant microbiomes and could be classified into three groups, e.g., rhizospheric, phyllospheric (epiphytic), and endophytic, according to the region they colonized. The plant microbiomes actively contribute to the maintenance of global nutrient balance and ecosystem function (Yadav et al. 2017). Among these microbes, bacteria and fungi are the most abundant, and some of them have a key role in the enhancement of plant growth. Therefore, they are referred to as biostimulants or plant growth-promoting agents or plant growth-promoting rhizobacteria (PGPR). Although PGPR are effective at improving plant stress tolerance (Etesami and Beattie 2017; Etesami 2018), their ability to transform nutrients and increase plant tolerance to abiotic stress is influenced by environmental conditions (Giongo et al. 2008). For example, phosphorus solubilization by some microorganisms is strongly related to environmental conditions and especially affected by stress factors (Yoon et al. 2001; Sánchez-Porro et al. 2009). Moreover, some PGPR were reported to lose their plant growth-promoting capacity in vitro under saline conditions (Upadhyay et al. 2009). Furthermore, rhizobacteria from saline habitats showed better efficacy in enhancing plant tolerance to salt stress than PGPR from nonsaline habitats (Paul and Nair 2008; Egamberdieva and Kucharova 2009; Khan et al. 2016). Thus, mining halophyte-associated microorganisms that are expressing both salt tolerance and PGP traits could be promising prospects for alleviating salinity stress in sustainable agriculture (Zhu et al. 2011).

Plant-associated extremophiles showed high diversity that covers the three domains of life: archaea, bacteria, and eukarya of different phylum/groups, e.g., *Actinobacteria*, *Ascomycota*, *Bacteroidetes*, *Basidiomycota*, *Crenarchaeota*, *Euryarchaeota*, *Firmicutes*, and *Proteobacteria* (α/β/γ/δ). Similar to plants growing in extreme environments (extremophytes), extremophilic rhizobacteria also evolve various strategies to survive under harsh conditions. Some microbes isolated from extremophytes had the ability to help in plant growth and adaptations under harsh conditions of temperatures, salinity, pH, and drought stresses. Hence, these microbes have been the subject of many studies involved in the assessment of crop

enhancement attributes in sustainable agriculture. Likewise, the root of halophytes was reported as a potential reservoir for the study of salt-tolerant bacteria, which ameliorate the salinity tolerance of these plants and stimulate their growth under saline stress (Kumar et al. 2019; Rodríguez-Llorente et al. 2019; Alishahi et al. 2020). Nevertheless, the application of extremophiles for agricultural purposes may still be limited to environment or/and species dependence (Etesami and Beattie 2018; Riva et al. 2019). Indeed, some bacterial strains were reported to specifically promote plant growth only under water stress but remain ineffective under optimal irrigation conditions (Rolli et al. 2014; Chen et al. 2017).

## *5.3 By-Products of Extremophiles*

From chemical perspectives, unusual microbes represent new biochemical resources with potential medicinal, industrial, and agrochemical applications. To this end, many studies were aimed to investigate extreme habitats for producers of novel interesting bioactive metabolites (Frisvad 2005). The application of extremophilic microbes in agrobiology includes difficulties associated with environment context differences. Therefore, the application of metabolites from extremophiles rather than uses of microorganisms themselves, represents a promising alternative in sustainable agriculture.

Extremozymes, exopolysaccharides (EPS), biosurfactants, biopolymers, and peptides, from extremophilic/extremotolerant microorganisms, have great economic-industrial potential (Raddadi et al. 2015). In agriculture, biosurfactants could substitute chemical adjuvants in herbicide and pesticide formulations, enhance bioremediation of soils, stimulate plant defenses and control phytopathogens, and ameliorate the soil quality in arid regions by improving wettability in arid zone soils (Sachdev and Cameotra 2013). Biosurfactant-producing genera like *Pseudomonas*, *Bacillus*, *Flavobacterium*, and *Rhodococcus* were reported from saline and arid soils (Bodour et al. 2003; Gesheva et al. 2010; Simpson et al. 2011).

In addition to biosurfactants, some enzymes isolated from arid extreme environments were revealed efficient in bioremediation of polluted soils (Mapelli et al. 2012; Soussi et al. 2016).

Extremophilic fungi (Fenice and Gooday 2006; Zhao et al. 2018), yeast (Vero et al. 2013; Sangorrín et al. 2014), bacteria (Núñez-Montero and Barrientos 2018), and especially *Actinobacteria* (Bibi et al. 2018; Santos et al. 2020) were also reported to be excellent reservoirs for antibiotics and some secondary metabolites with strong antimicrobial activities against a broad spectrum of phytopathogens. These antagonistic compounds, mainly from psychrophilic and halophilic microbes, could be used in the formulation of phytosanitary products to reduce the use of synthetic agrochemicals. Even though numerous extremophiles have been reported as attractive sources for new molecules and different bioactivities, their anti-phytopathogenic substances are still not well explored.

## 6 Key Features of Plant Growth-Promoting Extremophiles

In general, PGPR/PGPs exhibit beneficial effects on plants through direct and indirect mechanisms including the improvement of nutrient uptake and assimilation, the alleviation of abiotic and biotic stresses, the amelioration of soil texture, and induction or modulation of extracellular molecules secretion (e.g., hormones, secondary metabolites, antibiotics, and various signal compounds). Abiotic stresses such as water shortage and salinity of soil and/or water are among the most important challenges nowadays for agriculture. Regarding continuous decrease of agricultural soils and fertile lands due to global climate changes, advances in sustainable and eco-friendly agriculture propose several strategies for future exploitation of the affected lands. The use of PGPs in modern agriculture to cope with abiotic stresses is among the most prospering prospects. Although extremophilic PGPR have been usually exploited for their capacity to enhance abiotic stress tolerance in plants, their PGP action can be attributed to other traits including enhancement of soil fertility and biocontrol.

### *6.1 Abiotic Stress Tolerance*

Plants growing under naturally prolonged abiotic stresses such as water limitation, salt accumulation, cold/hot temperatures, and unfavorable acid/alkaline soils have also developed specific physiological and molecular strategies to survive under harsh conditions. In response to these growth-limiting factors, a special root-associated microbial community is enrolled by the plant in order to benefit from their PGP traits. Plant microbiome undergoes adaptation to harsh environmental conditions with their hosts over long evolutionary periods of time and likely contribute to stress adaptation in plants (Fig. 12.2). Indeed, bacterial capacities to solubilize phosphorus, form biofilms, and tolerate high salt concentrations and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity were reported to be significantly higher in stressed environments (Timmusk et al. 2011). Since the ability of microorganisms in extreme environments to resist abiotic stress is of some use to their host plants, extremophilic microbes have been harnessed to be applied for agricultural purposes in order to enhance crop production in stressed lands.

#### 6.1.1 Drought Stress

Drought is the major challenge limiting world agriculture production. Arid lands are characterized by various harsh conditions including soil deficiency in water and nutrients, hypersalinity and soil alkalinity, low rate of infiltration low precipitation, and strong sunlight leading to high temperatures and UV irradiation (Whitford 2002; Ortiz et al. 2000). All organisms living under these extreme conditions,

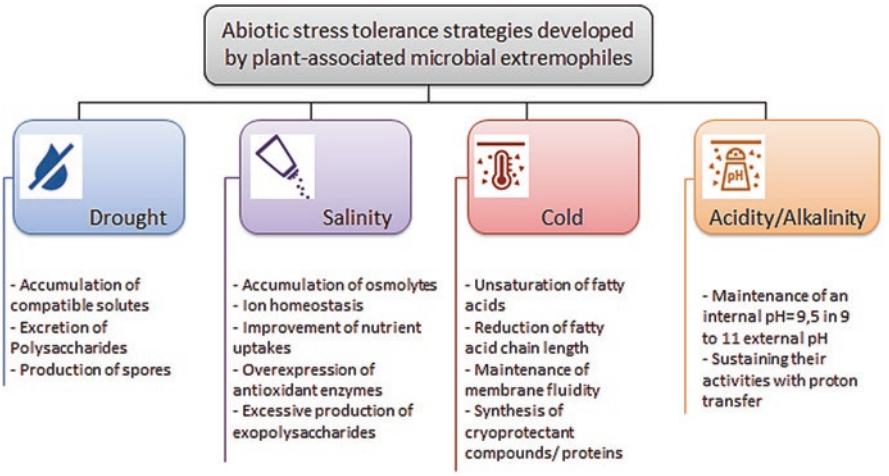


**Fig. 12.2** Main adaptive strategies developed by microbes associated with plants under different abiotic stresses

including plants and bacteria, develop complex strategies to survive abiotic stresses. Bacterial communities of lithic substrates in arid lands and deserts across the world were dominated by cyanobacteria (DiRuggiero et al. 2013), then *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Chanal et al. 2006; Jorquera et al. 2012; Neilson et al. 2012; An et al. 2013).

To resist drought, soil bacteria employ a variety of physiological mechanisms involving the accumulation of compatible solutes, the excretion of exopolysaccharides, and the production of spores (Conlin and Nelson 2007; Schimel et al. 2007; Allison and Martiny 2008; Bérard et al. 2015). Some of these strategies can also be adopted by plants to cope with drought. Indeed, compatible solutes like proline and glycine betaine that help bacteria to withstand drought stress were also reported in plants thriving under drought stress (Ngumbi and Kloepper 2016).

Arid soils are likely favoring the selection of bacteria able to alleviate plant drought stress (Marasco et al. 2012, 2013; Shelef et al. 2013). Therefore, the possibility of their application in arid agricultural systems was evaluated by many studies. For example, the inoculation of maize plants with an indigenous drought-tolerant *P. putida* strain FBKV2 from stressed ecosystems exhibited better growth in terms of shoot, root length, and dry biomass (Vurukonda et al. 2016). Another study reported plant growth-promoting activities of *Streptomyces* strains, isolated from soils of Moroccan desert, on maize plants in terms of N fixation, solubilization of P and K, and production of indole acetic acid (IAA) and siderophores (Nafis et al. 2019).

Marasco et al. (2013) observed that pepper plants treated with bacterial isolates from plants cultivated under desert farming were more tolerant to drought stress, compared with the untreated control. This amelioration is likely mediated by bacterial stimulation of plant root systems (up to 40%) and enhancement of plant ability

to uptake water from dry soils. Furthermore, arid environments are likely still not enough explored and must be considered as potential sources for new species. As a matter of fact, a novel species *Pantoea alhagistrain* LTYR-11ZT was isolated from surface-sterilized leaves of *Alhagi sparsifolia* Shap. (Leguminosae) from Taklamakan Desert in the northwest of China. Strain LTYR-11ZT led to increased accumulation of soluble sugars, decreased accumulation of proline and malondialdehyde (MDA), and decreased degradation of chlorophyll in leaves of drought-stressed wheat plants and thus promoted their growth (Chen et al. 2017).

Although these studies were conducted in the laboratory and require validation in the field, they provided initial evidence in terms of cross-compatibility between PGP extremophiles and different plant models, other than the one of original isolation, at least on a short-term. Thus, exploring extremophiles from arid and/or semi-arid environments can be a very useful approach for the development of bioinoculants for drought stress management in crops.

### 6.1.2 Salinity Stress

Soil salinity is an important limiting factor in agriculture especially in arid and semiarid regions of the world which are increasing by an average of 10% per year (Abbas et al. 2019). Climate change and human activities such as saline water and chemical fertilizers used in agriculture are some other sources of soil salinity.

Under salinity stress, reactive oxygen species (ROS) are accumulated in plant tissues, and consequently, the photosynthetic apparatus and cellular membranes are being damaged (Bose et al. 2014; Oukarroum et al. 2015). In response to salinity stress, halophytes and halotolerant plants developed several mechanisms such as excluding salts from roots and shoots, synthesis of the compatible solutes (e.g., glycine betaine and proline), and recruitment of microbial communities with salt alleviation abilities. The rhizosphere of halophytes is a rich source of osmotic stress-tolerant bacteria. Rhizobacteria isolated from saline habitats have been reported to be more efficient at enhancing plant tolerance to salt than PGPR isolated from non-saline habitats (Etesami and Beattie 2018). Besides the general mechanisms of salt stress attenuation in plants (such as production of IAA and ACC deaminase), halophilic PGPR developed their own strategies to help plants in salinity tolerance, including accumulation of osmolytes, ion homeostasis, improvement of nutrient uptakes, overexpression of antioxidant enzymes, and excessive production of exopolysaccharides (EPs) (Saghafi et al. 2019). These exopolysaccharides are used to be involved in biofilms to help bacterial binding to surfaces (Mah and O'Toole 2001). Nevertheless, biofilms were reported to not only protect the microbe from the environmental stresses but also to maintain moisture in roots and protect the plants from infection by soil borne pathogens (Mu'minah et al. 2015).

*Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, and *Achromobacter* were the most reported genera for enhancing the productivity of diverse crops under salt stress (Sharma et al. 2016; Singh and Jha 2016; Sarkar et al. 2018; Fazeli-Nasab and Sayyed 2019; Kusale et al. 2021). Various halophytes and

halotolerant plants such as *Suaeda* spp. (Alishahi et al. 2020), *Phragmites australis*, *Sesbania cannabina*, *Chrysanthemum indicum*, *Metaplexis japonica*, *Suaeda glauca*, *Lycium* Linn, *Spartina alterniflora*, *Artemisia* Linn (Gong et al. 2018), mangrove plants (Ramadoss et al. 2013), *Salicornia brachiate* (Jha et al. 2012), *Acacia* spp. (Boukhatem et al. 2012), *Sesuvium portulacastrum* (Anburaj et al. 2012), *Rosa rugosa* (Bibi et al. 2011), *Salicornia bigelovii* (Rueda-Puente et al. 2010), *Halocnemum strobilaceum* (Al-Mailem et al. 2010), and *Avicennia marina* (El-Tarabily and Youssef 2010) were mined for PGP-associated bacteria. The PGP activities of the majority of these bacteria were also expressed, under salt stress, with other plant species different from their original hosts. Furthermore, halophilic and halotolerant bacteria were able to perform PGP activities under different abiotic stress rather than saline conditions. Likewise, bacterial microbiome of *Salicornia* plants grown under hypersaline ecosystems in Tunisia displayed various PGP traits and higher root colonization under salinity and drought condition as well (Mapelli et al. 2013).

Outstandingly, bacteria associated with marine species such as coral (Ocampo-Alvarez et al. 2020) and mangroves (Ramadoss et al. 2013; Suksaard et al. 2017; Gong et al. 2018) were also able to enhance the growth of crop plants. Like their terrestrial homologues, some of the marine strains have shown some PGP activities including phytohormones production, nitrogen fixation, phosphate solubilization, siderophores production, and ethylene overproduction decreasing via the enzyme ACC deaminase (Rashad et al. 2015). Taken together, these data indicate that marine environments and coastal salt marsh habitats can be considered as important sources of new undescribed PGP species and their associated secondary metabolites.

### 6.1.3 Cold Stress

Among the various abiotic stresses that crops encounter, cold stress is the major environmental factor that limits agricultural yields. Plants are affected by cold stress at different levels including poor germination, stunted seedlings, yellowing of leaves (chlorosis), reduced leaf expansion, and wilting and may lead to the death of tissue by necrosis. To cope with cold stress, plants involve specific signaling and regulation of the transcriptome managed by cold-regulated genes (Yadav 2010). As for microorganisms, cold tolerance mechanisms include unsaturation of fatty acids, reduction in the average fatty acid chain length, maintenance of membrane fluidity, synthesis of several cryoprotectant compounds, cold acclimation proteins (Caps), cold-shock proteins (Csps), ice nucleators and antifreeze proteins, cold-adapted enzymes, and RNA degradosomes are some of the cold tolerance mechanisms (Mishra et al. 2010). Several studies explored the use of microbial strains to alleviate the impact of cold on plants. Some psychrotrophic/psychrophilic microorganisms were described to have potential PGP activities such as the production of stimulatory phytohormones, phosphate solubilization, IAA production, siderophore secretion (Katiyar and Goel 2004), and antagonism toward soil-borne plant pathogens (Misaghi et al. 1982) that make them useful in agricultural system under

low-temperature environments (Trivedi et al. 2007; Yadav et al. 2015). Moreover, some studies pointed out the fact that bacterized plants showed significantly higher levels of starch, proline, and phenols, comparing to the non-inoculated controls. These increases correlated with root growth enhancement and augmentation of dry biomass (Barka et al. 2006; Mishra et al. 2009). Although *P. putida* UW4 ACC deaminase-producing strain was reported to promote canola plant growth at low temperature under salt stress (Cheng et al. 2008), the role of ethylene and ACC-producing bacterial strains in plant growth promotion under cold temperature conditions still needs to be proved (Mishra et al. 2010).

Given the importance of temperature in decomposition processes, the identification of cold active decomposing microorganisms, in soils, could be another interesting perspective. According to Mishra et al. (2010), succeeding the identification of potential decomposer consortia that retain their enzymatic potential at lower temperatures could have an immense application to cope with cold stress in agriculture.

Apart from degradation, cold regions inhabiting microbial communities were reported as being able to improve crop growth at low to extremely low-temperature conditions (Yadav and Sayyed 2019). Psychrotrophic/psychrophilic microorganisms from Himalaya showed potential in plant growth promotion activities. *Rhodococcus* (Trivedi et al. 2007), *Pseudomonas* (Suyal et al. 2014), *Stenotrophomonas* (Kumar et al. 2019), *Stagonosporopsis*, *Bionectria*, *Aspergillus* (Arora et al. 2019), and *Penicillium* (Pandey et al. 2008) were the most described genera as promising PGP candidates isolated from Himalayan cryosphere.

### 6.1.4 Acid/Alkaline Stress

Soil quality deterioration is becoming a serious issue for agriculture worldwide. Although soil alkalinization is always correlated with soil salinization, drawbacks of soil alkalinization due to $NaHCO_3$ and $Na_2CO_3$ on crops sometimes become more serious than the problem of soil salinization caused by the neutral salts, such as NaCl and $Na_2SO_4$ (Shi and Sheng 2005). About 3% of the world's total geographical area is recognized as saline-sodic soils and is particularly common in arid and semiarid regions (Singh 2016). Permeability of alkaline soils is decreased by $Na_2CO_3$ and $NaHCO_3$ salts which impact plant growth. Soil alkalinity can generate many damages in plants at different levels such as root system, nutrient uptake, ionic balance, relative water content, photosynthetic pigments, total soluble sugar, etc. that eventually may lead to plant mortality (Dixit et al. 2020). Besides physical and chemical properties deterioration, alkaline soils undergo changes in their biological activity through microbial diversity variations under the action of $Na_2CO_3$ and $NaHCO_3$ salts. pH is the primary determinant of the bacterial community structure in acid/alkaline soils with enrichment of acidophilic bacteria in low pH soils (Jones et al. 2009; Chu et al. 2010; Shen et al. 2013; Wu et al. 2017; Kalam et al. 2020). To thrive under alkaline conditions, alkaliphilic bacteria maintain their internal pH of about 9.5 in the 9–11 external pH, and the proton transfer systems in their

cytoplasmic membrane (ATP pump and sodium-proton antiporter) sustain their activities (Horikoshi 1999).

Alkaliphilic and acidophilic microorganisms have an important role in the improvement of biological activities of sodic and acidic soils, such as the enzyme profile, which creates better environment for plant growth. Therefore, alkaline/acidic environments were subjected to many studies looking for PGP candidates in order to alleviate sodic/acidic stress in cultures. Most of these studies focused on alkaline environments that are also under high saline stress (haloalkaline). This dual haloalkaliphilic characteristic makes their inhabitant microbes interesting for application in plant growth enhancement with different environmental stresses. Haloalkaliphilic bacteria were recovered from hypersaline environments in India and showed PGP attributes (Sahay et al. 2012). According to the same reference, the amount of ammonia-producing isolates was highest (56%) when compared to those of producing ACC deaminase (53%), IAA (50%), hydrogen cyanide (28%), siderophore (21%), and solubilizing phosphate (34%). Haloalkaliphilic bacteria from different soils of Khorasan Razavi Province (Iran) were also reported to have PGP attributes, including ammonia and IAA and ACC deaminase on greenhouse conditions to reduce damage caused by alkaline salt stresses on wheat (Torbaghan et al. 2017). Recently, Dixit et al. (2020) described the role of alkalotolerant *Alcaligenes* and *Bacillus* strains in the alleviation of alkaline stress in *Zea mays*.

## *6.2 Soil Fertility and Bioremediation*

Soil fertility is "the inherent capacity of a soil to provide the essential plant nutrients in adequate amounts and proper proportions for plant growth" (Bharti et al. 2017) and depends on three major components: biological, chemical, and physical features. Soil biological fertility refers to the microbial metabolisms and interactions responsible for most of the nutrient flux. Microbes inhabiting the soil can have different functions like decomposition of cellulose, protein, and lignin; nitrogen fixation; ammonification; oxidization (iron, hydrogen, and sulfur); phosphorus solubilization and denitrification; or humus, nitrate, and nitrite formation. Hence, their attributes in soil fertility amelioration can be through many mechanisms including nutrients released from organic matter, atmospheric nitrogen fixation, increase in phosphorus availability, pesticide degradation, soil structure improvement, and soil-borne pathogen control. Biotechnology opened up new perspectives for the management of telluric microbes to enhance soil fertility.

Different groups of extremophilic microbes have been reported as potential candidates for soil fertilization especially in regions under abiotic stresses. These extremophiles were able to enhance nitrogen fixation and solubilize insoluble compounds such as phosphorus, potassium, zinc, and silicon (Ghorbanpour et al. 2016; Vaishnav et al. 2017). In arid ecosystems, emerging molecular tools have identified associations between phototrophic and chemolithoautotrophic communities in the soil which represent the sole sources of carbon and nitrogen for the vegetation

(Agarwal et al. 2014). In cold desert of northwestern Indian Himalayas, the solubilization activities of zinc, phosphate, and potassium have been reported in psychrotrophic *Bacillus* recovered from the soil (Yadav et al. 2016). In alkaline/acidic environments, halophilic archaea strains have been described for their role in P solubilization and mobilization (Yadav et al. 2015).

Adaptation of extremophiles to polluted extreme habitats was also explored for bioremediation of contaminated soils where different extreme conditions coexist. Extremophilic microbes can be applied in soil bioremediation directly through their bioconversion/biodegradation capacities (Margesin and Schinner 2001; Peeples 2014) or indirectly as PGPR for plants used in phytoremediation cultures (Radwan et al. 1998; Singh et al. 2019). The increasing number of patented hydrocarbon biodegraders emphasizes the importance of the commercial application of biosurfactants from extremophilic microorganisms in environmental friendly bioremediation of polluted soils (Margesin and Schinner 2001; Peeples 2014).

## *6.3 Biocontrol*

Many extremophilic microorganisms have shown antagonistic activity against deleterious microbes. In agriculture, extremophile environments have been screened for biocontrol agents that could enhance crop growth through their suppression of phytopathogens. Antagonistic PGP extremophiles encounter the impact of phytopathogens on the plant health via different mechanisms including mainly antibiotics, lysing enzymes, competition for nutrients and spaces, hydrogen cyanide (HCN) production, inducing systemic resistance (ISR), quorum quenching, inactivation of virulence factors, and siderophores release.

With the prosperity of molecular technologies, extreme environments have turned out to be potential and untapped sources of new pharmaceutical compounds. Extremophilic *Actinobacteria* and archaea have received particular attention in drug discovery for a new class of antibiotics, immunosuppressive treatments, anticancer drugs, and other biologically active compounds such as alkaloids, angucycline, macrolide, and peptides (Bérdy 2005; Cragg et al. 2005). However, screening of antibiosis in extreme environments for agricultural application is almost based on cultural approaches which prevent the discovery of valuable novel compounds. Among harnessed extremophiles for the suppression of plant diseases, psychrophilic strains of *Trichoderma* (McBeath 1995), *Pseudomonas* (Negi et al. 2005), and *Streptomyces* (Malviya et al. 2009) have been reported for potential biocontrol activities, especially under low temperatures. Saline soils were also described as promising sources of micro-antagonists against several phytopathogens (Príncipe et al. 2007; Sadfi-Zouaoui et al. 2008; Upadhyay et al. 2011; Etesami and Beattie 2018). In recent years, marine environments received significant interest in the search for antagonist candidates to control plant diseases (Kong 2017). Kurniawan et al. (2019) reported the potential of marine chitinolytic *Bacillus* isolates as biocontrol agents of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium solani*, and *Penicillium*

*chrysogenum*. Indigenous desert microorganisms were also described to promote plant health in arid agro-ecosystems through the suppression of phytopathogens (Koberl et al. 2011). Although extremophilic microorganisms showed promising results in the control of plant diseases, their efficiency under natural conditions still needs to be proven.

## 7 Challenges and Perspectives of PGP Extremophile Use in Agriculture

Over the last decade, scientists have been fascinated by potential of microbes inhabiting extreme environments. To thrive under conditions which for other terrestrial life forms are intolerably hostile or even lethal, extremophile microbes had developed many mechanisms that challenge the paradigms of modern biology and the fundaments of life. These microbial features made extreme environments a pivot of many groundbreaking discoveries that were adopted in mainly biotechnological, pharmaceutical, and agricultural applications.

After the Green Revolution of the twentieth century and the effects of climate change, new visons in agriculture were raised to sustain the food, fiber, and fuel needs of a growing global population, with reduced environmental impact. Among these innovations in crop production, microbe-based agriculture has received more attention regarding its ability to improve soil fertility and increase crop yields. This strategy is based on the fact that microbial communities associated with plant roots showed crucial role in plant establishment through many mechanisms such as assisting the plant nutrient uptake, stimulation of root development, managing abiotic stresses, and control of deleterious soil-borne microbes. In this context, many microorganisms were isolated mainly from rhizospheres of various plant species and were used to enhance crop production.

Although most of the harnessed microbes were from agro-ecosystems, recently, extreme environments gained attention as unexplored sources of potential biofertilizers and biocontrol agents. Nevertheless, the challenges of applying extremophile microbes in the enhancement of plants' growth and health include some difficulties, which are basically related to differences in ecological contexts. Therefore, the strategies of extremophile application in agriculture were mostly based on abiotic stress alleviation, and thus, the explored extreme environments were selected according to which problem the crops are exposed (salinity, drought, cold, etc.). In fact, the risk of PGP agents' inefficiency under field conditions is a common problem of microbial inoculants, even with indigenous species. As most of the studies proceed with soil sterilization before the inoculation which impacts the physical-chemical characteristics of the soil; the pertinence of the laboratory results may not be warranted under field conditions. To withstand soil and rhizosphere conditions, the introduced strain must compete with the indigenous microbiome for space and nutrients, establish positive signals with plant roots, and get accustomed to the new

niches' parameters and their fluctuations as well. All these aspects should be considered when PGPR candidates are screened for bioformulation. Therefore, commercial products developed on the basis of single- or multi-species inoculum may refer to some carriers and additives in the formulations to enhance the shelf life and the inoculum fate in the soil (Nakkeeran et al. 2005; Arora et al. 2010). Treatments based on repeated application or simply PGPR by-products could be also considered. For example, EPS and biosurfactants from extremophile bacteria may be used in biocontrol and/or in soil quality improvement. These biomolecules can replace harsh chemical products used in pesticide industries.

Overall, to estimate the real efficacy of any PGP contributor under field conditions, it is necessary to carry out large-scale experiments under different climates, with various plant species/varieties and for a long-term period. A better understanding of the molecular basis of plant-PGPR interaction and the resulting physiological changes may also favor optimal selection of extremophile candidates for the enhancement of crop production. New "omics" approaches are proving their efficiency in extending our knowledge about physiological pathways involved in the plant-microbe interactions in general (Imam et al. 2016) and plant-PGPR interactions (Basu et al. 2018) specifically under saline stress (Bakka and Challabathula 2020). Thus, large-scale omics tools such as transcriptomics, proteomics, and metabolomics are strongly recommended in future extremophile PGPR targeting.

Limits of PGPR extremophile application may include also difficulties of their recovery from natural environments. Problems related to culture methods, unculturability, low growth rates, and low biomass yields for some species are often reported (Merino et al. 2019). Nevertheless, metagenomic tools offer many openings into a broadened view of the uncultivable fraction in extreme-environment communities and understanding their biological activities.

