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Editors

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From Theory to Practices

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1 Plant Growth-Promoting Bacteria: Strategies to Improve Wheat Growth and Development Under Sustainable Agriculture

Éva Abod, Éva Laslo, Sarolta Szentes, Szabolcs Lányi, and Gyöngyvér Mara

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Abstract

A part of rhizospheric bacteria are considered plant growth-promoting bacteria (PGPB) due to their positive effect on the plant growth and development. Plant growth-promoting bacteria based on their metabolic activity can be grouped as biofertilizers, fitostimulants, or biopesticides. These efficient bacteria due to various direct or indirect effects exerted on plants have crucial role in agricultural sustainability. Recently were reported diverse genera as PGPB like *Acetobacter*, *Achromobacter*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Frankia*, *Phyllobacterium*, *Pseudomonas*, *Serratia*, and *Rhizobium*. Bacterial strains for this study were isolated from a natural habitat (raised bog) and agricultural environment. Selected bacterial strains based on 16S rRNA gene sequence analysis were identified as *Achromobacter spanius*,

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Delftia lacustris, *Pseudomonas protegens*, *P. jessenii*, and *Acinetobacter lwoffii*. These bacterial strains have different plant growth-promoting (PGP) activities like multi-stress resistances (temperature, pH, salinity) and others such as cellulose, phytin, and lecithin degradation, alkaline phosphatase and alkaline protease activity, and siderophore production. The selected strains were tested on plants either alone or in consortia. Based on the reports, it was confirmed that *Delftia lacustris* BI5, *P. jessenii* BI7, bacterial strains, and the bacterial consortia *P. jessenii* BI7 and *A. lwoffii* BI13 showed positive effect due to their PGP characteristics on wheat shoot growth under laboratory conditions. These promising strains have potential as inoculation agents in eco-friendly crop production contributing to environmental sustainability.

Keywords
Rhizosphere · Microorganisms · Plant growth · Synergy · Crop production

1.1 Introduction

Plant growth-promoting bacteria (PGPB) are soil and rhizosphere bacteria that can promote plants growth through various direct and indirect mechanisms. These bacteria based on their effect on plant growth and development, due to different metabolic activities, can be grouped as biofertilizers, phytostimulants, and biopesticides.

Bacteria with role in biofertilization can provide inaccessible nutrients for plants, due to atmospheric nitrogen fixing and phosphorus, iron or potassium solubilizing. They are able to increase the availability of nutrients through the decomposition of organic compounds, expected to enzymes such as phytase, acid or alkaline phosphatase, and esterase (Lü et al. 2005; Sarikhani et al. 2010). In iron insufficiency conditions, iron could be solubilized by the production of iron-binding molecules like siderophores which can form Fe-siderophore complex, readily available to plants (Kumar et al. 2017b). Phytostimulants produce different phytohormones (auxins, gibberellins, cytokinins, and ethylene) and fulfil a role in plant growth promotion (Shukla 2019). Due to the synthetized hormones, these microbes can also improve plant tolerance in various abiotic stress circumstances (Gupta et al. 2015). Biopesticides are able to control the growth of deleterious microorganisms due to the deliberation of different secondary metabolites or extracellular cell wall decomposing enzymes (cellulose, alkaline or neutral protease, siderophores, antibiotics, HCN, and induced systemic resistance) (El-Sayed et al. 2014; Akram et al. 2017; Barnawal et al. 2017).

From the recent observations were reported diverse genera as PGPB with important role in different crop or vegetable nutrition like *Agrobacterium*, *Acetobacter*, *Achromobacter*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Frankia*, *Herbaspirillum*, *Klebsiella*, *Kluyvera*, *Paenibacillus*, *Phyllobacterium*, *Pseudomonas*, *Proteus*, *Serratia*, *Rahnella*, and *Rhizobium* (Babalola 2010; Ahemad and Kibret 2014; Chatterjee et al. 2017; Shukla 2019).

The current agricultural practice due to intensive use of agrochemicals faces difficulties due to the pollution and nonrenewable resource use, having a significant effect on the state of the environment. The solution relies on a more resource-preserving and environmentally friendly practice so-called sustainable agriculture. In sustainable agricultural practice, the maintenance of soil health and its microbial community is crucial. Microbial products contribute to the plant nutrient status without pollution and depletion of natural resources and also protect plant under stress conditions (Bhattacharyya et al. 2016; Prasad et al. 2019). These microbes with plant nutrition enhancement, phytostimulation, or biocontrol effect can replace or complete the chemicals used in agriculture. Microbial inoculants are getting focus and are widely accepted in sustainable development of agriculture (Bhattacharyya et al. 2016; Prasad et al. 2019).

PGP bacteria from different ecological habitats, regions, and plant rhizosphere were described for their beneficial activities and impact on plant growth in order to be used in sustainable agriculture. Crop and wild plants and their rhizosphere represent a potential origin of new PGP bacterial strains. The wild plant rhizosphere, due to the harsh environment, is considered as a good source for competitive PGP bacteria (Gopalakrishnan et al. 2015). Nevertheless, a high percentage of studied PGP bacteria were isolated from crop plants as soybean (Sugiyama et al. 2014), pea (Meena et al. 2015), wheat (Majeed et al. 2015), and maize (Shahzad et al. 2013; Qaisrani et al. 2014). Data on PGP bacteria isolated from wild plants are deficient; several findings were published regarding the native plant-associated rhizobacteria from Saudi Arabia (El-Sayed et al. 2014) and India (Singh et al. 2015). It was observed also that the performance of the PGPB varies due to environmental factors and local conditions (Shukla 2019). The aim of the research was to isolate PGP bacteria from wild and crop plants adapted to local conditions in order to be used in microbial inoculants in this region.

This chapter presents a comparative study of plant growth-stimulating aspects (production of siderophores, protease, and phosphatase and degradation of cellulose, phytin, and lecithin) of bacterial strains originated from natural and agricultural ecosystems. Furthermore, the plant growth-promoting potential of the strains used either single or in consortia was assessed in vivo on wheat growth.

The present study identified novel PGP characters for *Achromobacter spanius*, *Acinetobacter lwoffii*, *Delftia lacustris*, *Pseudomonas jessenii*, and *P. protegens* strains. *D. lacustris* BI5, *P. jessenii* BI7, and *A. lwoffii* BI13 bacterial strains were found to be efficient on wheat plant growth based on a single and multistrain microbial formulation, making them good candidates to be used as microbial inoculants.

1.2 Strain Identification and Characterization

These efficient bacterial isolates were isolated from soil and rhizosphere of *Carex* sp. from Borsáros raised bog natural reserve (Harghita County, Romania, GPS coordinates: 46°18′37.6″ N, 25°50′24.8″ E) and from soil and rhizosphere of *Zea mays* from Cristuru Secuiesc (Romania, GPS coordinates: 46°28′62.4″ N, 25°03′85.3″ E).

Among 13 isolates studied, 7 (53.8%) were sequestered from natural raised bog environment, whereas the remaining 6 (46.2%) from agricultural environment.

The 13 bacterial isolates were identified by their 16S rRNA gene sequence. Genomic DNA was isolated from strains after cultivation of cells on King's B agar for 24 h. The 16S rRNA gene sequence was amplified by PCR using primers 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1401r (5′-CGGTGTGTACA AGGCCCGGGAACG-3′) and purified by PCR-MTM Clean-Up System (Viogene, Sunnyvale, USA). The partial 16S rRNA gene sequence was obtained by sequencing the gene in both directions (AmpliTaq® FS Big Dye TM Terminator sequencing kit, Applied Biosystems). The bacterial isolates were identified using partial 16S rDNA gene sequence alignment to database (Table 1.1) as follows: *Achromobacter spanius* BI1 and BI4; *Delftia lacustris* BI2, BI5, and BI6; *Pseudomonas protegens* BI3; *P. jessenii* BI7; and *Acinetobacter lwoffii* BI8, BI9, BI10, BI11, BI12, and BI13 strains.

The strains isolated from the natural raised bog environment showed higher taxonomic diversity, being identified to belong to three different genera (*Achromobacter*, *Delftia*, and *Pseudomonas*), whereas from the agricultural area, *Acinetobacter* sp. strains were isolated. The capacity to grow at different temperatures (4, 24, 25, 26, 28, 32, and 37 °C), various salinities (0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 g L^{-1} NaCl), and pH (pH 6, 6.5, 7, 7.5, and 8) was tested in flasks containing 20 mL tryptic soy broth (TSB) incubated at 28 °C (4–37 °C for temperature preference analysis) on 150 RPM. Cellular morphology and cell diameter of the strains were determined from overnight culture using optical microscopy (Olympus, BX53). The morphological, physiological, and biochemical profile of the isolated strains was realized. The *Achromobacter spanius* BI1 and BI4; the *Delftia lacustris* BI2, BI5, and BI6; and the *Pseudomonas protegens* BI3 (Table 1.1) bacterial strains were Gram-negative, nonspore-forming, short rods with 2.2 ± 0.5 μm length. Growth occurred between 4 and 37 °C with an optimum growth at 25 °C, salinity from 0 to 12 g L^{-1} NaCl with an optimum between 4 and 6 g L^{-1}, and pH values from 6 to 8 with an optimum between pH 7.0 and 7.5. The abovementioned bacterial strains proved to be aerobe and oxidase positive and were able to degrade glucose. *Achromobacter spanius* sp. nov., originated from medical samples, was first reported by Coenye et al. (2003) as Gram-negative, oxidase-positive bacteria, with optimal growth temperature range between 28 and 37 °C and salinity between 0 and 4.5 g L^{-1} NaCl. *Delftia lacustris* sp. nov. was first isolated from freshwater environment by Jorgensen et al. (2009). It was described as rod-shaped bacteria, with the same optimal growth as in the current study (25 °C); the growth to 12 g L^{-1} NaCl salinity was supported by the strains isolated from raised bog environment (BI2, BI5, and BI6), whereas in case of the first described strain, growth to 6 g L^{-1} NaCl salinity was observed (Jorgensen et al. 2009). Similar morphological and biochemical characteristics were observed in case of *Pseudomonas protegens* sp. nov. isolated from tobacco roots (Ramette et al. 2011) and *Pseudomonas protegens* BI3 strain isolated from raised bog environment. The isolate *Pseudomonas jessenii* BI7 was proved to be Gram-negative, nonspore-forming, oxidase-positive aerobic bacteria, and it was first isolated and described showing similar growth parameters and biochemical

Table 1.1 The resulted morphological, biochemical, and PGP characteristics of the isolates

Strain code	Identified strain, similarity (%), the 16S rDNA sequence length (base pair)	Oxidase test	Siderophore production	Lecithin degradation	Cellulose degradation	Phytin degradation	Alkaline protease activity (mol tyrosine/h)	Alkaline phosphatase activity (μ mol p-NP/h)
BI1	*Achromobacter spanius* LMG 5911(T) AY170848, 99.7%, 484 bp	+	+	+	+	−	0.13 ± 0.04	ND
BI2	*Delftia lacustris* DSM 21246(T) EU888308, 98.9%, 482 bp	+	+	−	+	+	0.08 ± 0.05	ND
BI3	*Pseudomonas protegens* CHA0(T) AJ278812, 99.1%, 483 bp	+	+	+	−	−	ND	0.24 ± 0.05
BI4	*Achromobacter spanius* LMG 5911(T) AY170848, 100%, 483 bp	+	−	−	−	+	ND	0.12 ± 0.03
BI5	*D. lacustris* DSM 21246(T) EU888308, 98.9%, 479 bp	+	+	−	+	+	0.24 ± 0.02	0.25 ± 0.04
BI6	*D. lacustris* DSM 21246(T) EU888308, 98.9%, 478 bp	+	+	−	+	+	0.21 ± 0.03	ND
BI7	*Pseudomonas jessenii* CIP 105274(T) AF068259, 99%, 467 bp	+	+	−	+	−	0.33 ± 0.01	ND
BI8	*Acinetobacter lwoffii* strain MBW4 JX966447.1, 99%, 482 bp	−	+	−	+	−	ND	ND
BI9	*A. lwoffii* strain MBW4 JX966447.1, 99%, 474 bp	−	+	−	−	−	0.04 ± 0.02	ND

(continued)

Table 1.1 (continued)

Strain code	Identified strain, similarity (%), the 16S rDNA sequence length (base pair)	Oxidase test	Siderophore production	Lecithin degradation	Cellulose degradation	Phytin degradation	Alkaline protease activity (mol tyrosine/h)	Alkaline phosphatase activity (μ mol p-NP/h)
BI10	*A. lwoffii* strain MBW4 JX966447.1, 99%, 474 bp	–	+	–	–	–	ND	1.12 ± 0.03
BI11	*A. lwoffii* strain MBW4 JX966447.1, 99%, 480 bp	–	+	–	–	–	ND	1.43 ± 0.04
BI12	*A. lwoffii* strain MBW4 JX966447.1, 99%, 481 bp	–	+	–	–	–	ND	1.75 ± 0.01
BI13	*A. lwoffii* strain MBW4 JX966447.1, 99%, 480 bp	–	–	–	–	–	ND	1.83 ± 0.05

"–" negative result, "+" positive result, all bacterial isolates having aerobic and glucose-degrading capacity as well as negative in Gram staining along with no spore forming ability

characteristics from mineral water by Verhille et al. (1999). The six studied *Acinetobacter lwoffii* strains isolated from agricultural area (BI8, BI9, BI10, BI11, BI12, and BI13) were Gram-negative, nonspore-forming, short rods with 1.73 ± 0.45 μm length. Growth occurred between 3 and 37 °C with an optimum growth at 32 °C, salinity from 0 to 12 g L^{-1} NaCl with an optimum between 10 and 12 g L^{-1}, and pH values from 6 to 8 with an optimum at pH 7.5–8.0. The *Acinetobacter* strains were aerobe and oxidase negative and were able to degrade glucose. *Acinetobacter lwoffii* sp. nov. was first described by Bouvet and Grimont (1986) as rods with similar characteristics, differences in growth on various temperature were observed among strains, and those isolated in the present study were able to grow on lower temperature ranges (3–15 °C).

1.3 Siderophore Production

Siderophores are low molecular weight organic compounds produced by bacteria and fungi to enhance the iron uptake and are believed an efficient iron source also for the plants, therefore promoting plant growth (Saha et al. 2016). For siderophore production screening chrome azurol S (CAS) plates were used (Oldal et al. 2002). The plates were point inoculated and incubated for 24 h at 28 °C.

Eleven strains from the studied 13 (~85%) were able to produce siderophore, a high-affinity iron-chelating compound. Two strains, *A. spanius* BI4 isolated from raised bog environment and *A. lwoffii* BI13 isolated from agricultural environment, showed no siderophore production ability (Table 1.1). Although *Achromobacter* sp. strains are widely described as potential human pathogens, they were isolated also from rhizosphere environment, and PGP characteristics such as phosphate solubilization, plant hormone production ability, acetylene reduction, and direct plant growth promotion have been recently described (Gopal 2013; Abdel-Rahman et al. 2017). The study is the first record of siderophore production ability of an *A. spanius* BI1 strain. Morel et al. (2011) report *Delftia* sp. strains as having siderophore and indole acetic acid (IAA) production capacity, but *Delftia lacustris* strains were not mentioned previously as siderophore producers. *D. lacustris* strains were previously reported as having biocontrol potential against fungal pathogens (Janahiraman et al. 2016). *P. protegens* strains were previously described as siderophore producers (Ruiz et al. 2015; Sexton et al. 2017). No data about the siderophore production potential of *P. jessenii* was found in the literature; it was reported as capable of phosphate solubilization (Valverde et al. 2007). *Acinetobacter* sp. strains were described by Trotel-Aziz et al. (2008) as biocontrol agents and by Farokh et al. (2011) showing PGP characteristics as siderophore and P-solubilization.

As the result of the screening for PGP potential according to our best knowledge, this is first recorded for siderophore-producing ability of *A. spanius*, *D. lacustris*, and *P. jessenii*.

1.4 Organic Compound (Cellulose, Phytic Acid, and Lecithin) Degradation

The cellulose degradation potential of the isolated bacterial strains was exploited on carboxymethylcellulose (2% CMC, minimal salt media) containing agar plates using clearing assay. Bacterial strains were point inoculated on agar plates in triplicate and incubated for 5 days at 28 °C. To visualize the producing halos around the bacterial culture, plates were stained with 0.1% Congo red dye. The cellulose degradation ability was recorded if a clear zone around the colonies was observed. Five strains (71.4%, *A. spanius* BI1; *D. lacustris* BI2, BI5, and BI6; and *P. jessenii* BI7) isolated from natural raised bog environment and one strain (16.66%, *A. lwoffii* BI8) isolated from agricultural environment (Table 1.1) were able to degrade CMC.

Screening methods were used in order to elucidate the organic phosphorus-degrading capacity of the strains. The analysis of the phytic acid utilization was conducted on Sperber agar (Sarikhani et al. 2010), while the lecithin degradation was performed on egg yolk agar (Lü et al. 2005). Each bacterial strain was point inoculated in triplicate on agar plates. After incubating at 28 °C for 5 days, the phytic acid or lecithin degradation was recorded for each strain that produced a clearing zone.

Lecithin degradation was observed only for two bacterial strains (15.3%): *A. spanius* BI1 and *P. protegens* BI3 isolated from the natural raised bog environment. The lecithinase activity of a *P. protegens* strain isolated from tobacco roots was previously described by Ramette et al. (2011). Phytic acid degradation was detected in four bacterial strains (30.76%): *D. lacustris* BI2, BI5, and BI6 and *A. spanius* BI4. The six studied *A. lwoffii* strains were unable to degrade any of the phosphorus-containing organic compounds. In case of *Achromobacter* sp., *Pseudomonas jessenii*, and *Acinetobacter* sp., only inorganic phosphate solubilization was previously reported (Valverde et al. 2007; Farokh et al. 2011; Abdel-Rahman et al. 2017); no data on phosphorus-containing organic compound degradation were found in literature. We provide new evidence of organic matter decomposing activity of the two *Achromobacter* and one *Acinetobacter* strain. *A. spanius* BI1 strain was able to decompose lecithin and cellulose, and *A. spanius* BI4 utilized phytic acid, whereas in case of *A. lwoffii* BI8 strain, cellulose-degrading capacity was detected.

1.5 Alkaline Protease and Phosphatase Enzyme Assays

The bacterial strains were grown in culture broth containing casein as substrate (Adinarayana et al. 2005). After incubation on a rotary shaker (28 °C, 140 RPM) for 24 h, the culture media was centrifuged at 10000 RPM for 10 min, and the supernatants were gathered for enzyme assay. The absorbance of the resulted tyrosine was determined using a microplate reader (Fluostar Optima, BMG Labtech), and from the absorbance values, the protease enzyme activities (mol tyrosine/mL/h) of the strains were determined. The alkaline protease enzyme activity was proved for six (*D. lacustris* BI2, BI5, BI6, *A. spanius* BI4, *P. jessenii* BI7, *A. lwoffii* BI9) bacterial

strains (Table 1.1). The protease enzyme activities ranged from 0.04 ± 0.02 mol tyrosine/mL/h to 0.33 ± 0.01 mol tyrosine/mL/h.

In most studies published in the last years, the protease activity of the PGP bacterial strains was detected on skim milk agar (Hantsis-Zacharov and Halpern 2007; Suresh et al. 2010; Yuttavanichakul et al. 2012; Sadeghi et al. 2014; Masciarelli et al. 2014). Quantitative determinations of the protease enzyme activities were performed for several PGP bacterial strains as follows: *Bacillus subtilis* 333 (0.162 mmol tyrosine/h), *Tatumella ptyseos* (0.162 mmol tyrosine/h), *B. megaterium* 817 (0.157 mmol tyrosine/h), *Acinetobacter* sp. 378 (0.065–0.126 mmol tyrosine/h on different pH values) (Rodarte et al. 2011), *P. putida* MSC1 (0.0057 mol tyrosine/h), *P. pseudoalcaligenes* MSC4 (0.012 mol tyrosine/h) (Saraf et al. 2013), and *B. cereus* PM2 strain (0.0029 mmol tyrosine/h) (Anwar et al. 2014). No previous evidence for the alkaline protease activity of the studied taxa, *Delftia lacustris*, *Achromobacter spanius*, *Pseudomonas jessenii*, and *Acinetobacter lwoffii*, was found in the scientific literature. Alkaline protease activity of strains affiliated to *Acinetobacter* sp. genera was determined by Rodarte et al. (2011).

Phosphatase activity was tested by using a chromogenic substrate p-nitrophenyl phosphate (pNPP) (Wu et al. 2007). The bacterial strains were grown in pNPP-containing broth in an incubator shaker (24 h, 28 °C, and 140 RPM). Cells were lysed by sonication, and the debris was separated by centrifugation at 10000 RPM for 10 min at 25 °C. The absorbance of the resulted p-nitrophenol was quantified using microplate reader (Fluostar Optima, BMG Labtech), and from the absorbance values, the phosphatase enzyme activities (μmol p-NP/mL/h) of the strains were determined. In case of eight bacterial strains (*D. lacustris* BI2 and BI5, *P. protegens* BI3, *A. spanius* BI4, *A. lwoffii* BI10, BI11, BI12, and BI13), the alkaline phosphatase enzyme activity was determined, varying between 0.12 ± 0.03 and 1.83 ± 0.05 μmol p-NP/mL/h. The values obtained for the PGP bacteria and presented here are higher than those reported previously. Alkaline phosphatase enzyme activity varied between 1.41 and 2.15 μmol p-NP/mL/h in case of four bacterial strains (*Bacillus brevis* 2W4W1, *B. polymyxa* 1W5W5, *B. thuringiensis* 2P1M3, *Xanthomonas maltophilia* R85) isolated from wheat and pea plants (De Freitas et al. 1997). Viruel et al. (2011) determined values between 0.24 and 4.92 μmol p-NP/mg protein/h enzyme activities in soil with small Pi values for four bacterial strains (*Serratia marcescens* EV1, *Pantoea eucalypti* EV4, *Pseudomonas tolaasii* IEXb, *Enterobacter aerogenes* IEY). Rana et al. (2012) investigated the effects of PGP bacterial strains on wheat plants and observed in case of inoculation with two bacterial consortia (*Bacillus* sp. AW1 and *Brevundimonas* sp. AW7 consortia, *Providencia* sp. AW5 and *Brevundimonas* sp. AW7 consortia, respectively) 1.4–1.94 μmol p-NP/g soil/h alkaline phosphatase enzyme activities in soil. Kang et al. (2013) determined 0.022 and 0.13 μmol p-NP/g soil/h alkaline phosphatase enzyme activities from soil inoculated with *Bacillus pumilus* WP8 and *Pseudomonas chlororaphis* RA6 bacterial strains.

This study confirms the alkaline phosphatase activity of *Delftia lacustris*, *Achromobacter spanius*, *Pseudomonas protegens*, and *Acinetobacter lwoffii*. Acid and alkaline phosphatase activity of *Delftia lacustris* strain when first described was

also mentioned by Jorgensen et al. (2009), but no activity was measured. According to our best knowledge, we provide new evidence of alkaline protease activity for *A. spanius*, *P. jessenii*, *D. lacustris*, and *A. lwoffii* and alkaline phosphatase activity for *A. spanius*, *P. protegens*, *D. lacustris*, and *A. lwoffii* strains. In case of two *D. lacustris* strains (BI1, BI5) and one *A. spanius* BI4 strain, both alkaline phosphatase and protease activity was observed.

1.6 Growth-Promoting Effect of Bacterial Treatment on Wheat

Seeds of the same weight (0.3–0.4 g) of *Triticum aestivum* (wheat) were surface sterilized and germinated on filter paper for 48 h. The seedlings (1.5–2 cm shoot length) were transferred into autoclavable polypropylene boxes (size 34 × 23 × 16 cm) with lid. In these boxes steel sieves were made (25 × 15 cm, height 2 cm), with 4-mm-diameter holes, and the distance between the holes were 20 mm. The boxes with the sieves were sterilized by autoclaving at 121 °C, 15 min. Seedlings were wrapped in sterile buds and were placed 4 cm from each other (20–30 seedlings/box). For plant growth a nutrient solution was used containing the minimum necessary elements as follows: macroelements, 1.85 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1.66 g L^{-1} $CaCl_2 \cdot 2H_2O$, 5 g L^{-1} peptone, and 2.5 g L^{-1} phytic acid sodium salt as organic nitrogen and phosphorus source, and microelements, 0.44 mg mL^{-1} $MnSO_4 \cdot 4H_2O$, 0.16 mg mL^{-1} H_3BO_3, 0.15 mg mL^{-1} $ZnSO_4 \cdot 7H_2O$, 0.08 mg mL^{-1} KI Fe-EDTA, 3.73 mg mL^{-1} Na_2EDTA, and 2.78 mg mL^{-1} $FeSO_4 \cdot 7H_2O$.

Bacterial cultures were grown for 24 h in a rotary shaker (150 RPM) in 100 mL flasks filled with 25 mL TSB broth. Following the seedling transplant, each was inoculated with 1 ml bacterial inoculum (10^8 CFU mL^{-1}) of the selected bacterial strains or bacterial consortium. Plants were placed in an environmental growth chamber (Sanyo MLR-351) at 25 °C, 70% relative humidity using a lighting program of 12 h/day with 2500 lx. After 11 days of growth, plants were harvested, and the shoot and root length and wet and dry biomass were determined. The tests were carried out in 8–15 replicates for each plant. Data obtained were compared to uninoculated control, using PAST statistical program.

The treatment of wheat plants with three selected isolates resulted different effects on dry and wet biomass production. Table 1.2 presents the results of the bacterial inoculation experiment on plant growth and biomass under gnotobiotic conditions. Bacterial isolates *D. lacustris* BI5 and *P. jessenii* BI7 significantly increased the shoot length of plants compared to the uninoculated plants; the relative increase was of 33.05% (11.43 ± 1.32 cm) and 25.27% (10.76 ± 1.4 cm), respectively. The inoculation with *A. lwoffii* BI13 strain showed no significant effect on the shoot length of wheat plants. However, *A. lwoffii* BI13 strain used in consortia with *P. jessenii* BI7 strain showed a higher increase on wheat shoot length (43.48%) than the *P. jessenii* BI7 strain alone (Fig. 1.1a). Regarding the total weight of the plants, the inoculation with *D. lacustris* BI5 slightly stimulated (9.37%), whereas *P. jessenii* BI7 strain had no influence on the plant growth (Fig. 1.1b).

Table 1.2 Influence of the bacterial treatments on wheat growth

Bacterial strains	*D. lacustris* BI5	*P. jessenii* BI7	*A. lwoffii* BI13	*P. jessenii* BI7 + *A. lwoffii* BI13
Total weight (g)	0.084 ± 0.012*	0.080 ± 0.01	0.068 ± 0.01	0.083 ± 0.009*
Shoot length (cm)	11.43 ± 1.32*	10.76 ± 1.40*	8.44 ± 1.81	12.32 ± 1.02*
Wet weight of the shoot (g)	0.058 ± 0.01	0.058 ± 0.01	0.046 ± 0.011	0.0616 ± 0.0068*
Dry weight of the shoot (g)	0.010 ± 0.001*	0.010 ± 0.001*	0.007 ± 0.001	0.009 ± 0.001*
Wet weight of the root (g)	0.021 ± 0.007	0.017 ± 0.006	0.018 ± 0.005	0.018 ± 0.006
Dry weight of the root (g)	0.004 ± 0.001	0.002 ± 0.000	0.003 ± 0.001	0.004 ± 0.001

*Significantly different from the control for $p < 0.05$

Regarding the total weight, the result of inoculation showed similar effect as for the shoot length; the inoculation with *A. lwoffii* BI13 used in consortia with *P. jessenii* BI7 showed an increase of 8.85% compared to the control.

Only the *P. jessenii* BI7 and *A. lwoffii* BI13 bacterial consortia showed beneficial effect on wet weight of the shoot (increase of 15.78%). No significant differences between the inoculated and uninoculated control plants wet and dry root biomass were observed (Fig. 1.1c, d). The shoot dry weight was significantly increased in plants inoculated with *D. lacustris* BI5 (0.010 ± 0.001 g, 25.98%), *P. jessenii* BI7 (0.010 ± 0.001 g, 25.98%), and *P. jessenii* BI7 + *A. lwoffii* BI13 consortia (0.009 ± 0.001, 11.96%) (Table 1.2, Fig. 1.1f).

The treatment of wheat plants with the bacterial strains had significant effect mostly on shoot growth: the *D. lacustris* BI5, the *P. jessenii* BI7, and the *P. jessenii* BI7 + *A. lwoffii* BI13 bacterial consortia showed growth promotion on shoot length and shoot dry weight. The *P. jessenii* BI7 + *A. lwoffii* BI13 bacterial consortia had also significant beneficial effect on shoot wet weight. In this research work, the co-inoculation of *P. jessenii* BI7 strain isolated from raised bog environment with *A. lwoffii* BI13 strain isolated from agricultural environment was found to be more efficient on wheat growth than single treatment with either strain.

Bacillus sp., *Azospirillum* sp., *B. megaterium*, *Paenibacillus polymyxa*, and *Raoultella terrigena* bacterial strains isolated from the wheat rhizosphere exhibited stimulatory effects on grain yields (Khalid et al. 2004) and notable increase in uptake of nutrients of grain, leaf, and straw part of the plants (Turan et al. 2010). Wheat plants showed better growth and higher biomass when inoculated with *Azospirillum brasilense*, *Bacillus subtilis*, and *Arthrobacter* sp. in pot experiments and also on field (de Souza et al. 2015). It was observed that certain co-inoculations caused synergy due to improvement of the performance of one bacterial strain by another, so-called helper bacteria (Gopalakrishnan et al. 2015). Bacteria belonging to *Azospirillum* sp., *Azotobacter* sp., *Bacillus* sp., *Pseudomonas* sp., *Serratia* sp., and *Enterobacter* genera were found successfully co-inoculated with *Rhizobium* sp. (Gopalakrishnan et al. 2015). Triple combinations of PGP rhizobacteria based on

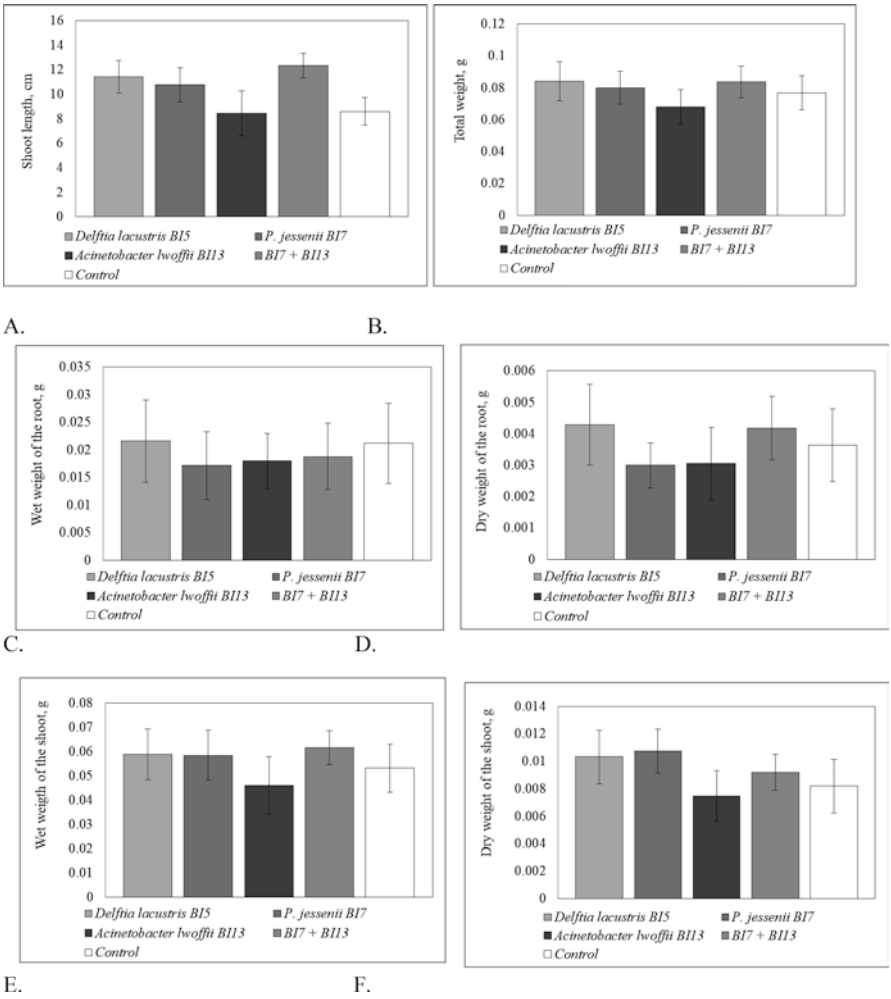

Fig. 1.1 The influence of plant growth-promoting bacteria *D. lacustris* BI5, *P. jessenii* BI7, *A. lacustris* BI13, and *P. jessenii* BI7 + *A. lacustris* BI13 bacterial consortia on wheat plants: (**a**) shoot length (cm); (**b**) total weight (g); (**c**) wet weight of the root (g); (**d**) dry weight of the root (g); (**e**) wet weight of the shoot (g); (**f**) dry weight of the shoot (g)

Bacillus sp., *Stenotrophomonas* sp., and *Acetobacter* sp. (Kumar et al. 2014) and *Serratia* sp., *Microbacterium* sp., and *Enterobacter* sp. strains (Kumar et al. 2017a) promoted plant growth and yield of wheat plant under pot and field experiment. *Bacillus subtilis* and *Arthrobacter* sp. efficiency was higher under co-inoculation conditions (de Souza et al. 2015). A commercial biofertilizer based on a consortium of *Azospirillum* sp., *Azoarcus* sp., and *Azorhizobium* sp. proved to be effective on wheat growth and grain yield (Dal Cortivo et al. 2017). It was previously mentioned that *Pseudomonas jessenii* was more efficient regarding to plant growth of chickpea

in co-inoculation (Valverde et al. 2007). The study is the first report on wheat growth-promoting *Delftia lacustris*, *Pseudomonas jessenii*, and *Acinetobacter lwoffii* strains used either alone or as co-inoculants.

1.7 Conclusion and Future Perspectives

The use of microbial inoculants in agricultural practice is an ecologically advantageous technique that is effective not only for crop production but also maintains soil fertility. The local environmental factors (temperature, humidity, edaphic aspects) have influence on the microbial processes and accordingly the effectiveness of the agriculturally important strains. Therefore it is important to carry out studies in order to isolate and select locally adapted, suitable bacterial strains for agricultural sustainability.

This study shows that there is a high potential in rhizosphere bacteria as plant growth promoters being original either from natural or from agricultural ecosystem. A number of 13 PGP bacterial strains isolated from natural raised bog and agricultural environment, belonging to *Achromobacter*, *Delftia*, *Pseudomonas*, and *Acinetobacter* genera, were characterized based on morphology, physiology, biochemical profiles, and their PGP potential. The most efficient bacterial strains (*D. lacustris* BI5, *P. jessenii* BI7, *A. lwoffii* BI13) were selected and tested on wheat plant growth. The bacterial strains alone either in consortia showed significant increase on wheat shoot growth. Higher efficiency in case of co-inoculation for *P. jessenii* and *A. lwoffii* was observed compared to single inoculation and control. The synergy was observed between a bacterial strain originated from a natural raised bog environment and another from an agricultural area. Due to the novel plant growth-promoting characters, observed in synergy between strains, we consider the abovementioned bacterial strains promising for sustainable agriculture.

Despite the evidence on the effectiveness of PGPB in crop production, their use in agricultural practice needs to be encouraged. Future research is needed for new and more efficient formulations of microbial inoculants even using a multidisciplinary approach.

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2 Rhizospheric Microbiomes: Biodiversity, Mechanisms of Plant Growth Promotion, and Biotechnological Applications for Sustainable Agriculture

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Abstract

Soil consists of diverse microscopic life forms such as actinomycetes, algae, bacteria, fungi, nematodes, and protozoans. But, the rhizospheric region is the most widely colonized regions of the soil due to the secretion of various nutrients by plant roots which attract microbes toward it with bacteria being the dominant one in this region. The bacteria in the rhizospheric region are highly beneficial for the plants as they directly or indirectly stimulate growth of the plants by nitrogen fixation; production of various phytohormones including auxins, cytokinins, and gibberellins; solubilization of phosphorus; production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), siderophores, HCN, ammonia, and various lytic enzymes; and induction of systemic resistance. These plant growth-promoting bacteria of rhizospheric region are referred to as plant growth-promoting rhizobacteria (PGPR). The phyla involving major groups of PGPR include *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* belonging to different genera *Acetobacter*, *Achromobacter*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Exiguobacterium*, *Flavobacterium*, *Gluconacetobacter*, *Herbaspirillum*, *Methylobacterium*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Staphylococcus*. Furthermore, the use of PGPR offers an eco-friendly and an attractive way of replacing the chemical fertilizers, pesticides. In fact, there are many reports on use of rhizobacteria for improving the productivity and also protection of plants against pathogens and pests. In this way, benefits of using PGPR for sustainable agriculture is gaining a greater attention as well as acceptance worldwide, and the progress that has been made to date in using the rhizospheric bacteria with various applications, for agricultural improvement with reference to plant growth-promoting mechanisms, has been summarized and discussed in the present chapter.

Keywords

Abiotic stress · Biodiversity · PGPR · Plant growth promotion · Sustainable agriculture

2.1 Introduction

In the last 10,000 years, human population has increased geometrically ten times from less than ten billion to more than six billion to ten billion soon. Most of the caloric intake making that possible has come from three major crops which include wheat, maize, and rice. Approximately 50% of the human calories are provided by wheat along with maize as well as rice being also a critical food source in the regions which have a rapid population growth including Asia, Africa, and the Middle East. But, the gap between the current global yields of wheat and maize and that achievable through best management practices is large. It is expected that this gap can be reduced by the manipulation of various soil processes especially focusing on those which involve microbial ecology. Developing a predictive understanding between soil biology, agronomy, and crop performance would be the first step so as to improve the yields of the intensive cereals, and it would have a great impact on the global food production.

Globally, the expectations are high that the productivity of the crops could be increased by manipulations of the soil biology through crop management and genetics (Morrissey et al. 2004; Welbaum et al. 2004). One of the worthwhile ways is to look into the rhizospheric region of the soil and exploring those microbes which are residing in rhizospheric region in close proximity of plants and is a justified move so as to attain this target. Rhizosphere is defined as the narrow zone of the soil surrounding the roots. It is known to be one of the largest ecosystems on earth with high energy flux with bacteria being dominant in this region.

The associations of rhizobacteria with the roots of the legumes for the fixation of the nitrogen, crop species rotation for pathogen control, as well as mycorrhizal associations are obvious examples that clearly had productivity benefits. The interactions of rhizobacteria with the plants are dynamic and intricate. Rhizobacteria play different roles; some of them makes the availability of the nutrients to the plants, thereby maintaining the health of the plants such as phosphorus solubilizers which includes *Rhizobium* sp., *Pseudomonas* sp., *Micrococcus* sp., *Flavobacterium* sp., *Erwinia* sp., *Chryseobacterium* sp., *Burkholderia* sp., *Bacillus* sp., *Agrobacterium* sp., *Aerobacter* sp., and *Achromobacter* sp. Some play a vital role in stimulating the plant growth by producing various phytohormones including IAA, cytokinins, and gibberellins or by suppressing pathogens, thereby acting as biocontrol agents. *Pseudomonas* sp. has been reported to be dominant among PGPR which act as the biocontrol agent against different phytopathogenic fungal species including *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Pythium*, *Erwinia*, and *Macrophomina* (Defago et al. 1990; Garbeva et al. 2004; Gupta et al. 2001; Validov et al. 2005; Yadav and Yadav 2018a).

Further, various strains of *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus pasteurii*, *Bacillus pumilus*, *Bacillus sphaericus*, and *Bacillus subtilis* have been known to be elicitors of induced systemic resistance (ISR), thereby reducing the incidence or severity of various diseases on diverse hosts (Kloepper et al. 2004). Using PGPR as biostimulant or bioprotectant is a potent as well as eco-friendly strategy to protect the crops from damage by pathogens as well

as for enhancing the yield and productivity. Even, the application of PGPR in diverse crops has been reported in different studies to approximately increase the yield by 20–40% all over the world (Aeron et al. 2011).

The knowledge about the populations of bacteria which are associated with the roots comes either from the plants that are grown in the pots or simple laboratory conditions. The stimulation of the growth by PGPR is well demonstrated in a number of the cereals, pulses, vegetables, various plantation crops, and even some trees. PGPR are known to enhance the germination percentage, seedling vigor, biomass of the plants, and ultimately the productivity. Majority of PGPR belongs to genera *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Chitinophaga*, *Delftia*, *Dyella*, *Enterobacter*, *Erwinia*, *Exiguobacterium*, *Flavobacterium*, *Klebsiella*, *Methylobacterium*, *Micrococcus*, *Pseudomonas*, *Paenibacillus*, *Pseudomonas*, *Rhodobacter*, *Salmonella*, *Serratia*, *Sphingobium*, and *Staphylococcus* (Chaiharn and Lumyong 2011; Lavania et al. 2006; Verma et al. 2016a, 2016b). PGPR have been formulated, produced, and marketed to be used as bio-inoculant and applied successfully to a wide range of agro-economically important plants including leguminous and non-leguminous crops.

This chapter provides an overview of rhizospheric diversity of bacteria associated with diverse crops and an important role they play in the rhizospheric region with particular reference to the various direct as well as indirect mechanisms used by PGPR for improving the growth of the plants and suppression of the diseases. Further, the chapter also focuses on the applications of PGPR for alleviation of different abiotic stresses.

2.2 Isolation and Characterization of Rhizospheric Bacteria

Microbial diversity has been widely studied by diverse techniques. A number of culture media have been designed for cultivating and isolating diverse groups of microbes. Besides, the traditional methods and molecular techniques such as polymerase chain reaction (PCR) or real-time polymerase chain reaction (RT-PCR), which target specific DNA or RNA, are helping in better way to study the microbial diversity. The PCR products can be used in preparation of the clone libraries which are very helpful in identification and characterization of dominant bacterial genera in the soil or can be used for fingerprinting techniques. Furthermore, amplified rDNA restriction analysis (ARDRA), density gradient gel electrophoresis or temperature gradient gel electrophoresis, and ribosomal intergenic spacer length polymorphism (RISA) (Ranjard and Richaume 2001) are some of other techniques to study microbial diversity. For identification of microbes, genomic DNA can be isolated using Zymo Research Fungal/Bacterial DNA MicroPrep™ following the standard protocol prescribed by the manufacturer. Different primers can be used for amplification of 16S rRNA gene for archaea and bacteria while 18S rRNA gene for fungi. PCR amplified 16S/18S rRNA genes have to be purified and sequenced. The partial 16S or 18S rRNA gene sequences should be compared with sequences available in the NCBI database. The phylogenetic tree can be constructed on aligned data sets using the neighbor-joining (NJ) method and the program MEGA 4.0.2.

To know the plant growth-promoting capability and other agricultural and biotechnological applications of plant growth promoting microbes, standard methods could be used for screening for PGP attributes including the production of plant growth regulators including indole-3-acetic acid (Bric et al. 1991), gibberellic acid (Brown and Burlingham 1968), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Jacobson et al. 1994); solubilization of phosphorus (Pikovskaya 1948), potassium (Hu et al. 2006), and zinc (Fasim et al. 2002); production of ammonia (Cappucino and Sherman 1992), HCN (Bakker and Schippers 1987), and Fe-chelating compounds (Schwyn and Neilands 1987); hydrolytic enzymes production (Yadav et al. 2016a); and biocontrol against different microbial pathogens (Sijam and Dikin 2005) as well as for the production of secondary metabolites.