Although the choice of extreme environments for extremophiles PGP/PGPR screening was always based on the abiotic stress in question for the culture, many studies reported the isolation of candidates from different stress contexts. Likewise, bacteria from cold regions or hypersaline biotopes showed multiresistance capacity to other harsh conditions (i.e., high temperature, osmotic stress, heavy metal contamination) and were able to confer a certain level of stress tolerance to the inoculated plants.

By considering the ecological and physiological importance of extremophiles, it is important to increase investigations of this group because they represent potential candidates as crop enhancement contributors through different mechanisms that still need to be more clarified.

## 8 Conclusion

To conclude this chapter on the importance of extreme environments as potential sources for the isolation of PGPR/PGPs, it is considerable to notice different aspects of this approach. While these environments are of a great biological diversity and

may harbor some microorganisms with relevant biological activities which have been never described before, compatibility issues may arise regarding the use of these microbes in agriculture. The fact that conditions in original biotopes of micro-extremophiles are quite different than those in agro-ecosystems, their survival and establishment within the plant may be critical to their attributes in improving crop growth and health. The key concept in using extremophilic PGPR/PGPs is that the microbiome of plants thriving in extreme environments ranges from valuable candidates for improving crop tolerance to abiotic stresses such as salinity, drought, and cold which help in promoting agriculture under these unfavorable conditions. Even though mining microbiomes of plants from extreme environments for abiotic stress alleviation in cultures seems to be a good strategy, more knowledge of environmental traits that influence this microbial activity is still required to reduce variation in PGP efficacy. Thus far, plant-microbe interactions in extreme environments need to be clarified with the underlying physiological and molecular mechanisms contributing to enhanced plant growth under abiotic stress. Further studies on the microbial diversity of the different communities in the rhizosphere and endosphere of extremophilic plants would clarify these ecological associations. Hence, insights into the optimization of the inoculum conception and the final product formulation could provide a better performance for micro-extremophile-based agriculture.

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# Chapter 13
# Commercial and Technological Aspects of *Bacillus* spp. PGPR


**Aurelio Ortiz, Estibaliz Sansinenea, Noshin Ilyas, and R. Z. Sayyed**

**Abstract** Plant growth-promoting rhizobacteria (PGPR) induce plant growth through different mechanisms such as producing various compounds, including growth regulators (phytohormones), siderophores, and organic acids, fixing atmospheric nitrogen, solubilizing phosphorus, and producing antibiotics to suppress harmful rhizobacteria. Numerous PGPR-based biocontrol and plant growth-promoting products are available on the market, and more are in the process of development. Most of these products are based on gram-positive microbes such as *Bacillus*, since these species produce spores and can be formulated in an easy way. However, knowledge of marketing and target diseases, cost of mass-scale production, and registration procedures also need to be revised to raise the market status of these biocontrol entities. A successful commercialization needs improvements in production and formulation processes which are dependent on some technological aspects. In this chapter, the commercial and technological aspects of *Bacillus* as plant growth-promoting rhizobacteria are reviewed for the use of these microbes in the agriculture sector.

**Keywords** PGPR · Biotechnology · Biopesticides · Commercialization · *Bacillus* sp

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## 1 Introduction

The global population is increasing; therefore, the demand for agricultural crops is also increasing, so the productivity of the crops should be improved. For many years, chemical fertilizers and pesticides have been used for this purpose, causing great environmental damage, creating pest resistance, and leading to many human health problems. The research has been directed to study more ecofriendly alternatives for the management of plant pathogens and for plant growth promotion. The use of biofertilizers or biopesticides has opened a new way to improve the yield of crops (Sarkar et al. 2021).

Plant growth results from the interaction of the roots with the environment. Therefore, the roots and their surrounding environment are the key to understanding how plants can benefit with this ecological niche. The rhizosphere is the part of the soil surrounding plant roots and is the habitat for millions of microbes exerting a potential impact on plant health and soil fertility. The bacteria colonizing this habitat are called rhizobacteria (Jabborova et al. 2020). Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, isolated from the rhizosphere, which enhance the growth of the plant (Basu et al. 2021) and reduce the damage from soil-borne plant pathogens when applied to seeds or crops (Zhou et al. 2015). PGPR can enhance plant growth by employing multiple mechanisms (Suriani et al. 2020), which can be acting simultaneously at different stages of the plant's growth and assuring the availability and uptake of certain macronutrients as well as micronutrients to the plant (Jaisingh et al. 2016). PGPR can produce different compounds that help the growth of the plant using different mechanisms, such as antibiosis against plant pathogens, fixing atmospheric nitrogen, secreting growth-regulators hormones, and solubilizing iron and phosphorus (Sharma et al. 2013).

Among the bacterial genera that are used as PGPR can be found *Bacillus*, which can suppress pathogens and promote plant growth. *Bacillus* can act using different direct and indirect mechanisms, which can be acting simultaneously in the plant growth. The direct mechanisms include the ability to obtain nutrient supply, such as nitrogen, phosphorus, potassium, and minerals, or modulate plant hormone levels. The indirect mechanisms include the secretion of antagonistic substances to inhibit plant pathogens or the induction of resistance to pathogens (Sansinenea 2019). *Bacillus* sp. genus has the capacity to secrete different chemical compounds, this characteristic being an advantage for these bacteria. In this chapter, the commercial and technological aspects of *Bacillus* as plant growth-promoting rhizobacteria are reviewed for the use of these microbes in the agriculture sector (Nithyapriya et al. 2021).

## 2 Application of *Bacillus* in Agriculture

*Bacillus* spp. have been widely used on the biopesticide market around the world because of its capacity to produce many important products for food, pharmaceutical, and environmental and agricultural industries with high impact on human activities. Recent studies have shown that these aerobic spore formers can produce fine chemicals with interesting biotechnological applications that open perspectives for new biotechnological applications of *Bacillus* and related species. The members of the genus *Bacillus* are often considered as microbial factories to produce a vast array of biologically active molecules, some of which are potentially inhibitory for fungal growth (Ortiz and Sansinenea 2019), as shown in Fig. 13.1.

*Bacillus* species have a good secretion system and produce a variety of extracellular enzymes for the detergent, textile, food, feed, and beverage industries. Among the enzymes of interest are amylases, pullulanases, and β-glucanase employed in the brewing and bakery industries; β-galactosidase applied in beet sugar and pulp and paper industries; cellulases and xylanases in paper and pulp industry; chitinases used in food industry; and esterases and lipases used in detergent industry. However, agriculture is the field where most have been applied *Bacillus* sp.

It is worth mentioning that *B. thuringiensis* is the best known and studied entomopathogenic bacterium that produces parasporal protein crystals, which are selectively toxic to different species of several invertebrate phyla being safe to people, beneficial organisms, and the environment. Microbial *B. thuringiensis* biopesticides contain a mix of bacterial spores and δ-endotoxin crystals, produced in fermentation tanks, and formulated into solid powdery presentation or liquid sprays. The

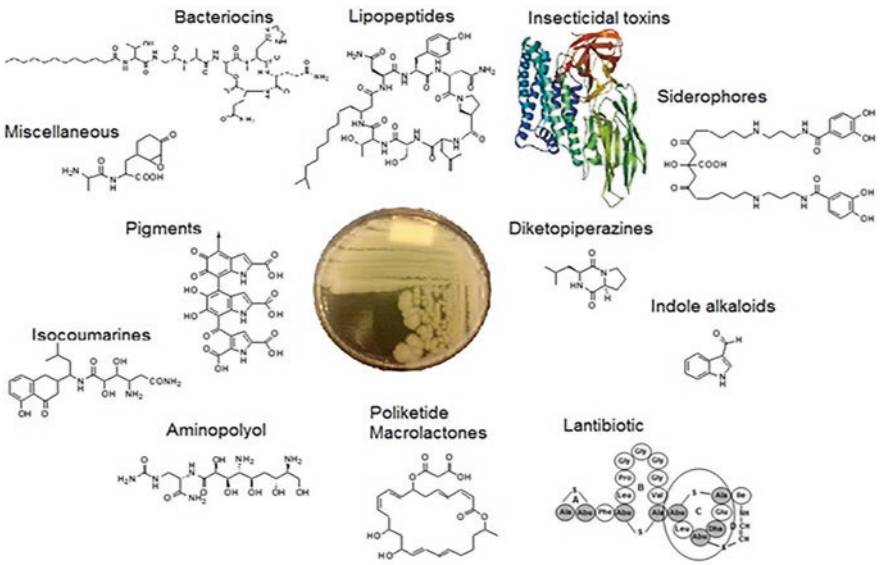


**Fig. 13.1** *Bacillus* sp. secondary metabolites

spore-crystal complex must be carried by a suitable inert substance that can function to protect the spore-crystal complex or to increase availability to insects. Because of their high specificity and their safety for the environment, crystal proteins are a valuable alternative to chemical pesticides for the control of insect pests in agriculture. This has been a strategy to control pests from a large number of crops, such as cabbage and cotton tobacco among others. *B. thuringiensis* has been used as a biopesticide in agriculture, forestry, and mosquito control. Its advantages are specific toxicity against target insects, lack of polluting residues, and safety to nontarget organisms such as mammals, birds, amphibians, and reptiles. Although, several proteins and other compounds produced by *B. thuringiensis* contribute to its insecticidal activity, by far the most important components are the proteins that form parasporal crystalline inclusions during sporulation. Transgenic crops based on insecticidal crystal proteins of *B. thuringiensis* are now an international industry with revenues of several billion dollars per year (Sansinenea 2019).

However, *B. thuringiensis* is not the only specie which has been applied to agriculture. In fact, several *Bacillus* species have been identified as plant growth-promoting bacteria since they suppress pathogens or promote plant growth. The suppression of pathogens is due to the production of antifungals that cause antagonism of pests and pathogens. These compounds seem to play an important role in the biological control of plant pathogens (Ortiz and Sansinenea 2019).

Many antifungal compounds (Chowdhury et al. 2015) isolated from these bacteria have been identified such as mycobacillins, iturins, plistatins, bacillomycins, surfactins, mycosubtilins, fungistatins, and subsporins (Koumoutsi et al. 2004; Madonna et al. 2003; Nihorimbere et al. 2012; Nishikiori et al. 1986; Pathak et al. 2012; Pecci et al. 2010; Peypoux et al. 1999). Other metabolites, including chitinases and other cell wall degrading enzymes and compounds, are also produced by *Bacillus* spp. (Chaaboni et al., 2012). A key issue arising from the patent activity for biocides is the wider impact of compounds, including antibiotics, on biodiversity and human health (Gilbert and McBain 2003).

## 3 Commercialization of *Bacillus*

PGPR production from laboratory to farmer requires careful and extensive study and market survey; it has to pass through different stages before appearing on the farmer's shelf. The process to select a specific strain or consortia for commercial availability depends on the crop requirement (Bhardwaj et al., 2014).

Presently, there are over 400 of *B. thuringiensis*-based formulations that have been registered in the market (Abdullah 2012; Sansinenea 2016). Most of the *B. thuringiensis* formulations are used to control many common leaf-feeding caterpillars. To control lepidopteran pests, there are many commercial products including Dipel®, Javelin®, Thuricide®, Worm Attack®, Caterpillar Killer®, and Bactospeine®, although many small companies sell similar products under a variety of trade names. Dipel® is a biological insecticide containing the naturally occurring

microorganism *Bacillus thuringiensis* subspecies *kurstaki* (Btk). For the manufacture of Dipel®, VBC selected a proprietary, high-yielding Btk strain (ABTS-351). Globally, Dipel® has become a cornerstone insecticide in many IPM programs as it offers high-quality, cost-effective, broad-spectrum caterpillar control on more than 200 crops, including vegetables, fruits, nuts, vines, cotton, oil palm, and corn. Specific registrations vary by country. The active ingredient in Dipel® consists of an optimized blend of four potent Bt protein toxins and a spore. Many other Bt strains lack the volume and balance of Bt toxin proteins that Dipel® delivers. Bt subsp. *kurstaki* toxins have distinct modes of action, unlike any chemical insecticide, providing a perfect tool for insect pest control programs which employ tank mix or rotation for insecticide resistance management. Dipel® has never shown cross-resistance with any chemical insecticide. Dipel® biological insecticide is non-toxic to pollinators and other beneficial insects. Similarly, Javelin® contains naturally occurring Bt *kurstaki* strain and is a biological insecticide specific for use against the lepidopterous larvae. Javelin® must be eaten by the larvae to be effective. Since Javelin® is most effective against small, newly hatched larvae, an early scouting program to determine early infestations is recommended. After consuming a lethal dose of Javelin®, larvae stop eating within an hour, but may remain on the foliage until they die, usually within several days. Affected larvae move more slowly and tend to become shriveled and discolored before dying. Agree® is a biological insecticide, based on *Bacillus thuringiensis aizawai,* specific for use against the lepidopterous larvae. Agree® must be eaten by the larvae to be effective. Since Agree® is most effective against small, newly hatched larvae, an early scouting program to determine early infestations is recommended. After consuming a lethal dose of Agree®, larvae stop eating within an hour, but may remain on the foliage until they die, usually within several days. Affected larvae move more slowly and tend to become shriveled and discolored before dying. Novodor® contains a bacterium, *Bacillus thuringiensis tenebrionis*, whose toxins (protein crystals) destroy the intestinal tract of potato beetle larvae (Sanahuja et al. 2011; Sansinenea 2016).

Many commercial products have been marketed as bio-fungicides and are based on various *Bacillus* species such *as B. amyloliquefaciens, B. licheniformis, B. pumilus*, and *B. subtilis* (Fravel 2005). They are employed to control fungal diseases. For example, *Bacillus subtilis* B246 was commercially registered as Avogreen and used as a biocontrol agent against avocado pre- and post-harvest anthracnose disease. The formulated product resulted in significant control of anthracnose caused by *Colletotrichum gloeosporioides* fungus (Demoz and Korsten 2006). Serenade® ASO fungicide (from Bayer) is a powerful tool designed to protect against the effects of soil and foliar bacterial and fungal diseases. The active ingredient in Serenade ASO fungicide, *Bacillus subtilis* strain QST 713, is a beneficial bacterium. The beneficial bacteria in Serenade® ASO act as small factories, producing bioactive compounds in addition to those in the product. These bioactive compounds provide three different effects important for healthy, high-yielding plants. Applied at planting or through chemigation, Serenade quickly builds a disease-protection zone around the seed or transplant. As the plant's roots grow, the beneficial bacteria in Serenade ASO formulated and provided at optimized levels grow with them,

expanding the disease protection zone and attaching themselves, like armor, to the roots of the plant. Applied as a foliar spray, Serenade ASO protects crops against diseases caused by fungi such as *Botrytis*, *Sclerotinia, Xanthomonas*, *Erwinia, Rhizoctonia, Fusarium, Phytophthora*, and other pathogens. Besides, it activates the plant's natural defenses by inducing systemic responses in the plant. It can be effective in different crops, such as broccoli, cabbage, cauliflower, onions, garlic, corn, carrots, and potatoes among others (Lahlali et al. 2011). Ballad Plus and Sonata were two marketed products from Bayer Crop Science based on *B pumilus* (strain QST 2808). Ballad Plus and Sonata produce an antifungal amino sugar compound which disrupts cell metabolism and destroys cell walls, killing plant pathogens. They create a zone of inhibition on plant surfaces, preventing pathogens from becoming established on the plant. This novel mode of action not only creates an effective fungicide but also makes it very difficult for diseases to develop resistance when rotated in a control program with other registered fungicides. Ballad Plus and Sonata are broad-spectrum products for the control or suppression of many important plant diseases and fit well into both conventional and organic production. They have the additional benefits of resistance management, short 4-hour restricted entry intervals, compatibility with other products, no residue restrictions for export, and safety to beneficial insects. In addition, these broad-spectrum fungicides have flexible uses (R1 or R3 stages) and can be tank-mixed with other crop-protection products (Serrano et al. 2013).

The product-line RhizoVital offers a range of bio-stimulating microbial inoculants, containing spores of the naturally occurring soil bacteria *Bacillus velezensis* (synonym *B. amyloliquefaciens* ssp. *plantarum*) or *Bacillus atrophaeus*. It is successfully commercialized as a biofertilizer by AbiTEP GmbH (Chowdhury et al. 2013). The bacteria germinate in the soil and release enzymes which stimulate nutrient mobilization. RhizoVital supports the availability of plant nutrients which can lead to an increase in yield response. Tolerance toward stress caused by unfavorable climatic conditions and field management can be improved. Use RhizoVital as an integral part of a future-oriented production strategy. RhizoVital has to be applied as early as possible in plant development. The good miscibility with crop protection products and fertilizers facilitates combination with almost all application processes. Thus, the product can be applied using different application methods like seed treatment, drenching, spraying (on soil surface), mixing into soils and substrates, injection into hydroponic and fertigation systems, and root dipping. The advantages of this product include: (a) Favors plant nutrient mobilization and promotes plant growing; (b) Increased crop yields possible through better plant nutrient availability; (c) Compatible with most fertilizers and plant protection products; (d) Complements conventional production strategies; (e) Easy to apply and store (min. 2 years at room temperature); and (f) Fully compatible with organic and residue-free production. It can be applied to crops such as lettuce, carrots, tomatoes, potatoes, cotton, or cereals like corn or rice, and to fruit trees such as apple or apricot.

## 4 Technological Aspects

The formulations of Bt have some problems such as narrow host range, low persistence on plants, and the inability of the foliar application to reach the insects feeding inside the plants; therefore, some formulations have a little effectiveness in the field owing to variable environmental stress (Kaur 2007). Some improvements have been developed with the help of genetic engineering. Therefore, to encourage the commercial production of *B. thuringiensis* biopesticides, the utilization of less expensive material is advisable, and several raw materials (industrial and agricultural by-products) have been tested as alternative culture media for entomotoxin production (Brar et al. 2006; Tirado-Montiel et al. 2001). One of the promising sources of cheap material is the utilization of wastewater. The exploitation of sewage sludge for entomotoxin production by *B. thuringiensis* and application to agricultural crops and forests for pest control seem to be fully compatible with current sludge disposal practices (Tirado-Montiel et al. 2001). As it has been mentioned, genetic engineering may play a complementary role in the development of more efficient formulations by increasing toxin production, broadening the host range, and enhancing germination and sporulation.

Topical Bt sprays are advantageous in terms of their safety, specificity, and potency compared to chemical sprays, and are also biodegradable, which provides for a large and competitive market. The spore-crystal complex is the active ingredient in commercial formulations, which is more effective to use and cheaper to obtain than the crystals alone and must be helped by a suitable inert substance that can function to protect the spore-crystal complex or to increase availability to insects. *B. thuringiensis* sprays are used sporadically and typically over small areas over cotton, fruit, and vegetable crops (Fig. 13.2). However, the use of *B. thuringiensis* spray as an insecticide has several disadvantages: (1) *B. thuringiensis* spray cannot be applied uniformly to all parts of the plant, (2) it cannot be applied inside plant tissues, and (3) *B. thuringiensis* is susceptible to rapid degradation by UV light and removal by water runoff. Therefore, multiple applications are required to provide extended pest protection. New *B. thuringiensis* formulations have consistently come to vegetable markets over the last number of years (Cerón 2001).

Usually, Bt is applied when early instar larvae are present because older larvae are more tolerant. Bt sprays persist for only a few days on the leaf surface because UV light, weather, the chemical environment of the leaf surface, and the presence of proteinases contribute to the degradation of Cry proteins. Therefore, the efficacy of *B. thuringiensis* microbials applied to the surface of leaves is limited by the fact that the formulation can be washed off by rain, and the Cry proteins are inactivated by sunlight within a few days of application (Federici and Siegel 2008). To solve the problem of the damage of UV irradiation to *B. thuringiensis*, some chemical screens have been used. However, these chemical screens have some negative impacts on the environment. As crystal proteins are even more vulnerable to degradation than the spores, Cry proteins have been encapsulated in the bacterium *Pseudomonas fluorescens* (e.g., in the Mycogen products, MVP which targets lepidopteran pests,

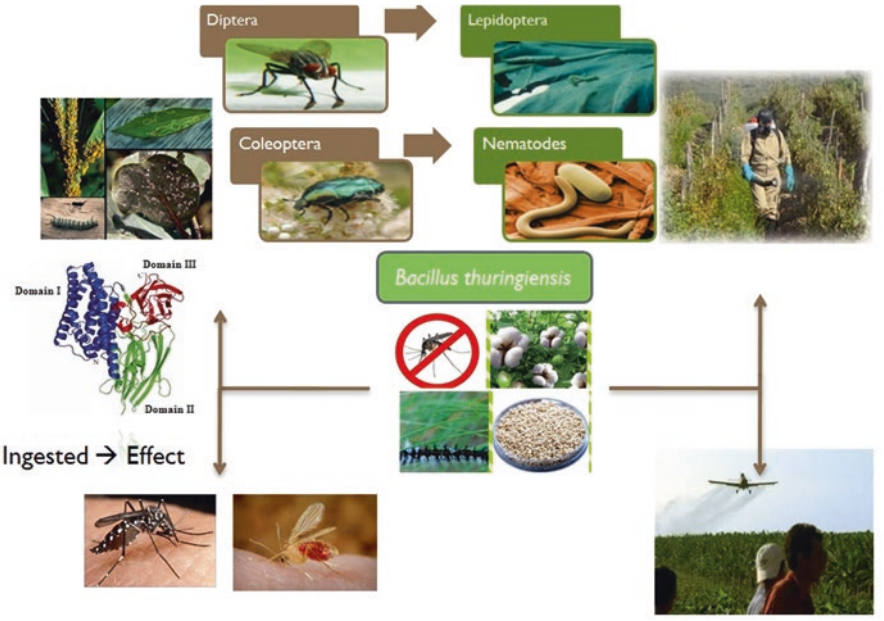


**Fig. 13.2** Application of Bt formulations to crops

and M-Trak which targets coleopteran pests). This encapsulation strategy protects the Cry protein from UV light and chemical degradation and allows large amounts of each Cry protein to be produced using high-yielding expression constructs, but the bacteria do not persist in soil or water for as long as Bt spores (Sanahuja et al. 2011). Another strategy involves melanin which is a natural pigment that is easily biodegradable in nature and can absorb radiation; therefore, it is a perfect photoprotective agent, which has been used to protect *B. thuringiensis* formulations from UV light (Sansinenea and Ortiz 2015; Sansinenea et al. 2015).

The problems of field application of *B. thuringiensis* biopesticides have been overcome by *B. thuringiensis* transgenic crops. Transgenic Bt-crops have been genetically modified by inserting a *Bacillus thuriengensis* gene, so the plant expresses a Cry toxin aimed for insect crop pests. Non-target soil invertebrates are particularly recognized for their contribution to plant nutrient availability and turnover of organic matter, and it is, therefore, relevant to protect these invertebrate taxa. The total acreage of transgenic crops has been steadily increasing with commercial cultivation of transgenic crops on 140 million hectares in 2010 (James 2010). The most widely grown *B. thuringiensis* crop is cotton (*Gossypium hirsutum* L.), accounting for 64% of the global cotton area devoted to *B. thuringiensis* crops, followed by corn (*Zea mays* L.) accounting for 29% of global corn area. It has been studied the effect of Bt crops on soil invertebrates, and the results indicate that there was no significant effect of Cry on soil invertebrates (Krogh et al. 2020). While research in different countries has shown that Bt crops adoption reduces farmers'

chemical pesticide use and increases crops yield and profits; opponents of transgenic technology have raised concerns about potential health and environmental risks. There have been several studies about the risk assessment of transgenic crops related to environmental safety and human health (Kaur 2012). One of the latest works is about the relation of transgenic Bt cotton with farmer's health in Pakistan (Kouser et al. 2019), which concludes that the employment of Bt cotton is safe and is associated with health cost savings. Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins can provide significant economic and environmental benefits and were planted on more than 100 million hectares worldwide in 2018. However, the evolution of practical resistance to Bt crops, which is field-evolved resistance with practical consequences for pest management, has occurred in at least nine major insect pests in six countries and is accelerating. With the idea to mitigate the effects of resistance to Bt corn pest rootworm, *Diabrotica virgifera virgifera*, crop rotation of Bt corn with a nonhost crop such as soybean has been applied (Carrière et al. 2020).

## 5 Conclusion and Future Prospects

Every year, plant pathogens cause great losses of food crops worldwide (Arora et al. 2021). The development of insect and weed-resistant varieties, human and animal health concerns, and environmental safety concerns have brought PGPR under remarkable consideration (Kour et al. 2021). PGPR are applied in various ways to control plant pathogens (Hamid et al. 2021). Several studies have demonstrated that PGPR-based formulations improve the growth attributes of the subjected plant (Moradzadeh et al. 2021), such as shoot elongation, yield, plant biomass, seed germination, seedling vigor, plant height, fresh and dry weight, and leaf area of economically important crops, including rice, tomato, soya bean, and wheat (Tabassum et al. 2017; Backer et al. 2018). PGPR-based formulations not only help protect plants from several pathogens by acting as biocontrol agents but also trigger different biological promotion effects in various plant growth parameters (Kusale et al. 2021). Effective utilization of PGPR for disease reduction or crop protection in the future will demand a rational choice of the organism as well as technical improvements in upscaling and formulation techniques. To generate PGPR-based products, formulations must be developed that allow for even distribution in the field. Alternatively, liquid inoculants can be sprayed onto seeds, prior to sowing or dripped into the seed furrow at the time of sowing. Signal molecules are probably best applied as liquid sprays, although slow release solid formulations could also be investigated. Storage and product lifespan are important considerations that need to be determined for a given product, to ensure microbial survival and/or bioactivity of the strain or compound of interest. As the product nears the marketplace, it is necessary to have approval for registration.

In this sense, *Bacillus* sp. species have been extensively used in agriculture as biocontrol agent using several mechanisms to promote plant growth. During last

decades *Bacillus* spp. have been successfully exploited and commercialized applying them to several crops against several plant pathogens. Some problems have been to be overcome and to improve their efficacy. Currently, the regulatory procedures for the registration and commercialization of biostimulants are complex. Genetic engineering has been a modern technique to accentuate these mechanisms; however, it is necessary to control the commercialized products, their results and risk evaluation for better employment of these products. There is even a need for methods of optimization of fermentation and formulation processes to improve their introduction in the agriculture industry. Every step in the process from microbe isolation to licensing is laborious, expensive, and requires time. Collaboration between industrial, academic, and government research should become an important part of the product development process.

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# Chapter 14
# Siderophores and Their Applications in Sustainable Management of Plant Diseases


**Gadde Venkata Swarnalatha, Vedavati Goudar, Eddula Chengal Reddy Gari Surendranatha Reddy, Abdel Rahman Mohammad Al Tawaha, and R. Z. Sayyed**

**Abstract** Microbes and microbial products application are increasing every day. Application of biopesticides and biofertilizers is a sustainable way of managing pests and diseases of plants. Siderophores are low molecular weight organic chelating agents produced by microbes that have specific affinity for iron. The iron in the environment exists in ferric form which is insoluble. This form is also inaccessible at normal physiological pH of 7.33–7.4. Thus, the microorganisms can be able to synthesize siderophores which have high affinity towards ferric iron. First the siderophore–ferric iron complex is transported to cytosol, where the ferric iron gets reduced. The reduced ferrous iron in cytosol is easily accessible to microorganisms. Siderophores have various applications in diverse fields like microbiology, agriculture, ecology, biosensor, and bioremediation. Thus, scientists are paying attention towards the use of siderophores in agriculture. Siderophores increase the yield of several plant species by enhancing the Fe uptake to plants. Siderophores are ecofriendly, as these act against phytopathogens that are harmful and also substitute hazardous pesticides. Siderophore-producing microbial antagonists deprive iron

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from being available to plant pathogens. In this chapter, we have discussed on the role of siderophores and their characterization, synthesis, and yields besides plant disease management. The role of siderophores and their application aspects as an integral part of sustainable agriculture are also discussed in our present review.