2.3 Diversity and Distribution of Microbes Associated with Different Crops

Recently, the rhizobacteria and their association with the crops and their proliferation of the rhizosphere are proving to very effective for most of the cereals. Some rhizobacteria possess capability to improve plant growth or influence root health by solubilizing phosphorus, producing phytohormones, or acting as biological control agents, while some are symbiotic plant colonizers which fix the atmospheric nitrogen though some free-living have also been known for nitrogen fixation. The interaction and diversity of PGPR with different crops certainly depend on environmental conditions. The diverse groups of microbes that have been reported from rhizospheric microbiomes include phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Fig. 2.1). Overall the distribution of microbes varied in all bacterial phyla; *Proteobacteria* has been most dominant followed by *Firmicutes*. The least number of microbes was reported from phyla *Bacteroidetes* and *Actinobacteria* (Fig. 2.2). On review on different research on diversity of rhizospheric microbiomes, it can be concluded that *Pseudomonas* was the most dominant genus followed by *Bacillus*, *Pantoea*, *Methylobacterium*, *Azotobacter*, *Paenibacillus*, *Enterobacter*, *Azospirillum*, *Staphylococcus*, *Sphingobium*, *Serratia*, *Rhizobium*, *Klebsiella*, *Flavobacterium*, *Exiguobacterium*, *Rhodobacter*, *Herbaspirillum*, *Gluconacetobacter*, *Erwinia*, *Burkholderia*, *Azomonas*, *Arthrobacter*, *Ochrobactrum*, *Kocuria*, *Dyella*, *Duganella*, *Dietzia*, *Delftia*, *Cronobacter*, and *Xanthomonas* (Fig. 2.2).

On review of different crops chickpea, maize, rice, soybean, sugarcane and wheat, it was found that rhizospheric microbes were most predominant and microbes belonged to different phylum. Along with common and predominant microbes, many host-specific rhizospheric microbes have been reported, i.e., *Duganella*, *Planococcus*, *Planomicrobium*, *Rhodobacter*, *Salmonella*, *Sporosarcina*, and *Achromobacter* from wheat; *Arenimonas*, *Beijerinckia*, *Bradyrhizobium*, *Chitinophaga*, *Dyella*, *Erwinia*, *Lysobacter*, *Massilia*, *Methylocella*, *Methylocystis*, *Myroides*, *Ohtaekwangia*, *Proteus*, *Ralstonia*, *Rhodovulum*, and *Variovorax* from maize; *Brevibacterium*, *Chryseobacterium*, *Ochrobactrum*, and *Phosphobacteria*

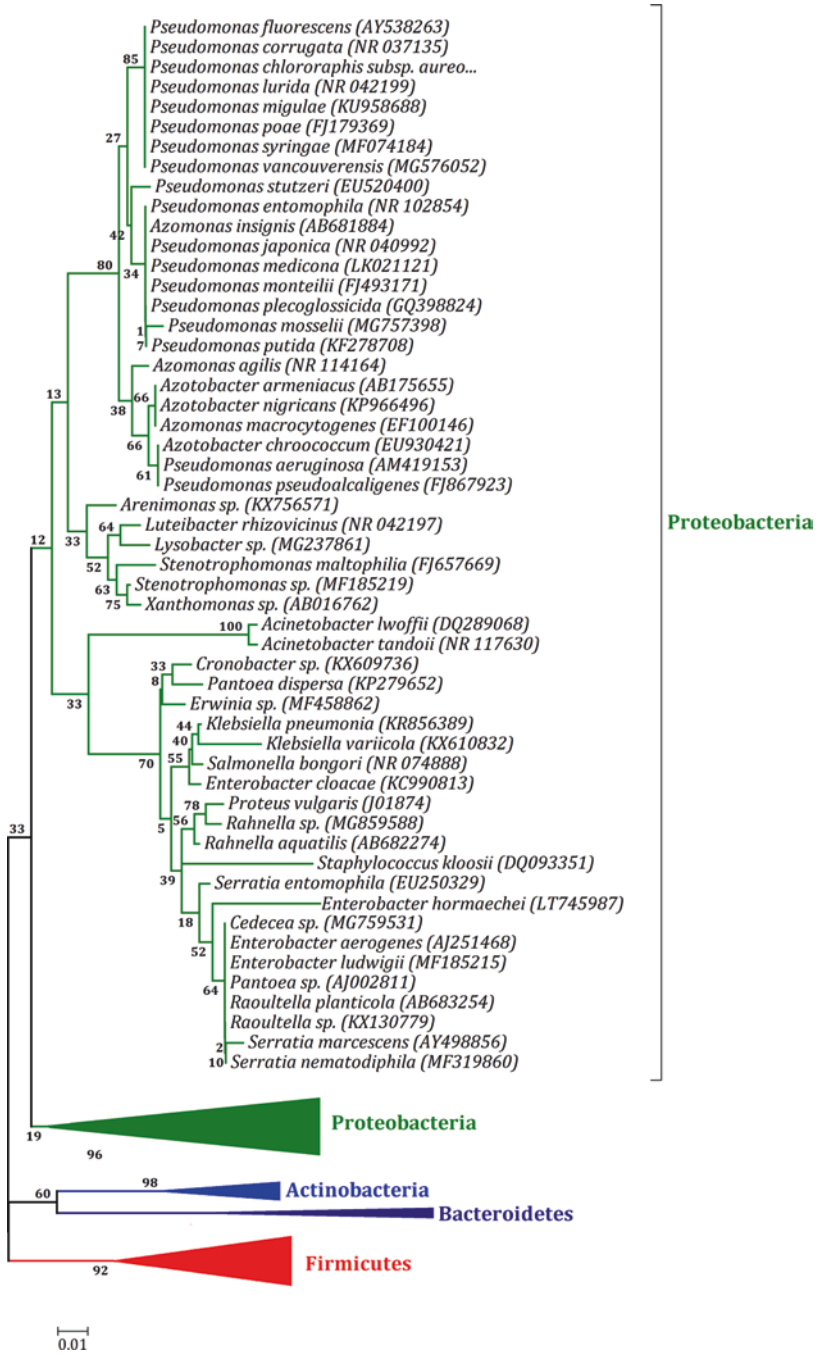
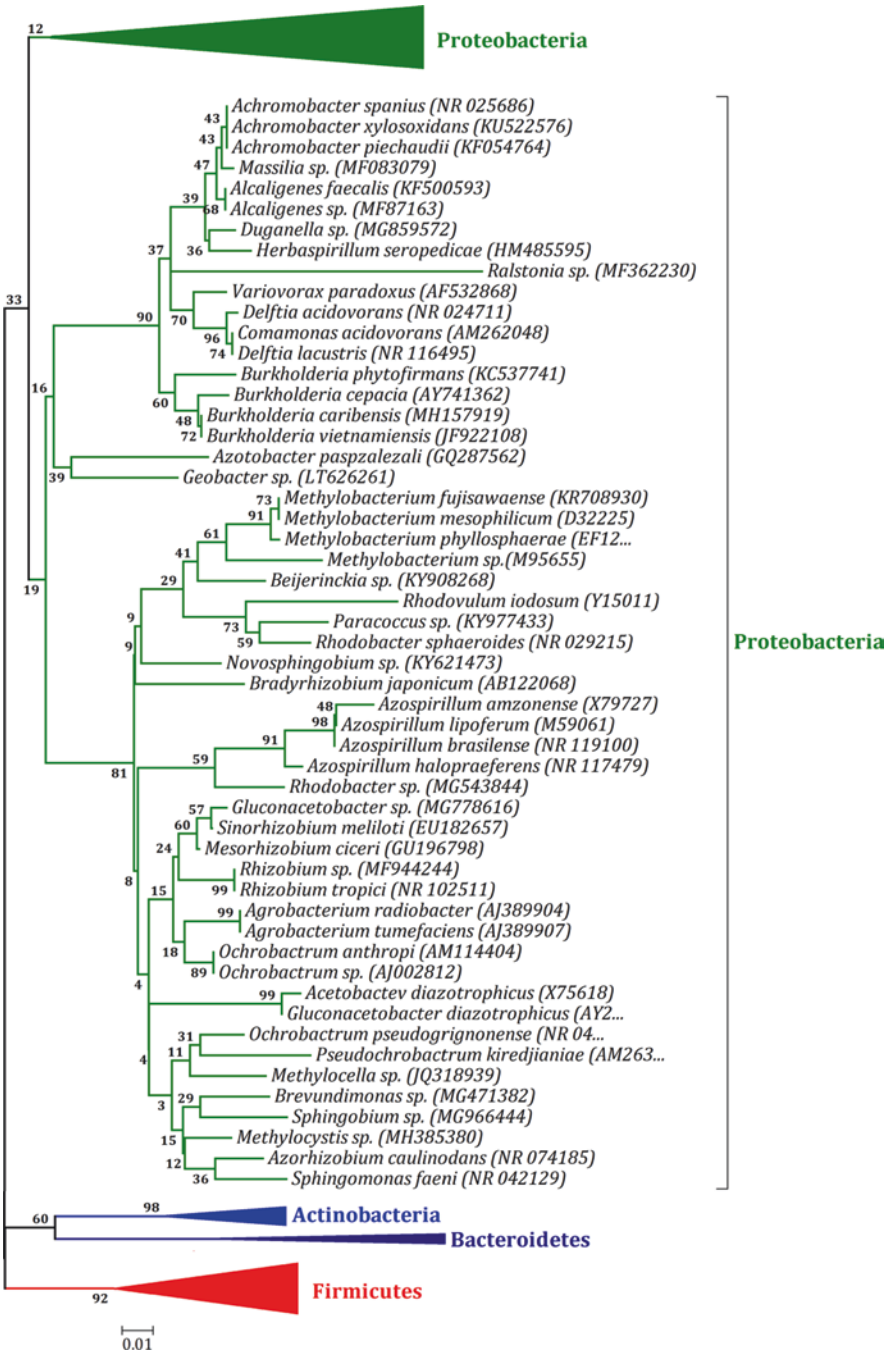


Fig. 2.1 Phylogenetic tree showed the relationship among different groups of microbes isolated from diverse sources worldwide



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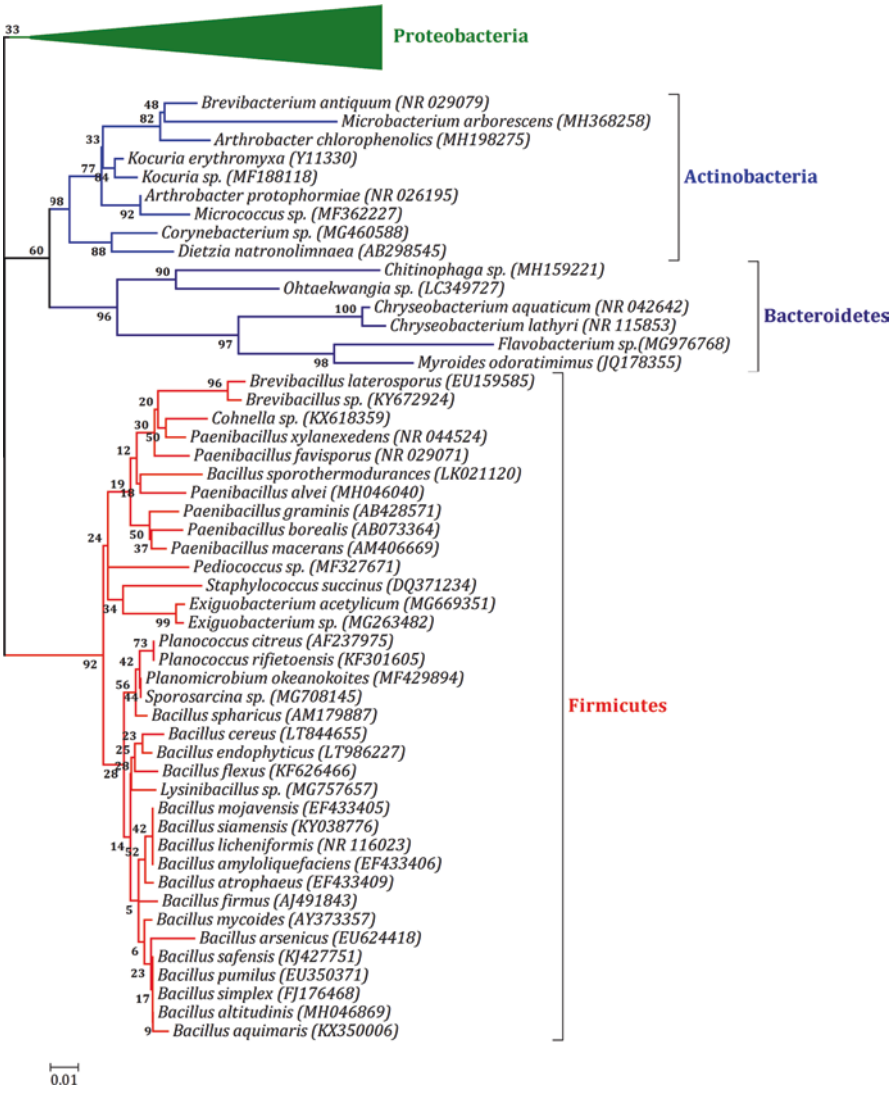


Fig. 2.1 (continued)

from rice; *Brevibacillus*, *Gluconacetobacter*, *Novosphingobium*, and *Pediococcus* from sugarcane; *Rahnella* and *Raoultella* from soybean; and *Brevundimonas* and *Corynebacterium* from chickpea (Fig. 2.2c). There are many reports on niche-specific microbiomes from different habitats, e.g., from cold habitats (Yadav et al. 2017d), hot springs (Kumar et al. 2014; Sahay et al. 2017), saline (Yadav et al. 2015c), drought (Verma et al. 2014, 2016b), host-specific plant microbiomes (Verma et al. 2016a; Yadav and Yadav 2018b), and soil specific (Biswas et al. 2018)

2.3.1 Wheat (*Triticum aestivum*)

Wheat is one of the most important crops grown around the world and one of the widely consumed crops by human population. Wheat is used to make a variety of foods including pasta, breakfast cereal, noodles, cakes, bread, etc. Recent research indicates that wheat is rich in antioxidants which are contained in the grain seeds that contribute to suppressing free radical damage and protect humans from chronic diseases such as cancer. The species of wheat can be classified into many different groups, such as hard, soft spring, or winter wheat, completely depending on seed quality, color, and pattern of growth. It is grown in about 100 countries throughout the world. In India, the production of wheat is expected to increase by ~4% every year. It basically grows in the temperate climate and is a

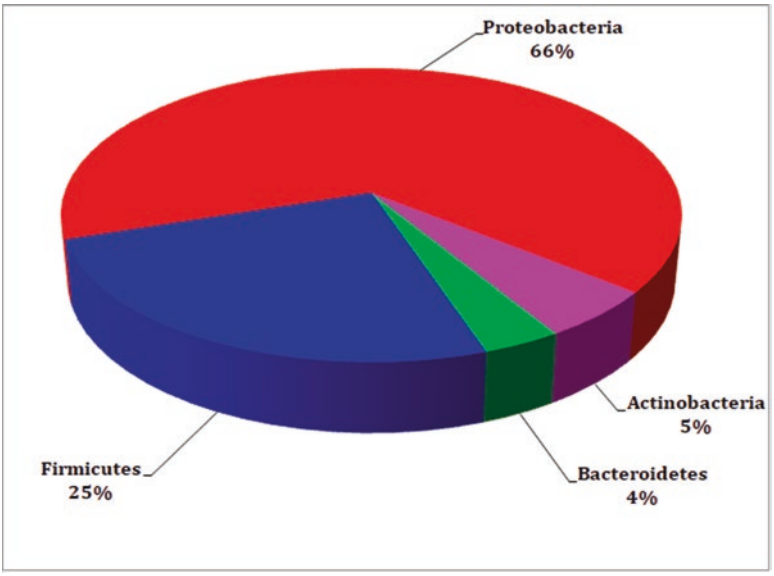
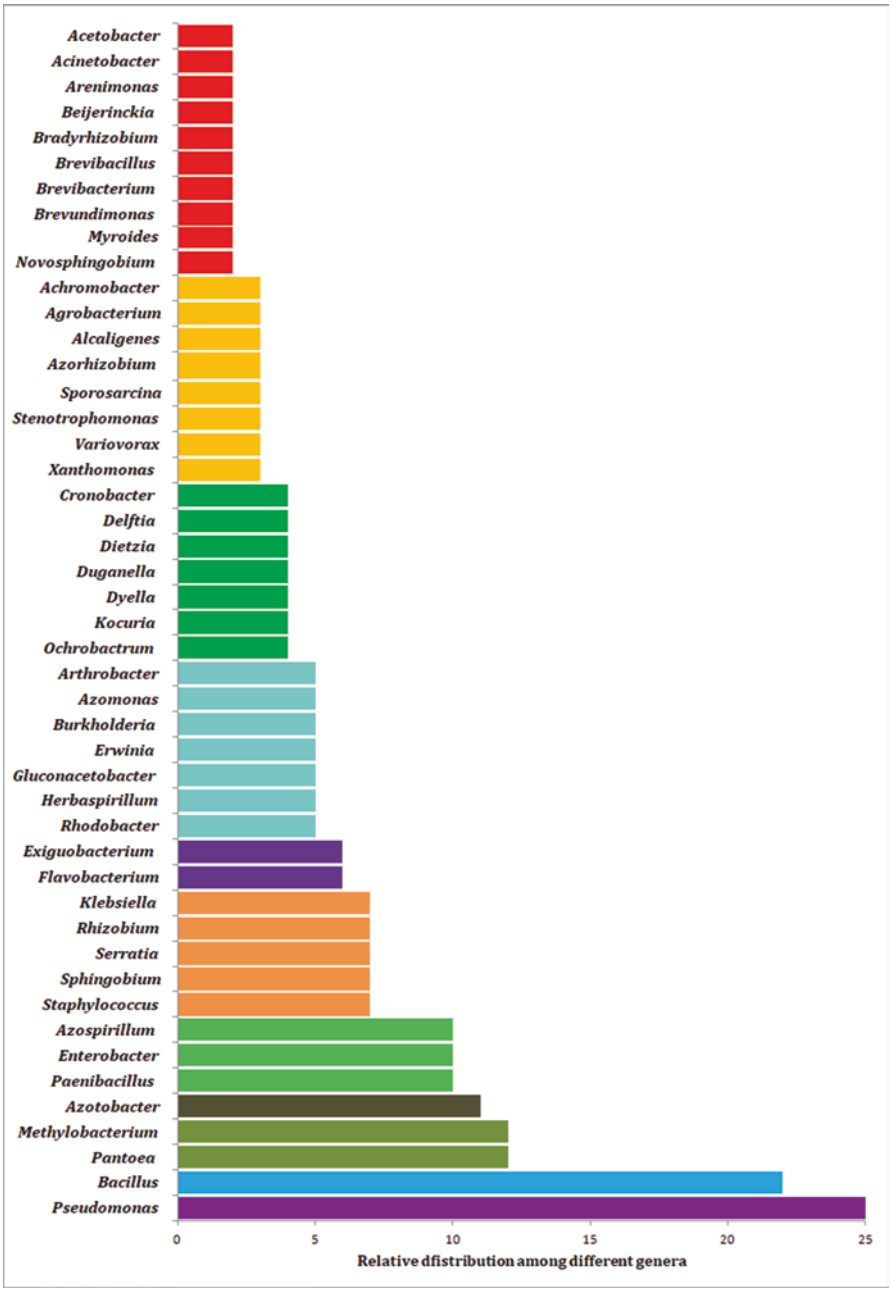


Fig. 2.2 (**a–c**) Abundance, diversity, and richness of microbiomes belonging to diverse phyla and genera isolated from diverse sources worldwide.
Wheat (Beneduzi et al. 2008; Joshi and Bhatt 2011; Majeed et al. 2015; Velazquez-Sepulveda et al. 2012; Verma et al. 2014, 2016a, 2016b); **Maize** (Baldani and Baldani 2005; Chauhan et al. 2011; Da et al. 2008; García-Salamanca et al. 2013; Li et al. 2014; Pereira et al. 2011; Roesch et al. 2008; Taiwo et al. 2017; Vardharajula et al. 2011); **Rice** (Arjun and Harikrishnan 2011; Bal et al. 2013; Gandhi Pragash et al. 2009; Gopalakrishnan et al. 2011; Hingole and Pathak 2016; Joshi et al. 2011; Rameshkumar et al. 2014; Samuel and Muthukkaruppan 2011; Sarkar et al. 2018a; Shrivastava 2013; Tripathi et al. 2002); **Sugarcane** (Beneduzi et al. 2013; Lamizadeh et al. 2016; Pisa et al. 2011; Rameshkumar et al. 2014; Ratón et al. 2012); **Soybean** (Jain et al. 2016; Sibponkrung et al. 2017; Sugiyama et al. 2014; Wahyudi et al. 2011); **Chickpea** (Belimov et al. 2001; Dubey et al. 2013; Kaur and Sharma 2013)



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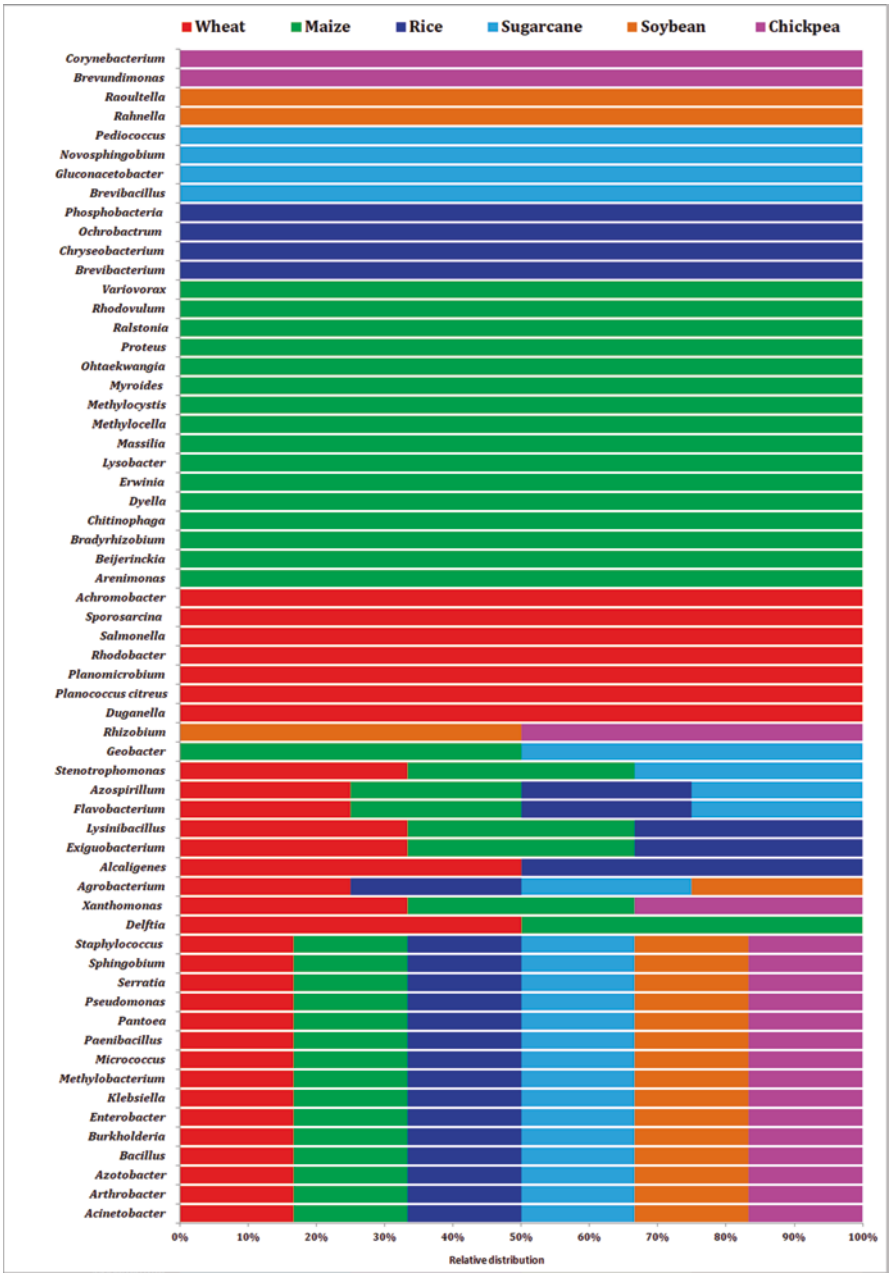

Fig. 2.2 (continued)

staple food for about 35% of the human population. To meet the demand and to provide the food security to the growing population, greater agricultural production is a pressing need in the twenty-first century. In the developing countries, to increase the productivity of the wheat, various chemicals are used for the elimination of the diseases caused by the different pathogens, but the use of chemicals causes damage to the environment as well as to the human health (He et al. 2005).

In this regard, rhizospheric microbial communities play an important role in limiting or inhibiting the growth of the pathogens. Furthermore, studies also show that PGPR can reduce the use of the chemicals in the crops (Adesemoye et al. 2009). The microbial community in the root zone is dependent on certain factors such as the age of the plant, plant species, root type, soil type as well as the other selection pressures. A number of bacterial species belonging to genera *Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Methylobacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia* have been recovered from the rhizosphere of wheat (Rana et al. 2018; Verma et al. 2017b; Yadav et al. 2018b).

Germida and Siciliano (2001) analyzed the rhizospheric diversity of wheat by using fatty acid methyl esterified method and found *Pseudomonas*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Xanthomonas*, *Agrobacterium*, and *Enterobacter* to be the predominant genera. Beneduzi et al. (2008) studied genetic as well as phenotypic diversity of bacilli isolated from rhizospheric and bulk soil of wheat fields in Southern Brazil. In the study, 311 putative nitrogen-fixing bacilli were isolated, and strains belonging to numerous species were grouped into 40 different nif H-RFLP-PCR profiles. The genus *Paenibacillus* was found to be the most prominent group in both the rhizospheric soil (77.8%) and bulk soil (79%). In a study of Verma et al. (2014), rhizospheric bacteria associated with wheat from central zone of India belonged to genera, namely, *Acinetobacter*, *Bacillus*, *Duganella*, *Exiguobacterium*, *Kocuria*, *Lysinibacillus*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas*. In another study of Verma et al. (2016a), the molecular diversity from rhizosphere of wheat from six agroecological zones of India was done in which bacterial communities including *Bacillus endophyticus*, *Paenibacillus xylanexedens*, *Planococcus citreus*, *Planomicrobium okeanokoites*, *Sporosarcina* sp., and *Staphylococcus succinus* were reported for the first time.

2.3.2 Maize (*Zea mays*)

Maize is one of the most important crops with great value to humanity. It is utilized in human diet in its both fresh and the processed forms and further also finds applications in the production of ethanol, industrial starches, and even oils. In Asia, Latin America, and Africa, maize is the only source of proteinaceous diet particularly in weaning of food for babies. The total production of the maize around the world ranges up to about 58 million tons with the main producer being Brazil, China, India, Mexico, and the United States.

The rhizospheric microbial community of maize has been thoroughly investigated (Aira et al. 2010; Castellanos et al. 2009; Chelius and Triplett 2001; Dohrmann et al. 2013). Different studies have been carried out which clearly reveal that there are specific microbial communities in association with the maize depending on the properties of the soil (Castellanos et al. 2009), genotypes, crop management such as the use of fertilizers (Aira et al. 2010), and stages of its growth (Cavaglieri et al. 2009). The study of Gomes et al. (2001) suggested that the *Arthrobacter* sp. decreases as the age of the plant increases. On the other hand, there are reports which suggest that the composition of the microbial community of maize is completely independent of the cultivar (Dohrmann et al. 2013; Schmalenberger and Tebbe 2002), stage of growth (Gomes et al. 2002), and genotype (Schmalenberger and Tebbe 2002). It is suggested that such discrepancy in the microbial responses in the rhizosphere may be due to various reasons such as the differences in the plant species and soil types or it can also be due to the different methodologies used. Furthermore, some studies suggest that stage of plant may be another reason influencing root physiology which in turn may affect the quality as well as the quantity of the root exudates ultimately exerting a selection on the rhizospheric microbial community (Dunfield and Germida 2003; Houlden et al. 2008), while some studies suggest that seasonal variations that affect activity as well as relative abundance of the bacterial communities in the rhizosphere are completely plant dependent (Dunfield and Germida 2003; Houlden et al. 2008; Mougel et al. 2006; Smalla et al. 2001). Cavaglieri et al. (2009) pointed out that microbial communities in maize plant show structural alteration over time in maize plant.

The taxonomic affiliation of the bacteria associated with maize reveals that there is a high dominance of *Actinobacteria* and *Proteobacteria* (Chelius and Triplett 2001; Roesch et al. 2008). Da et al. (2008) studied the genetic diversity of the *Paenibacillus polymyxa* populations from the rhizosphere of four cultivars of maize. On the basis of biochemical tests, 67 isolates were identified as *Paenibacillus polymyxa* which were further analyzed for DNA polymorphism, and also the amplification of repetitive DNA by sequencing methods and result demonstrated that 54 genotypic groups showed a high level of genetic polymorphism among strains of *Paenibacillus polymyxa*. Roesch et al. (2008) studied diversity of diazotrophic bacteria within rhizosphere soils, roots, and stems of field-grown maize. α-Proteobacteria and β-proteobacteria were most abundant in the rhizospheric soil and stem samples, and γ-proteobacteria dominated rhizospheric soil samples. The study revealed rhizospheric soil samples to possess greater diversity of diazotrophic bacteria. *Azospirillum* and *Azotobacter* were found in almost all samples at an abundance. *Beijerinckia* sp., *Delftia* sp., *Geobacter* sp., *Gluconacetobacter* sp., *Methylobacterium* sp., *Methylocella* sp., *Methylocystis* sp., and *Rhodovulum* sp. were mainly restricted to rhizospheric soil. Da et al. (2008) reported that the bacterial community associated with maize harbors multiple orders including *Actinomycetales*, *Burkholderiales*, *Clostridiales*, *Rhizobiales*, *Rubrobacteriales*, and *Xanthomonadales*. The members of the genera *Azospirillum*, *Herbaspirillum*, and *Burkholderia* and other free-living bacteria are diazotrophs also associated with maize (Baldani and Baldani 2005; Roesch et al. 2008).

Chauhan et al. (2011) evaluated the diversity of bacteria from rhizospheric region of maize using culture-independent method. *Proteobacteria* and *Actinobacteria* were found to be the second most dominating group in clone library. García-Salamanca et al. (2013) studied the bacterial diversity from the rhizospheric region of maize and the surrounding carbonate-rich bulk soil. *Pseudomonas* and *Lysobacter* were found to be the predominant genera in the rhizospheric region. In a study of Li et al. (2014), diversity of bacteria in the rhizosphere of maize cultivar was studied, and the dominant genera found included *Burkholderia*, *Chitinophaga*, *Dyella*, *Massilia*, *Ralstonia*, and *Sphingobium*, and the study also suggested that the rhizospheric bacterial community structures considerably changed through different stages of growth. *Arenimonas*, *Flavobacterium*, *Massilia*, and *Ohtaekwangia* were relatively abundant at early growth stages, while genera *Bradyrhizobium*, *Burkholderia*, *Chitinophaga*, *Dyella*, *Ralstonia*, *Sphingobium*, and *Variovorax* were dominant at later stages.

2.3.3 Rice (*Oryza sativa*)

Rice is another major food crop consumed by nearly half of the world's population. It is one of the nutritious crops for humans and caloric intake providing near about one-fifth of the calories consumed worldwide by the humans (Center 2010). Rice can be grown in different environments depending on the availability of the water (Maclean et al. 2002). Further, nutrient requirement is very high with nitrogen being most essential for their growth, development, and grain production. Rice crops remove around 16–17 kg nitrogen for the production of each ton of rough rice including straw. But, most of the soils around the world for growing rice are deficient in nitrogen and nitrogen fertilizers so as to meet a rice crop's nitrogen demand. Urea is most commonly applied as the N source for production of rice. But the efficiency of added urea-N is generally very low, often only 30–40%, and in some cases even lower. This low N use efficiency is mainly attributed to denitrification, NH_3 volatilization, and leaching losses (Choudhury and Kennedy 2005).

The aerobic bacteria are mostly associated with the upland rice for the fixation of the nitrogen, whereas in wetland cultures both the aerobic and the anaerobic bacteria fix the atmospheric nitrogen. Aerobic bacteria such as the *Azotobacter* live in the oxygenated rhizosphere of the rice plant and fix atmospheric N, and anaerobic bacteria, *Clostridium*, live in the reduced layer of the soil and fix atmospheric N. Further, the wetland ecosystem is a favorable habitat for the aquatic biota such as blue green algae and *Azolla*. *Azolla* in symbiotic association with *Anabaena* fix a substantial amount of nitrogen.

Tripathi et al. (2002) studied the diversity of salt-tolerant rhizobacteria associated with rice, and the isolates were identified as *Pseudomonas aeruginosa*, *Serratia marcescens*, *Alcaligenes xylosoxidans*, and *Ochrobactrum anthropi*. Gopalakrishnan et al. (2011) isolated *Pseudomonas plecoglossicida*, *Brevibacterium antiquum*, *Bacillus altitudinis*, *Enterobacter ludwigii*, *Acinetobacter tandoii*, and *Pseudomonas monteilii* from rice rhizosphere. In a study of Samuel and Muthukkaruppan (2011),

the diversity of rhizospheric bacteria associated with the rice were *Azotobacter* sp., *Azospirillum* sp., *Bacillus* sp., *Phosphobacteria* sp., and *Pseudomonas* sp. Arjun and Harikrishnan (2011) did the metagenomic analysis of the bacterial diversity in the rhizosphere of the rice; the bacterial taxa associated were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Acidobacteria.* Joshi et al. (2011) recovered 335 isolates from rhizosphere of irrigated and rainfed rice plants to study bacterial diversity. Approximately 27% of the isolates were *Bacillus* sp. which were followed by 26% *Pseudomonas* sp. which was followed by *Azotobacter* sp. (5%), *Flavobacterium* sp. (7%), *Serratia* sp. (4%), and *Klebsiella* sp. (6%). The other genera observed in low frequency included *Enterobacter* sp., *Micrococcus* sp., and *Staphylococcus* sp. Shrivastava (2013) isolated *Pseudomonas*, *Klebsiella*, *Azotobacter*, *and Agrobacterium* from rhizospheric region of rice from Nepal. *Pseudomonas plecoglossicida*, *Pseudomonas monteilii*, *Pseudomonas mosselii*, *Pseudomonas libanensis*, and *Pseudomonas aeruginosa* have been isolated from rhizosphere of rice grown in Sholavandan by Rameshkumar et al. (2014).

2.3.4 Sugarcane (*Saccharum officinarum*)

Grasses comprise of an important source of food all over the world. Particularly, grasses, including wheat, maize, rice, sorghum, and sugarcane, currently have much of their nitrogen needs being fulfilled by costly mineral fertilizers. Sugarcane is a semi-perennial grass which belongs to the Poaceae family and is extremely adapted to the tropical climate. It is one of the important crops grown around the world with Brazil being the leading producer. Even it was the first economically important crop which has been grown in Brazil since the sixteenth century and still plays a central role in economy of Brazil. In Brazil, the production of sugarcane is the chief source of employment simultaneously being an inducer of scientific development. In 2009, the annual production of sugarcane was more than 500 million tons in Brazil (http://www.unica.com.br/). In the last few years, culture of sugarcane is gaining attention in countries such as Brazil, the United States, and China because of bioethanol production (Qiu et al. 2010; Walter et al. 2008). In Khuzestan, culture of sugarcane has a major role in providing sugar, and the industry developed in this region is the producer of more than half of the country's sugar.

Microorganisms which are associated with sugarcane play an essential role in maintaining fertility of soil as well as health of plant. Many of these associated microbes can act as biofertilizers increasing the competence of absorption of nutrients by the plants and producing various plant growth-promoting substances additionally also increasing tolerance to various abiotic and biotic stresses. So, a basic understanding of microbial communities associated with sugarcane is very important for the instant application.

Pisa et al. (2011) studied the diversity of bacteria from rhizospheric soil of sugarcane at different times and under different nitrogen fertilization conditions. The predominating phylum was *Proteobacteria* (29.6%), which was followed by *Acidobacteria* (23.4%), *Bacteroidetes* (12.1%), *Firmicutes* (10.2%), and

Actinobacteria (5.6%). Ratón et al. (2012) isolated and characterized aerobic endospore-forming bacilli from rhizospheric region of sugarcane and further selected the strains with agriculture potentialities. A total of 18 strains were isolated on N-free medium. On the basis of phenotype and analysis of the 5′ end hypervariable region sequences of 16S rRNA, seven strains belonging to *Bacillus* (*Bacillaceae* family), four belonging to *Paenibacillus*, six belonging to *Brevibacillus*, and one strain of *Cohnella* belonging to *Paenibacillaceae* family were identified. Lamizadeh et al. (2016) isolated and identified plant growth-promoting rhizobacteria from the rhizosphere region of sugarcane in saline and non-saline soil. The isolates identified from saline soil included *Bacillus* sp., *Corynebacterium* sp., *Enterobacter* sp., *Micrococcus* sp., *Paenibacillus* sp., *Pediococcus* sp., and *Pseudomonas* sp., whereas *Arthrobacter* sp., *Bacillus* sp., *Paenibacillus* sp., and *Pseudomonas* sp. were identified from non-saline soils.

2.3.5 Soybean (*Glycine max*)

Soybean occupies an important place among different crop ecosystems. It is consumed by humans as it is a rich source of protein. It is a subtropical legume requiring a temperature of 25–30 °C for optimum growth, nodulation, and fixation of the nitrogen. Suboptimal root zone temperature below 25 °C can badly affect the growth of soybean. Further, it is also prone to many diseases among which the major ones include collar rot, charcoal rot, bacterial pustule, anthracnose, powdery mildew, etc. Diversity of rhizobacteria with different plant growth-promoting attributes associated with soybean has been studied.

Wahyudi et al. (2011) isolated *Bacillus* sp. from the rhizospheric region of the soybean and also studied the plant growth-promoting traits of the isolates. A total of 118 *Bacillus* sp. were isolated, and among 118 isolates 90, 12, and 11 produced phytohormones, siderophores, and solubilized phosphorus, respectively. Three isolates inhibited the growth of *Fusarium oxysporum*, nine inhibited the growth of *Rhizoctonia solani*, and one inhibited the growth of *Sclerotium rolfsii*. Sugiyama et al. (2014) studied the changes in the rhizobacterial community associated with soybean with growth. The physiological properties were studied by a community-level substrate utilization assay with BioLog Eco plates, whereas the composition was studied by gene pyrosequencing. By pyrosequencing, it was demonstrated that in the rhizospheric region, *Proteobacteria* increased from vegetative to maturity stage, while *Acidobacteria* and *Firmicutes* showed a decrease in rhizospheric soil during growth. Analysis of operational taxonomic units revealed that the bacterial communities in the rhizospheric region showed a considerable change with *Bacillus*, *Bradyrhizobium*, and *Rhizobium* being abundant plant growth-promoting rhizobacteria. Jain et al. (2016) isolated *Bacillus* sp. associated with rhizosphere of soybean as well as studied the plant-promoting attributes. Among ten isolates, nine solubilized phosphorus, five produced IAA, and three showed nitrogen-fixing capability. The bacterial strain was also used as inoculant for soybean, and the results indicated enhancement of shoot and root length, as well as the shoot and root biomass.

2.3.6 Chickpea (*Cicer arietinum*)

Chickpea is the major legume crops belonging to the family Leguminosae which is grown widely in tropical, subtropical, and temperate regions of the world. It is an important source of the dietary protein consumed by different preparations as supplementary food. Even though it has high nutritional quality, it also maintains the fertility of the soil through its symbiotic nitrogen fixation in association with *Mesorhizobium* species. It is also among the major export commodities with significant export market option among the field crops. Joseph et al. (2012) isolated *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Rhizobium* from rhizosphere of chickpea and characterized them for different plant growth-promoting attributes. All the isolates of *Bacillus*, *Pseudomonas*, and *Azotobacter* showed IAA-producing capability, whereas 85.7% of *Rhizobium* produced IAA. 95% of *Bacillus* sp. followed by 94.2% of *Pseudomonas* sp. and 74.2% of *Rhizobium* and 45% of *Azotobacter* produced ammonia, and all isolates were catalase positive. Kaur and Sharma (2013) characterized *Pseudomonas* sp. on the basis of morphological and biochemical characteristics from the rhizosphere of chickpea and screened for multiple plant growth-promoting activities including IAA production, P-solubilization, and production of ammonia, HCN, and siderophores, as well as studied the antibiotic resistance spectra. IAA production was in the range of 66.79 µg/ml to 70.05 µg/ml, 70% isolates solubilized phosphorus, and two among them produced ammonia, HCN, and siderophores. 70% of the isolates showed resistance to ampicillin. Further two isolates improved the seed germination in the two varieties of the chickpea.

2.4 Biotechnological Agricultural Applications of PGP Microbes for Alleviation of Abiotic Stress in Plants

Further, the researches on the microbial diversity of the rhizospheric region have been divided into different areas of interest. Some studies have been conducted for the determining and classifying rhizospheric communities; some researchers have focused on the outcomes of inoculating the plants with PGPR. Orhan et al. (2006) studied the effects of two plant growth-promoting *Bacillus* strains, one capable of fixing nitrogen (OSU-142) and another one possessing capability to fix atmospheric nitrogen as well solubilizing phosphorus (M3) alone as well as in combination on organically grown primocane-fruiting raspberry. The results demonstrated the increase in the shoot length and crop yield and improvement of the fruit quality. Son et al. (2006) studied the effect of inoculating soybean in rotational system with *Bradyrhizobium japonicum* and phosphate-solubilizing bacteria, *Pseudomonas* sp. The results demonstrated that application of *Bradyrhizobium japonicum* and *Pseudomonas* sp. can increase the number of nodules, dry weight of nodules, yield of grains, yield components, nutrient availability in soil, and also uptake by soybean crop. El-Azeem et al. (2007) inoculated faba bean with different strains of PGPR in a greenhouse experiment. The study observed increase in the biomass straw, seeds, and total yields, respectively.

Shaharoona et al. (2008) studied the effect of inoculating wheat with *Pseudomonas fluorescens*; the increase in the root weight, number of tillers, grain yield, and straw yield was observed. Akhtar et al. (2009) observed the increase in growth and yield of wheat when PGPR and compost in mixture with chemical fertilizer were used. Gholami et al. (2009) inoculated maize with *Azospirillum brasilense*, *Azospirillum lipoferum*, *Pseudomonas fluorescens*, and *Pseudomonas putida*. The results revealed increase in seed germination, seedling vigor of maize, leaf and shoot dry weight, leaf surface area, plant height, seed weight, number of seed per year, and leaf area. Hassen and Labuschagne (2010) found increase in the plant shoot weights, root weights, yield, cane length, number of clusters per cane, and number of berries per cane in wheat when inoculated with *Bacillus cereus*, *Bacillus megaterium*, *Bacillus simplex*, and *Paenibacillus alvei* singly as well as in combination.