**Keywords** Iron chelating compounds · Iron nutrition · Rhizobacteria · Plant growth promotion · Biocontrol · Plant disease

## 1 Introduction

Iron is the most significant essential part for the expansion and development of all living microorganisms. It acts as a catalyst in protein synthesis, electron transfer, and deoxyribonucleic acid and ribonucleic acid synthesis (Aguado-Santacruz et al. 2012). Iron is not simply accessible to microorganisms. Iron at biological pH scale and aerobic conditions gets change to insoluble oxyhydroxide polymers. One in every of the vital ways that include siderophores mediates acquisition of iron through specific receptors and transport system. Siderophores are compounds derived from the Greek words *Sidero* that means "iron" and *Phore* that means "carriers." Siderophores are low-molecular-weight (<10 kDa) iron-chelating, secondary metabolites created by "Rhizospheric bacteria" below iron-restricted condition (Sah et al. 2015). Iron (Fe) is the fourth most voluminous part within the earth's crust. Secondary metabolites were first used to chelate the metal iron (Fe III) from aquatic and terrestrial habitats. This boosts the plant's growth by scavenging iron from the setting and creating the mineral accessible to the cell close to the foundation (Ahmed and Holmstrom 2014). Siderophores are excreted below iron starvation by numerous microorganisms like bacteria and fungi and also by some plants. All aerobic and facultative anaerobic microbes (except Lactobacilli) synthesize siderophores. Recently some class of siderophores are according (Devireddy et al. 2010). Marine organisms like flora and *Eubacterium* also can also produce siderophores. The most role of siderophores is to chelate the ferric iron. However, the different types of complexes with alternative essential components, i.e., MO, Mn, Co, and Ni, within the setting make them accessible for microorganism cells (Loper and Buyer 1991). Different types of siderophores help within the uptake of iron for numerous functions. These are principally hydroxamate, catecholate, and carboxylates supporting their chemical structures and functions. Another cluster of siderophores are microorganism, fungi, actinomycetes, and plant (Sandy and Butler 2009). Siderophores are created by rhizosphere inhabitants, and they do not seem to only improve rhizosphere organization, but, however, also help in the iron nutrition of plant and antagonism against phytopathogens. It is also concerned in acquisition from ferric citrate, ferric phosphate, metal transferring, and iron certain to plant flavones pigment, sugars, and glycosides (Winkelmann 2002). The acquisition of iron starts with binding of excreted siderophores advanced with metal iron forming a ferri–siderophore

complex. The complex goes and binds to a particular receptor macromolecule, its gift on the microorganism cell surface. The advanced gets translocated by transport, and it is discharged within the cell. A number of microorganisms synthesize one or a lot of siderophores which might be used by alternative microorganism for iron. This property of siderophore redoubled application in virulence mechanisms in plants and animals. The assembly of siderophores decreases with a rise in iron concentration within the surroundings (Singh et al. 2008). Siderophores play a crucial role in recent years due to their potential roles and application in numerous areas of environmental analysis. Siderophores function as biocontrols, biosensors, bioremediation, and chelating agents. The aim of this chapter is to stipulate and discuss the siderophores and its varieties, significance, and vital role in enhancing the plant growth and application in property management of disease.

## 2 Characteristic Features of Siderophores

A. Siderophores are low relative molecular mass coordination molecules.

- They bind and transport the iron molecules.
- They are extremely specific iron ligands.
- Siderophores are synthesized by variety of fungi and microorganism.
- Siderophores promote the plant growth.
- Siderophores act as a possible bio-management agent.
- Siderophores help within the bioremediation method.
- Siderophores play a crucial role in microorganism physiology and their role in biotechnology.
- Siderophores improve soil fertility.
- Siderophores are also utilized in clinical applications.
- It helps in growth, organization, and a gamogenetic asexual reproduction.
- It has high affinity system $Fe^{3+}$ acquisition, utilization, and storage.

## 3 Classification of Siderophores Based on Chemical Structure

The great variation is seen in siderophore structure from one species to a different species. Siderophores are classified on the idea of coordinating teams that chelate the Fe (III) particle. The foremost vital coordinating groups are catecholates, hydroxamate, and carboxylate (Ali et al. 2013). Some totally different category of siderophores is "Mixed Ligands" having coordinating teams. Recently (Winkelmann and Dreschel 1997), three categories of bacterial siderophores are added: amide, mycobactin, and citrate hydroxamate. Additionally, flora siderophores have been classified into five categories: ferrichrome, coprogens, rhodotorulic acid, fusarinines (fusogens), and rhizoferrin. The diagrammatical illustration of siderophore classification is shown in Fig. 14.1 and Table 14.1.

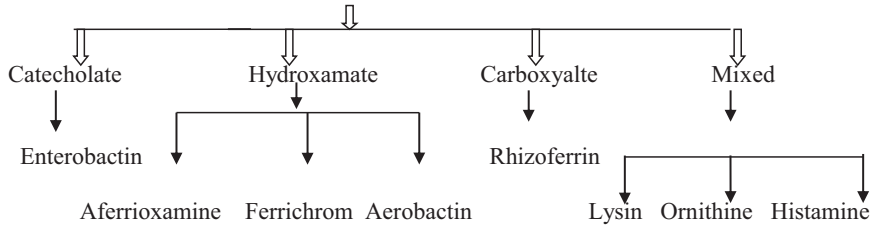


**Fig. 14.1** Types of siderophores

**Table 14.1** Classification of siderophores based on chemical structure

| Types of siderophores | Function | Bacterial source | References |
|---|---|---|---|
| 1. Catecholate | | | |
| (a) Enterobactin | Iron-chelating compound and used in agriculture | Family Enterobacteriaceae, e.g., *E. coli* | Pollack and Neilands (1970) and Walsh et al. (1990) |
| 2. Hydroxamate | | | |
| (a) Ferrioxamines | Used medically for the binding of excess blood iron in the treatment of thalassemia | *Streptomyces* and *Nocardia* | Gregory et al. (2012) and Sah et al. (2015) |
| (b) Ferrichrome | Microbes growth factor | Basidiomycetes producing fungi species | Gregory et al. (2012) and Sah et al. (2015) |
| (c) Aerobactin | Sequester iron in iron-poor environments (Urinary tract) | *Klebsiella pneumonia*, *Aerobacter aerogenes* | Gregory et al. (2012) and Sah et al. (2015) |
| 3. Carboxylate | | | |
| (a) Rhizoferrin | Metal-binding properties and used in biotechnology filed mainly degradation of metals | Fungi (Zygomycetes members) | Gregory et al. (2012) and Sah et al. (2015) |
| 4. Siderophores with mixed ligand | | | |
| 1. Lysine derivative | | | |
| Mycobactin | Chemotaxonomic markers for identification of mycobacterium up to species | *M. tuberculosis*, *M. smegmatis* | Sah et al. (2015) |
| 2. Ornithine derivative | | | |
| Pyoverdine | Inhibition of pathogenic bacterial growth | *Pseudomonas aeruginosa* | Sah et al. (2015) |
| 3. Histamine derivative | | | |
| Anguibactin | Inhibits iron uptake by living cells | *Vibrio anguillarum* | Sah et al. (2015) |

## *3.1 Types of Siderophore*

(a) **Catecholate Siderophore**

Catecholate siderophore is found only in bacterium. It consists of two teams: catecholate and hydroxyl radical groups; it binds $Fe^{3+}$ with adjacent hydroxyl radical or catechol ends. Its dihydroxybenzoic acid (DHBA) coupled to associate aminoalkanoic acid. Its lipophilicity advanced stability and resistance to environmental pH scale are its distinctive characteristics (Winklemann 2002).

(b) **Hydroxamate Siderophores**

This sort of siderophore is made by bacterium and fungi. Hydroxamate siderophores typically show robust absorption between 425 and 500 nm on bounding to iron. Ferrichrome made by the plant life genus *Ustilago sphaerogena* was the primary siderophore to be isolated and shown to be a protein for different microorganisms (Messenger and Ratledge 1985).

(c) **Carboxylate Siderophore**

This type of siderophores are produced by few bacteria like rhizobactin made by the bacteria genus meliloti and fungi like members of fungus order mucorales belonging to Zygomycotina. These siderophores have each carboxyl and hydroxyl radical for iron acquisition (Winklemann 2002). These siderophores are found within the kingdom of bacterium likewise within the realm of fungi. Curiously, each fungi and bacterium manufacture rhizoferrin. The fungi manufacture only R, R-rhizoferrin, and bacterium manufactures enantio-rhizoferrin S, S-Rhizoferrin (Munzinger et al. 1999).

(d) **Pyoverdin**

Pyoverdin is the by-product of aminoalkanoic acid. Pyoverdin it a water-soluble siderophore. It contains 6–12 amino acids, counting on the strain with a dihydroxyquinoline fluorescent group. Bacteria genus aeruginosa manufacture pyoverdin (Sah et al. 2015).

(e) **Mycobactin**

Mycobactin is the by-product of the essential amino acid. Mycobactins are 2-hydroxyphenyloxazoline containing siderophore molecules for the acquisition of iron. From the m*ycobacterium tuberculosis*, two chemical structures of siderophores are made (Sah et al. 2015).

(f) **Aerobactin**

Aerobactin is a bacterial iron-chelating agent found in *E. coli*. This can be the kind of hydroxamate siderophore of Pseudomonas, *K. pneumonia*, *A. aerogenes*, *E. coli*, and different bacteria (Winklemann 2002).

(g) **Ferrichrome**

It is a kind of hydroxamate siderophores. Ferrichromes are cyclic hexapeptide siderophores composed of three N-acyl-N-hydroxyl-L-ornithine and two variable amino acids and aminoalkanoic acid coupled by method of amide bonds**.**

(h) **Ferrioxamine**

Linear trihydroxamate siderophore is made by actinomycete and *Nocardia*, mainly utilized for medical purpose.

## 4 Siderophore Classification Based on Their Source

### *4.1 Fungal Siderophores*

It is also a vital siderophore-producing organism next to bacterium. A number of the vital siderophore-producing fungi include *Aspergillus nidulans*, *A. versicolor*, *Penicillium chrysogenum*, *P. citrinum*, *Mucor*, and *Rhizopus* and genus *Saccharomyces cerevisiae*, *Rhodotorula minuta*, and *Debaryomyces* species (Chincholkar et al. 2007). Recently, an updated list of some fungal siderophores is detailed in Table 14.3 (Ali et al. 2013).

### *4.2 Bacterial Siderophores*

Bacteria chiefly synthesize four varieties of siderophores: hydroxamate, catecholate, salicylate, and carboxylate. This area unit chiefly helps within the further cellular solubilization of iron from minerals or organic substances. Some vital siderophore-producing bacteria include Eubacterium infectious disease and bacteria genus *Azotobacter*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Salmonella*, *Enterobacteria pneumoniae*, *Aeromonas*, *Aerobacter aerogenes*, *Yersinia*, and *Eubacteria*. *Escherichia coli* Gram-negative facultative bacterium is the foremost wide-studied bacterium for siderophore production. It produces enterobactin, a catechol siderophore with the highest affinity towards metal (III) particle than the other famous siderophores (Raymond and Dertz 2003). Some of the bacterial siderophores are listed in Table 14.2 (Ali et al. 2013).

### *4.3 Cyanobacterial Siderophores*

Schizokinen, a dihydroxamate kind of siderophore made by *Anabaena sp.*, is reported to facilitate iron uptake. *Anabaena flosaquae* and *Anabaena cylindrica* form siderophores that accumulate copper (Chincholkar et al. 2007). Iron uptake is

**Table 14.2** Recently updated list of bacterial siderophores

| Siderophores | Siderophore-producing bacteria | Type |
|---|---|---|
| Agrobactin | *Agrobacterium tumefaciens* | Catechols |
| Enterobactin | *E. coli* | Catechols |
| Chrysobactin | *Erwinia chrysanthemi* | Catechols |
| Pyochelin | *Pseudomonas aeruginosa* | Catechols |
| 2,3 Dihydroxybenzoic acid | *Azotobacter vinelandii* | Catechols |
| Azotochelin | *Azotobacter vinelandii* | Catechols |
| Aminochelin | *Azotobacter vinelandii* | Catechols |
| Anguibactin | *Vibrio anguillarum* 775(PJM) | Catechols |
| Cepabactin | *P. cepacia* | Catechols |
| Parabactin | *Paracoccus denitrification* | Catechols |
| Staphyloferrin A | *Staphylococcus hyicus* | Catechols |
| Pyoverdin | *Pseudomonas* spp.<br>*Pseudomonas fluorescens*<br>*Pseudomonas aeruginosa*<br>*Pseudomonas putida* | Pyoverdin |
| Rhizobactin | *Rhizobium meliloti* | Unknown |
| Azotobactin | *Azotobacter vinelandii* | Unknown |
| Anthranilic acid | *R. leguminosarum* | Unknown |
| Citric acid | *Bradyrhizobium japonicum* | Unknown |
| Amonabactin | *Aeromonas hydrophila* | Hydroxamate |
| Vulnibactin | *Vibrio vulnificus* | Hydroxamate |
| Catechol and hydroxamate | *Azotobacter chroococcum* | Hydroxamate |
| Acinetobacter | *Acinetobacter baumannii* | Hydroxamate |
| Arthrobactin | *Arthrobacter* spp. | Hydroxamate |
| Corynebactin | *Corynebacterium glutamicum* | Hydroxamate |
| Desferrioxamine B & E | *Streptomyces viridosporus* | Hydroxamate |
| Aerobactin | *Erwinia carotovora,*<br>*Enterobacter cloacae*<br>*Pseudomonas* spp. | Hydroxamate |
| Yersiniophore | *Yersinia enterocolitica* | Hydroxamate |
| Yersiniabactin | *Yersinia enterocolitica* | Hydroxamate |
| Protochelin | *B. bronchiseptica* | Hydroxamate |
| Alcaligin | *Bordetella pertussis* | Hydroxamate |

mediated by the FutA/IdiA – based alphabet transporter system genes that are found in 28 unicellular cyanobacteria thing *Eubacterium* genomes of *Prochlorococcus* and *Synechococcus* (Rivers and Jakuba 2009). Siderophore made by *Anabaena oryzae*, a typical rice paddy *Eubacterium*, acts as biological sequestering agent for mitigation of metallic element metal ions, and it helps within the improvement in crop productivity. *Anabaena cylindrica* produces anachelin as a catecholate type of siderophores.

## 4.4 Actinomycete Siderophores

Actinomycetes are anaerobic Gram-positive filament-like bacterium with high G + C content and fissiparous spores. These are characterized with substrate and aerial mycelium growth and tolerate bound metals at high concentrations. A number of the siderophore-producing actinomycetes embody *Actinomadura madurae*, *Nocardia asteroids*, and *Streptomyces griseus*. Actinomycetes synthesize hydroxamate and salicylate kind of siderophores (Chincholkar et al. 2007).

## 4.5 Mammalian Siderophores

Recent study targets the invention of mammalian siderophores. Mammalian cells might contain siderophores. These show structural similarity with microorganism siderophores as studied in FL5.12/EC-24p3 murine interleukin-3 (IL-3)-dependent peo-B white blood cell lines (Devireddy et al. 2010). In mammals, lipocalin 24p3 binds enterobactin that successively binds iron in cells. 2,5 DHBA found in mammals is associated with iron-binding moiety of microorganism enterobactin (a pair of,3 DHBA) that binds to 24p3 in the absence of iron and is therefore thought of a class siderophore that binds to metal iron (III) and also facilitates mitochondrial iron uptake (Singh et al. 2008) (Table 14.3).

**Table 14.3** Recently updated list of fungal siderophores

| Siderophores | Siderophore-producing fungi |
|---|---|
| Ferrichrome | *Penicillium parvum* |
| Ferrichrome A | *Ustilago sphaerogena* |
| Ferrichrome C | *Neurospora crassa* |
| Ferrioxamine B | *Streptomyces* spp. |
| Ferrioxamine E | *Erwinia herbicola* |
| Ferricrocin | *Microsporum canis* |
| Asperchrome A, B, and C | *Aspergillus ochraceus* |
| Malionichrome | *Fusarium roseum* |
| Rhizoferrin | *Rhizopus microspores*<br>*Rhizopus arrhizus* |
| Canadaphore | *Helminthosporium carbonum* |
| Fusarinine A and B | *Fusarium roseum* |
| Rhodotorulic acid | *Rhodotorula piliminae* |

### *4.6 Phytosiderophores/Plant Siderophores*

Plants may also produce siderophores. Phytosiderophores are the members of poaceae; it belongs to the mugineic acid family that forms hexadentate Fe–PS complex. Phytosiderophores are $Fe^{3+}$-chelating compounds that are secreted by gramineous plant and form specific robust complexes with $Fe^{3+}$. These plant siderophores have hexadentate ligands that coordinate $Fe^{3+}$ with their amino and carboxyl groups (Singh et al. 2008). The coordinating groups of those siderophores are two amine, two carboxylate, and one α-hydroxycarboxylate site that form a decent octahedron during which the central metallic element Fe (III) atom remains certain. Phytosiderophores are organic substances like nicotinamide, mugineic acids, and avenic acid made by plants. The metallic iron element deficient condition results in increase of iron movement in soil (Ueno et al. 2007).

### *4.7 Characteristic Features of Phytosiderophores*

- The plant releases phytosiderophore at higher amounts.
- They are of crucial importance for iron and iron transport in soils.
- Fe chelates are extremely soluble and stable over a good hydrogen ion concentration.
- The plant siderophores mobilize micronutrients and metallic elements, such as Zn, Mn, and copper, from the soils to plant in deficient condition.
- Phytosiderophores are secreted from plant roots, and it is a lifesaving mechanism in plants.
- It enhances the plant nutrient uptake and improves the soil health.
- It plays a very important role in Fe and Zn element uptake for the crop plant**.**

## 5 Biosynthesis of Siderophores

Siderophores do not seem to be made at high iron concentration as a result of binding of the Fur macromolecule on the siderophore sequence promoter. Low concentration, conformational modification within the fur macromolecule causes its detachment from the siderophore sequence promoter, reestablishing the transcription of the sequence and also the ultimate synthesis of siderophores (Wani et al. 2016). Hence, iron-bound siderophores are mediated by membrane receptors, periplasmic membrane proteins, and TonB-dependent transporters, and during acquisition, ferric iron is reduced to its metallic ferrous form (Sandy and Butler 2009). Fur is expounded to the repression of genes concerned in synthesis export and import of siderophore.

## 6 Applications of Siderophores

Siderophore is a biological molecule made by numerous microorganism having wide application in numerous fields like agriculture to enhance soil fertility, biocontrol, and environmental application.

### *6.1 Plant Growth Promotion*

Siderophores' main vital role is to produce and store the iron in cell. Iron is a crucial substance, and it is needed for physiological activities, chlorophyll synthesis, and redox reactions in plant. Siderophores are used as bioinoculum to crops that may scale back the employment of chemical fertilizers and pesticides. This converts the insoluble variety of iron into soluble kind. These are eco-friendly, safe for crops, and farmer-friendly as they originate from nature. Natural potential of siderophore utilization assist the natural iron supply to soils (Munzinger et al. 1999). Endophytes use siderophores to enhance plant iron uptake from soil and help in the production of indole-3-acetic acid that could be a plant endocrine. The rhizobacterium *Cellulosimicrobium* spp. helps in the plant growth. Pyoverdine production increased iron nutrition of tomato plants. *Bacillus* species are found to be plant growth-promoting bacteria (Basu et al. 2021). Siderophores *Bacillus* species are the simplest performers in wheat plants. It helps in the plant growth and health (Luis and Serrano 2017). For plants, inoculation of soil with genus *Pseudomonas putida* that produces pseudobactin will increase the expansion and yield of varied plants (Kloepper et al. 1980). Pseudomonas enhances plant growth by manufacturing pyoverdine siderophores. Pseudomonas and different microorganism found within the rhizosphere region of margosa produce ferrioxamine siderophores that transfer iron to the plant for the expansion and development of shoot and root. The study found that excessive accumulation of serious metals is noxious for many of the plants responsible for the contamination of soil that decreases the soil fertility and soil microorganism activity. During this state of affairs, hydroxamate form of siderophores play a very important role in immobilizing the metals in soil (Singh et al. 2008). Fluorescent pseudomonads are a kind of siderophores that help improve plant growth through management of vesicatory organisms within the soil. Mycorrhizal fungi also synthesize siderophores to boost the plant growth. For example, mycorrhizal sorghum plants were shown to require higher concentrations of metallic element than non-mycorrhizal fungi. Once the bioavailability of metallic element is low in this condition, the vital potential mechanism is that plants may acquire metallic element from microorganism siderophores (Ahmed and Holmstrom 2014).

The two mechanisms are as follows:

1. Microbial siderophores with high reaction potential may be reduced to gift metallic element (III) to the transport system of the plant.
2. Microbial siderophores will chelate metallic Fe from soils and so do a substance exchange with phytosiderophores.

## 6.2 Biocontrol Agent/Suppress the Plant Diseases

Siderophore-producing bacteria play a vital role within the biological management against certain phytopathogens. Siderophores are concerned within the biocontrol of many plant diseases like plant disease of wheat, potato seed piece decay, stem rot of peanut, and damping off cotton. Microorganisms produces siderophores which binds with iron powerfully and build it unobtainable for the plant pathogens and also plays a vital role in inhibiting the expansion of phytopathogens (Whipps and John 2001). Siderophores act as growth inhibitors of varied phytopathogenic fungi like *Phytophthora ultimum* and *Fusarium oxysporum* var. *dianthi* and fungus sclerotiorum. The created siderophores were antagonistic to flora pathogens like *Fusarium oxysporum* and *Sclerotium rolfsii*. Bacillibactin siderophore acts as biocontrol agent in agriculture to boost the plant growth. *Acinetobacter calcoaceticus* siderophores increased iron solubilization within the plant (Patel et al. 2018). Siderophore production by strains of *Pseudomonas* spp. as a constituent of biological merchandise for disease management is of nice interest (Whipps and John 2001). The present analysis is the detection of production and optimization condition of siderophore by bacteria genus fluorescens and its biocontrol effectivity (Prema and Selvarani 2013). Siderophores of fluorescent pseudomonads has the capacity to inhibit germination of chlamydospores of *F. oxysporum*. Kloeeper et al. (1980) demonstrate the importance of siderophore production as a mechanism of biological management of Enterobacteria carotovora by bacteria genus glow strains A1, BK1, TL3B1, and B10. Siderophore-created bacteria genus species are wide studied as biological agents and manages the phytopathogenic organism in agriculture. Completely different species of bacteria are concerned within the management of wilt diseases of potato caused by the *Fusarium oxysporum* by production of pyoverdine siderophores, and also it suppresses the expansion of *Gaeumannomyces graminis* in wheat, barley, peanuts, and maize. Some samples of siderophore manufacturing bacteria genus that are planned as biocontrol agents soil-borne plant diseases embody *P. fluorescens* CHA0, *P. putida* WCS strains, and *P. syringae* pv. *syringae* strain 22d/93. Most significantly, studies have shown that coinculation of bacteria genus strains with *Bradyrhizobium* and *Ralstonia solani* strains extremely promoted legume growth and fully suppressed illness} disease below procedure and. also synthesizes differing kinds of siderophore that have a big role for the biocontrol of *F. oxysporum* in pepper (Ali and Vidhale 2013). The fungi *Trichoderma harzianum* recorded most hydroxamate and process production. These fungi not only scavenge

**Table 14.4** Important siderophores in plant disease control

| Siderophores | Target pathogen/disease | Crop |
|---|---|---|
| *P. fluorescence* | *Erwinia carotovora*<br>*G. graminis*<br>*Fusarium glycinia*<br>*Sarocladium oryzae* | Potato<br>Wheat<br>Wheat<br>Soybean, rice |
| *P. putida* | *Fusarium* spp. Wilt<br>*Fusarium solani*<br>*Erwinia carotovora* | Radish, Cucumber<br>Beans<br>Potato |
| *P. cepacia* | Fusarium oxysporum | Onion |
| *P. aureofaciens* | *G. graminis* var. *tritici* | Wheat |
| *Bacillus subtilis* A-13 | *Rhizoctonia solani* | Wheat |
| *B. pumilus* | *Gaeumannomyces graminis* var. *tritici* | Wheat |
| *Enterobacter aerogenes* | *P. cactorum* | Apple |
| *E. cloacae* | *S. homeocarpa* | Turf grass |
| *Bradyrhizobium* spp. | *Fusarium solani*<br>*Rhizoctonia solani* | Sunflower<br>Mung bean |
| *Rhizobium meliloti* | *Macrophomina phaseolina* | Groundnut |

iron but also result in the inactivation of microorganism enzymes. Iron plays the crucial role as a compound that provides that iron to the host plant leading to plant growth promotion. It has an eco-friendly nature and helps in crop improvement and enrichment of iron to the plant (Koeeper et al. 1980). Important siderophores in plant disease control are listed in Table 14.4 (Ali et al. 2013).

## 7 Conclusion

In recent days, people specialize in organic farming, microbe diversity, and soil health that have gained sizeable attention. Plant growth and crop yield are incredibly vital aspects currently these days. Siderophores manufacturing microorganism are the main gift within the rhizosphere region. It had the potential to chelate $Fe^{3+}$ ions and scale back to $Fe^{2+}$ by siderophore and supplement to the crop plant. Siderophores stop the expansion of the soil-borne phytopathogens. Siderophore application is widespread in numerous areas of medication and environmental and biological science. Henceforward, novel siderophores may be biosynthesized and used for the biocontrol of microorganism as flora pathogens. It will be vital to take advantage of molecular techniques to review the expression in plants. Recent studies specialize on discovery of potential class siderophores. The analysis focus on siderophore activity and its application to the environment and medicine. Understanding the chemical structures of various siderophores and therefore the membrane receptors concerned in metallic element uptake has opened new areas for analysis.

**Conflict of Interest** The authors declare that they have no conflict of interest.

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# Chapter 15
# Hydrolytic Enzyme Producing Plant Growth-Promoting Rhizobacteria (PGPR) in Plant Growth Promotion and Biocontrol


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**Abstract** A wide array of enzymes is produced by rhizospheric microbial communities that are hydrolytic in nature. Further, these hydrolytic enzymes produced by rhizobacteria can potentially degrade cell wall components of plant pathogenic origin. The main function of these hydrolytic enzymes is to break down the glycosidic linkages of cell wall polysaccharides. Plant growth-promoting rhizobacteria (PGPR) is one such beneficial group of microbes that promote plant growth and yields besides contributing to plant disease management through direct and indirect mechanisms. Enzyme-based lysis of plant pathogens by PGPR is also reported in several crops. The main hydrolytic enzymes are chitinase, glucanase, protease, and cellulose. In this regard, the production of PGPR hydrolytic enzymes plays an important role in the phytopathogen-controlling mechanism. Further, these enzymes are involved in sustainable plant disease management by breaking down the fungal pathogens cell wall and causing cell death. Our review critically discusses on

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various enzymes produced by PGPR strains and their application in sustainable management of phytopathogens.

**Keywords** Chitinase · Glucanase · Hydrolytic enzymes · Phytopathogen · Protease

## 1 Introduction

Plant pathogens are a serious problem worldwide amongst crop cultivars. Soil-borne phytopathogens are responsible for causing major plant diseases; plants suffer from infectious diseases caused by different pathogens; amongst them, fungi are responsible for majority of infectious plant diseases (Shaikh and Sayyed 2015b). It mainly effects on productivity and economic values. Plant growth-promoting rhizobacteria (PGPR) or fungi control over phytopathogens is an excellent biocontrol approach. Biocontrol helps in suppressing the growth of phytopathogens as well as reducing the use of chemical fungicides (Sayyed et al. 2010). The rhizosphere is rich in nutrients, energy, carbohydrates, and amino acids, and it contains a variety of microorganisms. The bacteria residing in this region are called rhizobacteria. The PGPR are beneficial and agriculturally important bacteria having symbiotic relationships with plants, and they also enhance plant growth and health by suppressing plant pathogens with the help of hydrolytic enzymes (Tariq et al. 2017). Recently, more researches are being conducted on these hydrolytic enzymes and their role in suppressing the phytopathogens, their mechanisms, and solutions to molecular level of gene coding to control the pathogens. These enzymes with rhizospheric microbes play an important role in controlling plant diseases. Pathogenic fungi are continually becoming resistant to existing fungicides; therefore, other methods of disease control are highly desirable (Hasan et al. 2014). The application of hydrolytic enzymes producing rhizospheric microbes is an ecofriendly solution to the environment. Recent studies describe that hydrolytic enzymes help in the control of plant pathogens (Shaikh and Sayyed 2015a). The chitinase, protease, and glucanase enzymes are responsible for disease resistance in plants and are an alternative available to be included in integrated disease management and also which are safer and relatively less expensive. This chapter discusses on the various enzymes produced by PGPR strains and their application in sustainable management of phytopathogens.