In a study of Abbasi et al. (2011), a significant increase in all the studied parameters was observed in PGPR-inoculated wheat plants. Further, combination of the nitrogen and PGPR increased the yield and nutrition in the treated plants. Rokhzadi and Toashih (2011) studied the effects of *Azospirillum*, *Azotobacter*, *Mesorhizobium*, and *Pseudomonas* singly as well as in consortium on the uptake of the nutrient, the growth, as well as the yield of the chickpea under field conditions. The maximum dry weight of root nodules as well as enhanced phosphorus concentration was observed in consortium. Each inoculation statistically increased the grain yield, biomass dry weight, and nitrogen and phosphorus uptake of grains as compared to the control plants. Rafi et al. (2017) inoculated foxtail millet with *Azospirillum lipoferum* and PSB alone as well as in combination, and a noteworthy increase in the plant height, root and shoot dry weight, and panicle and seed weight was observed.

Nagaraja et al. (2016) investigated antifungal efficiency of *Azotobacter nigricans* on *Fusarium* infection, total seedlings mass, root and shoot length, and seed germination on maize, sorghum, and wheat. The results demonstrated reduction in growth of *Fusarium equiseti*, *Fusarium graminearum*, *Fusarium poae*, and *Fusarium sporotrichioides* and up to 50% decrease in incidence of *Fusarium* infection in all the three cereals under treatment. Further, a twofold increase in the total mass of the maize seedlings was also observed. The highest vigor index was recorded as 11,616.7, 1321, and 1584.8 in sorghum, maize, and wheat against *Fusarium acuminatum*, *Fusarium crookwellense*, and *Fusarium sporotrichioides*, respectively. The germination incidence was 67%, 64%, and 56% in sorghum, maize, and wheat, respectively. Kumar et al. (2017a) inoculated wheat with phosphate-solubilizing and nitrogen-fixing rhizobacteria including *Serratia marcescens*, *Microbacterium arborescens*, and *Enterobacter* sp. alone as well as in combination to study their effect on growth promotion, yield, and nutrient uptake. Co-inoculation of three rhizobacteria showed best results in all the studied parameters.

The rhizospheric microbiomes may be used for mitigation of abiotic stress in plants such as high/low temperatures, alkaline/acidic, and drought and saline environments. There are many reports on plant growth promotion by rhizospheric microbiomes with multifarious PGP attributes under the normal as well as under the abiotic stress condition, e.g., for alkalinity (Rajput et al. 2013; Srivastava et al.

2013), drought (Verma et al. 2016b), saline environments (Saxena et al. 2016), high temperature (Ali et al. 2011; Verma et al. 2016b), low temperature (Yadav et al. 2017e), and drought (Kour et al. 2017b; Yadav and Yadav 2018a) (Table 2.1). There are vast numbers of reports of rhizospheric microbiomes for plant growth, crop yield, and mitigation of abiotic stress using single inoculums (Table 2.2) or by microbial consortium (Table 2.3).

2.5 Mechanisms of Plant Growth Promotion

Rhizosphere is basically the narrow zone surrounded and influenced by plant roots and is a hot spot for many organisms. It is one of the most complex ecosystems on the earth (Hinsinger et al. 2009; Hinsinger and Marschner 2006; Pierret et al. 2007; Saxena et al. 2016). Numbers of organisms are found in the rhizosphere including bacteria, fungi, oomycetes, protozoa, algae, viruses, and archaea (Biswas et al. 2018; Gaba et al. 2017; Kumar et al. 2017c; Suman et al. 2016a; Yadav 2009; Yadav et al. 2015c). The microbes play an important role in nitrogen, sulfur, and phosphorus cycling, further also making contribution to the stabilization of the soil structure, accumulation of organic residue, fixation of nitrogen, and removal of toxins. Further, they also contribute in maintaining the health of the crops. Additionally, they promote plant growth and also protect plants from the attack of the pathogens by different mechanisms such as biofertilization, stimulation of the root growth, rhizoremediation, control of the abiotic stress, and disease control. They are regarded as the most sensitive biological indicators for monitoring the soil quality changes (Niemi et al. 2001).

Though a variety of organisms are found in the rhizosphere, most studies on the rhizospheric microbiology especially those describing cooperative microbial interactions have focused mainly on bacteria and fungi. The prokaryotic bacteria and eukaryotic fungi have different trophic habitats, and a variety of non-symbiotic as well as symbiotic relationships both detrimental (pathogenic) and beneficial (mutualistic) have been described. Rhizospheric soil is well known to host a variety of plant growth-promoting rhizobacteria (PGPR). The range of rhizobacteria which have been reported to increase growth of the plants and also control various pathogens of plants includes *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Mesorhizobium*, *Pseudomonas*, *Rhodococcus*, *Serratia*, and *Streptomyces* (Yadav et al. 2018a, 2018b) (Verma et al. 2017a). PGPR may be defined as the rhizobacteria inhabiting the roots and exerting a positive effect either through direct or the indirect mechanisms on the plant. PGPR are also termed as plant health-promoting rhizobacteria (PHPR) or nodule-promoting rhizobacteria (NPR). PGPR offer an environment-friendly means for increasing the crop productivity as well as sustainability in agriculture. There are lot of PGPR inoculants that have been commercialized and seem to promote the growth through either of the following mechanisms:

Table 2.1 Plant growth-promoting rhizobacteria associated with diverse host plant with multifarious PGP attributes

Rhizospheric microbiomes	Stress	IAA	P	N_2	ACC	Sid	References
Amaranthus							
Pseudomonas sp.	Low temp	+	+	–	–	+	Mishra et al. (2011)
Brassica							
Pseudomonas koreensis	Low temp	+	+	–	–	+	Mishra et al. (2011)
Pseudomonas putida	Low temp	+	+	–	–	+	Mishra et al. (2011)
Cabbage							
Pseudomonas sp.	Low temp	+	+	–	–	+	Mishra et al. (2011)
Canola							
Pseudomonas fluorescens	Salinity	+		–	+	–	Akhgar et al. (2014)
Foxtail millet							
Enterobacter hormaechei	Drought	–	–	–	+	–	Niu et al. (2017)
Pseudomonas fluorescens	Drought	–	–	–	+	–	Niu et al. (2017)
Pseudomonas migulae	Drought	–	–	–	+	–	Niu et al. (2017)
Garlic							
Pseudomonas jessani	Low temp	+	+	–	–	+	Mishra et al. (2011)
Maize							
Bacillus amyloliquefaciens	Drought	+	+	–	–	+	Vardharajula et al. (2011)
Bacillus licheniformis	Drought	+	+	–	–	+	Vardharajula et al. (2011)
Pseudomonas entomophila	Drought	+	+	–	–	+	Sandhya et al. (2010)
Bacillus subtilis	Drought	+	+	–	–	+	Vardharajula et al. (2011)
Bacillus thuringiensis	Drought	+	+	–	–	+	Vardharajula et al. (2011)
Paenibacillus favisporus	Drought	+	+	–	–	–	Vardharajula et al. (2011)
Pea							
Pseudomonas fluorescens	Low temp	+	+	–	–	+	Mishra et al. (2011)
Pseudomonas lurida	Low temp	+	+	–	–	+	Mishra et al. (2011)
Pearl millet							
Pseudomonas stutzeri	Drought	+	+	–	–	+	Sandhya et al. (2010)
Rice							
Alcaligenes sp.	Salinity	–	–	–	+	–	Bal et al. (2013)
Bacillus sp.	Salinity	–	–	–	+	–	Bal et al. (2013)
Enterobacter sp.	Halophilic	+	+	–	–	–	Hingole and Pathak (2016)

(continued)

Table 2.1 (continued)

Rhizospheric microbiomes	Stress	IAA	P	N_2	ACC	Sid	References
Enterobacter sp.	Salinity	+	+	–	+	+	Sarkar et al. (2018a)
Ochrobactrum sp.	Salinity	–	–	–	+	–	Bal et al. (2013)
Wheat							
Achromobacter spanius	High temp	+	+	+	–	+	Verma et al. (2016b)
Alcaligenes faecalis	High temp	+	+	+	–	+	Verma et al. (2016b)
Arthrobacter sp.	Salinity	+	+	–	–	+	Upadhyay et al. (2009)
B. sporothermodurans	Salinity	+		–	–	+	Upadhyay et al. (2009)
Bacillus altitudinis	High temp	+	+	–	–	+	Verma et al. (2016b)
Bacillus amyloliquefaciens	Low temp	+	+	–	–	+	Verma et al. (2015b)
Bacillus aquimaris	Salinity	+		–	–	+	Upadhyay et al. (2009)
Bacillus arsenicus	Salinity	+	+	–	–	+	Upadhyay et al. (2009)
Bacillus cereus	Low temp	–	–	–	–	+	Verma et al. (2015b)
Bacillus cereus	Salinity	+	+	–	–	+	Upadhyay et al. (2009)
Bacillus flexus	High temp	–	+	–	–	+	Verma et al. (2016b)
Bacillus flexus	Low temp	–	+	–	–		Verma et al. (2015b)
Bacillus licheniformis	High temp	–	+	+	–	–	Verma et al. (2016b)
Bacillus megaterium	Low temp	+	+	–	–	+	Verma et al. (2015b)
Bacillus mojavensis	High temp	+	+	+	–	–	Verma et al. (2016b)
Bacillus pumilus	Salinity	+		–	–	+	Upadhyay et al. (2009)
Bacillus siamensis	High temp	+	–	–	–	–	Verma et al. (2016b)
Bacillus subtilis	High temp	+	+		–	+	Verma et al. (2016b)
Bacillus subtilis	Salinity	+	+	–	–	–	Upadhyay et al. (2009)
Bacillus thuringiensis	Low temp	–	+	–	–	+	Verma et al. (2015b)
Delftia acidovorans	High temp	+	+	+	–	+	Verma et al. (2016b)
Delftia lacustris	High temp		+	+	–	+	Verma et al. (2016b)
M. mesophilicum	High temp	+	+	–	–	+	Verma et al. (2016b)
M. phyllosphaerae	Low temp	+		–	+	+	Verma et al. (2015b)
Planococcus rifietoensis	Alkalinity	+	+	–	+	–	Rajput et al. (2013)
Pseudomonas aeruginosa	Low temp	+		–	+	+	Verma et al. (2015b)
Pseudomonas fluorescens	Low temp	+	+	–	+	+	Verma et al. (2015b)
Pseudomonas japonica	High temp	–	+	–	–	–	Verma et al. (2016b)
Pseudomonas medicona	Salinity	+		–	–	+	Upadhyay et al. (2009)
Pseudomonas poae	High temp	+	+	+	–	+	Verma et al. (2016b)
Pseudomonas stutzeri	High temp	+	+	–	–		Verma et al. (2016b)
Rhodobacter sphaeroides	High temp	+	–	–	–	–	Verma et al. (2016b)
Salmonella bongori	High temp	+	–	–	–	+	Verma et al. (2016b)
Staphylococcus succinus	High temp	–	–	–	–	+	Verma et al. (2016b)

IAA indole acetic acids, *P* phosphorus solubilization, N_2 nitrogen fixation, *ACC* 1-aminocyclopropane-1-carboxylate deaminase, *Sid* siderophores

Table 2.2 Agricultural applications of PGP microbes for alleviation of diverse abiotic stress

PGPR	Crop inoculated	Stress ameliorated	Effect	References
Achromobacter piechaudii	Tomato	Salinity	Fresh and dry weight	Mayak et al. (2004)
Arthrobacter	Tomato	Salinity	Seed germination, vigor index, biomass	Fan et al. (2016)
Arthrobacter protophormiae	Wheat	Salinity	Photosynthetic efficiency, IAA content,	Barnawal et al. (2017)
Arthrobacter sp. SU18	Wheat	Salinity	Dry biomass, total soluble sugars, and proline content	Upadhyay et al. (2012)
Azospirillum brasilense	White clover	Salinity	Shoot/root length, biomass, leaf area, and chlorophyll	Khalid et al. (2017)
Azospirillum brasilense	Wheat	Drought	Mg, Ca, K content	Creus et al. (2004)
Bacillus amyloliquefaciens	Wheat	Cold stress	Growth and alleviation	Verma et al. (2015a)
Bacillus aquimaris DY-3	Maize	Salinity	Chlorophyll content, leaf relative water content	Li and Jiang (2017)
Bacillus atrophaeus EY6	Strawberry	Salinity	Growth, chlorophyll content, nutrient uptake, and yield	Karlidag et al. (2013)
Bacillus megaterium	Tomato	Salinity	Seed germination, seedling length, vigor index	Fan et al. (2016)
Bacillus megaterium	Wheat	Cold stress	Dry weight	Turan et al. (2012)
Bacillus mojavensis	Wheat	Salinity	Biomass, chlorophyll content, and nutrient uptake	Pourbabaee et al. (2016)
Bacillus pumilus	Rice	Salinity	Germination, survival, dry weight, plant height	Jha and Subramanian (2013)
Bacillus safensis	Wheat	High temperature	Chlorophyll content, accumulation of osmolytes	Sarkar et al. (2018b)
Bacillus sp.	Sorghum	Drought	Shoot length, root biomass, chlorophyll content	Grover et al. (2014)
Bacillus sp.	Potato	Salinity	mRNA expression levels, proline content	Gururani et al. (2013)
Bacillus sp.	Potato	Heavy metal	mRNA expression levels, proline content	Gururani et al. (2013)
Bacillus sp.	Potato	Drought	mRNA expression levels, proline content	Gururani et al. (2013)

(continued)

Table 2.2 (continued)

PGPR	Crop inoculated	Stress ameliorated	Effect	References
Bacillus sphaericus	Strawberry	Salinity	Growth, chlorophyll content, nutrient uptake, and yield	Karlidag et al. (2013)
Bacillus subtilis LDR2	Wheat	Drought	Photosynthetic efficiency, IAA content	Barnawal et al. (2017)
Bacillus subtilis EY2	Strawberry	Salinity	Growth, chlorophyll content, nutrient uptake, and yield	Karlidag et al. (2013)
Bacillus subtilis SU47	Wheat	Salinity	Dry biomass, total soluble sugars, and proline content	Upadhyay et al. (2012)
Bacillus sp.	Maize	Drought	Proline, sugars, free amino acids	Vardharajula et al. (2011)
Bradyrhizobium japonicum	Soybean	Salinity	Antioxidant activity, proline	Methé et al. (2005)
Burkholderia phytofirmans PsJN	Wheat	Drought	Ionic balance, antioxidant levels, NPK uptake	Naveed et al. (2014)
Dietzia natronolimnaea STR1	Wheat	Salinity	Photosynthetic efficiency, IAA content	Barnawal et al. (2017)
Enterobacter aerogenes	Maize	Salinity	Growth and yield	Nadeem et al. (2007)
Enterobacter cloacae ZNP-3	Wheat	Salinity	Biomass and chlorophyll content	Singh et al. (2017)
Enterobacter cloacae ZNP-3	Wheat	High temperature	Biomass and growth	Singh et al. (2017)
Enterobacter cloacae HSNJ4	Canola	Salinity	Increased IAA content and reduced ethylene emission	Li et al. (2017)
Enterobacter sp. P23	Rice	Salinity	Growth and yield	Sarkar et al. (2018a)
Enterobacter sp. S16–3	Canola	Osmotic stress	Root volume	Oskuei et al. (2017)
Exiguobacterium acetylicum	Pea	Cold stress	Germination and early growth parameters	Selvakumar et al. (2009)
Klebsiella sp.	Oat	Salinity	Shoot /root length, biomass and relative water content	Sapre et al. (2018)
Klebsiella sp.	Wheat	Drought	Water status, membrane integrity	Gontia-Mishra et al. (2016)
Klebsiella sp. IG 3	Oat	Salinity	Plant growth, genes expression	Sapre et al. (2018)

(continued)

Table 2.2 (continued)

PGPR	Crop inoculated	Stress ameliorated	Effect	References
Klebsiella variicola	Soybean	Flooding	Quantum efficiency of chlorophyll	Kim et al. (2017)
Klebsiella variicola F2	Maize	Drought	Accumulation of choline	Gou et al. (2015)
Kocuria erythromyxa EY43	Strawberry	Salinity	Growth, chlorophyll content, nutrient uptake	Karlidag et al. (2013)
Kocuria erythromyxa	Radish	Salinity	Biomass, chlorophyll content, relative water content	Yildirim et al. (2008)
Pantoea dispersa	Wheat	Cold stress	Growth and nutrient uptake	Selvakumar et al. (2008)
Planococcus rifietoensis	Wheat	Salinity	Shoot, root length, biomass, growth, and yield	Rajput et al. (2013)
Pseudochrobactrum kiredjianiae	Wheat	Cold stress	Physiological parameters	Qin et al. (2017)
Pseudomonas aeruginosa	Wheat	Heavy metal	Uptake of NP, leaf chlorophyll, total soluble protein	Islam et al. (2014)
Pseudomonas aeruginosa PRR1	Rice	Salinity	Germination percentage, shoot and root length	Kumar et al. (2017b)
Pseudomonas fluorescens	Maize	Salinity	Growth and yield	Nadeem et al. (2007)
Pseudomonas fluorescens	Foxtail millet	Drought	Soil moisture, root adhering soil/root tissue ratio	Niu et al. (2017)
Pseudomonas fluorescens YX2	Maize	Drought	Accumulation of choline	Gou et al. (2015)
Pseudomonas lurida	Wheat	Cold stress	Growth and nutrient uptake	Selvakumar et al. (2011)
Pseudomonas pseudoalcaligenes	Rice	Salinity	Germination, survival, dry weight, plant height	Jha and Subramanian (2013)
Pseudomonas putida Rs-198	Cotton	Salinity	Biomass, absorption of the micronutrients	Yao et al. (2010)
Pseudomonas putida GAP-P45	Sunflower	Drought	Plant biomass, and root adhering soil/ root tissue ratio	(Sandhya et al. 2009)
Pseudomonas putida N_{21}	Wheat	Salinity	Shoot/ root length, grain yield, chlorophyll content	Zahir et al. (2009)
Pseudomonas putida AKMP7	Wheat	Heat stress	Biomass, chlorophyll, sugars, amino acids	Ali et al. (2011)

(continued)

Table 2.2 (continued)

PGPR	Crop inoculated	Stress ameliorated	Effect	References
Pseudomonas sp.	Pea	Drought	Shoot, root length, biomass, grain yield	Arshad et al. (2008)
Pseudomonas sp.	Maize	Drought	Proline, sugars, free amino acids	Sandhya et al. (2010)
Pseudomonas sp.	Asparagus	Drought	Enhanced growth	Liddycoat et al. (2009)
Pseudomonas sp.	Wheat	Low temperature	Chlorophyll, total phenolics, and relative water content	Mishra et al. (2011)
Pseudomonas syringae	Maize	Salinity	Growth and yield	Nadeem et al. (2007)
Pseudomonas vancouverensis	Wheat	Cold stress	Germination	Mishra et al. (2008)
Pseudomonas sp. AKM-P6	Sorghum	High temperature	Proline, chlorophyll, sugars, amino acids, and proteins	Ali et al. (2009)
Raoultella planticola YL2	Maize	Drought	Accumulation of choline	Gou et al. (2015)
Serratia nematodiphila PEJ1011	Pepper	Low temperature	Growth, shoot, root length, biomass	Kang et al. (2015)
Sphingomonas faeni	Finger millet	Cold stress	Shoot, root length, biomass, antioxidant activity	Srinivasan et al. (2017)
Staphylococcus kloosii	Radish	Salinity	Biomass, and relative water content	Yildirim et al. (2008)
Staphylococcus kloosii EY37	Strawberry	Salinity	Growth, chlorophyll content, nutrient uptake, and yield	Karlidag et al. (2013)
Stenotrophomonas maltophilia	Wheat	Salinity	Shoot/root length, chlorophyll content	Singh and Jha (2017)

Suppression of the various plant diseases; in this context they are referred to as the bioprotectants.
Improved nutrient acquisition where they are referred to as the biofertilizers.
The phytohormone production where they are known as biostimulants (Kour et al. 2017a, b).

2.5.1 Biological Nitrogen Fixation

Nitrogen is the major limiting factor for plant growth; the application of N_2-fixing bacteria as biofertilizer has emerged as one of the most efficient and environmentally sustainable methods for increasing the growth and yield of crop plants and an attractive way of replacing chemical fertilizers (Ashrafuzzaman et al. 2009).