## 2 Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria are a group of bacteria that enhances plant growth. These PGPR are beneficial micro-organisms which help in agriculture and have increased globally (Etesami and Maheshwari 2018). PGPR are well

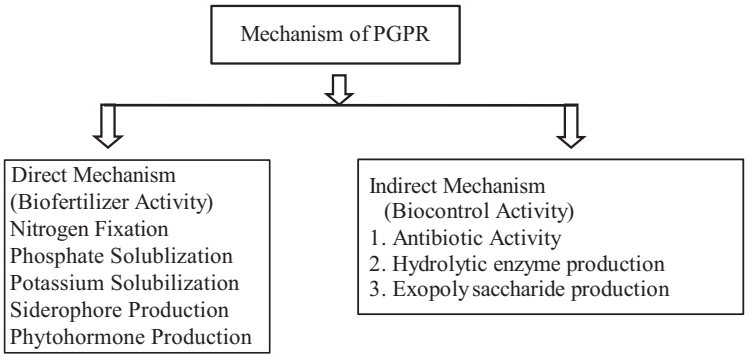


**Fig. 15.1** Mechanism of PGPR

recognized as biofertilizers and efficient soil microbes for sustainable agriculture and hold great promise in the improvement of agriculture yields (Singh et al. 2013).

PGPR help in plant growth by direct and indirect mechanisms (Fig. 15.1). They enhance plant growth and can help in sustainability of safe environment and crop productivity (Benaissa et al. 2019). Several investigations are conducted on PGPR's role and mechanism for further research. They also play an important role in suppressing plant pathogens, fungi, viruses, and nematodes and even against abiotic stresses (Basu et al. 2021).

The uses of PGPR are shown in Fig. 15.2. The PGPR act as biofertilizers and also help in the phosphate solubilisation, uptake of nutrients, and in the enhancement of root growth and root system (Miransari et al. 2014). PGPR secret extracellular metabolites called as siderophores, which mainly help in the phytopathogen suppression and also in the uptake of iron to the plant growth and mechanism (Prema and Selvarani 2013). They also help in the production of antibiotics, act as biocontrol agents, and produce phytohormones that promote plant growth and yield. These siderophores are eco-friendly in nature, sustainability of safe environment (Vessey 2003). PGPR can be very effective and are potential microbes for enriching the soil fertility, soil environment, and phytopathogen suppression (Glick 2012). PGPR diversity in the rhizosphere along with their colonization ability of a wide range of cultivated plants and mechanism of action should facilitate a sustainable agriculture system. The role of PGPR in various mechanisms is listed in Table 15.1.

## 3 Hydrolytic Enzymes

A rhizospheric microbe produces hydrolytic enzymes which help in inhibiting the growth of phytopathogens through hydrolysis of their cell wall, proteins, and DNA. Hydrolytic enzymes of microorganisms play a very important role against pathogen suppression. Mainly it helps in crop protection, plant growth, and crop yield (Sayyed et al. 2010). These enzymes exhibit hyper-parasitic activity, attacking

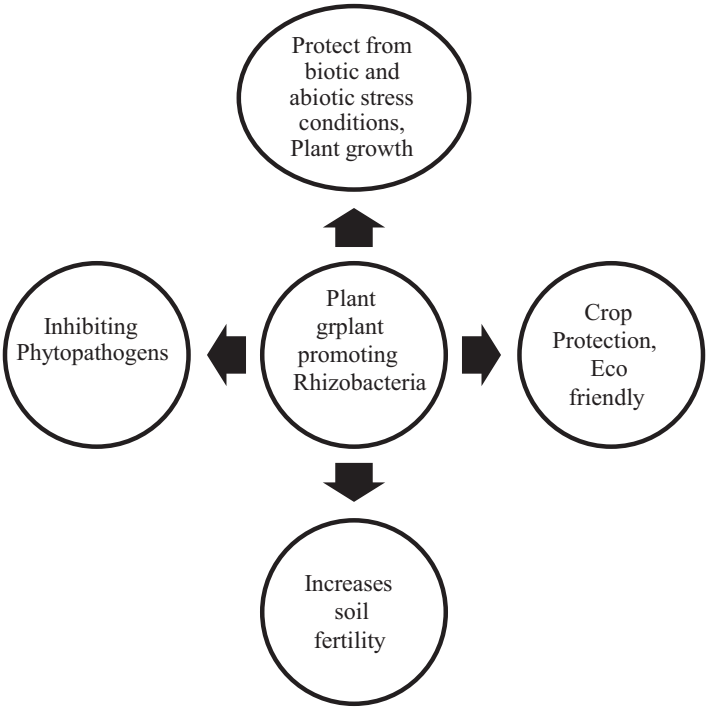


**Fig. 15.2** Uses of plant growth-promoting rhizobacteria

**Table 15.1** PGPR microbe's role in various mechanisms

| Mechanism | PGPR | Application | Reference |
|---|---|---|---|
| 1. *Phytohormone production* | *Acetobacter diazotrophics*, *Azospirillum* sp., *Azospirillumlipoferum*, *Azospirillum brasilense* | Helps in the plant growth | Sharma et al. (2019) and Singh et al. (2013) |
| 2. *Crop and fruit yield through PGPR* | *Rhizobium leguminosarum*, *Pseudomonas putida*, *Pseudomonas fluorescence*, *Azotobacter*, *Azospirillum* | Direct growth promotion and growth of wheat and maize plants, improved seed germination | Singh et al. (2013) and Sharma et al. (2019) |
| 3. *Biocontrol agent* | *Bacillus* sp., *B. subtilis*, *Pseudomonas fluorescens*, *Bacillu* sp. | Protect plants against various diseases | Sharma et al. (2019) and Singh et al. (2013) |
| 4. *Nitrogen fixation* | *Azospirillum*, *Azotobacter*, *Frankia*, *Pseudomonas* | Enhance plant growth and suppress the attack of phytopathogens | Nandal and Hooda (2013) and Sharma et al. (2019) |
| 5. *Siderophore production* | *Aerobacteria*, *Pseudomonas florescence* | Phytopathogen suppression and plant growth | Kenneth et al. (2019) |
| 6. *Phosphate solubilisation* | *Bacillus*, *Azotobacter*, *Pseudomonas*, *Rhizobium* | Increase plant growth | Singh et al. (2013) and Sharma et al. (2019) |

pathogens by excreting cell wall hydrolyses. PGPR enzyme activity plays a very significant role in plant growth promotion particularly to protect them from biotic and abiotic stress by the suppression of pathogenic fungi including *Fusarium oxysporum, Sclerotium rolfsi*, *Rhizoctonia solani,* and *Phthium ultimum* (Upadyay et al. 2012). These enzymes either digest the enzymes or deform components of the cell wall of fungal pathogens. It is also one of the important mechanisms for environment-friendly control of soil-borne pathogens (Neeraja et al. 2010). Hydrolytic enzymes help in the decomposition of non-living organic matter and plant residues to obtain carbon nutrition. Hydrolytic enzymes directly contribute in the parasitisation of phytopathogens and rescue plants from biotic stresses.

## *3.1 Role of Hydrolytic Enzyme*

1. Ability to control plant pathogens.
2. These are able to degrade the fungal cell wall and cause the cell lysis of fungal pathogens.
3. Gives plant protection.
4. Through these enzymes plant growth-promoting rhizobacteria help in plant growth promotion.
5. Particularly to protect from biotic and abiotic stresses by suppression of pathogenic fungi.

## *3.2 Mechanism of Hydrolytic Enzymes Against Phytopathogens*

The mechanism involves four major steps, which are as follows:

1. Niche competition – In this, the phytopathogens growth occurs. They adjust to the rhizospheric environment in the soil or host tissue.
2. Mycoparasitism – Leading to the lysis of fungal pathogen.
3. Production of antibiotics – That interfere with the metabolism of phytopathogen.
4. Production of hydrolytic enzymes – That degrade the cell wall of phytopathogens (Sayyed et al. 2013).

## *3.3 Types of Hydrolytic Enzymes*

The enzymes involved in pathogenesis are as follows:

(a) Chitinase
(b) Pectinase

(c) Cellulase
(d) Glucanse
(e) Proteinase

(a) *Chitinase* – Chitinases are chitin degrading enzymes and play an important role in biological control and plant defense mechanisms against phytopathogens. Chitinase lyses the fungal cell wall through degradation of chitin polymer present in the cell wall of fungal phytopathogens. Chitinase produced by rhizobacteria exhibits antagonism in vitro against fungi. The bacteria produce chitinase enzymes, which are *Xanthomonas, Serratia, Chromobcaterium, Klebsiella, Pseudomonas, Aeromonas,* and *streptomyces.* Fungal chitinases are produced from *Trichoderma, Penicillum, and Agaricus* (Sharma et al. 2011). The chitinase enzyme or purified chitinase proteins through manipulation of gene coding for chitinase are used in the biocontrol of microorganisms. In this way, chitinase degrades and suppresses the phytopathogens (Kim et al. 2003). Chitin participates in plant defense system by stimulating their physical, chemical, biological, and kinetic properties. It helps in chitin degradation by the cleavage of glycosidic bond between C1 and C4 carboxylic position of two N-acetyl–D-glucosamine monomers of chitin. Chitinase is gaining importance by researchers, as they show a prime role in the plant defense system and managing fungal infections (Jalil et al. 2015).
(b) *Pectinase* – Pectic substances are the components of the middle lamella, and they protect as an intracellular cementing material between plant cells. It forms a large portion of the primary cell wall. It forms space between cellulose microfibrils. Plant pectin is a polysaccharide composed of galacturonic acid residues. It helps in the successful entry of pathogens into the host cells, and it degrades the cell wall easily.
(c) *Cellualse* – The entry of host cell in the pathogen should break the thick cellulosic cell wall present around the cell. Cellulase enzyme production is a major characteristic of plant pathogens of bacterial and fungal origin. Cellulases hydrolyze the 1,4 β-glucosidic linkages in cellulose, help in the recycling of this polysaccharides, and complete degradation of cellulose, which involves an interaction between different celluloytic enzymes like cellulose, exo- cellobiohydrolae into β-glucose (Lynd et al. 2002; Jadhav and Sayyed 2016).
(d) *Glucanse* β-1,3-Glucanase found in plants, bacteria, and fungi. Enzymes help in the degradation of cell walls of fungi and yeast. These enzymes can hydrolyze the substrates by two mechanisms, one by hydrolysing the substrate by sequentially cleaving glucose residues from the non-reducing end and the other by cleaving linkages at random sites along the polysaccharide chains (Jadhav and Sayyed 2016).
(e) *Protease* – It helps in the lysis of cell wall of phytopathogenic fungi. The protease enzymes break down major proteins of phytopathogens into peptides chains and further to aminoacids. They destroy the pathogenic protein action to act on plant cells. Protease helps in the inactivation of extracellular enzymes of pathogenic fungal species. Protease of trichodermasp also plays a siginificant role in

the lysis of cell walls of phytopathogenic fungi and also helps in inactivating extracellular enzymes and finally destroys the phytopathogens (Xia 2004).

## 4 Importance of Rhizospheric Microbial Enzymes

A rhizospheric microbe helps in the biocontrol of phytopathogens and also produces cell wall degrading hydrolytic enzymes. Many rhizobacteria are capable of synthesizing these extracellular enzymes. It hydrolyzes a variety of polymeric compounds like chitin, proteins, cellulose, hemicelluloses, and DNA of phytopathogens (Kobayashi et al. 2002). The microbial strains like *S. marcescens, B. Subtilis, B. cereus, and B. thurigensis* produce hydrolytic enzymes that control phytopathogens like R. solani, F. Oxysporum, S. Rolfsii, and P. ultimumetc (Prasannath 2017). In recent years, the most valuable and important rhizospheric microbes are *Pseudomonas fluorescens* and *Trichoderma* sp. The *Psesudomonas fluorescens* is one of the potential biological control agents due to its ability to colonize the rhizosphere and protect plants against a wide range of important fungal diseases, namely, black root rot of tobacco, root rot of mustard, and damping-off of sugar beet in field condition (Arora et al. 2008; Kumar et al. 2000). Lytic enzyme produced by myxobacteria is effective in the suppression of fungal plant pathogens (Bull et al. 2002). Antagonistic bacteria Serratia marcescens reduce mycelia network of Sclerotium rolfsii by expressing chitinase enzymes. Lysobacter is capable of producing glucanase that is involved in the control of diseases caused by bioplaris and Pythium sp. (Palumbo and Yaen 2005). The inoculation of plants with arbuscular mycorrhiza also improves plant growth. Some strains of Trichoderma sp have been widely used as biological control agents as well as plant growth promoters. Some of the rhizospheric micro-organisms showing hydrolytic activity are listed in Table 15.2.

## 5 Conclusion

Plant growth-promoting rhizobacteria produces hydrolytic enzymes. For control of phytopathogens, hydrolytic enzyme usage is one of the best alternatives compared to chemical fungicides. Applications of these rhizobacterial enzymes help to improve in crop protection, plant growth, and high yield. The study of these hydrolytic enzymes of rhizobacteria will help in manipulating the bacteria community with biological control and plant growth ability in the rhizospheric zone of different sites. The application of efficient rhizobacterial strain secreting various hydrolytic enzymes will help to reduce the liberal use and dose of agrochemicals. Research is going on the PGPR and hydrolytic enzymes to improvement in the crops yield and role of hydrolytic enzymes in suppress the pathogens.

**Table 15.2** List of micro-organisms showing hydrolytic activity

| Microbes showing hydrolytic activity | Hydrolytic enzymes produced | Target phytopathogens | Reference |
|---|---|---|---|
| *S. marcescens* | *Chitinase* | *R. solani* and *R. oxysporum* | Someya et al. (2000) and Jadhav et al. (2017) |
| *B. subtilis NPU 001* | *Chitinase* | *F. oxysporum* | Chang et al. (2010) and Jadhav et al. (2017) |
| *S. plymuthica C48* | *Chitinase* | *Botrytis cinerea* | Frankowski et al. (2001) and Jadhav et al. (2017) |
| *Paenibacillus* sp. *Strain300* and *Streptomyces* sp. *strain 385* | *β-1,3-glucanase* | *F. oxysporum* | Jadhav et al. (2017) |
| *Bacillus coagulans* | Carboxymethylcellulase and polygalacturonase | – | Odeniyi et al. (2009) and Jadhav et al. (2017) |
| *Bacillus subtilis YJ1* | *Cellulase* | – | Li-Jung et al. (2010) and Jadhav et al. (2017) |
| *Cellulomonas* sp. *ASN2* | *Cellulase* | – | Basavaraj et al. (2014) and Jadhav et al. (2017) |
| *P. aeruginosa PGPR2* | *Protease* | *Macrophomina* sp., *Rhizoctonia* sp. and *Fusarium* sp. | Illakkiam et al. (2013) and Jadhav et al. (2017) |
| *Bacillus subtilis PE-11* | *Alkaline protease* | – | Adinarayana et al. (2003) and Jadhav et al. (2017) |
| *Paenibacillus* and *Streptomyces* | – | *F. oxysporum* | Compant et al. (2005) and Jadhav et al. (2017) |
| *B. cepacia* | – | *R. solani*, *P. Ultimum* and *S. Rolfsii* | Compant et al. (2005) and Jadhav et al. (2017) |
| *P. fluorescens LRB3W1* and *S. marcescens B2* | – | *F. oxysporum* | Someya et al. (2007) and Jadhav et al. (2017) |

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# Chapter 16
# Fungal Hydrolytic Enzymes Produced by Plant Growth-Promoting Rhizobacteria (PGPR)

**Lucky Duhan, Deepika Kumari, Rohit Verma, and Ritu Pasrija**

**Abstract** Roots are the lifeline of plants and besides anchorage, they are a source of nutrients incorporation from soil. Although health-giving roots safeguard plants' fitness, but adjacent soil is also a dwelling place for various microbial pathogens, which might attack the roots. To neutralize this, soil has plant growth-promoting rhizobacteria (PGPR), which are generally free-living and populate around plants' roots. They defend plants from various biotic and abiotic stresses, as well as enhance soil texture for superior plant growth. The PGPR involves various species, like *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, *Enterobacter, Agrobacterium, Serratia,* etc., but *Bacillus* and *Pseudomonas* are most predominant. They encourage robust plant growth, in both direct and indirect manner. The direct mechanism refers to nutrient uptake, release of siderophores, seed germination, etc. While indirect mechanisms include release of enzymes like chitinase, protease/elastase, cellulase, catalase, β-(1,3)-glucanase, etc., and hydrogen cyanide, and antibiotics. The hydrolytic enzymes synthesis/secretion is under stringent regulation and shields the roots from pathogens attack, including fungi. The enzymes targeting fungal microbes, either generate disturbance in the cell wall structure, interfere with membrane composition, impede hyphal formation, cause myco-parasitism, etc., leading to fungal cell death. Indirect mechanisms also involve induced systemic resistance (ISR) and reinforce the roots by evolving physical and chemical barriers to withstand adverse conditions.

The PGPR-mediated fungal biocontrol suggests their imperative role in sustainable pathogen management and ultimately supporting plants' well-being besides yield. This chapter summarizes the PGPR role in fungal control, especially through their hydrolytic enzymes.

**Keyword** Hydrolytic enzymes · Induced systemic resistance (ISR) · MOA · Myco-parasitism · PGPR · Phytopathogens

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## 1 Introduction

In the modern era, one of the biggest challenges before mankind is feeding the expanding population; this has placed a substantial liability on farmers and governments to increase the yield and crops' quality. To achieve this goal, various modern agricultural tools and hybrid seed varieties are developed and employed to significantly escalate the agricultural production. However, despite the deployment of these practices, different plant diseases cause significant reduction (~30%) in yields, which puts a huge economic burden on the producers and country (Sayyed et al. 2012). To combat these plant diseases, cultivators often turn towards chemical pesticides that are overpriced and have detrimental after-effects on the ecosystem as well. So, to circumvent these drawbacks of chemicals-based pesticides, renewed attempts involve inculcation of a safer and inexpensive practice involving the Plant Growth-Promoting Rhizobacteria (PGPR) that boost seed germination, root development, water utilization, resistance development against plant pathogens, etc., which finally promote plant growth and yields.

In 1904, a German scientist named Hiltner, coined the term "rhizosphere" referring to the soil around the plants' roots, which is rich in varied bacterial population density (100–1000 folds) than bulk soil. These bacteria form micro-colonies and constitute ~15% of the root surfaces (Gray and Smith 2005). "PGPR" refers to a heterogeneous group involving several bacterial species that populate the rhizosphere and promotes plant growth through separate mechanisms. Thus, it is predictable that the rhizosphere is a region of immeasurable microbes' interactions with plant roots, as root secretions act as a major nutrient source for these microbes and support efficient geo-cycling of nutrients. In general, PGPR can perform functions as biofertilizers, biostimulator, rhizo-mediator, and biopesticides (Table 16.1).

PGPR classification: Different criteria can be used for their classification and these are discussed here.

***Based on Location*** Depending upon the interrelation with plant roots, PGPR can be categorized into two types: the first is **extracellular PGPR** (ePGPR), which are

**Table 16.1** Classification of PGPR according to their use and mechanism of action (MOA)

| Class | Description | Mechanism of Action (MOA) |
|---|---|---|
| Biopesticide | PGPR improve plant growth and yield by inhibiting the phytopathogens. | By production and release of hydrolytic enzymes, antibiotics, siderophores, hydrogen cyanide (HCN), induced systemic resistance (ISR), etc. |
| Biofertilizer | PGPR improve plant growth and yield by supplying growth nutrients. | By nitrogen fixation and utilization of insoluble nutrients from the soil |
| Phyto-stimulator | PGPR improve plant growth and yield by supplying of different phytohormones for various functions in plants. | By production of phytohormones i.e., indole acetic acid (IAA), gibberellic acid (GA), cytokinesis, ethylene, jasmonic acid (JA), etc. |

Source: Adapted from Shah et al. (2018)

found predominantly inside the rhizosphere or in between root cortex cells. These include species of *Agrobacterium*, *Arthobacter*, *Bacillus*, *Caulobacter*, *Erwinia*, *Micrococcus*, *Pseudomonas*, *Serratia,* etc. The second group is **intracellular PGPR** (iPGPR), restricted to specific sections in root nodules (Gray and Smith 2005). In particular, Rhizobacteriaceae family bacteria reside in these zones, which contain *Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, *Frankia,* etc. Experiments have validated the contribution of both these categories in improving the yields, by generating resistance in plants that too without any side effects (Vessey 2003; Gray and Smith 2005).

***Based on Mechanisms*** Apart from location, direct or indirect impact is also a valid criterion to categorize PGPR, as shown in Fig. 16.1. Direct impacts involve nitrogen fixation, phosphorous and potassium solubilization, release of siderophores, seedling enhancement, etc., promoting nutrient uptake and growth of the plants. On the contrary, indirect influences comprise antagonistic compounds production like antibiotics, hydrolytic enzymes, etc., that provide resistance, especially against fungal phytopathogens, as compiled in Table 16.1. Additionally, fungal pathogens are rendered ineffective due to the mycoparasitism (parasite to fungi) activity of PGPR, and ultimately protecting the plant roots (Woo and Lorito 2007). Besides, rhizobacteria also augments the plant defense called "Induced Systemic Response (ISR)" by activation of a latent resistance system containing physical and chemical barriers (Loon et al. 1998). Intriguingly, the enhancement is not restricted to the nodular area but also protects the distal parts of plants. This response involves signaling pathways and employs compounds such as jasmonic acid (JA), ethylene and other components like antibiotics, siderophores, and hydrolytic enzymes, which exhibit synergism in inducing ISR against the phytopathogens. The next section explains

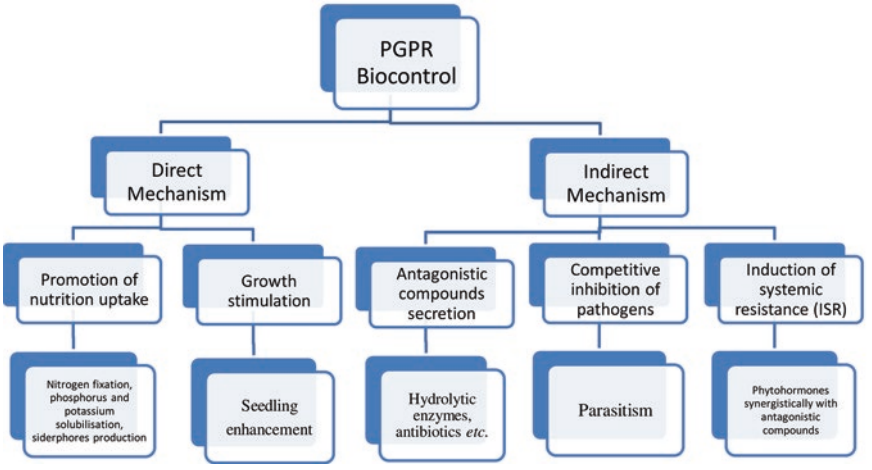


**Fig. 16.1** Various mechanisms involving PGPR-mediated biocontrol of phytopathogens. Biocontrol may be done by one or more than one mechanism acting in synergism

the various components of PGPR secretions, with major emphasis on hydrolytic enzymes.

## 2 PGPR Secretions

PGPR emancipate various metabolites and hydrolytic enzymes, which doesn't allow the fungal phytopathogens to carry out a successful attack.

### *2.1 Hydrolytic Enzymes*

The PGPR are efficient in production of several different hydrolytic enzymes i.e., chitinase, glucanase, protease/elastase, cellulase, catalase, etc. These enzymes have activities against several phytopathogens including fungi, thereby restricting several plant diseases. Although the hydrolytic enzymes perform their function via various mechanisms, but the major one remains degrading the glycosidic bonds in fungal wall chief component chitin. This inhibits hyphal formation in fungi, a crucial step in deeper fungal penetration in plant tissues.

Besides enzymes, various antibiotics, toxins, or volatile compounds are also synthesized by PGPR, which are extremely target specific and thus prevent varied pathogens from attacking plant root nodules. It is reported that physical factors such as pH, temperature, and moisture content influence antibiotic production (Shanahan et al. 1992). *Pseudomonas* secretes lipopeptides, hydrogen cyanide (HCN), phenazines, pyoluteorins, etc. (Haas and Keel 2003). Alongside, different antibiotics, antibacterial, antivirals, and cytotoxic agents effective against insects like antifeedant and anti-helminthic molecules are also produced.

### *2.2 Antibiotics*

PGPR-mediated antifungal activity is also due to the release of antibiotics (Haas and Keel 2003). These antibiotics are a heterogeneous group of organic, low-molecular-weight compounds (Duffy et al. 2003). The *Bacillus* strains are associated with production of more than 20 different antibiotics, most important being Kanosamine, Zwittermycin A, Iturin A (Cyclopeptide), Bacillomycin, Plipastatins, etc. (Volpon et al. 2000). Haas and Defago categorized the antibiotics into two subclasses: (1) diffusible antibiotics, which involve five classes – phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, and cyclic lipopeptides, and (2) volatile antibiotics, like hydrogen cyanide (HCN) (Haas and Defago 2005).

### *2.3 Siderophores*

PGPR produce siderophores, which are low-molecular weight and high-affinity iron chelating compounds that help in ion uptake through channels across cell-membrane. Their importance can be estimated from the fact that iron in soil is actually present in bound state, which is inaccessible for plants' usage and can lead to iron deficiency and reduced yield. The siderophores facilitate iron solubilization and absorption by its chelation from various organic and inorganic sources (Wandersman and Delepelaire 2004). These compounds bind easily to insoluble ferric ions ($Fe^{3+}$) and facilitate their absorption as soluble form. Siderophores are broadly divided into four classes – hydroxamates, carboxylate, catecholates, and mixed type.

## 3 PGPR-Mediated Biocontrol

In general, biocontrol refers to the use of living organisms to suppress the growth of pathogen by either direct (parasitism, hyper-parasitism, commensalism), indirect (competition, systemic acquired or ISR), or hybrid antagonistic modes (like production of antibiotics, lytic enzyme, siderophores, volatile organic substances, etc.) (Heimpel and Mills 2017). Free-living PGPR restrain bacterial, nematode, viral, and fungal pathogens by controlling microbial balance near plant roots (Kenawy et al. 2019).

Fungal pathogens cause a lot of diseases in plants and their effective control is required for improved harvest and quality. The common fungal pathogens attacking plants are summarized in Table 16.2. The biocontrol of fungal phytopathogens through the PGPR involves metabolites secretion including hydrolytic enzymes, which play the central role in suppressing the fungal infections (Gangwar et al. 2016).

The fungal pathogen exists in diverse morphological forms, such as spores, hyphae, or fruiting bodies. Thus, an effective response requires rhizobacteria to recognize all these structures. The fungal phytopathogen exists in close proximity to the rhizobacteria, enabling direct target recognition, penetration, and lysis of pathogenic cells (Shaikh and Sayyed 2015). Although, the attachment can either be direct connection of bacteria and target cell, or entrapment of phytopathogen into the rhizobacterial biofilms.

Here, although lysis seems the only method in biocontrol, but various other mechanisms exist in combating these plant fungal pathogens. Some of major mechanisms in effective biocontrol are following:

(a) *Niche Competition* – The PGPR contest with fungal pathogens for the niche and outcompetes the fungal phytopathogens from plant tissue and soil (Loper and Henkels 1997).
(b) *Antibiotics, Siderophores Production* – These compounds manipulate the metabolism of fungal phytopathogen and restrict pathogen growth (Beneduzi et al. 2012).

**Table 16.2** Various fungal pathogens causing plant diseases

| Fungal pathogen | Target plant species | Disease | References |
|---|---|---|---|
| *Syncephalastrum racemosum* | Potatoes, onions, carrots, fleshy organs, etc. | Soft rots | Misra et al. (2016) |
| *Phytophthora sp.* | Jarrah | Root rots | Rea et al. (2010) |
| *Puccinia* | Wheat, oats, rye, barley | Rusts | Uchida et al. (2006) |
| *Alternaria solani* | Potato | Early blight | Abuley and Nielsen (2019) |
| *Phytophthora infestans* | Potato | Late blight | Small et al. (2015) |
| *Gibberella circinata* | Woody plants | Cankers | Wingfield et al. (2002) |
| *Claviceps purpurea* | Wheat, rye, barley, and other grasses | Ergots | Giesbert et al. (2008) |
| *Rhizoctonia solani* | On the whole lawn irregularly | Brown and yellow patches | Giesler and Yuen (1998) |
| *Fusarium sp.* | Potatoes, onions, carrots, fleshy organs | Dry rots | Heltoft et al. (2016) |
| *Fusarium sp.* | Mango | Leaf spot | Sultan et al. (2019) |
| *Phragmidium satoanum* | Rose | Leaf rust | Ono and Wahyuno (2019) |
| *Puccinia arachidis* | Groundnut | Leaf rust | Sathiyabama and Balasubramanian (2018) |
| *Uncinula necator* | Onions,cucumbers, Grains, alfalfa | Powdery mildew | Doster and Schnathorst (1985) |
| *Erwinia amylovora* | Pea and apple | Fire blight | Braun-Kiewnick et al. (2011) |
| *Fusarium graminearum* | Wheat, rye, barley, potatoes | Scab | O'Donnell et al. (2000) |
| *Uromycladium tepperianum* | Sengon | Gall rust | Lestari et al. (2013) |
| *Xanthomonas oryzae* | Rice | Leaf blight | Wongkhamchan et al. (2018) |
| *Bipolaris maydis* | Maize | Leaf blight | Kumar et al. (2016) |
| *Fusarium oxysporum* | Potatoes, alfalfa | Wilts | Pietro et al. (2003) |
| *Erwinia amylovora* | Apple | Fire blight | Gaucher et al. (2013) |
| *Phomopsis sp.* | Various plants | Seed decay | Li et al. (2015) |
| *Pythium and Fusarium* | Various plants | Damping off | Mao et al. (1997) |
| *Glomerella cingulata* | Apple | Leaf spot | Liu et al. (2016) |

(c) *Hydrolytic Enzymes Production* – Hydrolytic enzymes refer to various proteases and lipases, which together work and degrade the cell wall of fungal phytopathogens (Sayyed et al. 2013).

(d) *Mycoparasitism* – Refers to parasitism on fungal pathogen (Woo and Lorito 2007).

(e) *Induction of ISR* – ISR improve antifungal activity of plants by strengthening the physical and chemical barriers (Beneduzi et al. 2012).

## *3.1 The PGPR Released Hydrolytic Enzymes*

PGPR secretes various extracellular hydrolytic enzymes i.e., chitinase, protease, cellulose, β-(1,3)-glucanase, etc., which play a key task in the inhibition of fungal phytopathogens growth (Wang et al. 2019). These hydrolytic enzymes cleave the cell wall units of fungi, including, chitin, proteins, cellulose, hemicelluloses, glucans, etc., thus inhibit the hyphal formation and penetration deep into plant tissues.

### 3.1.1 The Fungal Cell Wall: Weaker Link

The cell wall is a protective barrier in fungi and guards against external environmental stresses, but also controls morphogenesis, as well as helps in plant-fungal interaction (Latge and Beauvais 2014). The criticalness of cell wall in maintaining the integrity of the fungal cell and is regarded as an outstanding target for antifungal compounds (Geoghegan et al. 2017). The fungal cell wall is made up of approximately 80% of the fibrillar cross-linked polysaccharides. The major components are chitin, glucans, mannans, polyphosphate, and glycoproteins. These are cross-linked together and build the skeleton of the cell wall (Bowman and Free 2006), (Geoghegan et al. 2017). The fibrillar polymers are surrounded with the complex gel-like matrix including polyglucuronic acid, xylomannoprotiens, polyphosphate, etc. (Table 16.3).

About 20–30% of the proteins exist as glycoproteins and either form the structural framework of the cell wall (Srinorakutara 1998) or perform various functions like aiding in water movement, preventing desiccation, or signaling proteins (receptors) involved in regulation, etc. (Cox and Hooley 2009). Therefore, disturbing the homeostasis or degrading the integrity is one of the most employed mechanisms of hydrolytic enzymes mediated fungal combating.

Hydrolytic enzymes have the capacity of destroying the fungal cell wall structure and integrity (Budi et al. 2001). They function by breaking or disturbing the glycosidic bonds forming the chitin polymers and results in lysis of cell walls, inhibition of germ tubes, and hyphae formation (Shaikh and Sayyed 2015; Kim et al. 2003). Wu et al. studied the control of paper seedling wilt disease, caused by a thread-like fungus *R. solani,* which is confronted by various hydrolytic enzymes, namely, chitinase, β-1,3-glucanase, peroxidase, catalase, superoxide dismutase (SOD), polyphenol oxidase, phenylalanine ammonia lyase, etc., from *Bacillus subtilis* SL-44 (PGPR). These enzymes fracture the mycelia and thus result in leaking of cell material, ultimately leading to fungal cell death in the pepper plant (Wu et al. 2019). Hydrolytic enzymes also act synergistically with other anti-fungal by-products of PGPR. Someya et al. demonstrated the synergistic effects of hydrolytic enzymes of *Serratia marcescens* B2, with efficacy of *Pseudomonas fluorescens* LRB3W1 antifungal compounds, against cabbage *Fusarium* yellows, caused by *F. oxysporum*. Reportedly, the fungal cell wall and hyphae degradation were more effective than PGPR hydrolytic enzymes alone (Someya et al. 2007). In the next section, some major hydrolytic enzymes and their mode of action are discussed in detail.

**Table 16.3** List of major components of fungal cell walls

| Fungal classification | Fibrous polymers | Gel-like polymers |
|---|---|---|
| Basidiomycota | Chitin β-(1–3), β-(1–6) glucan | Xylomannoproteins α (1–3) glucan |
| Zygomycota | Chitin chitosan | Polyglucuronic acid, glucuronomannoproteins, polyphosphate |
| Ascomycota | Chitin β-(1–3), β-(1–6) glucan | Galactomannoproteins α (1–3) glucan |
| Chytridiomycota | Chitin glucan | Glucan |
| Oomycetes | β-(1–3), β-(1–6) glucan cellulose | Glucan |

Adapted from Gow and Gadd (1995)

## Chitinase

Chitinase [EC 3.2.1.14] is the chief hydrolytic enzyme released by PGPR. Its antifungal activity is well-known and as the name suggests, it acts on polymer chitin, present in fungal cell wall. Chitin polymer is formed by β-1,4 linkages between N-acetyl-D-glucosamine (NAG or GlcNAc) subunits, as shown in Fig. 16.2 (Pillai et al. 2009). The purified enzyme works as efficiently as chitinase coding genes in bacteria (Kim et al. 2003). In general, chitinases are found in a number of chitin-containing microbes like insects, crustaceans, yeasts, and fungi, and also in many non-chitin synthesizing cells of bacteria, higher plants, viruses, animals, etc. (Sharp 2013). Table 16.4 is a compilation of chitinase released by various PGPR, which suppress fungal phytopathogens effectively.

Besides bacteria, the cloning and purification of *CHIA* (Chitinase Acidic) gene, encoding chitinase has also been tried for controlling fungal phytopathogens. Oppenheim and Chet effectively controlled the *S. rolfsii* and *R. solani* fungal pathogens, by cloning, expressing, and purifying *CHIA* gene (*S. marcescens)* product in *E. coli* (Oppenheim and Chet 1992). Similar results were obtained in producing chitinase, chitosanase (chitosan), and protease enzymes from *B. cereus* QQ308, which suppressed spore germination and tube formation in *F. oxysporum, F. solani,* and *P. ultimum* on Chinese cabbage plant (Chang et al. 2007). Jones et al. rather followed the forward genetics approach and inactivated the *ChiA* gene in *S. marcescens* to make chitinase mutants and studied its effect on growth of *F. oxysporum* in pea plants (Jones et al. 1986). These various studies prove that indeed chitinase enzyme can be used as controlling means against fungal phytopathogen.

*Types of Chitinase enzymes* – The chitinase can be divided into two main groups.

1. *Endo-chitinases* – Cause random cleavage of chitin polymer at internal positions of linear chitin polymer which produce the diacetylchitobiose dimer, as well as GlcNAc soluble multimers like chitotriose and chitotetraose (Sahai and Manocha 1993).
2. *Exo-chitinases* – These are further sub-divided into two.

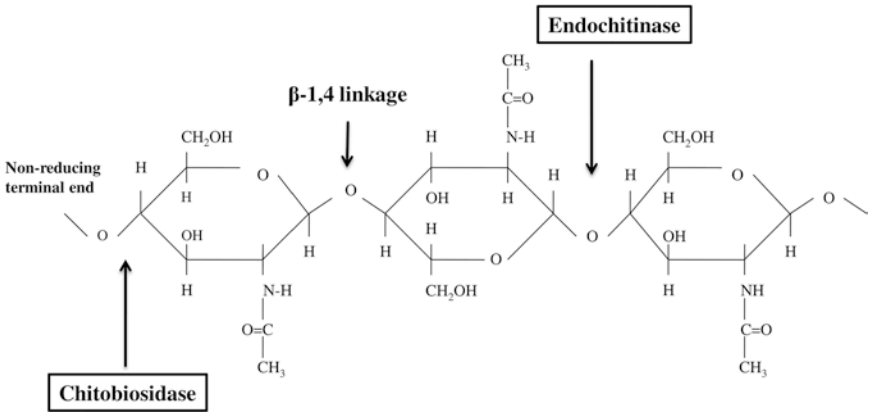


**Fig. 16.2** Sites of chitinase enzyme on chitin polymer in cell wall of fungal phytopathogens. Endochitinase catalyses random splitting of chitin polymer at internal positions. Chitobiosidase catalyses release of di-acetylchitobiose in chitin microfibril, starting from non-reducing end. 1–4-β-glucosaminidases splits the endochitinases and chitobiosidases oligomeric products, generating monomers of GlcNAc

(a) Chitobiosidases (E.C.3.2.1.29) – Catalyse the release of diacetylchitobiose of chitin microfibril from the non-reducing end.
(b) 1–4-β-glucosaminidases (E.C.3.2.1.30) – Split endochitinases and chitobiosidases, generating monomers of GlcNAc (Sahai and Manocha 1993).

### Glucanase

Glucanases refer to a category of hydrolases that breaks the glucosidic bond in glucans, a polysaccharide made of glucose monomers. Among these, β-1,3-glucanases [EC 3.1.1.6] are found in various microbes like bacteria, fungi, and higher plants (Simmons 1994). β-1,3(1,6)-glucans polysaccharides are a major structural framework component, having β-1,3-linked backbone and β-1,6-linkages in the fungal cell wall. The β-1,3-glucanase hydrolytic enzymes are released from various PGPR and cause lysis of β-1,3(1,6)-glucans polysaccharides thus, inhibiting the hyphal cell growth, ultimately leading to their death (Goswami et al. 2016; Fridlender et al. 1993) (Fig. 16.3). These are further sub-divided into two divisions.

1. *Exo-1,3-glucanases (EC 3.2.1.58)* – Catalyse hydrolysis of the fungal cell wall via sequential breakdown of glucose residues from the non-reducing end of glucan polysaccharides (Mouyna et al. 2013).
2. *Endo-1,3-glucanases (EC3.2.1.39)* – Catalyse hydrolysis via random breakdown of the glucan polysaccharide into oligosaccharides units (Mouyna et al. 2013).

Various groups took efforts to study glucanases in detail and made successful attempts at their purification. β-1,3 glucanase from *Pseudomonas cepacian* was

**Table 16.4** Various microbes showing hydrolytic antifungal cell wall lysis activities in different host plants

| Microbes releasing hydrolytic enzymes | Hydrolytic enzyme | Host plant | Target fungus species | References |
|---|---|---|---|---|
| *S. plymuthica C48* | Chitinase | Mustard crop | *Botrytis cinerea* | Frankowski et al. (2001) |
| *S. marcescens* QMB1466 | Chitinase | Pea | *F. oxysporum* | Jones et al. (1986) |
| *S. marcescens* | Chitinase | Cotton | *Sclerotium. rolfsii* and *R. solani* | Oppenheim and Chet (1992) |
| *Bacillus cereus* QQ308 | Chitinase, chitosonase, protease | Chinese cabbage | *F. oxysporum, F. solani, Pythium. Ultimum* | Chang et al. (2007) |
| *Bacillus* strain EBS8 | Chitinase | Maize | *F. verticillioides* | Abiala et al. (2015) |
| *S. marcescens* | Chitinase (into the *Rhizobium meliloti*) | Alfalfa | *R. solani* | Sitrit et al. (1993) |
| *B. subtilis* 30VD-1 | Chitinase, Protease | *Pea* | *Fusarium* sp. | Khan et al. (2018) |
| *Streptomyces griseus* | Chitinase | Cotton | *F. oxysporum, A. alternata, R. solani, F. solani* | Anitha and Rabeeth (2010) |
| *Paenibacillus sp.* strain 300 and *Streptomyces sp.* strain 385 | β −1,3-glucanase, Chitinase | Cucumber | *F. oxysporum* f. sp. *cucumerinum* | Singh et al. (1999) |
| *P. cepacia* | β-1,3-glucanase | Soil borne | *R. solani*, *S. rolfsii*, *P. ultimum* | Fridlender et al. (1993) |
| *B. subtilis* NSRS 89–24 | β-1,3-glucanase | Rice | *P. grisea* and *R. solani* | Leelasuphakul et al. (2006) |
| *P. aeruginosa* PGPR2 | Protease | Mung-bean | *Macrophomina sp., Rhizoctonia sp.* and *Fusarium sp.* | Illakkiam et al. (2013) |
| *B. subtilis* SL-44 | Lytic enzymes include chitinase and β-1,3-glucanase | Pepper | *R. solani* | Wu et al. (2019) |
| *P. fluorescens* LRB3W1 | Chitinase | Cabbage | *F. oxysporum* | Someya et al. (2007) |
| *Paenibacillus jamilae* HS-26 | Cellulase, Chitinas, protease, glucanase | Cucumber | *Fusarium* sp., *Alternaria* sp., *R. solani,* etc. | Wang et al. (2019) |

purified, by growing it on a laminarin (in brown algae) as a carbon source and found to be active (pH 5.0) (Fridlender et al. 1993). The β-1,3-glucanase of *Bacillus subtilis* NSRS 89–24 was even cloned and purified, having a molecular weight of 95.5 kDa. The optimal activity at pH 6.5–9.5 and 50 °C (Leelasuphakul et al. 2006). However, β-1,3-glucanase from *Trichoderma harzianum* is reported to be around

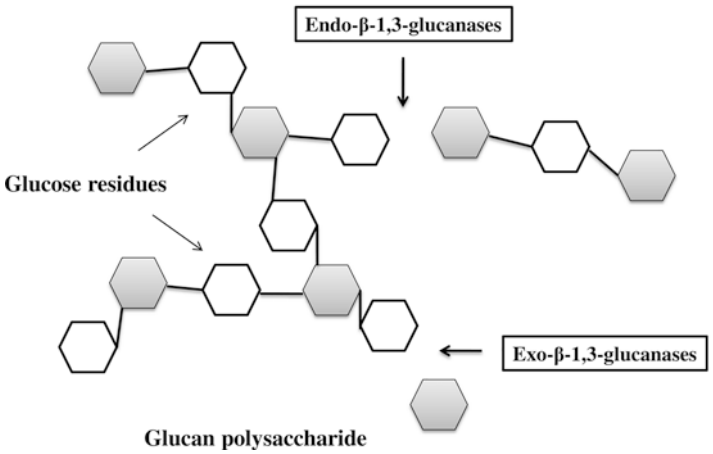


**Fig. 16.3** The mechanism of action of different glucanases on β-1,3-glucans. Exo-1,3-glucanase cause sequential breakdown of glucose residues of glucan polymers. Endo-1,3-glucanase cause random breakdown of the glucan polymers

29 kDa and active at pH 4.4 and 50 °C. Its $K_M$ and $V_{max}$ are 1.72 mg/ml and 3.10 U/ml, respectively, with laminarian as substrate (Noronha and Ulhoa 2000).

The inhibitory effects of β-1,3-glucanases from different PGPR are reviewed in Table 16.4. β-1,3-glucanase and chitinases from *Paenibacillus* sp. 300 and *Streptomyces* sp. 385, against *F. oxysporum* f. sp. *cucumerinum*, instigated cucumber' vascular wilt (Singh et al. 1999). β-1,3-glucanase from *Pseudomonas cepacia* cause fungal cell wall lysis in phytopathogens-: *R. solani*, *S. rolfsii*, and *P. ultimum;* and thus reduce diseases progression by 85%, 48%, and 71%, respectively (Fridlender et al. 1993). The inhibitory effects of β-1,3 glucanase from *Bacillus subtilis* NSRS 89–24 contained *Pyricularia grisea* and *R. solani* with MIC values of 12.5 mU/ml and 3.13 μg/ml, respectively. Further, β-1,3 glucanase act synergistically with antibiotics and show better results together than alone (Leelasuphakul et al. 2006).

## Protease

Fungal cell wall possesses various proteins and peptide units to provide essential structural framework. The PGPR proteases are extracellular and its intervened hydrolysis is not a mere theoretical possibility to disturb the cell wall integrity, but indeed substantiated with experiment based studies (Jadhav et al. 2017). Proteases [E.C. 3.4.24] play an important role in the phytopathogenic fungi biocontrol, as either alone or in synergism with other PGPR secretions. Although several microbes produce proteases, but it is the PGPR secreted proteases only which are primary in biocontrol activities against *Aspergillus flavus, A. niger, A. wentii, A. alternata, Byssochlamys fulva,* etc. (Sayyed et al. 2019; Tewari et al. 2019). It is reported that

extracellular proteases are chiefly released by *Bacillus sp.* (Sookkheo et al. 2000). Scientists claim that protease also display slight specificity against specific protein structures in cell wall of fungi.

The function of the proteases is shown in Fig. 16.4. Different studies of proteolytic enzymes active against fungal phytopathogens are compiled in Table 16.4.

The protease enzyme from *P. aeruginosa* mungbean rhizosphere was purified and molecular weight of purified protease is around 33 kDa, with activity of 235 U/mL at pH 6.0 and 40–70 °C temp (Illakkiam et al. 2013). However, Sookkheo et al. (2000) could purify three extracellular proteases from *B. stearothermophilus* strain TLS33, naming them as S, N, and B, with weights of 36, 53, and 71 kDa respectively. The optimal activities is rather reported at varied pH of 8.5 (72 °C), 7.5(78 °C), and 7.0 (90 °C) respectively (Sookkheo et al. 2000). It again reaffirms protease to be broad category.

The *P. aeruginosa* PGPR2 protease has antifungal activities against *Macrophomina sp., Rhizoctonia sp.,* and *Fusarium sp.*, and maximum activity is reported against *M. phaseolina* in agar disc diffusion assay with a distinct inhibition zone at pH 6.0 (Illakkiam et al. 2013). The protease, chitinase, and chitosanase from *B. cereus* QQ308 are active against *F. oxysporum, F. solani,* and *P. ultimum* infections in Chinese cabbage (Chang et al. 2007). Proteases released by PGPR are mainly extracellular; the quantity of proteases is very high and shows hydrolytic activity under harsh environmental conditions as well.

## Cellulase

Celluloses are microfibrils, rigid, insoluble, and crystalline structures. Cellulase enzyme system mainly involves the combination of the three major hydrolytic enzymes, involving endo-1,4-β-glucanase enzymes [EC 3.2.1.4], exo-1,4-β-glucanase enzymes [EC 3.2.1.91], and β-glucosidases [EC 3.2.1.21], which can break the glycosidic linkages (Lynd et al. 2002). They hydrolyse the 1,4-β-D-glucosidic linkages in cellulose, and thus recycle this polysaccharide (Jayasekara

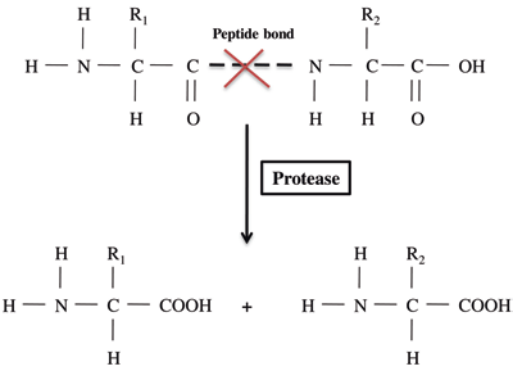


**Fig. 16.4** Actions of protease on peptide bond of protein molecule causing proteolysis

and Ratnayake 2019). Although there are not enough conclusive evidences to prove fungal cell wall degradation by cellulase enzymes alone, but rather studies support the synergistic participation of glucanase other hydrolytic enzymes with cellulase. Many PGPR release cellulolytic enzymes and thus help in the breakdown of cellulose in microbial cell wall (Tang et al. 2020). Some studies involving role of cellulase enzyme in antifungal activities are discussed here as well (Table 16.4). Wang et al. reported various lytic enzymes, break the fungal cell wall (*Fusarium* spp., *Alternaria* sp., *R. solani,* etc.), from the *P. jamilae* HS-26 (rhizobacteroid) strain, both qualitatively and quantitatively. The mixture of enzymes, after 3 days, show cellulase, glucanase, and protease enzymes level reaching up to 62.76 ± 1.35 U/mL, 4.13 ± 0.53 U/mL, and 15.56 U/mL, respectively (Wang et al. 2019). This study endorses synergistic roles of cellulase in the degradation of fungal cell wall. Another mechanism of fungal inhibition with cellulolytic enzymes is its synergism with mycoparasitism in *Phytophthora* and *Pythium spp.* (Picard et al. 2000). These reports conclude that either the exact mechanism is still unknown and needs to be explored or separate ways could be employed for effective biocontrol by cellulases.

Limited literature is available on cellulases and one study involves purification from *B. licheniformis* (Isolate 380) with 20 kDa size, and maximum activity of this carboxymethyl cellulase is 0.14 UEA mL-1 min-1 (Marco et al. 2017). Cellulase enzyme from *B. subtilis* YJ1 have a molecular mass of 32.5 kDa and appear to be an endo-1,4-glucanase enzyme at 6.0 pH and 50–60 °C temp, (Yin et al. 2010).

## 4 Mycoparasitism in Antifungal Response

Mycoparasitism is an indirect mode of inhibition of fungal cells and refers to obtaining nutrients from living fungal cells. It involves different phases, starting with: attachment, detection, contact, and penetration, followed by nutrient acquisition as shown in Fig. 16.5 (Woo and Lorito 2007). Mycoparasitism activity can be shown in two ways: necrotrophic and biotrophic. Necrotrophic mycoparasites destroy the host mycelium in the early stages of parasitism and use the nutrients that are released from dead host cells. Necrotrophic mycoparasites are more hostile and violent in comparison to biotrophic parasites. These mycoparasites show a broad range of host choices and infinite mode of parasitism. The parasitic activity of necrotrophic parasites is due to the secretion of hydrolytic enzymes, antibiotics, and other antagonistic compounds (Sahai and Manocha 1993). On the other hand, in biotrophic parasitism, the biotrophs fulfil their need of nutrient from living host instead of dead cell (Scott 1976). Biotrophic mycoparasites show a narrow host range, and implicate haustorial structures development for nutrients uptake from the host fungal cells (Sahai and Manocha 1993).

Mycoparasitism property of PGPR, especially by actinomycetes, could act as a game changer in the field of biocontrol of fungal phytopathogens (Barnett and Binder 1973). For direct physical attachment, PGPR can recognize various forms of fungi, involving spores, fruiting bodies, hyphae, etc. The mycoparasitism normally

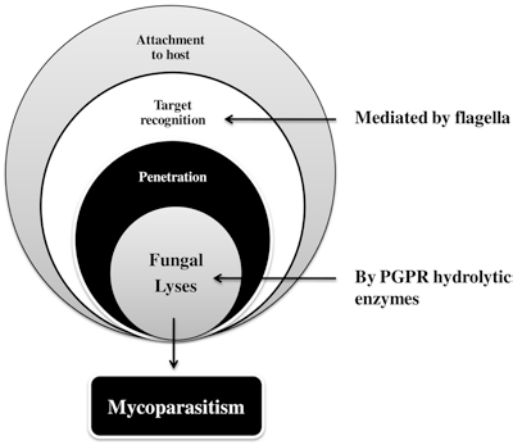


**Fig. 16.5** PGPR parasitism on fungal pathogen. PGPR hydrolytic enzymes show lytic activities against cell wall of fungal phytopathogens

involves help of various compounds involving hydrolytic enzymes such as chitinases, proteases, glucanases, cellulose, etc., along with other PGPR products (Fig. 16.5) (Chet et al. 1990). Chet et al. reported the release of lytic enzymes from *S. marcescens* which caused inhibitory activity against *S. rolfsii* in beans and *S. solani* in cotton, for effective biocontrol. The cloned and purified chitinase enzyme extracted from *S. marcescens* caused effective outburst of hyphal tips of *S. rolfsii* (Chet et al. 1990). Bolwerk et al. reported that *P. fluorescens* WCS365 and *P. chlororaphis* PCL1391 showed parasitism on *F. oxysporum* hyphae with the help of phenazine-1-carboxamide (PCN) and other lytic secretions, thus helped in the biocontrol of foot and root rot in case of tomato plants (Bolwerk et al. 2003).

## 5 Induced Systemic Resistance (ISR) in Combating Fungi

Induced Systemic Resistance (ISR) is an acquired process to expand plants' defensive competency manifolds against various biotic infections and other environmental challenges (Loon et al. 1998). This defensive ability is called systemic because it increases plants' endurance at not infection site, but also at rest other sites, and protect from any future attack from fungi or other phytopathogens.

It's warranted that various PGPR products should act synergistically and induce ISR in plants. These include siderophores, pyoverdin, antibiotics, and hydrolytic enzymes. The role of enzymes in the induction of ISR has not been studied in detail, and limited reports exist. The ISR associated enzymes are chitinase, β-1,3-glucanase, peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT), lipoxygenase (LOX), ascorbate peroxidase (APX), phenylalanine ammonia-lyase (PAL), protease, etc. (Annapurna et al. 2013). Lawrence et al. report three moderately early blight resistant tomato varieties that have higher chitinase and

β-1,3-glucanase (antifungal isozymes) levels; compared to the non-resistant varieties for *A. solani* (Lawrence et al. 2000). Dumas-Gaudot et al. recorded the short-term increase of the chitinase and sometime β-1,3-glucanase activities as an induced defense response in plants towards fungal phytopathogens (Dumas-Gaudot et al. 1996). Bargabus et al. reported an increase in systemic resistance of sugar beet plant by chitinase and β-1,3-glucanase released from *Bacillus pumilus* (Bargabus et al. 2004) Therefore, it is conclusive that there is some involvement of PGPR hydrolytic enzymes in promoting ISR, but yet, there is a lot more scope in exploring the well-defined mechanism behind ISR induction activities of PGPR hydrolytic enzymes.

## 6 Conclusions and Future Prospects

The possibility of PGPR in protecting plants from attack of fungal pathogens, and thereby enhancing yield and quality of crops is feasible. It is promising due to release of several antifungal components, like hydrolytic enzymes, antibiotics, siderophores, defensive hormones, etc. All these factors play an important role in sustainable plant disease control, including fungal phytopathogens. Different hydrolytic enzymes target the multiple cell wall components i.e., chitinase, pectinase, glucanases, cellulases, and effectively guard from fungi attack. The PGPR maintain microbial balance in rhizosphere, enhance the seed, and ensure absorption of nutrients. Thus, it improves harvest and strength of plants cultivated for economic reasons. These hydrolytic enzymes effectively bring mycoparasitism of PGPR, along with increased ISR. The synergism between various PGPR released components further augments the affectivity. The natural biocontrol of fungal phytopathogens is promising as it can efficiently decrease the reliability on chemical fertilizers and promote organic farming, which is fast catching attention. Sikkim, in India, has already committed to 100% organic farming. The commercial production of PGPR secretions in combination with nanoparticles would be a splendid biofertilizer, as these would not result in acquired resistance in fungal species, like various chemical fertilizers.

However, like the two sides of the coin even PGPR have their own share of complications as well. PGPR exhibit some shortcoming as well, like cyanide can inhibit the growth of some plants. The auxins accumulation in rhizosphere can impede roots development. Some compounds of PGPR secretion negatively affect nodulation in plants or induces foliar chlorosis in soybeans. Therefore, we can conclude that responsible manipulation of PGPR has promising potential to act as an alternative to current agriculture practices, in controlling pathogens and ensuring plant health and productivity with sustainability. However, despite a lot of research on the production of hydrolytic enzymes in the last 40 years, the functioning of PGPR is still not fully understood and requires more efforts and support.