Table 2.3 Agricultural applications of microbial consortium for plant growth promotion and alleviation of abiotic stress

Consortium of PGPR	Crop inoculated	Stress ameliorated	Effect	References
Ochrobactrum pseudogrignonense RJ12 *Pseudomonas* sp. RJ15 *Bacillus subtilis* RJ46	*Vigna mungo*	Drought	Seed germination percentage, root, shoot length, dry weight, enzyme activity cellular osmolytes, chlorophyll content, relative water content, root recovery intension	Saikia et al. (2018)
Ochrobactrum pseudogrignonense RJ12 *Pseudomonas* sp. RJ15 *Bacillus subtilis* RJ46	*Pisum sativum*	Drought	Seed germination percentage, root, shoot length, dry weight, enzyme activity cellular osmolytes, chlorophyll content, relative water content, root recovery intension	Saikia et al. (2018)
Azospirillum brasilense Ab-V6 *Rhizobium tropici* CIAT 899	*Zea mays*	Salinity	Upregulation of antioxidant activity-related genes	Fukami et al. (2018)
Bacillus sp. AZ-1 *Enterobacter cloacae* AZ-3	*Cicer arietinum*	Heavy metal	Seed germination, shoot and root length, root and shoot fresh weight, number of seeds and weight of seeds	Amin and Latif (2017)
Bacillus pumilus *Pseudomonas pseudoalcaligenes*	*Oryza sativa*	Salinity	Germination, survival, dry weight, plant height	Jha and Subramanian (2013)
Bacillus cereus AR156 *Bacillus subtilis* SM21 *Serratia* sp. XY21	*Solanum lycopersicum*	Cold stress	Soluble sugar, proline, osmotin accumulation, antioxidant defense system, stress-related gene activation	Wang et al. (2016)
Bacillus amyloliquefaciens Bk7 *Brevibacillus laterosporus* B4	*Oryza sativa*	Cold stress	Growth, enzymatic activity	Kakar et al. (2016)

Nitrogen-fixing endophytic bacteria belonging to different genera including *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, and *Serratia* have been reported and well characterized for biological nitrogen fixation (Yadav and Saxena 2018; Yadav et al. 2017c, 2018c). Figueiredo et al. (2008) reported that use of PGPR for sustainable agriculture has tremendously increased in different parts of the world. Microbes are gaining a lot of importance in agriculture for promoting

circulation of various plant nutrients, thereby reducing the need of chemical fertilizers. There are a number of nitrogen-fixing bacteria in rhizospheric region which has been used in nonlegume crop species including rice, wheat, maize, sugarcane, sugar beet, etc. The nitrogen fixers include symbiotic nitrogen fixers like *Rhizobium* which are obligate symbionts in the leguminous plants and *Frankia* in non-leguminous plants and non-symbiotic nitrogen fixers which may either be free-living, associative symbiotic, or endophytic including *Cyanobacteria*, *Azotobacter*, *Azospirillum*, *Acetobacter diazotrophicus*, *Azoarcus*, etc. (Marag et al. 2015; Rana et al. 2017, 2016a, b; Saharan and Nehra 2011).

2.5.2 Symbiotic Nitrogen Fixers

The most commonly studied symbiotic nitrogen-fixing bacteria include *Rhizobium* and *Frankia*. In the last few years, a considerable change in the taxonomic status of *Rhizobia* has come out. The current status of taxonomy of *Rhizobia* has been outlined in a study of Sahgal and Johri (2003), according to which there are about 36 species of *Rhizobia* which are distributed among 7 different genera which are *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Methylobacterium*, *Rhizobium*, and *Sinorhizobium* derived based on the polyphasic taxonomic approach. *Rhizobium* basically forms a symbiotic association with the roots of the leguminous plants and forms nodules, and this relationship has coevolved for 10 millions of years. The process by which the nodules are formed involves a very complex array of the signaling molecules, molecular recognition, and regulation. Legumes basically secrete flavonoids which are secondary metabolites into the soil. *Rhizobia* being motile get attached to these flavonoids and ultimately to the rhizoplane. These flavonoids also induce bacteria to secrete specific signaling molecules which are known as the nod factors (Werner 2008), which are critical molecules for the nodule formation. These nod factors then bind to the receptors which are present in the root hair cell and cause root hair curling and ultimately the penetration of the bacterium into the root hair cell. The nod factor alone is not the only requirement for nodule formation, but various bacterial cell structures such as the lipopolysaccharides (LPS), β-glucans, exopolysaccharides (EPS), capsular proteins, and K antigen are also recognized by the plants and help in determining the host specificity (Fraysse et al. 2003; Mathis et al. 2005; Spaink 2000). The formation of the root nodules is mediated by nod genes. In these root nodules, the atmospheric nitrogen is fixed in which there is reduction of the molecular nitrogen to ammonia which is then utilized by the plants for the synthesis of various proteins, vitamins, and other nitrogen-containing compounds.

Frankia forms root nodules on about 280 species of the woody plants from 8 different families, but its symbiotic association is not well understood. *Frankia* are basically used in the land reclamation for timber and fuel wood production and in mixed plantations for the purpose of the windbreaks and for shelterbelts. An increase in the rhizospheric population has been reported after crop rotation with the

nonlegumes. Though their symbiotic associations are important, very limited information is available for inoculation practices and use.

2.5.3 Associative Symbiotic Nitrogen Fixers

The most commonly used associative symbiotic nitrogen fixers include *Azospirillum. Azospirillum* is basically facultative endophytic diazotroph and belongs to the family *Spirillaceae.* It includes different species *Azospirillum amazonense*, *Azospirillum halopraeferens*, and *Azospirillum brasilense* (Potrich et al. 2001). The members of this genus fix the atmospheric nitrogen under microaerophilic conditions. It has an ability to fix about 20–40 $kgha^{-1}$ nitrogen and additionally also produces some regulatory substances. It is mainly used for the crops such as the maize, sugarcane, pearl millet, sorghum, etc. It also helps in the development of root and shoots (González-López et al. 2005). They are associated mostly with the root and the rhizosphere of agriculturally important crops.

2.5.4 Free-Living Nitrogen Fixers

Azotobacter is the most extensively used free-living nitrogen fixer. It fixes the atmospheric nitrogen in nonlegumes which include mainly in maize, rice, cotton, vegetables, etc. The deficiency of the organic matter is basically a limiting factor for its proliferation. *Azotobacter* belongs to the family *Azotobacteriaceae* which comprises of two genera including *Azomonas* and *Azotobacter*. *Azomonas* is non-cyst forming which comprises of the three species which include *Azomonas agilis*, *Azomonas insignis*, and *Azomonas macrocytogenes*, whereas *Azotobacter* is cyst forming and comprises of the following six species including *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Azotobacter beijerinckii*, *Azotobacter nigricans*, *Azotobacter armeniacus*, and *Azotobacter paspali* (Saharan and Nehra 2011). *Azotobacter* fixes atmospheric nitrogen in nonleguminous plants such as the rice, cotton, and vegetables. They have the capacity to fix about 15–20 $kg.ha^{-1}$ of N per year. In addition to fixation of atmospheric nitrogen, *Azotobacter* also produces phytohormones such as indole acetic acids and siderophores such as the azotobactin.

2.5.5 Phytohormone Production

A wide range of microorganisms are found in the rhizosphere which produce various regulatory substances which are important for the growth and development of the plants. The various phytohormones which are produced by the rhizospheric microorganisms include auxins, gibberellins, and cytokinins. There are many reports on plant microbiomes producing phytohormones. The phytohormone-producing rhizospheric microbes, when inoculated to crops, help in plant growth

promotion, enhance yield, and increase soil fertility for sustainable agriculture (Singh et al. 2016; Yadav et al. 2015a, b, 2018c)

2.5.6 Indole Acetic Acid (IAA)

It is one of the most active auxins and it positively affects the growth of the roots. IAA affects cell division, extension, and differentiation and seed and tuber germination, controls various processes of vegetative growth, and increases the rate of xylem and root development, pigment formation, and resistance to various stressful conditions (Miransari and Smith 2014). Tryptophan is found in root exudates and is the precursor for the synthesis of IAA. There are Trp-dependent and Trp-independent pathways in plants and bacteria. Physiological evidence for different Trp-dependent pathways for synthesis of IAA has been reported in *Azospirillum brasilense* (Carreno-Lopez et al. 2000). Another important mechanism for the biosynthesis of IAA via the formation of the indole-3-pyruvic acid and indole-3-acetic aldehyde has been found in *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Enterobacter*, and *Klebsiella* (Shilev 2013).

The species of *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* despite of being the N_2 fixers have also been reported to produce IAA under in vitro conditions (Ahmad et al. 2008; Antoun et al. 1998; Wani et al. 2008a, b, c). Other PGPR strains including *Agrobacterium* sp., *Alcaligenes piechaudii*, *Bacillus*, *Comamonas acidovorans*, and *Pseudomonas* have also been reported to secrete IAA (Barazani and Friedman 1999; Rajkumar et al. 2006). In a study of Khalid et al. (2004), numerous bacterial isolates were recovered from rhizosphere of wheat which produced IAA under in vitro conditions, and further, supply of exogenous tryptophan enhanced auxin biosynthesis which was ultimately confirmed by high-performance liquid chromatography. Bottini et al. (2004) demonstrated the production of IAA and GA by P-solubilizers including *Enterobacter*, *Xanthomonas*, and *Pseudomonas* isolated from rhizosphere of sorghum plants. Joseph et al. (2012), while working on chickpea, found that all the isolates of *Azotobacter*, *Bacillus*, and *Pseudomonas* were capable of producing IAA, whereas only 85.7% of *Rhizobium* could produce IAA.

Chaiharn and Lumyong (2011) screened rhizobacteria for plant growth-promoting traits such as solubilization of inorganic phosphate and IAA production and further evaluated their effect on root elongation of bean and maize seedlings, and *Klebsiella* was found to be the best IAA producer. Further, some microbes possess the capability to catabolize IAA, and this characteristic feature has been well demonstrated in *Bradyrhizobium japonicum* (Jensen et al. 1995) and also in *Pseudomonas putida* 1290 (Leveau and Lindow 2005). *Pseudomonas putida* 1290 when co-inoculated in *Raphanus sativus* L. decreased negative effects of higher concentrations of IAA produced by pathogenic bacteria *Rahnella aquaticus* and *Pseudomonas syringae*. The catabolizing property of PGPR could have a positive effect on the growth of the plants and might prevent the pathogenic attack (Leveau and Lindow 2005).

2.5.7 Gibberellins and Cytokinins

There is a very little information which is available for the production of the gibberellins by microorganisms though it is known that symbiotic bacteria have the capability to produce gibberellins, auxins, and cytokinins but in very low concentrations when the formation of the nodules takes place as well as when there is high duplication rate (Atzorn et al. 1988). The gibberellins are rarely produced by PGPR with only *Bacillus pumilus* and *Bacillus licheniformis* known to produce gibberellins (Gutiérrez-Mañero et al. 2001). Production of cytokinins has been reported in fewer strains of PGPR as compared to auxins. Cytokinins have been demonstrated to be produced by strains of *Bacillus*, *Rhizobium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, and *Pseudomonas*. It has been shown that the inoculation of the cytokinin-producing bacterium, *Bacillus subtilis*, enhanced chlorophyll content and cytokinin accumulation and ultimately the shoot and root weight in lettuce (Kudoyarova et al. 2014; Arkhipova et al. 2007).

2.5.8 ACC Deaminase Activity

Ethylene is a key phytohormone and is known to possess wide range of biological activities such as it affects plant growth and development in different ways including promotion of root initiation, inhibition of root elongation, promotion of fruit ripening, stimulation of seed germination, promotion of leaf abscission, and activation of the synthesis of other plant hormones (Kang et al. 2010). There are a number of mechanisms that have been investigated to reduce the levels of ethylene, and one of the best mechanisms to reduce the levels of the ethylene in plants is by the activity of bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Farajzadeh et al. 2012; Glick 2005; Jalili et al. 2009). ACC deaminase basically metabolizes ACC which is the immediate precursor of ethylene into α-ketobutyric acid and ammonia (Arshad et al. 2007; Saleem et al. 2007).

PGPR including *Alcaligenes* sp., *Bacillus pumilus*, *Pseudomonas* sp., and *Variovorax paradoxus* (Belimov et al. 2001) as well as *Azoarcus*, *Azorhizobium caulinodans*, *Azospirillum* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillum* sp., *Burkholderia vietnamiensis*, and others (Dobbelaere et al. 2003) have been identified to show ability to grow on minimal media containing ACC as sole nitrogen source. Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Cronobacter sakazakii*, *Enterobacter*, *Halomonas*, *Klebsiella*, *Mesorhizobium*, *Methylobacterium fujisawaense*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Rhizobium*, *Variovorax*, and *Zhihengliuela* (Suman et al. 2016b; Verma et al. 2017b; Yadav and Yadav 2018b).

The inoculation of crops with PGPR showing ACC deaminase activity is more resistant to the stressful conditions (Zahir et al. 2008). In the study of Arshad et al. (2008), it was demonstrated that *Pseudomonas* sp. with ACC deaminase activity provided drought-tolerant *Pisum sativum*. Bal et al. (2013) evaluated that *Alcaligenes*

sp., *Bacillus* sp., and *Ochrobactrum* sp., possessing ACC deaminase activity, induced salt tolerance and improved the growth of the rice plants under salinity stress.

2.5.9 Phosphate Solubilization

Phosphorus is an important macroelement in the nutrition of the plants, next to nitrogen, and plays an important role in almost all the metabolic processes of the plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and respiration. It is abundantly present in the soil in both organic and inorganic forms. Plants are not capable of utilizing phosphate as 95–99% is present in the insoluble, immobilized, and precipitated form. Plants are able to utilize phosphate in two soluble forms, i.e., the monobasic (H_2PO_4) and the diabasic (HPO_4^{2-}) ions (Ahemad and Kibret 2014). There are a number of P-solubilizing bacteria (PSB) in rhizosphere which use different strategies so as to make unavailable forms of phosphorus available for plants so that it can be absorbed. The main mechanisms used by PGPR for the solubilization of phosphorus include releasing certain complexing or mineral-dissolving compounds, liberating extracellular enzymes, or releasing phosphate during substrate degradation (Pandey and Maheshwari 2007).

Phosphate-solubilizing PGPR have been included in the genera *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Serratia* which have attracted a great attention of agriculturists as soil inoculums so that plant growth and yield can be improved. A number of phosphorus-solubilizing microbes have been isolated from rhizosphere of different plants (Yadav et al. 2016b, 2017a, b). Chen et al. (2006) reported *Bacillus*, *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, and *Delftia* to be P-solubilizers. Qin et al. (2011) demonstrated the capability of rhizobia to solubilize inorganic phosphate is mainly associated with acidification of rhizosphere. Ambrosini et al. (2012) reported *Burkholderia* strains from sunflower plants to be predominant in solubilization of $Ca_3(PO4)^2$. de Souza et al. (2013) identified *Burkholderia*, *Cedecea*, *Cronobacter*, *Enterobacter*, *Pantoea*, *and Pseudomonas* possessing capability to solubilize tricalcium phosphate from rice. Yadav and Pandey (2018) isolated P-solubilizers including *Bacillus* sp., *Streptomyces* sp., and *Cronobacter* sp. from the rhizospheric region of tomato.

2.5.10 Siderophore Production

Iron is an essential growth cofactor for living organisms. Iron exists in two states in aqueous solution which are Fe^{2+} and Fe^{3+}, but Fe^{3+} forms cannot be utilized by the plants as well as the microorganisms as they form oxides and hydroxides which are insoluble and in turn limit the bioavailability (Desai and Archana 2011; Zuo and

Zhang 2011). When aerobic or facultative anaerobes grow in iron-deficient environment, they start synthesizing Fe^{3+} ion-specific chelating agents which are referred to as the siderophores which are basically peptide molecules and contain side chains as well as the functional groups which provide high affinity set of ligands to coordinate the ferric ions (Crosa and Walsh 2002). On the basis of these iron-coordinating functional groups, structural features, and types of ligands, siderophores have been divided into four categories which are carboxylate, hydroxamates, phenol catecholates, and pyoverdine (Crowley 2006). These bind Fe^{3+} ions and make siderophore-ferric complex to be transported into the cell. Siderophores mean the iron (Fe^{3+}) carrier. Roots can take up iron from the siderophore-Fe complex by different ways which include (a) chelate degradation, (b) direct uptake of the siderophore-Fe complex, and (c) ligand exchange reaction. *Azotobacter*, *Pseudomonas*, *Mycobacterium*, *Rhodococcus*, and many enterobacteria are known to produce peptidic siderophores, whereas *Agrobacterium*, several actinomycetes, *Burkholderia*, *Paracoccus*, and *Rhizobia* produce siderophores based on di- and tri-aminoalkane skeletons (Scavino and Pedraza 2013). *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Marinobacter*, *Ochrobactrum*, *Ralstonia*, *Rhizobium*, *Staphylococcus*, *Synechococcus*, and *Vibrio* produce citric acid-based siderophores (Budzikiewicz 2010).

Arora et al. (2001) isolated *Rhizobium meliloti* from medicinal plant, *Mucuna pruriens*, which produced siderophores which acted as biocontrol agent against *Macrophomina phaseolina* and also proved an efficient plant growth promoter that was evidenced by increased seedling biomass and fresh nodule weight over uninoculated controls. Sharma and Johri (2003) reported the significant increase in germination percentage and plant growth in maize with inoculation of seeds with siderophore-producing *Pseudomonas* sp. strains GRP3A and PRS. The maximum shoot, root length, and dry weight were observed with 10 μM Fe (III) along with bacterial inoculants.

The siderophore production is one of the most common characteristic features of the isolates associated with sunflower (Ambrosini et al. 2012) and rice (de Souza et al. 2013). The major function of the siderophores is to obtain the iron from the insoluble hydroxides or the oxides, but they can also extract iron from various soluble or insoluble compounds, such as the ferric citrate, ferric phosphate, ferritin, Fe-transferrin, iron bound to the sugars, plant flavone pigments, and glycosides, or even from the artificial chelators such as EDTA and nitrilotriacetate by Fe ligand exchange reactions. In fact, siderophores are not only directly involved in the solubilization of iron but indirectly also make iron available to both plants and microbes.

2.5.11 Biocontrol

Recently, the use of PGPR as biocontrol agents is on rise for the control of bacterial, viral, and fungal plant diseases. Treatment of the tomato seeds with PGPR strains *Bacillus subtilis* and *Bacillus amyloliquefaciens* protected the plants against the bacterial canker (Girish and Umesha 2005). *Pseudomonas corrugata*, *Bacillus*

megaterium, and *Flavobacterium* sp. efficiently controlled *Phytophthora capsici* and *Phytophthora* blight of pepper (Akgül and Mirik 2008) (Sang et al. 2008). In the study of Kirankumar et al. (2010), *Pseudomonas* B-25 has been conferred to promote the growth, the yield, as well as the nutrient uptake of the tomato in the presence of the tobacco mosaic virus (TMV).

2.5.11.1 Antibiosis

The production of the antibiotics by PGPR is one of the most powerful and most widely studied mechanisms against the phytopathogens. According to Haas and Défago (2005), there are six categories of the antibiotics which are basically related to the biocontrol of the root diseases; these include phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, and cyclic lipopeptides; and all of these are diffusible and hydrogen cyanide which is basically volatile in nature. Recently, lipopeptide biosurfactants have been implicated in the biocontrol which are produced by *Pseudomonas* and *Bacillus* species. These biosurfactants possess a potential positive effect on competitive interactions with organisms including bacteria, fungi, oomycetes, protozoa, nematodes, and plants (Arora et al. 2018; De Bruijn et al. 2007; Raaijmakers et al. 2010). There are various other antimicrobial compounds such as oligomycin A, kanosamine, zwittermicin A, and xanthobaccin which are produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* sp. which also prevents the proliferation of plant pathogens mostly the fungi. One of the effective and extensively studied antibiotics is 2,4-diacetylphloroglucinol (DAPG) which is produced by *Pseudomonads*, an effective and extensively studied antibiotic, causes membrane damage to *Pythium* sp., and is particularly inhibitory to zoospores of this oomycete (de Souza et al. 2003). Another compound phenazine, which is also produced by *Pseudomonads*, possesses redox activity and can suppress pathogens of plants such as *Fusarium oxysporum* and *Gaeumannomyces graminis* (Bloemberg and Lugtenberg 2003).

2.5.11.2 Lytic Enzymes

There are a number of enzymes which are produced by plant growth-promoting rhizobacterial strains such as chitinases, dehydrogenase, β-glucanase, lipases, phosphatases, proteases, etc. (Joshi et al. 2015; Lanteigne et al. 2012). PGPR through the activity of these enzymes play an important role in growth promotion mainly by protecting the plants from various pathogenic fungi such as *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* sp., *Rhizoctonia solani*, and *Pythium ultimum* (Hayat et al. 2010; Nadeem et al. 2013; Yadav et al. 2016a). Someya et al. (2000) studied the chitinolytic and antifungal activities of *Serratia marcescens* against *Rhizoctonia solani* and *Fusarium oxysporum*. When the mycelia of the fungal pathogens were co-inoculated with the strain, partial swelling in the hyphae as well as at the tip, curling of the hyphae, and bursting of the hyphal tip were observed. The strains of *Paenibacillus* and *Streptomyces* sp. have been reported to synthesize β-1,3-glucanase which is responsible for degradation of cell walls of pathogenic fungi including *Fusarium oxysporum* (Compant et al. 2005). *Bacillus cepacia* has been revealed to synthesize β-1,3-glucanase which degrades the cell

walls of various soil-borne phytopathogens including *Rhizoctonia solani*, *Pythium ultimum*, and *Sclerotium rolfsii* (Compant et al. 2005)

2.6 Conclusion and Future Prospects

The use of the plant growth-promoting rhizobacteria in agricultural production systems started long time ago, and the evidences of their benefits by diverse mechanisms are increasing day by day. Diverse groups of microbes with multifarious plant growth-promoting attributes have been identified to date. But, the benefits from PGPR still need to be explored more. The better understanding of the bio-inoculants in the uptake of the nutrients has to be maximized. Before application of the foreign bacteria, proper assessment is required so that the survival of the native bacteria is not challenged which can affect the plant growth. In spite of all lengthy research to the date, a lot more work is still to be done to open out hidden capabilities of PGPR to commercialize them and to make them a proficient technique for sustainable agriculture, and for this, proper formulations, strategies, and field trials are essential. All these strategies will add to sustainable development simultaneously influencing the economic development. Thus, the application of PGPR will surely prove a potent tool to reduce the use of chemical fertilizers which is already on rise to meet the demands of expanding population.

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Advances in the Application of Plant Growth-Promoting Rhizobacteria in Horticulture

3

Ragini Maurya, Shivani Verma, and Indra Bahadur

Contents

Abstract

To manage a soil biodiversity, significant role is played by microbes. In this context, free-living soil bacteria/rhizobacteria are beneficial for improving plant growth and development commonly termed as plant growth-promoting rhizobacteria (PGPR).