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# Chapter 17
# Selection of Carbon Sources by Rhizobacteria – A Muster of Signalling Factors Governing Carbon Catabolite Repression

**Akshita Champaneria and Shalini Rajkumar**

**Abstract** Rhizosphere is a dense dynamic area of soil around the roots harbouring plant-beneficial bacteria thriving on the nutrients obtained from plants. These bacteria prevent plants from pathogen attack by establishing themselves in the rich rhizospheric niche and competing for carbon and energy sources. The heterogeneity of carbon sources and the competition for survival is the impetus for Carbon Catabolite Repression (CCR) phenomenon where the utilization of preferred carbon source over the less preferred carbon substrate takes place. The genes for metabolism of less preferred carbon sources are repressed by the presence of the preferred carbon source or by the signalling and/or participating molecules of metabolism of the preferred carbon source. Plant root exudates are rich in different types of carbon sources like carbohydrates and sugars, organic acids, amino acids, acid alcohols, etc., urging the bacteria to choose its preferred one. Bacteria selectively utilize those which maximize their growth and colonization at minimum expense of energy. Different genera of bacteria have different transport systems and catabolism mechanisms for carbon sources which facilitate more than one type of bacteria to flourish in the niche. The phosphoenolpyruvate: carbohydrate phosphotransferase system (PEP-PTS) dictates CCR in enteric bacteria and firmicutes whereas in rhizobia and pseudomonads, there is no single governing pathway but a combination of various transport and catabolism pathways bringing about CCR. The signalling molecules dictating CCR have been studied in detail in the model organisms like *Escherichia coli, Bacillus subtilis,* and *Pseudomonas putida*. In this chapter, an attempt is made to understand and relate the different molecules and pathways responsible for successful catabolite repression to take place.

**Keywords** CCR · Carbohydrate transport · Rhizobacteria

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## 1 Introduction

Rhizobacteria are known for their beneficial traits for the plant's growth such as nitrogen fixation (Vocciante et al. 2022), siderophore production (Sayyed et al. 2019), salt stress tolerance (Kusale et al. 2021), production of hormones like ACC deaminase (Sagar et al. 2020), antibiotic production (Vinay et al. 2016), and mineral acquisition properties (Kapadia et al. 2021), which have made them suitable for applications in the field as biofertilizers (Najafi et al. 2021). However, the presence of different energy and carbon sources in the rhizosphere trigger the phenomenon of catabolite repression in the bacterial cell leading to compromise on some of the plant beneficial traits. This leads to their non-functionality and non-fulfilment to provide important macronutrients to the plants and hence failure as biofertilizers (Iyer et al. 2016).

In nature, bacteria are surrounded by various carbon substrates and most of them can assimilate these substrates for energy generation and colonization. The pattern by which carbon sources are utilized is varied and differs from bacteria to bacteria as they survive and adapt to various biotic and abiotic challenges. Some carbon sources are co-utilized whereas some are co-metabolized; some are also assimilated prior to others (Gorke and Stulke 2008). But competition dictates survival and hence utilization at minimal expense of energy becomes important. To support effective growth and proliferation with least expenditure of energy, some carbon sources are preferred over others leading to preferential utilization of the prior. This preference for the carbon sources is specific at the genus and species level of the organisms and is termed as Carbon Catabolite Repression (CCR) or Catabolite Repression Control (CRC) (Kremling et al. 2015). CCR is defined as the uptake and metabolism of preferred carbon source over less preferred carbon sources by preventing the expression of genes required for metabolism and uptake of less preferred sources of carbon by the presence of preferred substrate (Stulke and Hillen 1999). This leads to the sequential utilization; however, in the case of growth-limiting concentrations of the carbon sources, which is mostly encountered by the organisms in vivo, the bacteria concentrate solely on growth and proliferation rather than following CCR (Beisel and Afroz 2016). At such times, even the less preferred substrate is taken up along with the preferred carbon source. CCR is one of the oldest phenomena studied and is still in discussion owing to the fact that it is a complex mechanism governing not only the sequential utilization of carbon sources but also the functioning of the catabolism of the involved carbon source. CCR was first observed in the model organism, *E. coli*, by Monod (1942) where glucose repressed the uptake of lactose when the cells were made to encounter both glucose and lactose together, yielding a diauxic growth curve and was named as the glucose effect. The same glucose effect was also found to take place in other gram-positive organisms like *B. subtilis;* and hence, it was thought that glucose was the most preferred carbon source for the bacteria. However, as CCR was investigated in detail in other families of bacteria *Pseudomonas*, glucose was found to be the secondary carbon source while organic acids like succinate were the preferred substrate. Similar observations were made with *Rhizobium* species (Rojo 2010).

The response to various environmental conditions by adapting to uptake and assimilation of carbon sources for energy is under tight regulation with its transport system. It acts as a sensory system which responds to the stimulus by ultimately deciding the preferred carbon source uptake (Lengeler 1993). The diversity in metabolism in response to the ever-changing nutrient compositions is maintained by the intracellular signal transduction pathways communicating the energy-efficient uptake (Jeckelmann and Erni 2019). The bacteria try to maximize the nutrient/carbon source uptake by modulating the transporters present on its cell membrane. Different rhizobacteria have evolved various mechanisms and signalling pathways for transporters to be able to utilize a plethora of carbon sources. Described here are the classes of transport systems dictating the uptake in the rhizosphere in various bacteria.

## 2 Transport of Carbon Sources

### *2.1 Carbohydrate Transport*

Transport of carbohydrates and sugars in the bacterial cells takes place via one of the two mechanisms active transport pathway depending on ATP hydrolysis and phosphoenolpyruvate phosphotransferase system (PTS) (Kotrba et al. 2001). The ATP Binding Cassette (ABC) transporter mediates the unidirectional active transport of sugars where the sugar molecule is taken by the cell in an unaltered manner, i.e., it traverses the cell membrane and enters the cytosol without the addition of any new chemical groups. However, for this transport to take place, the cell has to spend energy metabolically. The ATP molecule binds the ATP-binding domain, brings conformational changes leading to the transport of sugar from outside to inside of the cell (Wilkens 2015). The other transport system is the PTS system which phosphorylates the sugar as it enters the cell. Here, the phosphate group is carried from the phosphoenolpyruvate molecule to the sugar molecule via a relay by the PTS components. PTS sugars are specific and have preference for utilization by the organism. The difference in the mechanisms of sugar uptake leads to sequential utilization when different sugars are present at the same time leading to the phenomenon of carbon catabolite repression (Erni 2013).

### *2.2 Dicarboxylate Transport*

Rhizobacteria like *Rhizobium, Sinorhizobium, Mesorhizobium* and *Bradyrhizobium* are involved in forming symbiosis with plants and fixing atmospheric nitrogen to more usable form, ammonium (Valentini et al. 2011). For effective nodule formation and nitrogen fixation, these bacteria prefer organic acids like dicarboxylates as the preferred carbon source in contrast to sugars and carbohydrates (Schultze and

Kondorosi 1998). For their uptake, Ronson and his team, in 1984, showed the presence of C4 dicarboxylate transporter (Dct) system. It is a tripartite system made up of DctA, DctB and DctD of which DctA is of the transporter family with specificities towards different acids. The genes, *dctB* and *dctD* code for a two-component regulatory system (DctB – sensor protein and DctD – regulatory protein) which regulates and transports C4 acids inside the cell (Yurgel and Kahn 2004). When organic acid is present outside the cell, DctB sensor activation takes place by autophosphorylation. Later, the translocation of this phosphate group to the response regulator DctD activates the transcription of dctA gene by recruiting the $\sigma^{54}$(RpoN) bound RNA polymerase. In absence of such organic acid, inhibition of autophosphorylation of DctB is said to take place due to no binding of the substrate to DctA (Ledebur et al. 1990; Giblin et al. 1995; Wang et al. 2003). In other rhizospheric bacteria too, like *Pseudomonas aeruginosa*, *Pseudomonas putida, Bacillus subtilis, Corynebacterium glutamicum*, and *Escherichia coli*, similar types of Dct transporter have been identified for the uptake and transport of C4 carboxylic acids (Davies et al. 1999; Asai et al. 2000; Teramoto et al. 2008). In *E. coli*, under anaerobic conditions, Dct system doesn't seem to work instead, its homologous DcuABC carriers bring about dicarboxylate transport (Six et al. 1994). The tripartite ATP-independent periplasmic (TRAP) carriers encoded by the genes *dctP, dctQ, dctM* are another class of dicarboxylate transporters found in the rhizobia, pseudomonads and some photosynthetic bacteria (Valentini et al. 2011).

The affinities of various transporters towards their specific substrates are different in each genera of bacteria. Also, the biotic and abiotic factors encountered by the bacterium to thrive dictate the preferential transport of carbon sources.

## 3 Carbon Catabolite Repression (CCR)

Carbon catabolite repression is the utilization of a preferred carbon source over less/non-preferred carbon sources by inhibiting the expression of metabolic genes of the latter. The mechanism of CCR was first observed in *E. coli* by Monod in 1942. He and his colleagues showed that in presence of glucose and lactose, *E. coli* would utilize glucose prior to lactose. This was based on the observation that the growth curve obtained was a diauxie, where in two log and two stationary phases were present. The first stationery marked the complete utilization of glucose and the second log phase, the synthesis of enzymes required for metabolism of lactose. Later, on investigating the mechanisms and pathways for metabolism of the two sugars, it was found that transport of the sugars was via different mechanism that is, glucose was taken up by the PTS system and lactose being a non-PTS sugar had its own transporters (Bruckner and Titgemeyer 2002). A similar preference of glucose utilization was observed in *Bacillus subtilis* too, and the mechanism was called the glucose effect (Magasanik 1961; Fujita 2009). In both the organisms, glucose was the most preferred sugar over the other sugars. But as investigations proceeded, it was found that there are other PTS sugars along with glucose that are utilized prior to the

non-PTS ones. However, in other bacteria like the pseudomonads, glucose utilization was repressed in presence of organic acids and the same was observed in rhizobia. In *Streptococcus thermophilus* and *Bifidobacterium longum* too, glucose uptake was repressed which came as a surprise as in the latter organism, lactose would repress glucose uptake. This phenomenon was termed as reverse glucose effect or more generally catabolite repression (Collier et al. 1996; Van den Bogaard et al. 2000; Parche et al. 2006).

CCR in all organisms have the same outcome, that is, preferred carbon source being utilized before the non-preferred ones. However, the mechanism of execution varies. In presence of preferred carbon source, some bacteria inhibit the transport and hence the gene transcription (in *E.* coli) while some inhibit the induction of expression of genes (in *B.* subtilis) required for metabolism of the less/non-preferred carbon sources. In pseudomonads, the repression is at the level of post transcription inhibition. In all the cases, growth curves obtained in presence of dual carbon sources exhibit a diauxie. The different mechanisms of CCR are briefly described here in this chapter.

## *3.1 CCR Signalling in* Escherichia coli

The model bacterium, *Escherichia coli* has been studied for many phenomena, and CCR is extensively unravelled. In *E. coli*, CCR is the glucose effect wherein glucose metabolism exerts repression on other carbon sources' metabolism via inducer exclusion. Internalization of the glucose molecules is via the phosphoenolpyruvate (PEP) phosphotransferase system (PTS) whereas that of the secondary preferred lactose is controlled by *lac* operon. PTS components- enzyme I (EI), histidine protein (HPr) and enzyme II (EII) and PEP to pyruvate ratio are the major factors contributing to CCR. In presence of glucose, PEP rapidly converts to pyruvate and phosphate is released. This phosphate group phosphorylates EI and is relayed from EI to HPR and finally to the A subunit of EII enzyme (EIIA). The phosphorylated sugar-specific EII enzyme is the major molecule governing CCR in this bacterium. The phosphate group is then transferred to the B subunit of EII (EIIB) making conformational change ultimately transporting glucose through EIIC (C subunit of EII). Glucose obtains the phosphate molecule from EIIB as soon as it enters the cell. In presence of glucose in bumper amounts, EIIA is continuously dephosphorylated, inhibiting the transporters of the non-PTS sugars (for example- lactose) in turn inhibiting the formation of respective inducers giving this mechanism the name of inducer exclusion. Upon complete exhaustion of glucose, decreased PEP to pyruvate ratio increases the phosphorylated EIIA concentration. This EIIA cannot block the transporters of secondary non-PTS sugars but in turn activates the adenylate enzyme bound to the inner wall of the cell membrane. ATP molecules are acted upon by adenylate cyclase enzyme converting it to cyclic AMP (cAMP) that binds to the cAMP receptor protein (CRP) inducing transcription of the lactose operon (Brucker and Titgemeyer 2002; Gorke and Stulke 2008). Catabolite repression is

also reported among secondary/non-preferred carbon sources, which are controlled by the cAMP-CRP complex owing to its difference in the affinities towards promoters of various secondary carbon sources (Aidelberg et al. 2014).

In the last two decades, other hierarchies have been established in *E. coli*, amongst lactose, arabinose and xylose, the non-PTS sugars where lactose utilization repressed the uptake of pentoses. However, the pentoses mildly inhibit the *lac* operon too. Also, concentration dependent crosstalk between the pentoses govern inhibition of the two over each other. This crosstalk is strong when arabinose metabolism represses xylose metabolism in presence of abundance of arabinose, but weak, vice versa. In addition to this, in *E. coli*, hierarchy of glucose and lactose has been questioned at the single cell level where no strict hierarchy of glucose over lactose is reported (Desai and Rao 2009; Koirala et al. 2016; Ammar et al. 2018).

## *3.2 CCR Signalling in* Bacillus subtilis

In *B. subtilis*, the principle of CCR is the inhibition of transcription initiation of genes of secondary carbon source metabolism. This mechanism, hence known as induction prevention, regulates transcription through the homo-hexameric catabolite control protein A (CcpA). Transport of sugars takes place via the relay of phosphate group by the PTS however, CCR is managed by the unique HPr having dual phosphorylation sites – His15 and Ser46 – phosphorylated by EI and HPr kinase/phosphorylase (HPrK) respectively. When the preferred glucose is present, high amount of glycolytic intermediate fructose-1,6-bisphosphate (FBP) is produced and when glucose is present along with other secondary source, this FBP triggers phosphorylation of HPr at Ser46 by HPrK. CcpA complexes with HPr(Ser-P) which binds to the catabolite responsive element (*cre*) site present in the promoter/transcription initiation of the genes for metabolism of secondary carbon sources leading to inhibition of the latter's transcription (Brucker and Titgemeyer 2002; Gorke and Stulke 2008).

Figure 17.1 shows the overview of the sugar uptake via PTS and its effect on CCR in *E. coli* and *B. subtilis*. The boxes display the CCR mechanisms in *E. coli* and *B. subtilis*. The effect of PTS sugar being utilized prior to non-PTS sugar in both the organisms is the same but the mechanisms by which CCR is brought about are different.

## *3.3 CCR Signalling in Pseudomonads*

Bacteria belonging to the *Pseudomonas* species are versatile in being found in various habitats, adapting to various conditions by utilizing a wide range of nutrients for survival. Some species are found to be pathogenic to plants and animals as well.

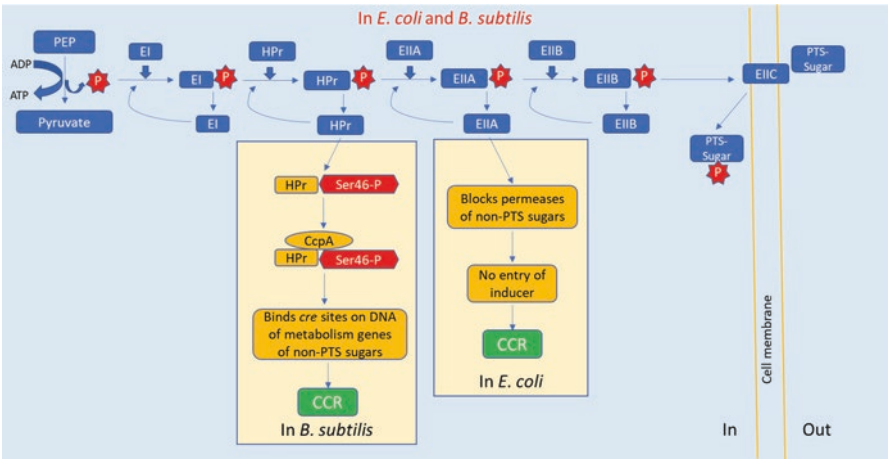


**Fig. 17.1** Transport of PTS sugars and CCR

However, rhizospheric niches are abundant in these species and they differ from *E. coli* and *B. subtilis* in preferring organic acids over glucose. The *crc* gene coding for the catabolite repression control (Crc) protein is the central regulatory molecule responsible for CCR in this class of bacteria which takes place at the post-transcriptional level. The presence of preferred and non-preferred carbon sources activates Crc protein to bind the mRNAs of the non-preferred carbon source at the promotor region hindering their translation. When the preferred carbon source is fully utilized or totally absent, a two-component regulatory protein CrcY/Z comes into the picture. The CrcZ sequesters the Crc bound to the promotors of mRNA of the non-preferred carbon sources and frees the site for the ribosomes for further translation. The RNA of CrcY/Z protein is expressed only in presence of the non-preferred carbon source (Rojo 2010).

## *3.4 CCR Signalling in Rhizobia*

Rhizobia are the soil-borne bacteria capable of fixing atmospheric nitrogen to ammonium in specialized root organs on the leguminous plants. Rhizobia are a class of bacteria studied extensively for their nodule formation and plant growth-promoting (PGP) characteristics (Zahran 1999) of which the two main PGP activities are N2 fixation and mineral phosphate solubilization. The elucidation of mechanisms and the principles of these traits have been the topic of interest for many decades now, but the complete understanding remains elusive (Gopalakrishnan et al. 2015). Rhizobia have also been studied as biofertilizers and have proved efficient in vitro and have benefits over the chemical fertilizers. But when subjected to the field applications, the traits may be compromised, as the understanding of the

organisms is not global and only limited to plant growth response in the field (Iyer et al. 2016). The basic understanding of the physiology in soil with respect to the utilization of carbon sources when present in many and response to the overall soil abiotic and biotic components have been superficially elucidated. For the application of these organisms as biofertilizers, it becomes imperative to know the thorough physiological mechanisms so as to harness their maximum potential and prudent use.

In soil, the microorganisms are subjected to a variety of carbon sources, which may or may not be in utilizable or assimilable form. But it is important for the microbes to flourish and colonize the rhizosphere and compete for the available nutrients so as to promote plant growth directly and/or indirectly (Joshi et al. 2019). When in soil, multiple carbon sources which are available at the same time due to the root exudates and due to the organic matter from plants and animals, rhizobia too like other organisms prefer particular carbon source over the others. Unlike *E. coli* and *B. subtilis* and like pseudomonads, rhizobia prefer organic acids over sugars and hence organic acids repress the utilization of sugars and bring about catabolite repression. Succinic acid is one of the major components of the root exudates represses sugar utilization; SMCR (succinate mediated catabolite repression) has been investigated and later, other organic acids mediated catabolite repression have been studied to some extent (Bringhurst and Gage 2002; Diab et al. 2006; Pinedo and Gage 2009). Along with these carbon sources, rhizobia encounter many other substrates like the sugars and amino acids in soil. The understanding of how different carbon sources affect each other and affect the growth, and PGP activities have not been fully explored. The major PGP activity of mineral phosphate solubilization is mediated via acid production by utilization of sugars, primarily glucose. Phosphate is the second most important molecule to the plant but its bioavailability is very poor as due to its high reactive nature, it is always found in complexed form. When glucose is utilized by rhizobia, a small amount of it is converted to gluconic acid via the periplasmic glucose dehydrogenase while the rest is taken up as glucose. This acidification leads to solubilization of the bound phosphate via cation exchange. The proton of the acid produced breaks the bound phosphate and forms $HPO_4^-$ which is the assimilable form of phosphate. The freed phosphate can then be assimilated easily by the plant. This acid production is one of the ways in which the phosphate is made available to the plants by bacteria contributing to plant growth promotion (Iyer and Rajkumar 2019; Joshi et al. 2019; Bharwad and Rajkumar 2019). However, root exudates of plants, especially legumes, have high amounts of organic acids. Bacteria like rhizobia preferring organic acids over sugars as preferred carbon source, inhibit sugar uptake and hence no periplasmic acid formation takes place leading to no phosphate solubilization and hence poor availability of phosphate to the plants and inoculants performing poorly as phosphate solubilizers (Iyer et al. 2017). This is one of the main reasons of potential P-solubilizers to being compromised as biofertilizers in fields (Iyer et al. 2016). Hence, it becomes very important to elucidate and understand the underlying mechanism and later harness the potential as each bacterial species follow a distinct metabolic network depending upon the available carbon source.

The repression of succinate on catabolizing glucose and other sugars has been observed in many *Rhizobium* species. Early studies on *Rhizobium meliloti* have shown that the enzymes of the ED pathway and TCA did not express in presence of succinate. Other studies show absence of β-glucosidase and β-galactosidase enzymes activities in presence of succinate in *S. meliloti* 2011 (Geddes and Oresnik 2014). The SMCR operates via different genes when different non-preferred carbon sources are present. Inducer exclusion is the reported mechanism for succinate to repress raffinose in *S. meliloti*. HPrK of this organism is homologous to that of firmicutes and was found to regulate CCR in presence of succinate for repression of sugars like lactose. Most of the mechanism of repressions by succinate are catabolite or operon specific (Mandal and Chakrabartty 1993; Pinedo and Gage 2009; Garcia et al. 2010). But no global mechanism has been elucidated in case of rhizobia as that for some members of firmicutes and enterobacteria. This lack of information about the basic functioning of the bacteria in presence of multiple carbon sources prove as a hurdle for these to be harnessed. Also, the biofertilizers, which are used to some extent, are not able to totally replace the chemical fertilizers as the effects are slow and the need for increasing the yield is always exponentially growing. The main advantage of studying CCR in rhizobia will be the thorough understanding of the underlying mechanisms and their physiology in soil in presence of multiple carbon sources, which can lead to coupling rhizobia with present bioinoculants for a more effective and quick-acting biofertilizer, thus decreasing the use of chemical fertilizers and improving the agricultural soil quality.

## *3.5 CCR Signalling in Different Bacteria Dictating Carbohydrate Preference*

As stated above, when grown in presence of preferred and non-preferred carbon sources, diauxic growth indicates the phenomenon of carbon catabolite repression in bacteria. CCR, being a global phenomenon, is controlled and influenced by several signalling factors including intrinsic traits of the bacterium, cellular signals or external factors. Internal factors modulating CCR can be the sensor molecules that detect the carbon sources, signalling pathways for phosphorylation-dephosphorylation, and the energy requirements of the cell (Wang and Lei 2018). The external factors, abiotic and biotic in nature, also affect the modulation of repression. pH of the soil is one of the main factors that changes the mobility and availability of carbon and energy sources influencing catabolite repression (Neina 2019). Another abiotic factor, salinity of soil, impacts the survival of the bacteria under stressful condition and compels them to smartly utilize easily assimilable carbon sources establishing colonization and outcompeting pathogens (Egamberdieva et al. 2019). Several of these factors that govern CCR in bacteria are presented in Fig. 17.2.

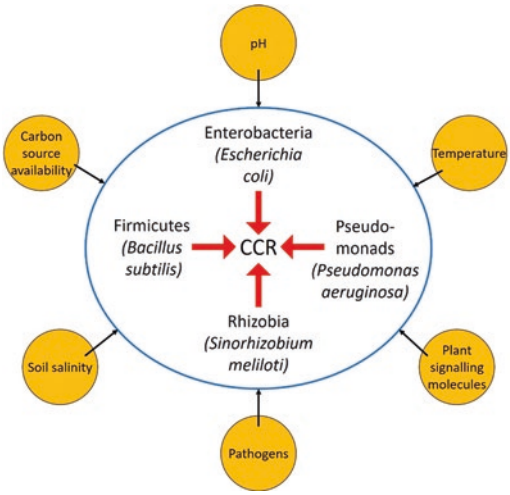


**Fig. 17.2** Factors influencing CCR in rhizobacteria

## 4 Conclusion

In prokaryotes, a selection of preferred carbon source is conferred upon carbon catabolite repression, which optimizes maximum growth while competing for resources with other bacteria. Rhizobacteria, due to their various direct and indirect beneficial PGP traits, are therefore harnessed as biofertilizers. Many experiments have been carried out for formulating best combination of plant growth-promoting rhizobacteria (PGPR) as biofertilizers (Basu et al. 2021). Use of *Pseudomonas* strains to fight sheath blight infection in rice crop (Reshma et al. 2018), consortium of rhizobacteria and piper leaf extract for biocontrol of blast pathogen (Luh Suriani et al. 2020), exopolysaccharide producing bacteria for overcoming drought stress in wheat crop (Ilyas et al. 2020) are some of the few examples where rhizobacteria were employed for improving growth, productivity and health of the crop (Hamid et al. 2021). Harnessing different bacteria for enhanced crop production has been in practice from very early times. Unfortunately, however, in vitro success of various rhizobacterial strains (to produce various plant promoting molecules) could not be achieved in fields. This is attributed to the lack of complete understanding of the physiology and interactions of these bacteria with the biotic and abiotic factors in vivo. The phenomenon of CCR plays a vital role in hindering the best results obtained for various bacteria in lab-controlled environment. The CCR signalling in the various rhizobacteria described above requires further research and extensive perusal which will disclose the global mechanisms of bacterial physiology and help to overcome the obstacles in achieving in vivo success. The use of selected rhizobacteria then, as green biofertilizers will certainly help substitute and overcome the chemical fertilizers to improve plant growth and crop yield.

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# Chapter 18
# Plant Growth-Promoting and Biocontrol Metabolites Produced by Endophytic *Pseudomonas fluorescence*

**P. Saranraj, R. Z. Sayyed, M. Kokila, A. Sudha, P. Sivasakthivelan, M. Durga Devi, Rabia Naz, and Humaira Yasmin**

**Abstract** Plants are generally associated with diverse microorganisms in its whole parts. Endophytic organisms are those microorganisms that colonize the plant internal tissue showing no external sign of infection or negative effect on their host. Endophytic bacteria have been isolated from a large diversity of plants species. Microorganisms like *Bacillus* sp., *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Burkholderia* sp., *Pantoea* sp., *Agrobacterium* sp. and *Methylobacterium* sp. constitute the endophytes commonly isolated from diverse plants such as rice, wheat, maize, cotton, clover, potato, sugarcane, tomato, cucumber and wild grasses. Only a few of these plants have ever been completely studied relative to their endophytic biology. Consequently, the opportunity to find new and beneficial endophytic microorganisms among the diversity of plants in different ecosystems is considerable. Based on types of activity, the role of endophytic microorganisms in plants can be divided into two categories viz., Growth promotion and Disease control. Other beneficial effects of endophytes to

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plants include by helping plants acquire nutrients, via Atmospheric nitrogen fixation, Phosphate solubilization, Iron chelation or siderophore production, increased drought resistance, thermal protection and Survival under osmotic stress. A particular bacterium may affect plant growth and development using one or more of these mechanisms and may use different ones at various times during the life cycle of the plant. In addition to these plant growth-promoting traits, endophytic bacteria must also be compatible with host plants and able to colonize the tissues of the host plants without being recognized as pathogens. The endophytic bacteria have a multitude of applications that enhance agricultural production. They enhance wheat growth through production of phytohormones, increase rice production by increasing mineral availability, increase cotton disease resistance, contribute to corn pest management, fix nitrogen in rice and wheat, decrease susceptibility to frost damage and increase potato tuber formation under heat stress conditions.

**Keywords** Endophytes · PGPR · *Pseudomonas fluorescens* · Induced Systemic Resistance (ISR) · Biocontrol agent · Crop response

## 1 Introduction

Plants are generally associated with diverse microorganisms in its whole parts. Endophytic organisms are those microorganisms that colonize the plant internal tissue showing no external sign of infection or negative effect on their host (Schulz and Boyle 2006). Endophytic bacteria have been isolated from a large diversity of plants species. Microorganisms like *Bacillus* sp., *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Burkholderia* sp., *Pantoea* sp., *Agrobacterium* sp. and *Methylobacterium* sp. constitute the endophytes commonly isolated from diverse plants such as rice, wheat, maize, cotton, clover, potato, sugarcane, tomato, cucumber and wild grasses (Bacon et al. 2006). Only a few of these plants have ever been completely studied relative to their endophytic biology. Consequently, the opportunity to find new and beneficial endophytic microorganisms among the diversity of plants in different ecosystems is considerable (Fallah et al. 2021).

Based on types of activity, the role of endophytic microorganisms in plants can be divided into two categories viz., Growth promotion and Disease control (Rosenbleuth and Martinez-Romero 2006). Endophytic bacteria are believed to elicit plant growth promotion in one of two ways:

1. Directly by producing Phytohormones such as Auxin or Cytokinin or by producing the enzyme 1 – aminocyclopropane – 1 – carboxylate (ACC) deaminase, which lowers plant ethylene levels.
2. Indirectly by preventing pathogen infections via Antagonistic antifungal or antibacterial agents, by outcompeting pathogens for nutrients by siderophore production, or by establishing the plant's Induced Systemic Resistance (ISR).

Other beneficial effects of endophytes to plants include by helping plants acquire nutrients, via atmospheric nitrogen fixation, phosphate solubilization, iron chelation

or siderophore production, increased drought resistance, thermal protection and survival under osmotic stress. A particular bacterium may affect plant growth and development using one or more of these mechanisms and may use different ones at various times during the life cycle of the plant. In addition to these plant growth-promoting traits, endophytic bacteria must also be compatible with host plants and able to colonize the tissues of the host plants without being recognized as pathogens (Rosenbleuth and Martinez-Romero 2006).

The endophytic bacteria have a multitude of applications that enhance agricultural production. They enhance wheat growth through production of phytohormones, increase rice production by increasing mineral availability, increase cotton disease resistance, contribute to corn pest management, fix nitrogen in rice and wheat, decrease susceptibility to frost damage and increase potato tuber formation under heat stress conditions (Sturz et al. 1997).

## 2 Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting Rhizobacteria (PGPR) were first defined by as the bacteria that colonize the roots of plants by following inoculation on to seed and that enhance plant growth. PGPR enhance plant growth by direct and indirect means, but the specific mechanisms involved have not been well characterized (Glick 1995). Direct mechanisms of plant growth promotion by PGPR can be demonstrated in the absence of plant pathogens or other rhizosphere microorganisms, while indirect mechanisms involve the ability of PGPR to reduce the deleterious effects of plant pathogens on crop yield. PGPR have been reported to directly enhance plant growth by a variety of mechanisms, viz., fixation of atmospheric nitrogen that is transferred to the plants, production of siderophores that chelate iron and make it available to the plant roots, solubilization of minerals such as phosphorous and synthesis of phytohormones (Saranraj et al. 2022).

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity (McCully 2005).

Bacteria associated with plants can be either harmful or beneficial plant growth-promoting rhizobacteria (PGPR) and may promote growth directly, by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators, phytohormones (Kloepper 1997). Some bacteria support plant growth indirectly by improving growth restricting conditions either via production of antagonistic substances or by inducing host resistance towards plant pathogens.

Since, associative interactions of plants and microorganisms must have come into existence as a result of convolution; the use of either former or latter groups as bio-inoculants forms one of the vital components for a long-term sustainable agriculture system (Tilak et al. 2005).

Rhizospheric bacterial communities have efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquio et al. 2000). Several bacteria have the ability to attach to the root surfaces (rhizoplane) making them to derive maximum benefit from root exudates. Few of them are more specialized, as they possess the ability to penetrate inside the root tissues (endophytes) and have direct access to organic compounds present in the apoplast. By occupying this privileged endophytic location, bacteria do not have to face competition from their counterparts as encountered in the rhizosphere or in soil (Patel et al. 2016).

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria known as plant growth-promoting rhizobacteria (PGPR). Plant growth-promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangarajan et al. 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg 2001). Among these organisms, Fluorescent *Pseudomonas* are considered to be the most promising group of plant growth-promoting rhizobacteria (PGPR) involved in biocontrol of plant diseases. They produce secondary metabolites such as antibiotics, phytohormones, volatile compound Hydrogen cyanide and siderophores. Plant growth-promoting ability of these bacteria is mainly because of the production of Indole-3-acetic acid, Siderophores and Antibiotics.

The genera of PGPR include *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas* sp., *Acetobacter* sp., *Burkholderia* sp., *Bacillus* sp., *Paenibacillus* sp. and some are members of the Enterobacteriaceae. Direct use of microorganisms to promote plant growth and to control plant pests continues to be an area of rapidly expanding research. Rhizosphere colonization is one of the first steps in the pathogenesis of soil borne microorganisms. It is also crucial for the microbial inoculants used as biofertilizers, biocontrol agents, phytostimulators and bioremediators. *Pseudomonas* sp. is often used as model root-colonizing bacteria (Lugtenberg et al. 2001).

The beneficial effects of these rhizobacteria have been variously attributed to their ability to produce various compounds including phytohormones, organic acids, siderophores, fixation of atmospheric nitrogen, phosphate solubilization, antibiotics and some other unidentified mechanisms (Glick and Ibid 1995). Motile rhizobacteria may colonize the rhizosphere more profusely than the non-motile organisms resulting in better rhizosphere activity and nutrient transformation. They also eliminate deleterious rhizobacteria from the rhizosphere by niche exclusion thereby better plant growth. Induced Systemic Resistance has been reported to be one of the mechanisms by which PGPR control plant diseases through the manipulation of the host plant's physical and biochemical properties.

The recognition of plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today

researchers are able to repeatedly use them successfully in field experiments. Increased growth and yields of potato, sugar beet, radish and sweet potato have been reported. Commercial applications of PGPR are being tested and are frequently successful; however, a better understanding of the microbial interactions that result in plant growth increases and will greatly increase the success rate of field applications (Farzana et al. 2009).

PGPR, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR. Plant Growth-Promoting Bacteria (PGPB) is reported to influence the growth, yield and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth (Himanshu Arora et al. 2021).

Direct enhancement of mineral uptake due to increase in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Bashan and Levanony 1991). PGPR strains may use one or more of these mechanisms in the rhizosphere. PGPR that synthesize auxins and cytokinins or those that interface with plant ethylene synthesis have been identified (Garcia et al. 2001; Glick 1995; Percello Carticaux et al. 2003). The indirect means by which PGPR enhance plant growth is through suppression of phytopathogens by a variety of mechanisms. These include the ability to produce siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize antifungal metabolites such as antibiotics, fungal cell wall lysing enzymes or hydrogen cyanide, which suppress the growth of fungal pathogens. The ability to successfully compete with pathogens for nutrients or to exclude specific niches on the root and the ability to induce systemic resistance in plants are the other mechanisms (Bloemberg and Lugtenberg 2001; Persello Carticaux et al. 2003).

Growth and yield of crop plants are influenced by a myriad of abiotic and biotic factors. While growers routinely use physical and chemical approaches to manage the soil environment to improve crop yields, the application of microbial inoculants for this purpose has gained significance in the recent past. The microbial inoculants that are used in agriculture include biofertilizers, biocontrol agents, plant growth-promoting rhizobacteria, etc. While the biofertilizer organisms make the nutrients available to plants, biocontrol agents protect the plants against the pathogenic organisms and insect pests, whereas the growth-promoting rhizobacteria enhance the plant growth by various mechanisms. These beneficial microorganisms can be a significant component of management practices to achieve sustainable yields (Bloemberg and Lugtenberg 2001).

The concept of biocontrol of plant diseases includes disease reduction or decrease in inoculum potential of a pathogen brought about directly or indirectly by other biological agencies (Johnson and Carl 1972). Outside the host, the biocontrol agent may be antagonistic and thereby reduce the activity, efficiency and inoculum density of the pathogen through antibiosis, competition and predation/hyper parasitism. This leads to a reduction in inoculum potential of the pathogens (Baker 1977). The biocontrol agent may operate primarily in the host tissue, there by indicating a

resistance response in the host, by transmitting factors that render the pathogens avirulent (Cook and Baker 1983). These interactions are mediated by environment and may have an overriding impact in determining whether biocontrol operates in a system or not.

## 3 Concept of Endophytes

The term endophyte (Greek – *endon*, within; *phyton*, plant) was first coined by De Bary (1866), and an endophyte is a bacterial or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- or intra-cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Wilson 1995). The presence of endophytes was reported by Vogl (1898) who revealed a mycelium residing in the grass seed *Lolium temulentum*. In the year 1904, in Germany, Freeman identified an endophytic fungus, in Persian darnel (annual grass).

Bacterial endophytes have been known for >100 years. The presence of bacteria resident within the tissues of healthy plants was first reported as early as 1926 (Hallman et al. 1997). In 1926, Perotti recognized endophytic growth as a particular stage in the life of bacteria, described as an advanced stage of infection and as having a close relationship with mutualistic symbiosis. Perotti (1926) was the first to describe the occurrence of non-pathogenic flora in root tissues, and Henning and Villforth (1940) reported the presence of bacteria in the leaves, stems and roots of apparently healthy plants. Since then, endophytes have been defined as microorganisms that could be isolated from surface-sterilized plant organs. Since 1940s, there have been numerous reports on endophytic bacteria in various plant tissues (Hallmann et al. 1997). In the 1980s, endophytic bacteria having nitrogen fixing ability were found in graminaceous plants (Reinhold-Hurek and Hurek 1998). These endophytic relationships may have begun to evolve from the time that higher plants first appeared on earth hundreds of millions years ago. Evidence of plant associated microbes has been discovered in the fossilized tissues of stems and leaves (Taylor and Taylor 2000). As a result of these long-held associations, it is possible that some of these endophytic microbes may have devised genetic systems allowing for the transfer of information between themselves and the higher plant and vice versa (Stierle et al. 1993).

The term endophyte refers to interior colonization of plants by bacterial or fungal microorganisms. Endophytes have been defined in several ways, and the definitions have been modified as the research in this field advanced. defined endophytes as microorganisms that are able to live inside plants without causing disease symptoms. ‘Endophytic bacteria’ are the population of bacteria that reside within the living organism without doing substantive harm or gaining benefit other than securing residency (Kado 1992).

Endophytic bacteria or fungi colonize the host tissue internally, sometimes in high numbers, without damaging the host or eliciting symptoms of plant disease

according to a widely used definition by Quispel (1992). Microorganisms living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence are defined as endophytes (Wilson 1993). Bacon and White (2000) defined endophytes as 'microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects'. According to Schulz and Boyle (2006) endophytic bacteria can be defined as those bacteria that colonize the internal tissue of the plants showing no external sign of infection or negative effect on their host.

Among the definitions given to endophytic bacteria the following by Hallmann et al. (1997) seems to be the most adequate. Hallmann et al. (1997) defined endophytic bacteria as all bacteria that can be detected inside surface sterilized plant tissues or extracted from inside plants and having no visibly harmful effect on the host plants. This definition includes internal colonists with apparently neutral behaviour as well as symbionts. It would also include bacteria which migrate back and forth between the surface and inside of the plant during their endophytic phase. The relationship between the endophytes and its host plant may range from latent phytopathogens to mutualistic symbiosis (Sturz et al. 1997).

In accordance with their life strategies, bacterial endophytes can be classified as 'obligate' or 'facultative'. Obligate endophytes are strictly dependent on the host plant for their growth, and survival and transmission to other plants occurs vertically or via vectors. Facultative endophytes have a stage in their life cycle in which they exist outside host plants. In the extreme view, bacterial phytopathogens might be included as (facultative or obligate) endophytes because they often occur in avirulent forms in plants. Avirulent forms of plant pathogens should thus be regarded as endophytes, whereas virulent forms of these organisms should not be included (Hardoim et al. 2008). The life cycle of facultative endophytes can be characterized as biphasic, alternating between plants and the environment (mainly soil). The vast majority of the microorganisms that can thrive inside plants probably have a propensity to this biphasic life style. In fact, the observed microbial diversities inside plants could be explained by the ability of diverse endophytes to enter into and persist in plants (Rosenbleuth and Martinez-Romero 2006).

## 4 Biodiversity of Endophytes

Soil microbial communities play an integral and often unique role in ecosystem functions and are among the most complex, diverse, and important assemblages in the biosphere (Zhou et al. 2003). It seems that the bacteria best adapted for living inside plants are naturally selected. Endophytes are recruited out of a large pool of soil or rhizospheric species and clones. Endophytic bacteria can actively or latently colonize plants locally or systemically and both intercellularly and intracellularly. Various reports indicate that these bacteria exist in a variety of tissue types within numerous plant species, suggesting a ubiquitous existence in most, if not all, higher

plants. Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species (Ryan et al. 2008).

Endophytic bacteria have been isolated from a large diversity of plants, which were reviewed by. Bacterial endophytes are found in a variety of plants, such as sugar beet (Dent et al. 2004), prairie plants, agronomic crops (Zinniel et al. 2002), potato varieties (Sessitsch et al. 2002), wheat (Germida and Siciliano 2001) and rice . Mavingui et al. (1992) found that there are different populations of *Bacillus polymyxa* in soil, rhizosphere, and rhizoplane and that wheat roots select specific populations as endophytes. Sturz et al. (1997) characterized 15 bacterial species from red clover nodules and estimated endophyte population densities to be in the range of $10^4$ viable bacteria per gram of fresh nodule and found that the endophytic population was less diverse than the root-surface population, and the endophytes appeared to originate from the latter (Zope et al. 2016).

Suman et al. (2001) isolated endophytic bacteria from several cultivars of Indian sugarcane on LGI medium. In a review by Lodewyckx et al. (2002), 81 different bacterial species were reported to form endophytic associations with plants. Zinniel et al. (2002) isolated 853 endophytic strains from aerial tissues of 4 agronomic crop species and 27 prairie plant species. A majority of the microorganisms isolated (689 strains) were from corn and sorghum; 45 strains were recovered from soybean and wheat, and 119 strains were obtained from 27 different host species of grasses, forbs, legumes and wildflowers. As a whole, fewer isolates were recovered from perennial plants than from the agronomic crops.

Surette et al. (2003) have reported the isolation of up to 360 endophytic microorganism strains from *Daucus carota,* which were classified into 28 genera*,* with *Pseudomonas, Staphylococcus* and *Agrobacterium* being predominant. Bacteria belonging to the genera *Bacillus* and *Pseudomonas* are easy to culture, and cultivation dependent studies have identified them as frequently occurring endophytes (Seghers et al. 2004). The presence and taxonomy of endophytic bacteria of the entire aerial parts of Crocus (*Crocus albiflorus*) was investigated by Sessitsch and Reiter (2006). Their results suggest that *Crocus* supports a diverse bacterial microbial communities resembling the microbial communities that have been described for other plants, but also containing species that have not been described in association with plants before. This study confirms that the culturable endophytes are a subset of total endophyte biodiversity.

The genotypic diversity of indigenous bacterial endophytes within stem of tropical maize (*Zea mays* L.) was determined in field and greenhouse experiments by Rai et al. (2007). Endophytes were found in most of the growing season at population ranging from 1.36 to 6.12 × $10^5$ colony, forming units per gram fresh weight of stem. *Bacillus pumilus, Bacillus subtilis, Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were the relatively more predominant group of bacterial species residing in maize stem. Thirty-two isolates of endophytic bacteria were obtained by Magnani et al. (2010) from Brazilian sugarcane. Most of the bacteria isolated from the sugarcane stem and leaf tissues belonged to Enterobacteriaceae and Pseudomonaceae, respectively, demonstrating niche specificity (Shaikh et al. 2018).

Pereira et al. (2011) investigated bacterial diversity associated with the roots of maize through the use of culture dependent and culture independent methods and showed that γ-Proteobacteria within *Enterobacter*, *Erwinia*, *Klebsiella*, *Pseudomonas* and *Stenotrophomonas* genera were predominant groups. The culturable component of the bacterial community revealed that the predominant group was Firmicutes, mainly of *Bacillus* genus, while *Achromobacter*, *Lysinibacillus* and *Paenibacillus* genera were rarely found in association with the roots. Only two genera within γ-Proteobacteria, *Enterobacter* and *Pseudomonas* were found in the culture collection.

Patel et al. (2012) isolated and characterized bacterial endophytes from root and stem of *Lycopersicon esculentum* plant, which were collected from different regions. Total 18 isolates of endophytic bacteria were selected in which, only HR7 endophyte of tomato was identified as *Pseudomonas fluorescens* by 16S rDNA analysis (Zope et al. 2019).

Endophytic bacteria have been isolated from both monocotyledonous and dicotyledonous plants, ranging from woody tree species, such as oak and pear, to herbaceous crop plants such as sugar beet and maize. Studies on the diversity of bacterial endophytes have focused on characterization of isolates obtained from internal tissues following disinfection of plant surfaces with sodium hypochlorite or similar agents (Miche and Balandreau 2001). In general, endophytic bacteria occur at lower population densities than rhizospheric bacteria or bacterial pathogens (Rosenblueth and Martínez-Romero 2004). The endophytic niche offers protection from the environment for those bacteria that can colonize and establish in plants. These bacteria generally colonize the intercellular spaces, and they have been isolated from all plant compartments including seeds (Posada and Vega 2005).

## *4.1* Pseudomonas fluorescens

Fluorescent *Pseudomonas* has emerged as the biggest and potentially the most promising group amongst the PGPR isolates involved in biocontrol of diseases (Suslow and Schroth 1982). Fluorescent *Pseudomonas* is Gram negative, aerobic rods, motile with polar flagella and has the ability to produce water soluble yellow green pigment (Palleroni 1984; Schippers et al. 1987). They are well adapted to rhizosphere and rhizoplane and have a fast growth rate in the rhizoplane (Bowen and Rovira 1976) and are able to utilize a large number of organic substrates including root exudates (Rovira and Davey 1974).

*Pseudomonas* sp. is a ubiquitous bacterium in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been Fluorescent *Pseudomonas* spp. Considerable research is underway globally to exploit the potential of one group of bacteria that belongs to fluorescent *Pseudomonas*. The *Pseudomonas fluorescence* helps in the maintenance of soil health and is metabolically and functionally most diverse (Lata et al. 2002). The presence of *Pseudomonas fluorescence* inoculant in the combination of microbial

fertilizer plays an effective role in stimulating yield and growth traits of chickpea. Isolates of *Pseudomonas fluorescence* from roots, shoots and rhizosphere soil provide significant increase in fresh and dry masses (Mehnaz et al. 2009). Field trials of a *Pseudomonas* strain lead to a great increase in yield of legumes (Johri 2001).

Specific strains of the *Pseudomonas fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields. This *Pseudomonas*, termed PGPR, rapidly colonize plant roots of potato, sugar beet and radish, and cause statistically significant yield increases up to 44% in field tests. The occurrence and activity of soil microorganisms are affected by a variety of environmental factors (e.g. soil type, nutrient abundance, pH, moisture content) as well as plant-related factors (species, age). So, while working on two winter wheat cultivars, it was found that the genus *Pseudomonas* show higher counts, thus the population size of bacteria of the genus *Pseudomonas* depends on the development phase of wheat plants (Wachowska et al. 2006).

*Pseudomonas* spp. are important plant growth-promoting rhizobacteria (PGPR) used as biofertilizers and are able to enhance crop yield by direct and indirect mechanisms (Walsh et al. 2001). Several researchers have shown that Fluorescent *Pseudomonas* is abundant in the rhizosphere of different crops (Kumar and Sugitha 2004). Effectively, they produce a variety of biologically active substances among which growth-promoting compounds represent a keen interest (Rodriguez 2006).

Fluorescent *Pseudomonas* is known to produce plant growth-promoting substances like, auxins, gibberrelins, cytokinines, etc. (Suneesh 2004). Thirty isolates of fluorescent *Pseudomonas* from wheat rhizosphere were found to produce 1.1–12.1 mg of auxin per liter of medium without tryptophan and 1.8–24.8 mg per liter with tryptophan (Khalid et al. 2004a, b). The strains of *Pseudomonas* are able to solubilize phosphorous in soil and increase its availability to plants (Sundara et al. 2002). Some strains of *Pseudomonas* produce chelating agents called Siderophores with high affinity for iron absorption. Microbial siderophores can enhance plant growth through increasing iron solubility in the plant rhizosphere. Such products are also able to alleviate the unfavourable effects of pathogens on plant growth (Kapadia et al. 2021).

The strains of *Pseudomonas* are able to solubilize phosphorous in soil and increase its availability to plants (Sundara et al. 2002). Some strains of *Pseudomonas* produce chelating agents, called Siderophores with high affinity for iron absorption. Microbial siderophores can enhance plant growth through increasing iron solubility in the plant rhizosphere. Such products are also able to alleviate the unfavorable effects of pathogens on plant growth (Sagar et al. 2020).

Mundt and Hinkle (1976) reported that the bacteria belonging to 19 genera and 46 species were recovered from seeds and from ovules; *Pseudomonas fluorescence* was recovered from 93% of the seeds tested. The populations of indigenous Gram negative bacteria, *Pseudomonas* sp. and of *Pseudomonas fluorescence* were larger on root infected by *Gaeumannomyces graminis* var. *Tritici* than on healthy roots (Brown 1974). In sugar beet, it was demonstrated that the *Pseudomonas fluorescens* colonized the roots immediately after plant emergence and continue to be in the developing root system throughout the season (Suslow and Schroth 1982).

Geels and Schippers (1983) isolated fluorescent *Pseudomonas* from potato tubers using modified King's 'B' medium supplemented with Cyclohexamide, Chloramphenicol and Antimycin and Hydroxyquinoline. Gould et al. (1985) formulate a new medium that provides a high degree of selectivity and detection of fluorescent *Pseudomonas* based on a detergent, sodium lauryl sulphate (sarcosine) and antibiotic trimethoprim. Mew and Rosales (1986) isolated fluorescent bacteria from rhizosphere of rice (Khan et al. 2021).

Chanway et al. (1989) reported that 32 bacterial strains representing *Pseudomonas putida, Pseudomonas fluorescens* and *Serratia* sp. were isolated from soil and were seen to colonize soya been roots in laboratory, green house and field assays when applied as seed inoculants. The colony forming units (CFU) ranged from 1–9 to 6.1 CFU/g of root. Misaghi (1990) isolated *Pseudomonas fluorescens* and *Pseudomonas putida* from the rhizosphere and rhizoplane of tomato, cucumber and alfalfa.

Kumar and Dube (1996) obtained strains of *Pseudomonas putida* from tomato root using modified King's 'B' medium incorporated with Cyclohexamide, Ampicillin, Chloramphenicol and Pentachloro benzene. Glick (1995) demonstrated a novel procedure for rapid isolation of plant growth-promoting *Pseudomonas* using 1-aminocyclopropane-1-carboxylate (Acc) as the sole source of nitrogen.

Benchabane (2004) isolated about 500 fluorescent strains of *Pseudomonas* from the rhizosphere of different plants, namely, tomato, potato, corn and vine, and suggested that the plant and the soil type play a considerable role in the distribution and the taxonomic diversity of fluorescent *Pseudomonas*.

Reddy et al. (2007) obtained 30 isolates of *Pseudomonas fluorescens* from rice rhizosphere and were tested for antifungal activity against *Magnaporthe grisea, Dreschelaria oryzae*, *Rhizoctonia solani* and *Sarocladium oryzae* that are known to attack rice plants. One *Pseudomonas fluorescens* isolate effectively inhibited the mycelial growth in all these fungi in dual culture tests (62–85%). The antifungal compounds were extracted with equal volume of ethyl acetate. The antifungal compounds from *Pseudomonas fluorescens* at 5% completely inhibited the pathogens. The antifungal compounds were tentatively identified on Thin Layer Chromatography (TLC) at Rf 0.22, 0.35, 0.42 and 0.51. These compounds were individually purified by Column chromatography and retested for antifungal activity (Bastamia et al. 2021).

Egamberdieva (2010) analyzed the plant growth-promoting bacteria for their growth-stimulating effects on two wheat cultivars. The investigations were carried out in pot experiments using calcareous soil. The results showed that bacterial strains *Pseudomonas* sp. and *Pseudomonas fluorescens* were able to colonize the rhizosphere of both wheat cultivars. Their plant growth-stimulating abilities were affected by wheat cultivars. The bacterial strains *Pseudomonas* sp. and *Pseudomonas fluorescens* significantly stimulated the shoot and root length and dry weight of wheat.

Maleki et al. (2010) isolated 144 bacteria from cucumber rhizosphere and screened as potential biological control agents against *Phytophthora drechsleri,* causative agent of cucumber root rot, in vitro and greenhouse condition. On the

basis of dual culture assays, eight isolates of *Pseudomonas fluorescens* were selected for root colonization, PGPR and greenhouse studies. Among these isolates, isolate *Pseudomonas fluorescens* CV6 exhibited the highest colonization on the roots and significantly promoted plant growth under in vitro condition.

Recently, Mahmoud Reza Ramezanpour et al. (2011) revealed that *Pseudomonas* have plant growth-promoting properties. Isolated strains showed high ability of IAA production, phosphate solubilization and siderophore production, while genotyping analysis showed that *Pseudomonas* isolated from the rhizosphere of rice are genetically diverse. Nevertheless, the strains were distributed into 11 genotypes, including 5 groups of fluorescent *Pseudomonas*.

### 4.1.1 Occurrence of *Pseudomonas fluorescence*

*Pseudomonas fluorescens* has emerged as the biggest, potentially the most promising group among *fluorescent Pseudomonads* and also involved in biocontrol of plant diseases (Suslow and Schroth 1982). Several hypotheses have been advanced to explain the beneficial association of *Pseudomonas fluorescens* with crop plants including aggressive colonization, host specificity and biocontrol activities (Glandorf et al. 1994). The cells of *Pseudomonas fluorescens* are Gram negative, aerobic rods, motile with polar flagella and have the ability to produce water soluble yellow green pigments in the medium under iron-free condition. The organism have fast growth rate in the rhizosphere when compared to other bacteria and are known to produce a variety of secondary metabolites with antagonistic characteristics viz., Siderophore (Neilands 1981, b) and Hydrogen cyanide.

The plant growth-promoting rhizopseudomonas strain *Pseudomonas fluorescens* was isolated from the roots of barley and under sub-optimal conditions. Seed or soil bacterization with this strain resulted in significant increases in dry weight ranging from 10% to 25% for various vegetables and cereals (Seong and Shin 1991). Furthermore, the strain promotes the germination of maize seed exposed to cold stress and also enhanced the seedling emergence (Hofte et al. 1991). *Pseudomonas fluorescens* strain effectively colonized the pistils of pear blossoms and controlled the blight caused by *Erwinia amylovora* (Wilson and Lindow 1993).

The rhizosphere occurrence and activities of *Pseudomonas fluorescens*, as PGPR, have been considered as an important component of sustainable agriculture due to their plant growth-promoting ability as well as their biocontrol potential against phytopathogens. The ubiquitous occurrence and activities of *Pseudomonas fluorescens* in the rhizosphere of many crop plants have already been reported (Lindow and Brandl 2003). The occurrence and activities of *Pseudomonas fluorescens* in the rhizosphere have been already reported in tobacco and tomato (Yan et al. 2002). Motility and chemotaxis, adhesion (Jana 1998), production of IAA, siderophores (Scher and Baker 1982) and antimicrobial substances play a key role in determining the degree of community population and the saprophytic competence of *Pseudomonas fluorescens* in the rhizosphere of crop plants (Dilfuza Jabborova et al. 2020).

## 4.2 *Plant Growth Promoting and Biocontrol Substances Produced by* Pseudomonas fluorescens

Plant hormones are chemical messengers that affect a plant's ability to respond to its environment. Hormones are organic compounds that are effective at very low concentration; they are usually synthesized in one part of the plant and are transported to another location. They interact with specific target tissues to cause physiological responses, such as growth or fruit ripening. Each response is often the result of two or more hormones acting together. Because hormones stimulate or inhibit plant growth, many botanists also refer to them as plant growth regulators. Botanists recognize five major groups of hormones: auxins, gibberellins, ethylene, cytokinins and abscisic acid (Saranraj et al. 2013).

### 4.2.1 Indole-3-Acetic Acid (IAA) Production

Bacterial production of plant growth substances occurs continuously in the ecosystem. Those with the plant roots or soils improve the plant growth by their direct effects on their metabolic processes. The phytohormone Indole-3-acetic acid (IAA) produced by bacteria is involved in several types of plant–microbe interactions (Morris 1986). In beneficial associations, bacterial IAA may modify the plants pool of growth regulators, resulting in stimulation of plant growth (Loper and Schroth 1986). There is firm evidence that Indole-3-acetic acid produced by plants are essential for their growth and development, which are also produced by various bacteria which live in association with plants (Scott 1972). There is also evidence that the growth hormones produced by the bacteria can in some instances increase growth rates and improve the yields of the host plants (Kour et al. 2021).