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Uses of efficient microbes inhabit the roots forming colonies in higher plants acting as a pipeline for nutrient supply and provide many beneficial compounds competent for boosting plant growth and development. PGPR are well-known plant growth promoter (PGP) native of rhizosphere, which is a substantial soil ecological, environmental and plant wellness for soil-plant-microbe interaction. Keeping in mind the above points, it was felt that they are very useful in horticultural plants. For that, this book chapter encompasses the works of various scientists/students/researchers and reviewed their work for the awareness and use of PGPR in horticultural crops.

Keywords
Rhizosphere · Environment · Plant growth promotion

3.1 Introduction

Agriculture has a major contribution to the share of national income. Nowadays population increases, and to feed the ever-growing population, much more effort and innovation will be urgently required to increase agricultural production. This may also be increase with decrease in loss of productivity to ensure that everybody should get nutritious food. Good management practices (GMPs) are needed to ensure agricultural production, growth of economy and maintenance and protection of biodiversity as well as meet the food necessity of global inflating population. Nowadays, world cultivation needs sustainable approach to the fulfilment of our future need without affecting present requirement and traditional agricultural practices (TAPs). It is a challenge to enhance the productivity along with maintaining environmental health's for that educating farmers give special importance of results of their present techniques of agriculture and encourage them to GMPs and TAPs. Efficient microbes that affect the health of plant called as pathogenic organisms are main causes for food and environmental sustainability.

Current synthetic chemicals along with cultural practices are mostly used (Parra and Ristaino 2001). If microbes are used along with GMPs, it enhances environment, economic stability and social importance to ensure long-lasting production of natural resources and maintain liveliness. Some of these synthetic products have caused manifold threats, i.e. ecosystem pollution and human health toxicity, and develop pesticide-resistant pests (Hernández-Castillo et al. 2005). Recently, there has been focused on organic and sustainable farming. There is an urgency to use organic methods from total dependence on chemicals only. In this setting, the bio-alternatives/bioagents/biopesticides are to be considered as a feasible choice for pest management; many efficient microbes including bacteria, fungi, protozoa and algae are already present in the rhizosphere.

Microbes inhabiting the rhizosphere zone can be differentiated according to their effects on plants and their interaction with roots and others beneficial effects. The agricultural soil influenced plant growth by abiotic and biotic factors. Rhizobacteria

not only have beneficial effects in phyllosphere but also possess its positive impact on rhizosphere of plant. PGPR will be one of the most widely accepted for managing diseases in plants of horticulture, forestry and agriculture. PGPR being a significant component in rhizospheric biota and when grown in symbiotic with the host plants stimulate its growth. Plant growth-enhancing species of bacteria includes those in the genera *Azospirillum*, *Pseudomonas*, *Klebsiella*, *Azotobacter*, *Alcaligenes*, *Enterobacter*, *Burkholderia*, *Arthrobacter*, *Serratia* and *Bacillus*.

3.2 Role of PGPR

PGPR plays a vital function in improving growth of plant through a diverse system of mechanisms. The mode of action of PGPR includes (1) tolerance to abiotic stress, (2) nutrient fixation, (3) production of regulators of plant growth, (4) siderophore formation and (5) volatile natural compound production and produces some protecting enzymes as ACC deaminase, glucanase and chitinase for elimination diseases of plant. Stresses come that affect the growth of plant in many ways, which is a significant limitation for long-term crop production. These extremes are classified into two groups, biotic stresses and abiotic stresses (Fig. 3.1).

3.2.1 Different Ways to Tolerate Abiotic Stress in Plant

There are many reasons responsible for yield loss of horticultural commodities at field conditions among which the abiotic stress is of prime importance. However, the effectiveness of these abiotic stresses depends upon the intensity of various edaphic factors (soil moisture, soil pH, imbalance in nutrition, etc.) and other factors (Nadeem et al. 2010). The findings of Pishchik et al. (2002) state that PGPR is helpful in reducing harmful effects of cadmium pollution on barley due to its capacity of the bacteria to adhere cadmium ions to soil molecule attraction theory,

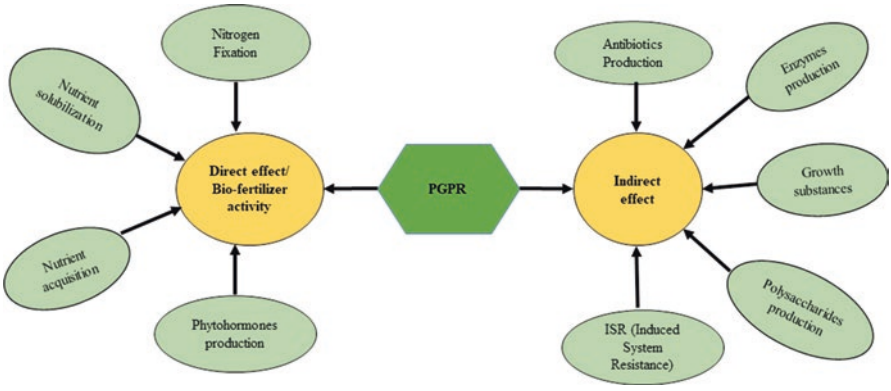

Fig. 3.1 Effect of PGPR on horticultural plants

therefore reducing down the available amount of cadmium in soil. However, Nautiyal et al. (2008) reported, the bacteria named *Bacillus lentimorbus* enhanced the antioxidant property of the edible parts of lettuce, carrots and spinach which leads to improve productivity. Findings of Naveed et al. (2014) reported that PGPR on plants grown in stressful abiotic conditions improve water status of leaves, particularly under stressful drought and salinity. *P. aeruginosa* isolate reported to enhance the growth under drought conditions mung beans (Sarma and Saikia 2014).

3.2.2 Soil Nutrients and Relative Plant Uptake

The findings of Choudhary et al. (2011) noted that PGPR facilitates the nutrient fixing due to some or other means and thereby increases the accessibility of concentration of nutrient in the rhizosphere, preventing them from leaching out. For example, nitrogen, which plays important role in formation of proteins and amino acids, is the major nutrient for plants. According to Tejera et al. (2005), there are very few free-living nitrogen-fixing organisms. The most famous one is *Azospirillum* which is often reported to be concomitant with cereals in temperate regions and reported to improvise the yield of rice.

3.2.3 Plant Hormones

The plant growth regulators (PGR), synthetic substances and phyto-stimulator are terms for promotion of growth by PGPR (Table 3.1). These efficient microbes have the ability to produce GA, IAA, ethylene and cytokinins (Lugtenberg et al. 2002; Somers et al. 2004). Auxin is an essential molecule for plant growth (Tanimoto 2005). PGPR which degrades ACC in the rhizosphere shortens the weakening cycle reconstructing healthy root system withstanding stress of environment. Glick (2014) illustrates that plant growth-promoting bacteria yields ACC deaminase and synthesize IAA promotes plant growth (Ahmad et al. 2013).

Table 3.1 Examples of different phytohormone produced by PGPR

Phytohormones	PGPR
Indole-3-acetic acid (IAA)	*Herbaspirillum seropedicae*, *Aeromonas veronii*, *Acetobacter diazotrophicus*, *Azospirillum brasilense*, *Enterobacter cloacae*, *Agrobacterium* spp., *Enterobacter* spp., *Alcaligenes piechaudii*, *Bradyrhizobium* spp., *Rhizobium leguminosarum*, *Comamonas acidovorans*
Cytokinin	*Rhizobium leguminosarum*, *Pseudomonas fluorescens*, *Paenibacillus polymyxa*
Zeatin and ethylene	*Azospirillum* spp.
Gibberellic acid (GA_3)	*Azospirillum brasilense*, *Bacillus* spp., *Azospirillum lipoferum*
Abscisic acid (ABA)	

3.2.4 Production of Siderophores

PGPRs secrete extracellular metabolites termed as siderophores. It has protein containing iron (Fe) compounds associated in the procedure of chelating ferric iron Fe^{3+}, and when Fe is finite, microbes of siderophores furnish iron by employing siderophores secreted by PGPR. Rhizobacteria have the capability of cross-utilization; few are skilled in utilization of siderophores within similar genus (Khan et al. 2014).

3.2.5 Volatile Organic Compound Production

Efficient PGPR produce volatile organic compounds (VOCs) which are involved in uplifting growth of plant and induced systemic resistance (ISR) against pathogens. Bacterial/rhizobacterial species from several genera include *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Stenotrophomonas* and *Serratia*. VOCs produced by *Bacillus* are best known and are accountable for noticeable enhancement in plant growth (Ryu et al. 2003).

3.3 Role as Biofertilizer

Biofertilizer is an essential part of organic farming in modern era. It is of significance for general agricultural production and economy on global scale. Biofertilizers are the products containing microbes in living state; if they incorporated in soil, plant surfaces, or seeds colonize the rhizosphere or plant's interior, by uplifting the primary nutrients accessibility promoting plant growth. A mixture of latent cells improves phosphate-solubilizing, nitrogen-fixing or cellulolytic microbes applicable to seed, soil, composting areas or roots improving a number of symbiotic microbes and uplifting the accessibility of nutrients which can be integrated and absorbed by the plants (Mishra et al. 2014). PGPM is classified in three major groups: (1) PGPR, (2) arbuscular mycorrhizal fungi (AMF) and (3) N-fixing rhizobia. PGPR is used globally as biofertilizers and biocontrol (Francis et al. 2010). PGPR in biofertilizers are beneficial in forming a proper rhizosphere for growth plant and altering important nutrients biologically, for example, increasing the inhibiting pathogen growth and enhancing nutrient availability. The survival rates of microbes enhanced soil fertility, and improved antagonistic biocontrol effects are due to high accessibility of nutrients (Yang et al. 2011). PGPR act as biofertilizers when used as a plant enrichment and nourishment source replenishing the nutrient cycle.

3.4 Application of PGPR in Horticulture

The PGPR is used for processes as promotion, propagation and biocontrol of growth and development in horticulture (Table 3.2).

Table 3.2 Plant growth-promoting rhizobacteria used in various horticultural plants and its beneficial effect

Rhizobium/arbuscular mycorrhizal strain	Effect	Horticultural plants	References
G. mosseae	Growth	Tomato	Gamalero et al. (2004)
R. leguminosarum	Root length, shoot height and dry weight	Pea	Kumar et al. (2001)
R. tropici	Growth	Field bean	Camacho et al. (2001)
Rhizobium	Siderophore production, protein production	Pepper, tomato, lettuce, carrot	Garcia-Fraile et al. (2012)
Sphingomonas	Plant height, gibberellin synthesis	Tomato	Khan et al. (2014)
Agrobacterium	Root inoculation increased fruit yield	Strawberry	Ipek et al. (2011)
B. subtilis	Enhanced diameter of trunk	Apple	Utkhede and Li (1989)
P. fluorescens	Decreased bacteria population and freeze injury + INA	Pear	Lindow et al. (1996)

3.4.1 Effects on Vegetative Propagation

The abundant of fruit species are heterozygous and majorities are self-sterile, resulting almost all commercially yielded fruit cultivars do not form true-to-type if propagated from seeds. Therefore, generative (seedling) methods of propagation are of no value. PGPR inoculation helps in propagation. Many bacteria in the genera of *Bacillus*, *Agrobacterium*, *Pseudomonas*, *Streptomyces* and *Alcali* induce formation of root in stem cuttings (Bassil et al. 1991; Rinallo et al. 1999). These bacteria generate IAA (Goto 1990). The rooting of bacteria-incorporated cuttings can be hastened by application of exogenous indole-3-butyric acid (IBA) (Falasca et al. 2000). Strawberries vastly multiply asexually via runners desiring efficient production, and quality of plant has a noticeable impact on growth and yield.

3.4.2 PGPR Used for Disease Management

PGPR improvises development and growth by indirect or direct effect techniques. Earlier, PGPR treatments were given to roots and, nowadays, sprayed to aerial part of the plant. Plant diseases in association with crop plants were managed by synthetic pesticides to enhance production of food. Regular use of pesticides has resulted in the outbreak of fungicide-resistant pathogens and environmental pollution. PGPR application either as single-strain or strain-mixture-based formulations stopped spreading of disease and increased yield and growth (Table 3.3).

Table 3.3 Use of PGPR in disease management of horticultural crop

PGPR	Crop	Disease
Actinoplanes spp.	Beetroot	*Pythium ultimum*
Pseudomonas fluorescens	Banana	Bunchy top virus, Panama wilt (*Fusarium oxysporum* f. spp. *Cubense*)
	Mulberry	Leaf spot
	Mango	*Anthracnose* (*Colletotrichum gloeosporioides*)
	Apple	Grey mould (*Botrytis cinerea*)
	Pear	Fire blight (*Erwinia amylovora*)
	Strawberry	Grey mould (*B. cinerea*)
	Potato	Bact. wilt, *Ralstonia solanacearum*, soft rot (*Erwinia carotovora*)
	Tomato	Cucumber mosaic virus, wilt (*F. oxysporum* f. spp. *Lycopersici*), bact. wilt (*R. solanacearum*)
	Brinjal	Blight (*Pythium vexans*), root rot (*Rhizoctonia solani*)
	Chilli	Powdery mildew (*Leveillula taurica*), fruit rot and dieback (*Colletotrichum capsici*), wilt (*F. oxysporum*)
	Pea	Damping off (*P. ultimum*), wilt (*F. oxysporum* f. spp. *Pisi*), root rot (*Aphanomyces euteiches*)
	Onion	Tip blight (*Alternaria* spp.)
	Radish	Wilt (*F. oxysporum* f. spp. *raphani*)
	Cucumber	Wilt (*F. oxysporum*), damping off (*Pythium aphanidermatum*), anthracnose (*Colletotrichum orbiculare*), angular leaf spot (*Pseudomonas syringae* pv. *Lachrymans*)
	Carnation	Wilt (*F. oxysporum* f. spp. *dianthi*)
P. syringae	Apple	Blue mould (*Penicillium expansum*), grey mould (*B. cinerea*)
	Peach	Brown rot (*Monilinia fructicola*)
P. aeruginosa	Tomato	Damping off (*P. aphanidermatum*)
	French bean	*B. cinerea*
P. putida	Tomato	Wilt (*F. oxysporum* f. sp. *lycopersici*)
	Pea	Damping off (*P. ultimum*)
P. corrugate	Cucumber	Damping off (*P. aphanidermatum*)
P. chlororaphis	Tomato	Damping off (*F. oxysporum* f. spp. *radicis-lycopersici*)
Bacillus subtilis	Apple	Blue mould (*P. expansum*),grey mould (*B. cinerea*)
	Peach	Brown rot (*M. fructicola*)
	Potato	Bact. wilt (*R. solanacearum*), scab (*Streptomyces scabies*)
	Tomato	Bacterial spot and late blight, wilt (*F. oxysporum* f.spp. *Lycopersici*), damping off (*P. aphanidermatum*)
	Brinjal	Collar rot (*S. sclerotiorum*)
	French bean	Root rot (*R. solani*)
	Lettuce	Root rot (*P. ultimum*)

(continued)

Table 3.3 (continued)

PGPR	Crop	Disease
B. pumilus	Strawberry	Grey mould (*B. cinerea*)
	Tomato	Tomato mottle virus, wilt (*F. oxysporum* f. spp. *lycopersici*)
B. coagulans	Mango	Bacterial canker (*Xanthomonas campestris* pv. *Mangiferaeindicae*)
B. amyloliquefaciens	Tomato	Tomato mottle virus
Enterobacter aerogenes	Apple	Collar rot (*Phytophthora cactorum*)
E. cloacae	Cucumber	Damping off (*P. aphanidermatum*)
	Lettuce	Root rot (*P. ultimum*)
Erwinia herbicola	Apple	*Erwinia amylovora*
Streptomyces griseoviridis	Cauliflower	Blight (*Alternaria brassicicola*)
	Narcissus	*F. oxysporum* f. spp. *narcissi*
Serratia marcescens	Cucumber	*R. solani*, wilt (*F. oxysporum* f. spp. *cucumerinum*)
Agrobacterium radiobacter K84, K1026	Peach	Crown gall (*Agrobacterium tumefaciens*)
Avirulent *Ralstonia solanacearum*	Ginger	Bacterial wilt (*R. solanacearum*)

3.4.3 Plant Protection from Insects

Now bio-management is needed for various pests like viral, insect, phytoplasma, bacterial, nematode, and fungal diseases of horticultural crops, viz. vegetables, fruits, spice, plantation, tuber crops. No doubt synthetic pesticides were effective and help in achieving the higher productivity. Nowadays, pressures on farmers to reduce and eradicate the use of chemicals in fruits and vegetable to reduce the effects of chemicals on humans because they are consumed afresh. So the problems were more and more severe in the coming days; this concern will encourage for better alternatives which are eco-friendly and cheaper than synthetic pesticides. For that PGPR is one of the best-known enterprises to maintain soil and crop health through a variety of techniques.

3.4.4 Stress Management

Stresses (abiotic and biotic) are major cork to horticultural crop yield. Drought and salinity is one of the important environmental elements of abiotic stress, checking the productivity and growth of various crops including fruit, vegetables and flowers, in semiarid and arid areas. It was estimated that >50% average production loss worldwide was due to abiotic stress; the requirement of vegetables is enhancing daily for balance nutrition for exponentially increasing population globally that also

pushes the vegetable and fruit production in the present and/or in the future. Enhanced means of the production of important vegetables like cucumber, onion, potato, carrot, eggplant, cabbage, cauliflower, lettuce, pepper, spinach, etc. has been developed by many scientists (Shivakumar and Bhaktavatchalu 2017).

3.5 Future Prospective and Conclusion

It was found that almost all the PGPR significantly increased growth and development of plant like plant height, volume and root length and dry matter production in many horticultural crop plants. There is an urgent need to develop stable microbial formulations for sustainable agricultural production system which replaces chemicals use in crops. PGPR has the capacity to replace the chemical fertilizers and pesticides from horticultural as well as agricultural use. PGPR applications may also enhance input efficiency for fertilizer especially under organic and sustainable growing conditions. Besides, these PGPR are used as safeguard to biological resources as well as natural environments. It is also an integral part of IPM-integrated pest management. It would be felt that in coming era, study needed on the action mechanisms of microbes for easy to combine different strains, bacteria with fungi or bacteria with bacteria to control pathogens in broader spectrum. Use of biotechnology can also be a very useful tool to improve qualities of strains by transgenic strain creation combining multiple action mechanisms.

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4 Agriculture Application of *Pseudomonas*: A View on the Relative Antagonistic Potential Against Pests and Diseases

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Contents

Abstract

Agriculture is an important factor for improving economy of the country. Productivity of the crop is drastically reduced due to the incidence of biotic factors such as pests, diseases and nematodes as their infestation causes huge economic loss to the farmers. Biocontrol agents are excellent candidates for the reduction of biotic stresses and effective alternative to the chemicals as chemicals cause a huge menace to the environment. Among biocontrol agents, plant growth-promoting rhizobacteria (PGPR) is important group of root-colonizing bacteria which help in the promotion of plant growth in addition to the suppression

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of pests and diseases. *Pseudomonas* is an important candidate belonging to PGPR which is a gram-negative and rod-shaped bacteria. Efficacy of various strains of these bacteria in enhancing the plant growth and suppression of pest and diseases were well proved. This chapter deals with the pioneering and recent works of *Pseudomonas* in the management of pests, diseases and nematodes. This review will help in the research work that involves *Pseudomonas* as a potential bioagent in the management of pests, diseases and nematodes.

Keywords
Pseudomonas spp. · Insect pests · Diseases · Nematodes · Biomanagement

4.1 Introduction

Agriculture is an inevitable source of livelihood for the people living all over the world. It increases economy of the country by providing raw materials to nonagricultural-based sectors, export of agricultural products and providing employment to vast uneducated people. Owing to increase in the population of underdeveloped and developing countries, demand for the food supply is increasing in a faster pace. With increased production of agriculture crops, increased level of infestations by the insects, diseases and nematodes were observed. These biotic factors reduce the crop yield both quantitatively and qualitatively. Nowadays, application of chemicals against pests and diseases is restricted due to its contamination in the soil and environment. Microbial bioagents are promising approach to address the problems with soil dwelling insects and diseases. Effectiveness of microbial agents increases in the subterranean region, since it provides more favourable environment for the microbes in contrast to aboveground habitats.

Biological control is an eco-friendly method to control insects, pathogens and nematodes. It involves the application of certain other living organisms like microbes, predators and parasitoides or their products to control pests and diseases. It keeps the population of pests and diseases below economic injury levels and does not produce any toxic problem to the soil. Efficacy of many microbial biocontrol agents were proved against pests, diseases and nematodes (Khan 2007). Plant growth-promoting rhizobacteria (PGPR) are one among the microbial bioagents widely being used for the suppression of pests and diseases (Khan et al. 2009; Upadhyay and Srivastava 2010). Besides, they trigger the latent defence mechanism in the plants against biotic stresses (Ryals et al. 1996). Even though the level of control provided by the rhizobacteria could not be compared with the chemicals, they offer an effective protection to the plants against the incidence of biotic problems.

PGPR are group of free-living bacteria that lives in the rhizosphere and colonize the root system aggressively. They are known for its plant growth promotion

and biocontrol potential against insects, diseases and nematodes. Often, some of the rhizobacteria are feared as human or plant pathogens such as *Pseudomonas aeruginosa*/*Pseudomonas syringae*, some other species of *Pseudomonas* group are well known for their biocontrol potential. Among them, *Pseudomonas fluorescens* is one of the most extensively used rhizobacteria which received particular attention due to the root-colonizing ability and capacity to produce plant growth regulators (Khakipour et al. 2008), enzymes and metabolites (Raaijmakers et al. 2010). *Pseudomonas* is an aerobic, gram-negative bacterium that can be mass produced in vitro. These bacteria mainly help in plant growth promotion by the secretion of growth hormones like auxins, gibberellins and cytokinins (Vidhyasekharan 1998). Besides, they help in the solubilization of minerals and nutrients in the soil to get them easily absorbed by the plant roots (Berg and Smalla 2009; Khan et al. 2009).