Indole- 3-acetic acid is a member of the auxins family of phytohormones that influence many cellular functions in plants and therefore are important regulators of plant growth and development. In addition to production in plant tissues, IAA synthesis was widespread among plant-associated bacteria (Patten and Glick 1996) and provides bacteria with a mechanism to influence plant growth.

IAA is the member of the group of phytohormones and is generally considered the most important native Auxin (Ashrafuzzaman et al. 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses and cellular responses, such as cell expansion, division, differentiation and gene regulation (Ryu and Patten 2008). Different biosynthesis pathways have been identified and redundancy for IAA biosynthesis was widespread among plant-associated bacteria. Interactions between IAA producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phytostimulation (Seema Sharma et al. 2013).

Isolates producing IAA have stimulatory effect on the plant growth. When the crop is inoculated with the isolates capable of IAA production significantly increases the plant growth by the Nitrogen, Phosphorous, Potassium, Calcium and Magnesium

uptake of sweet potato cultivar (Farzana and Radizah 2005). There was a significant increase in rooting and root dry matter of cuttings of eucalypts when grown on IAA producing rhizobacteria inoculated substrate. Some rhizobacterial isolates stimulate the Rhizogenesis and plant growth, maximizing yield of rooted cuttings in clonal nurseries (Teixeria et al. 2007). When cucumber, tomato and pepper are inoculated with different strains of PGPR which produce IAA, there was a significant increase in the growth of the vegetables (Kidoglu et al. 2007).

IAA of microbial origin plays a major role in promotion of orchid germination, at least when the bacterial strains are in tight association with the seeds. *Azospirillum brasilense* strain and *Bradyrhizobium japonicum* are able to excrete IAA into the culture medium, at a concentration sufficient to produce morphological and physiological changes in young seed tissues of Maize (*Zea mays* L.) and are responsible for their early growth promotion (Cassana et al. 2009) The use of PGPR isolates is beneficial for maize cultivation as they enhance the growth of rice by inducing IAA production (Jabborova et al. 2021).

Some microorganisms produce auxins in the presence of a suitable precursor such as L- tryptophan. The tryptophan increases the production of IAA in *Bacillus amyloliquefaciens*. Tien et al. (1979) showed that *Azospirillum* was able to produce auxins when exposed to tryptophan. Plants inoculated with the *Rhizobia* together with $Ag^{+}$ ion and L – tryptophan give the highest root dry weight and significantly increase the uptake of N, P and K compared to non-inoculated control plants.

Markus Beyeler et al. (1999) explained that the biocontrol strain *Pseudomonas fluorescens* produces small amounts of Indole-3-acetic acid via the tryptophan side chain oxidase and the tryptophan transaminase pathways. A recombinant plasmid (pME3468) expressing the tryptophan monooxygenase pathway was introduced into strain, this resulted in elevated synthesis of Indole-3-acetic acid in vitro, especially after addition of L-tryptophan.

Shino Suzuki et al. (2002) investigated the IAA biosynthesis in *Pseudomonas fluorescens*. After several repeated sub-cultures, the spontaneous IAA low-producing mutant was isolated. The IAA low production of the *Pseudomonas fluorescens* strain HP72LI was due to the low Tryptophan Side Chain Oxidase (TSO) activity. Colonization of *Pseudomonas fluorescens* strain HP72 on the bent grass root induced root growth reduction, while strain *Pseudomonas fluorescens* HP72LI did not induce such growth reduction. The colonization ability of strain *Pseudomonas fluorescens* HP72 on the bent grass root is higher than that of strain *Pseudomonas fluorescens* HP72LI. However, as for biocontrol ability, a significant difference in both strains was not detected.

Plant growth-promoting bacteria (PGPB) produce different plant growth regulators, among these the most significant one was Indole-3-acetic acid. This has been considered to be the predominant cause of plant growth improvement in comparison to the Nitrogen fixing capacity of diazotrophic PGPB strain, such as, *Azospirillum* (Malhotra and Srivastava 2006). Thirty isolates of fluorescent *Pseudomonas* obtained from wheat rhizosphere were found to produce 1.1–12.1 mg of auxin per/litre of medium together without supplementation of tryptophan and 1.8–24.8 mg per/litre with tryptophan (Khalid et al. 2004a, b). Suresh et al. (2004) reported that

fluorescent *Pseudomonas* strains, obtained from moist deciduous forest of Western Ghats, recorded Indole acetic acid and Gibberellic acid production in the range of 1.63–17.0 µg and 0.72–5.27 µg/ml respectively. Indole acetic acid and Gibberellic acid production by 52 fluorescent *Pseudomonas* have been found in the range from 80 to 760 µg and 24.82 µg per/litre of broth, respectively (Ni Suriyani et al. 2020).

Beom et al. (2006) reported that the *Pseudomonas fluorescens* and *Bacillus pumilus*, secreted higher levels of Indole-3-acetic acid in Tryptophan-amended medium at stationary phase. Yang et al. (2007) reported that the microbial IAA production through the IAM pathway which was considered to be a major pathogenicity determinant in gall and knot forming bacterial species. The IPyA pathway was found to be enhanced by their epiphytic fitness in plant root systems. (i) Reported that the *Pseudomonas fluorescens* and *Pseudomonas putida* are most important kinds of PGPR for the production of IAA (31.6 mg/L, 24.08 mg/L) which could stimulate the plant growth promotion and yield of many crop species.

Khakipour et al. (2008) evaluated the auxin productivity potential in *Pseudomonas fluorescens* through chromatography, using HPLC devise; comparing the methods used and appointing IAA synthesize method by the studied strains in the applied cultivars. In fact, a variety of auxins like indole-3-acetic acid (IAA), indole-3-pyruvic acid, indole-3-butyric acid and indole lactic acid; cytokinins and gibberellins are detected, with auxin production being quantitatively most important. *Azospirillum brasilense* strain has the potential to be a competent rhizospheric bacterium as it triggers the IAA accumulation under nutrient stresses, likely environmental fluctuations and long-term batch cultures and beneficially influences the growth of Sorghum (Imran Khan et al. 2020).

Prassana Reddy Battu and Reddy (2009) isolated twenty *Pseudomonas fluorescens* strains from rice growing soil samples and characterized. One of the *Pseudomonas fluorescens* isolated and identified from the dual culture test. It was fermented for secondary metabolite in a small scale and extracted with ethyl acetate. The isolated metabolite tested against plant fungal pathogens.

Karnwal (2009) obtained 30 fluorescent *Pseudomonas* isolates from different plant rhizosphere and were characterized on the basis of biochemical tests and plant growth-promoting activities. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* showed the best plant growth-promoting activity. These isolates were tested for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500 µg/ml. For both strains, indole production increased with increases in tryptophan concentration *and Pseudomonas aeruginosa* was less effective in production of indole acetic acid than *Pseudomonas fluorescens*. Inoculation of maize seeds with *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* showed a good level of indole acetic acid compared to uninoculated seeds (Reshma et al. 2018).

Khare and Arora (2010) proposed that the production of Indole-3-acetic acid, by Rhizobacteria, which resulted in plant growth promotion, especially root initiation and elongation. The isolate *Pseudomonas fluorescence* showed maximum production of IAA and also exhibits the biocontrol activity against Charcoal rot disease caused by *Macrophomina phaseolina* in chickpea, whereas the IAA-defective

mutant caused reduction in biocontrol and plant growth-promoting activity than wild isolate.

Jayasudha et al. (2010) studied the *Pseudomonas fluorescence* and quantitatively evaluated for Indole acetic acid producing ability in the presence (trypt$^+$) or absence (trypt$^-$) of tryptophan, and growth promotion in groundnut was analyzed in response to seed treatment with high IAA producers. It was found that more amounts of IAA were released in trypt$^+$ than the trypt$^-$. They revealed that IAA producers of the fluorescent *Pseudomonas* group showed significant plant growth promotion when compared with control, but plant growth was not greatly influenced by those organisms that produced high amounts of IAA. Antagonistic *Pseudomonas* sp. was able to release moderate or even low amounts of IAA, which may be better growth promoters.

Ramezanpour et al. (2011) reported the high level PGPR activities of fluorescent *Pseudomonas*, including, IAA biosynthesis, phosphate solubilization and siderophore production for the enhancement of plant growth stimulation and yield of rice plant. Further, the 16S rRNA assay confirmed that these strains were genetically diverse and mainly belonged to *Pseudomonas fluorescens, Pseudomonas putida* and *Pseudomonas aeruginosa,* respectively. Mandira Kochar et al. (2011) analyzed the biocontrol strain *Pseudomonas fluorescens* for indole-3-acetic acid (IAA) biosynthesis and studied the effect of its consequent manipulation on its plant growth-promoting potential. While the indole pyruvic acid pathway commonly associated with PGP bacteria was lacking, the indole acetamide pathway generally observed in phytopathogens was expressed in strain *Pseudomonas fluorescens*. Over expression of indole acetamide pathway genes *iaaM-iaaH*, from *Pseudomonas syringae* subsp. *savastanoi* drastically increased IAA levels and showed a detrimental effect on sorghum root development.

### 4.2.2 Siderophore Production

Iron is an essential growth element for all living organisms. The scarcity of bioavailable iron in soil habitats and on plant surfaces foments a furious competition (Whipps 2001). Under iron limiting conditions, PGPB produce low molecular weight compounds called siderophores to competitively acquire ferric ion. Siderophores (Greek: 'iron carrier') are small, high affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi and grasses (Miller and Marvin 2009). Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble $Fe^{3+}$ complexes that can be taken up by active transport mechanisms. Many siderophores are non-ribosomal peptides (Miethke and Maraheil 2007), although several are biosynthesized independently (Sayyed et al. 2019).

Iron is present as a co-factor in various enzymes, such as, peroxidase, aconitase, catalase, nitrogenase complex and ribonucleotide diphosphate reductase (Byers and Arceneaux 1977). Iron also plays a structural role in microorganisms. Crystals of magnetite ($Fe_3O_4$) giving magnetotactic characteristics in some bacteria so that they

orient themselves in weakly magnetic fields. The siderophores sequester ferric ions from the environment and the ferric siderophores are taken up by the microbial cell after specific recognition by membrane proteins. Then, it becomes available for metabolic function of microorganisms (Hofte et al. 1991).

The term "Siderophore" was proposed by Lankford (1973). Siderophore is defined as a low molecular weight (500–1000 Daltons) and virtually ferric specific ligand. The biosynthesis of Siderophore is carefully regulated by iron and its function is to supply iron to the cell. As iron is involved in several critical stages in metabolism, the microbes have evolved multiple system for acquisition. The high affinity system is comprised of the siderophores and the matching membrane associated receptors (Neilands 1981; 1984). The term "Siderophore" should be reserved for the metal free ligand and designated as "Deferrichrome" (Shaikh et al. 2018).

Microbial iron containing or iron binding compounds, most of which are classified as 'Siderophores' (Greek for Iron bearers). The siderophores, as chemical entities, display considerable structural variation, the majority of them are either hydroxamates or phenolates – catecholates and all exhibited a very strong affinity for Fe (III), the formation of a constant lying in the range of $10^{30}$ or higher. Neilands (1984) reviewed the iron metabolism of microorganisms in detail. Bacterial and fungal mechanisms of iron metabolism have been discussed extensively by (Patel et al. 2016).

Iron ($Fe^{3+}$) at physiological pH makes its acquisition by microbes difficult. In aqueous medium, iron exists as insoluble polymer ($Fe[OH]_3$) at neutral pH which has solubility constant $10^{-38}$ M, so that very little is available as soluble $Fe^{3+}$ (Spiro and Saltman 1969). Solubility of free iron at pH 7.0 is $10^{-17}$ M. Therefore, living organisms evolved efficient high affinity system for keeping iron in a soluble or at least accessible form from soil, phyllosphere, marine or fresh water environment (Messenger and Ratledge 1955). Components of a high affinity system, include, the synthesis and release of siderophore into the extracellular environment to chelate and solubilize Fe (III), the synthesis and deployment of specific membrane receptor proteins and, in gram negative bacteria, outer membrane receptor proteins for the ferrisiderophore complex (Neilands 1974, 1984). The biosynthesis of both the siderophores and their receptors is regulated by the cell and occurs only during iron limitation (Knosp et al. 1984).

Siderophores are also important for some pathogenic bacteria for their acquisition of iron. Siderophores are amongst the strongest binders to $Fe^{3+}$ known, with Enterobactin being one of the strongest of these (Raymond et al. 2003). Distribution of siderophore producing isolates according to Amplified Ribosomal DNA Restriction Analysis (ARDRA) groups, reveals that most of the isolates belong to Gram negative bacteria corresponding to the *Pseudomonas* and *Enterobacter* genera, and *Bacillus* and *Rhodococcus* genera are the Gram positive bacteria found to produce Siderophores.

Although, various bacterial siderophores differ in their abilities to sequester iron, in general, they deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity. Some PGPB strains go one step further and draw iron from heterologous siderophores produced by cohabiting microorganisms.

*Pseudomonas* sp. have the capacity to utilize siderophores produced by diverse species of bacteria and fungi, and *Pseudomonas putida* can utilize the heterologous siderophores produced by rhizosphere microorganisms to enhance the level of iron available to it in the natural habitat (Loper and Henkels 1999). The two strains of *Pseudomonas fluorescens* along with *Pseudomonas putida* produce maximum yield of hydroxamate type of siderophore in the modified succinic acid medium (Wani et al. 2016).

Soil bacteria isolates including *Azotobacter vinelandii, Pseudomonas fluorescens* and *Bacillus cereus* produce siderophores, and they can be used as efficient PGPR to increase the yield of the crop (Husen 2003). *Pseudomonas fluorescens* from rhizosphere is able produce siderophore, and thus it helps in the plant growth promotion and reduction of disease intensity. Specific strains of the *Pseudomonas fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields of various crops. These results prompted Kloepper et al. (1980) to investigate the mechanism by which plant growth was enhanced (Jadhav et al. 2017).

A previous study indicated that the PGPR increase plant growth by antagonism to potentially deleterious rhizoplane fungi and bacteria, but the nature of this antagonism was not determined. They presented evidence that PGPR exert their plant growth promoting activity by depriving native microflora of iron. PGPR produces extracellular siderophores which efficiently complex environmental iron, making it less available to certain native microflora. The siderophores production by *Bacillus* and *Pseudomonas* when assessed both in the presence and in absence of technical grade of herbicides show that the metabolic activities of plant growth-promoting rhizobacteria decline following herbicides application (Munees and Mohammad 2009).

Siderophores are low molecular weight (<10 kDa) iron binding compounds synthesized by microbes in large quantity under iron limited conditions. Siderophores chelate the ferric ions with a high specific activity and serve as vehicles for the transport of iron ($Fe^{3+}$) into the microbial cell. Most of the siderophores have either hydroxamate, catechol or carboxylate ligands (Hofte 1993).

Rhizosphere bacteria that promote plant growth have been shown that under iron limiting conditions they produce siderophores and corresponding membrane outer proteins for iron acquisition (Leong 1986). These high specificity, high affinity $Fe^{3+}$ specific ligands that serve as vehicles for iron transport exerted a strong influence on the microbial environment in the rhizosphere and have been implicated in plant-microbe interaction (Leong and Expert 1990). Iron regulation was mediated through an iron binding repressor protein, which under iron rich conditions inhibited the expression of genes required for synthesis of receptor protein (Neilands 1982). Iron formed as insoluble hydroxides at neutral and basic pH levels (Lindsay 1979).

Becker and Cook (1988) identified two Pseudobactin or Pyoverdin type Siderophores from *Pseudomonas fluorescens* whereas its mutant 5-2/4 showed 47% and 33% less production in both the type of Siderophores. Loper and Henkles (1997) suggested the siderophore mediated biocontrol activity of the fluorescent *Pseudomonas* suppress the *Pythium* species which significantly increased the growth of wheat. Mutants of *Pseudomonas fluorescens*, deficient in siderophore production were unable to control the damping off disease in cotton caused by *Pythium ultimum* when compared with the wild strain.

Manwar et al. (2000) reported the in vitro suppression of plant pathogens through siderophore production by fluorescent *Pseudomonas*. Kurek and Jaroszuk Scire (2003) reported that two *Pseudomonas fluorescens* strains synthesized $Fe^{3+}$ chelating compounds which inhibited the in vitro growth of *Fusarium culmorum* strain by competition for $Fe^{3+}$ utilization. Suryakala et al. (2004) suggested that trihydroxyamate siderophores might be the highly potent biocontrol compounds against plant pathogens. Suryakala et al. (2004) reported that siderophores exerted maximum biocontrol activity against *Fusarium oxysporum* than *Alternaria* sp. and *Colletotrichum capsici* (Dilfuza Jabborova et al. 2020).

Djibaoui Rachid and Bensoltane Ahmed (2005) tested the ability of *Pseudomonas* to grow and to produce siderophores, which are dependent on the iron content and the type of carbon sources in the medium. Under conditions of low iron concentration the *Pseudomonas* isolates studied produced yellow – green fluorescent iron – binding peptide siderophores and the biosynthesis of this siderophores was affected by several different environmental parameters. Four basal media, supplemented with different concentration of iron, were employed to study the effect of iron and different organic carbon sources on siderophore production in *Pseudomonas fluorescens*. The highest siderophores concentration was obtained in succinate medium. Ferric iron increased the growth yield and completely repressed siderophores production above 200 g/l, but had a positive effect below 160 g/l.

Urszula Jankiewicz (2006) tested the ability of six strains belonging to the genus *Pseudomonas* isolated from the rhizosphere of wheat to produce Pyoverdin. The studied strains demonstrated a varied level of production of the siderophore, depending on the culture conditions. The highest level of Pyoverdin was determined after 72 h of growth at 20–25 °C in iron-free medium supplemented with succinate. The synthesis of pyoverdin by all the strains studied was strongly repressed by the addition of iron ions (III) to the growth medium. Calcium, Cadmium and Magnesium ions stimulated the synthesis of the siderophore examined, whereas Zinc and Lead ions partially decreased its level. Enrichment of the growth medium in cobalt ions completely inhibited the synthesis of siderophores as well as growth of the bacteria.

Sayyed et al. (2005) reported that *Pseudomonas fluorescens* and *Pseudomonas putida* were able to give higher yields of hydroxamate type of siderophore (87% and 83% units) respectively in modified Succinic acid medium and under iron stress conditions. Increased in iron concentration up to 100 μM favoured growth but drastically affected siderophore production in both the strains. Saikia et al. (2006) reported that the PGPR viz., *Pseudomonas* mediated ISR in chickpea against the wilt pathogen (*Fusarium* sp.) is dependent on iron availability.

### 4.2.3 Phosphate Solubilization

Phosphorous is one of the major nutrient second only to nitrogen in requirement for plants. Most of the phosphorous in soil is present in the form of insoluble phosphates and cannot be utilized by plants (Pradhan and Sukla 2006). The ability of bacteria to solublize mineral phosphates has been of interest to agricultural

microbiologists as it can enhance the availability of phosphorous for plant growth PGPR which has been shown to solublize precipitated phosphates and enhance phosphate availability to rice that represent a possible mechanism of plant growth promotion under field condition (Verma et al. 2001).

The biological conversion of unavailable/fixed form of inorganic phosphorous into primary orthophosphate ($H_2PO_4^-$) and secondary orthophosphate $(HPO_4)_2$ are termed as 'Mineral Phosphate Solubilisation (MPS)' (Goldstein 1986). Involvement of microorganisms in the solubilization of insoluble phosphate was first demonstrated by Stalstorm (1903). Since then, lot of work has been done on the isolation, enumeration, efficiency screening, mechanisms of solubilization and crop response to their inoculation (Jadhav et al. 2020).

The calcium phosphate solubilization is an important criteria for the isolation and enumeration of phosphate solubilizing microorganisms (Sperber 1957). Among the different groups of Mineral Phosphate Solubilizing Bacteria (MPSB), the *Pseudomonas* are assumed as important one, since they are the most common and frequently occurring group in the plant rhizosphere and capable of utilizing a wide array of compounds as carbon and energy sources. They are also known to possess a wide range of plant growth-promoting activity by virtue of nutrient mobilization, Phosphate solubilization, production of plant hormones and biocontrol potential.

The improvement of soil fertility is one of the most common strategies to increase agricultural production. The biological nitrogen fixation is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, Phosphate solubilization is equally important. Phosphorus is major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic Phosphorus of soil and make it available to the plants. The ability of some microorganisms to convert insoluble Phosphorus to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields. The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth-promoting agent in agriculture (Sukmawati et al. 2021).

The use of phosphate solubilizing bacteria as inoculants increases the Phosphorus uptake by plants (Chen et al. 2006). Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate Solubilizing Microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the phosphorus demands of plants. These organisms in addition to providing Phosphorus to plants also facilitate plant growth by other mechanisms (Abhishek Sharma et al. 2020).

Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems (Zaidi et al. 2009). PSM include largely bacteria and fungi. The most efficient PSM belong to genera *Bacillus, Rhizobium* and *Pseudomonas* among bacteria, and *Aspergillus* and *Penicillium* among fungi. Within rhizobia, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Rivas et al. 2006). However, it is known

that every aspect of the process of nodule formation is limited by the availability of phosphorous (Afreen Khan and Sayyed 2019).

Illmer and Schinner (1992) reported that *Pseudomonas* sp. and *Penicillium* sp. were found to be solubilize high amount of insoluble inorganic phosphates in forest soils. Nahas (1996) studied the ability of 31 bacterial strains to solubilize rock phosphate and calcium phosphate in culture medium. Among them, *Pseudomonas cepacia* had the highest phosphate solubilizing activity. Di Simine et al. (1998) denoted the solubilizaiton of $ZnPO_4$ by a phosphate solubilizing *Pseudomonas fluorescens* occurring only in the presence of glucose, as the sole carbon source.

Gupta et al. (2002) developed heavy metal resistant mutants of *Pseudomonas* sp. as a potent phosphate solubilizer. Neelam and Meenu (2003) reported that the *Pseudomonas* sp. isolated from rhizosphere soil of *Trigonella* was found to be highly efficient in solubilizing the Tricalcium phosphate. Das et al. (2003) confirmed the Tricalcium phosphate solubilizing activity of *Pseudomonas fluorescens* wild and cold-tolerant mutant strains. They reported the cold-tolerant mutants were more efficient than their respective wild-type counterparts for Phosphate solubilization at low temperatures (Sagar et al. 2020).

### 4.2.4 ACC-deaminase Activity of *Pseudomonas fluorescens*

*Pseudomonas* contain the enzyme (ACC-aminocyclopropane-1-carboxylate) deaminase (*acd*), which hydrolyses ACC, the immediate precursor of the plant hormone ethylene. These bacteria, occurring on the root surface, degrade ACC to ammonium and α-ketobutyrate and are used as nitrogen source. Moreover, it could maintain the equilibrium of ACC concentration which exists between root, rhizosphere and bacterium. The bacterial uptake of ACC stimulated the plants, ACC efflux, which led to decrease in ACC concentration and ethylene elevation in plant root system (Belimov et al. 2001).

The positive effect PGPR inoculation having ACC-deaminase activity could promote root growth, root elongation and biomass production of different plant species, particularly when the plants were subjected to stress conditions (Belimov et al. 2001; Burd et al. 1998; Glick et al. 1997; Hall et al. 1996; van Loon and Glick 2004). The *acd* was first isolated from *Pseudomonas* sp. strain ACP in 1978, which was able to hydrolyze ACC to ammonia and α-ketobutyrate. The isolation of ACC-deamianse (acd) from *Pseudomonas putida* and *Pseudomonas fluorescens* has been frequently reported (Minami et al. 1998). The *acd* (ACC-deaminase) activity of *Pseudomonas putida* strains isolated from rhizosphere of bean, corn and clover, respectively has been reported by Shah et al. (1998).

*Pseudomonas fluorescens* strain, possessing *acd* (ACC-deaminase) activity enhanced the saline resistance in groundnut plant which ultimately resulted in increased yield. Inoculation of canola seedling with *Pseudomonas putida* has been found to increase the root and shoot dry weight of canola under cold and salinity stress conditions (Glick et al. 1997). Cheng et al. (2007) reported that the inoculation of *acd* (ACC-deaminase) producing *Pseudomonas putida* strain could protect

the plant under salinity stress. Glick et al. (1994) reported that *Pseudomonas putida* contain an enzyme, ACC-deaminase which hydrolyzed ACC to ammonia and α-ketobutyrate. This process eventually led to decreased level of ACC, and thereby reduced the level of endogenous ethylene. Thus the potential inhibitory effect of increased ethylene concentration could be eliminated (Yuhashi et al. 2000). Glick et al. (1999) isolated eight strains of *Pseudomonas putida* from the rhizosphere of pea. Among the eight strains, the most efficient strain was selected, based on their ability to utilize ACC, as sole source of nitrogen, for growth, production of siderophores and root length enhancement of canola under in vitro condition (Sadaf Kalam et al. 2020).

### *4.3 Aggressive Root Colonization*

The attachment of bacterial cells to the plant root is one of the early steps in the root colonization process (Howie et al. 1987). Several bacterial characteristics involved in this early event, including the presence of pili (Vesper 1987), a 33,000 molecular weight root-adhesive protein and surface charge properties have been described (James et al. 1985). In the natural environment, soil *Rhizopseudomonas* interact with each other to sustain their growth which is partly influenced by their ability to adhere to soil particles, roots or other nutrient rich substrates (Gannon et al. 1991).

Several surface proteins are potentially involved in surface hydrophobicity. Fewer studies reported the ecological significance of hydrophobic cellular interactions with various surfaces such as, soil particles, biological surfaces, or inert support (Schafer et al. 1998; Troxler et al. 1998). Adhesion of bacteria to biological surfaces is important for colonization, pathogenesis, and antagonistic interactions (Wisniewski and Delmotte 1996; Jana 1998). Considerable research has been done and emphasized the role of specific recognition (lectin mediated cell recognition) in root-bacterial interaction in the rhizosphere of many crop plants (Kapadia et al. 2021).

Adherence to surface is a general feature of microbial development in natural environments. Firm attachment to surface of all kinds is thought to confer nutritional advantages. Mechanism of adhesion is too many among bacteria involving fibrils, cell wall proteins, capsular and slime secretions, deposition of inorganic cements and holdfast microcapsular areas often localized on special structures of prostheca (Rao and Johri 1999).

Fibrillar attachment of the bacteria is primarily dependent on active bacterial metabolism; dead bacteria did not adsorb to roots while live bacteria attached to dead plant roots (Bashan and Levvanony 1988a, b). The colonization of *Azospirillum* sp. to roots of many cereal crops can affect the plant metabolism (Baldani et al. 1983; Dobereiner and Baldani 1979; Kapulnik et al. 1987; Okon and Kapulnik 1986). The mechanism involved in this was unknown (Patriquin et al. 1983; Schank et al. 1979; Umali-Garcia et al. 1980). Polar attachment of *Azospirillum* cells to roots was demonstrated by Patriquin et al. (1983) and Whallon et al. (1985) and the

same was later confirmed by Levanony and Bashan (1989). Gafni et al. (1986) described various modes of adsorption of *Azospirillum brasilense* to maize roots. Patriquin and Dobereiner (1978) reported the differences between various strains of *Azospirillum* in the degree of attachment to wheat root hairs. However, most of the root surface was colonized by bacteria in a horizontal and thermodynamically more stable position (Singh et al. 2021).

Adsorption of bacterium to a solid phase was known (Fletcher et al. 1980) and may give the rhizosphere bacteria nutritional and favourable microspace advantages. *Azospirillum* attachment to root surfaces occurs rapidly and was varying with bacterial growth phase and strain (Bashan and Levanony 1988a, b; Eyers et al. 1988; Gafni et al. 1986; Umali-Garcia et al. 1980). Bashan (1986) described the vertical transfer of *Azospirillum* from the growth tip to deeper soil layers.

Adsorption of bacteria to plant roots can be either passive or it may depend on the active metabolism of both partners (Fletcher et al. 1980). Shimshick and Hebert (1979) found that absorption of *Rhizobium japonicum* to soybean root was inhibited by killing the bacteria through heat or mercuric chloride. On the other hand, killing roots had no effect on binding.

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