Application of these bioagent brings about the natural suppressiveness to the soil. Some of the traits associated with *Pseudomonas* spp. in enhancing the biocontrol potential against pest, diseases and nematodes includes the fixation of atmospheric nitrogen, synthesis of phytohormones, solubilization of minerals, synthesis of antibiotics, secretion of iron-binding siderophores, production of secondary metabolites and enzymes and outcompeting pathogens for nutrients and niches. Brief descriptions on the different mechanisms of *Pseudomonas* were listed below:

4.2 Plant Growth Promotion (PGP)

It involves various mechanisms like nitrogen fixation; sequestration of iron by the production of siderophores; production of phytohormones like auxins, cytokinin and gibberellin; and lowering of plant ethylene level (Kavino et al. 2010). Presence of regulatory molecule, ACC deaminase, plays a significant role in the plant growth promotion. *Pseudomonas* that produce IAA increase the root surface area which enables the plant to access more nutrients from the soil (Barazani and Friedman 1999). Presences of ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) induce saline resistance in the plants and promote plant growth during the stress period (Saravanakumar and Samiyappan 2007).

4.3 Production of Siderophores

Pyoverdines, the yellow-green pigments produced by the *Pseudomonas*, are fluorescens under UV light and function as siderophores. Siderophores solubilize iron from their surrounding environment and form a ferric-siderophore complex which moves by diffusion to colonize the roots to exclude the invasion of deleterious microorganisms from the ecological niche (Haas and Defago 2005).

4.4 Production of Antibiotics

Antibiotics are low molecular weight compounds that are deleterious to the growth and metabolic activities of other microorganisms. Several strains of *Pseudomonas* spp. were found to produce wide array of antibiotics like 2, 4 diacetylphloroglucinol, hydrogen cyanide, kanosamine, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, pyocyanin and viscosinamide as well as several other uncharacterized moieties (Haas and Defago 2005).

4.5 Production of Lytic Enzymes

Lytic enzymes, viz. chitinase and ß-1, 3-glucanase, exhibit the biocontrol potential against plant pathogens and insect pests. They degrade the chitin and glucans in the fungal cell wall and associated with the osmotic disruption of the cellular membrane.

4.6 Induction of Systemic Resistance (ISR)

Induced resistance has been recognized as a vital tool in plant disease management. Plants establish multiple defence responses which include physical and chemical mechanisms (Pieterse et al. 2009) against the infestation of pests and diseases.

Biocontrol agents induce systemic resistance (ISR) in plants through fortifying the physical and mechanical strength of cell wall and by altering the physiological and biochemical reaction of the host against the infestation of pathogens. Defence reaction mainly occurs due to the accumulation of PR proteins (chitinase, ß-1, 3 glucanases), chalcone synthase, phenylalanine ammonia lyase, peroxidase, phenolics, callose, lignin and phytoalexins. These lytic enzymes help in the mycoparasitism to degrade the pathogen cell wall. The enzymes like chitinases and ß-1, 3 glucanases lyse the host cell wall and lead to the leakage of protoplasmic cell contents which can be used as a food source for the multiplication of antagonistic organisms.

4.7 *Pseudomonas* in the Management of Pests, Diseases and Nematodes

Detailed reports on the efficacy of *Pseudomonas* spp. against pests, diseases and nematodes were given below.

4.7.1 Management of Insect Pests Through *Pseudomonas* spp.

Pest control by the microbial bioagents received much attention towards the research side after the discovery of insecticidal toxins from the microorganisms like *Pseudomonas* spp. and *Bacillus* spp. Secondary metabolites produced by these microorganisms were associated with the insecticidal properties. Several strains of *P. fluorescens* were reported to exhibit insecticidal activity towards the agriculture pests such as aphids (Hashimoto 2002), phytophagous ladybird beetles (Otsu et al. 2004) and termites (Devi and Kothamasi 2009). In the same vein, combined formulation of two *P. fluorescens* strains was demonstrated to reduce the incidence of herbivorous insect, rice leaf roller (*Cnaphalocrocis medinalis*) and phytopathogenic fungus (*Rhizoctonia solani*) in rice under greenhouse and field conditions (Commare et al. 2002; Karthiba et al. 2010). Further, a number of *P. fluorescens* strains were reported to be effective against the common fruit fly, *Drosophila melanogaster*, and other widely used laboratory insect (De Lima Pimenta et al. 2003; Olcott et al. 2010). Protein extracts (Prabakaran et al. 2002) or secondary metabolites like HCN (Devi and Kothamasi 2009), lipopeptides viscosin (Hashimoto 2002) and orfamide (Jang et al. 2013) obtained from various strains of *P. fluorescens* were recorded with insecticidal properties. The following table describes in brief about the insecticidal potential of different strains of *Pseudomonas* against wide array of insect pests (Table 4.1).

4.7.1.1 Artificial Diet (D) or Plant Leaves (L)

P. aeruginosa is one of the commonly isolated bacteria from the insects. Pathogenicity of the bacterium is correlated with the production of proteolytic enzymes. Production of protease enzyme causes degenerative changes in haemocytes and digests certain specific insect haemolymph proteins (Lysenko and Kucera 1971). *P. maltophilia* retarded the growth of corn earworm, *Helicoverpa zea*, which reflected in the reduction of adult emergence of the pest (Bong and Sikorowski 1991). Application of *P. gladioli* affected the relative growth, consumption and digestibility of the feed by *Helicoverpa armigera* in cotton (Qingwen et al. 1998). Different strains of *P. fluorescens*, viz. Pf1, TDK 1 and PY 15, rendered notable reduction in the leaf folder damage in rice plants. Besides, their application increased natural enemy population (Saravanakumar et al. 2007). Tomato leaves treated with defence inducer, jasmonic acid (JA), along with *P. aeruginosa* recorded maximum larval mortality of *Spodoptera litura* under pot culture condition. It also recorded significant reduction of pupation rate, adult emergence and adult longevity of the pest. Activity of proteinase inhibitor, polyphenol oxidase (PPO) and lipoxygenase molecules was promoted by JA treatment (Melvin and Muthukumaran 2008) which partially contributed for the suppression of pest population.

In vitro, different *Pseudomonas* species and their metabolites exhibited insecticidal effect on various insect pests. Entomopathogenic activity of different *Pseudomonas* species was proved on the larvae and adults of alder leaf beetle, *Agelastica alni* (Sezen et al. 2004), and on the larvae of *Melolontha melolontha* (Sezen et al. 2007), *Phyllocnistis citrella* (Meca et al. 2009) and *Locusta migratoria*

Table 4.1 Insecticidal effect of different strains of *Pseudomonas* spp.

Strain and species	Target insect	Mode of application	References
Pseudomonas protegens			
CHA0	*Galleria mellonella*, *Manduca sexta*	Injection	Pechy-Tarr et al. (2008, 2013)
	Spodoptera littoralis	Feeding (D, L)	Ruffner et al. (2013)
	Heliothis virescens	Feeding (L)	Ruffner et al. (2013)
	Plutella xylostella	Contact (live cells)	Devi and Kothamasi (2009)
	Odontotermes obesus		
F6	*Myzus persicae*	Contact (purified metabolite)	Jang et al. (2013)
Pseudomonas chlororaphis			
30–84	*G. mellonella*	Injection	Ruffner (2013)
PCL1391	*S. littoralis*	Feeding (D, L)	Ruffner et al. (2013)
	H. virescens, *P. xylostella*	Feeding (L)	
AH1, FP7 and Pf1	*C. medinalis*	Feeding (L)	Commare et al. (2002)
HS870031	*Myzus persicae*, *Aphis gossypii*, *Aulacorthum solani*	Contact (purified metabolite)	Hashimoto (2002)
KPM-018P	*Epilachna vigintioctopunctata*	Feeding (oral injection, L)	Otsu et al. (2004)
Pseudomonas entomophila			
L48	*D. melanogaster*	Feeding (D)	Vallet-Gely et al. (2010) and Opota et al. (2011)
Pseudomonas syringae			
B728a	*Acyrthosiphon pisum*	Feeding (D, L)	Stavrinides et al. (2009)
Pseudomonas aeruginosa			
PA14	*G. mellonella*	Injection	Miyata et al. (2003)

(Mohandkaci et al. 2015). Formation of morphological defects in widely used laboratory insects was evident with the application of *P. fluorescens* (Flugge) strains (Pimenta et al. 2003). Application of *P. alcaligenes* caused septicaemia in the grubs of rhinoceros beetle under stress conditions (Gopal et al. 2002). *P. aeruginosa* isolated from dead grubs of epilachna beetle, *Henosepilachna vigintioctopunctata*, caused mortality of the grubs (Aswathy 2015). Haemocoelic injection of low dose of *P. fluorescens* CHA0 or Pf-5 was observed to induce mortal effects on the larvae of tobacco hornworm, *Manduca sexta*, and the greater wax moth, *Galleria mellonella* (Maria et al. 2008). *P. aeruginosa* and *P. putida* were reported to cause disease in spider mite, *Tetranychus urticae*. *P. entomophila* exhibited virulence against *Drosophila melanogaster* due to strong haemolytic activity exhibited due to the production of enzymes such as lipases, chitinases and/or hydrolases (Vodovar et al. 2006). Bacterial chitinases kill the insects by hydrolysing the chitinous exoskeleton of the insects (Kramer and Muthukrishnan 1997).

4.7.2 Management of Diseases Through *Pseudomonas* spp.

Application of *P. fluorescens* inhibited or displaced the soilborne pathogens at the root-soil interface in several the annual crops like cotton, potato, tobacco, flax, cucumber, sunflower, wheat and rice (Ganesan and Gnanamanickam 1987; Weller et al. 2002) and in the pulse crops like chickpea, pigeon pea and black gram (Jayashree et al. 2000). Incidence of anthracnose disease in mango got reduced, when *P. fluorescens* strain, FP7, was applied through foliar spray (Vivekananthan et al. 2004). Different strains of *Pseudomonas*, viz. Pf1, TDK1 and PY15, when applied combinedly, exhibited more effect in the reduction of sheath rot disease in rice under glasshouse and field conditions compared to their individual application (Saravanakumar et al. 2009). Similarly, chitin-based bioformulation of *P. fluorescens* enhanced the control against collar rot disease in groundnut (Senthilraja et al. 2010).

Enhanced resistance in the retardation of *Colletotrichum musae* infestation in the banana plants was recorded when the plants were treated with water in oil-based bioformulation of *P. fluorescens* (FP7) (Faisal et al. 2014). Even though the efficacy of *Pseudomonas* spp. has been proved against wide range of crops, their varied efficacy and consistency are still needed to be studied under complex environmental conditions. Application of fluorescent pseudomonads strengthened the cell wall structure of the plants to restrict invasion of the pathogens in the plant tissue (Chen et al. 2000). Induction of defence mechanism by *P. fluorescens* is the primary character of the bacteria against the diseases which was well observed in the *P. fluorescens* strain, Pf1, against the infestation of *F. oxysporum* f. sp. *lycopersici* in tomato (Manikandan and Raguchander 2014).

Antibiotic compounds produced by *Pseudomonas* have immense effect on the management of diseases. Production of siderophores by the bacteria plays a major role in disease management. Partially purified siderophore obtained from *Pseudomonas* strain, JAS-25, completely inhibited the spores of *F. oxysporum* f. sp. *ciceri*, *F. udum* and *A. niger* which completely degraded the mycelial hyphae of the phytopathogens (Sulochana et al. 2012). In a similar vein, hydrogen cyanide (HCN) obtained from the *Pseudomonas* strains exhibited bacteriostatic and antifungal action against phytopathogenic fungi (Alexandra et al. 2014). HCN obtained from *P. aeruginosa* (LES4) suppressed *F. oxysporum* f. sp. *radicis-lycopersici* in tomato (Sandeep Kumar et al. 2009). Cyanide producing pseudomonad, EA105, collected from the rice soil, effectively inhibited the growth and appressoria formation of *M. oryzae* (Spence et al. 2014).

In vitro, different strains of *P. fluorescens* suppressed the growth of fungus, by the production of one or more antifungal antibiotics (Whipps 2001). Antibacterial activity of DAPG/HCN produced by *Pseudomonas* sp. (LBUM 300) was recorded against bacterial canker of tomato (Lanteigne et al. 2012). Similarly, phenazine-1-carboxylic acid (PCA) obtained from *Pseudomonas* sp. (LBUM223) exhibited control against *Streptomyces scabies* (Arseneault et al. 2013).

Combined application of antibiotics obtained from the bioagents exerted greater effect over its individual inoculation. This concept was evident in the study

conducted against *R. solani* and *C. gloeosporioides* wherein production of the compounds, viz. phenazine-1-carboxylic acid, 2, 4 diacetylphloroglucinol and pyoluteorin by *P. aeruginosa* (FP6), exerted antifungal activity against those pathogens (Bakthavatchalu et al. 2013). Likewise, chitinase production from the *P. fluorescens* was recorded to induce antifungal activity against *Alternaria alternata*, *A. brassicicola* and *A. brassicaceae* (Ramyasmruthi et al. 2012).

4.7.3 Management of Nematodes Through *Pseudomonas* spp.

Plant-parasitic nematodes are microscopic organisms which occupy a diverse habitat on the earth. They are mostly subterranean in nature and affect the plant root system which deteriorates them. Mechanical damage caused by the nematodes paves way for the easy entry of secondary bacterial and fungal pathogens into the plants and results in disease complex which ultimately leads to death of the plants. Hence, disease complex should be taken into consideration for the development of effective management practices. The antibiotic, 2, 4 diacetylphloroglucinol (DAPG), produced by the *Pseudomonas* spp. reduced the mobility of nematode juveniles (Cronin et al. 1997) and induced systemic resistance in the plants by the synthesis and accumulation of peroxidase, chitinase and glucanase in the plant root system. Endophytic bacteria colonize the same root tissues where sedentary plant-parasitic nematodes feed. This made the bacteria, an excellent candidate, to work against nematodes. Production of siderophores (Siddiqui and Ehteshamul-Haque 2001), phenazine (Toohey et al. 1965), hydrogen cyanide (Voisard et al. 1989), ammonia (Gaur 1990) and pyrrolnitrin (Burkhead and Geoghegan 1994) by the bacteria contributed for the suppression of plant-parasitic nematodes (Khan 2007).

These secondary metabolic compounds suppressed nematode reproduction and survival, besides killing the nematodes directly by causing paralysis and convulsive movements (Siddiqui and Mahmood 1999). Production of phytohormones and cell wall lytic enzymes and increased activity of defence enzymes like PO, PPO, PAL and phenol are the major contributing factor of rhizobacteria that work against nematodes. *P. fluorescens* (Pf1) was adjudged as the best inducers of defence enzymes, chitinase and peroxidase, which is crucial for the induction of systemic resistance (Nandakumar et al. 2001) against nematode attack (Anita and Rajendran 2012).

Root-knot nematodes (*Meloidogyne* spp.) are the important group of sedentary endoparasites having a wide host range and cause serious damage to the solanaceous crops (Anamika et al. 2011; Sikora and Fernandez 2005). Second-stage juveniles (J2) of *Meloidogyne* spp. infect the plant roots and migrate to the vascular cylinder where it induces severe root galls (Karssen and Moens 2006). Even though the characteristic symptoms of the nematode occur belowground, in the aboveground, their infestation reflected in stunted growth, wilting and poor fruit yield.

Reduction in the multiplication of *M. incognita* with the application of *P. fluorescens* has been evident in many crops like tomato (Sankari Meena et al. 2002;

Hanna et al. 1999; Jothi et al. 2003), chickpea (Khan et al. 2001), turmeric (Srinivasan et al. 2001), banana, maize (Ashoub and Amara 2010; Siddiqui et al. 2007; Jonathan et al. 2006), chilli (Thiyagarajan and Kuppusamy 2014), mung bean (Khan et al. 2016) and tobacco (Khan and Haque 2011). *P. aeruginosa* (Rao 1990), *P. stutzeri* (Khan and Tarannum 1999) and *P. fluorescens* (Khan and Akram 2000) reduced the severity of root galls caused by *M. incognita/M. javanica* in various crops tested under controlled (pot culture) environment conditions. Reduction in the nematodes might be due to the induction of systemic resistance (Siddiqui and Shaukat 2003). Similarly, *P. putida* played a significant role in the reduction of *M. incognita* in okra (Rao et al. 2017) and pea (Akhtar and Panwar 2012; Siddiqui and Aakhtar 2008). The same bacteria recorded inhibitory effect on *M. javanica* in lentil (Siddiqui et al. 2007).

The antibiotic compound, 2, 4 diacetylphloroglucinol (DAPG), produced by *P. fluorescens* recorded to reduce the mobility of cyst nematode juveniles in potato (Cronin et al. 1997). *P. putida*, when applied along with *P. aurantiaca* through soil application, recorded to reduce the infestation of cyst nematode, *Globodera rostochiensis*, in potato. Since the expected level of control of cyst nematode could not be achieved with these bacteria, their effectiveness can be exploited through integrated control strategies (Trifonova et al. 2014).

Toxic compounds produced by the *Pseudomonas* spp. enhanced the mortality rate of the infective juveniles of *Hirschmanniella gracilis* infesting rice (Seenivasan and Lakshmanan 2001); lesion nematode, *Pratylenchus penetrans*, infesting banana (Senthilkumar et al. 2008); and *M. incognita* in vitro (Rajkumar et al. 2013; El-Hamshary et al. 2004). At very low concentration level (1%), the bacteria brought about nearly 77% mortality of root-knot nematode juveniles in vitro at the exposure period of 72 hours (Mane and Mhase 2017). This might be due to the presence of secondary metabolites in the culture filtrate (Hallamann et al. 2001; Ali et al. 2002).

4.7.4 Management of Disease Complex Through *Pseudomonas* spp.

Infestation of nematodes leads to the secondary infection by several soilborne fungal or bacterial pathogens which results in the disease complex in the plants (Taylor 1990). Several *Pseudomonas* spp. were identified with the properties to control the disease complexes. *P. aeruginosa* reduced the root-knot nematode (*M. javanica*) and fungal (*F. oxysporum*, *F. solani* and *Rhizoctonia solani*) disease complex in tomato (Siddiqui and Haque 2001). *P. fluorescens* (Pfbv 22 and Pf 1) reduced the disease complex caused by root-knot nematode, *M. incognita*, and wilt-inducing fungus, *F.* ox*ysporum*, in tube rose (Sankari Meena et al. 2016). Combination of *P. fluorescens* with *T. viride* and *P. lilacinum* brought about significant reduction of the disease complex caused by root-knot nematode, *M. incognita*, and wilt-inducing fungus, *F. oxysporum* f. sp. *conglutinans*, in cauliflower (Rajinikanth et al. 2013).

4.8 Compatibility of *Pseudomonas* with Other Microbial Agents/Chemicals

Pseudomonas spp. were compatible with many bioagents/chemicals. Combination of *Pseudomonas* spp. with other bioagents/chemicals brought about significant reduction of the pathogen load and nematode population in the plants. Effects of the bioagents were enhanced in the combination when compared with their individual effect. Application of *P. fluorescens* in combination with *Pochonia chlamydosporia* as seed treatment enhanced the growth of bell pepper and reduced the nematode infestation in the crop (Rao et al. 2004). *P. fluorescens* along with *T. viride* effectively reduced *M. incognita* population in mulberry and improved growth of the plant (Muthulakshmi and Devrajan 2015). The abovesaid combination had significant effect in the reduction of rhizome rot disease of turmeric (Surajit and Chowdhury 2008). The same bacteria, when combined with *T. harzianum*, retarded the growth of rice root-knot nematode, *M. graminicola* (Narasimhamurthy et al. 2017). Interestingly, when botanical product (seed powder of *Azadirachta indica*) and chemical nematicides (carbofuran and bavistin) were added with *P. fluorescens* and *T. harzianum*, a significant reduction in the disease complex due to *M. incognita* and *F. oxysporum* in green gram, *Vigna radiata* cv ML-1108, was noticed (Haseeb et al. 2005). In the same vein, combination of *P. chlororaphis* (PA23) and *B. subtilis* (BSCBE4) induced resistance of hot pepper to *P. aphanidermatum* (Nakkeeran et al., 2006).

4.8.1 Commercial Formulation of *Pseudomonas* spp.

Many strains of *Pseudomonas* species were already being marketed as commercial products against wide range of pests, diseases and nematodes. Some of the commercial products available in India are mentioned in the table below (Table 4.2).

4.8.2 Enhancement of Shelf Life of Bioagents

Addition of different chemical amendments to the bioformulation enhanced the viability of the bioagents in the formulation. Increased viability of *P. fluorescens* (Pf 1) in liquid formulation was recorded when the formulation was amended with glycerol (10 mM) (Sankari Meena et al. 2014). Reports of Manikandan et al. (2010) and Chavan and Kadam (2009) supported the above finding where they recorded increased the viability of *Pseudomonas* spp. and *Verticillium lecanii* with the addition of glycerol to the medium. Similarly, addition of ammonium molybdate to the bacterial formulation enhanced biocontrol potential of the inoculants and reduced the quantity of inoculant required to suppress the root-knot nematode population in the soil (Hamid et al. 2003).

Table 4.2 Commercial products of *Pseudomonas* sp.

Biocontrol agent	Product name	Product form	Manufacturer
Pseudomonas fluorescence	Monas	Talc	K.N. Biosciences Pvt. Ltd., Hyderabad, Telangana, India
Pseudomonas fluorescence	Ecomonas	Talc	PJ Margo Pvt. Ltd., Bengaluru, Karnataka, India
Pseudomonas fluorescence	Bio protector	Talc	Manidharma Biotech Pvt. Ltd., Chennai, Tamil Nadu, India
Pseudomonas fluorescence	*Pseudomonas fluorescence* 1% wp	Talc	Genuine Fert. and Pest Pvt. Ltd., Bengaluru, Karnataka, India
Pseudomonas fluorescence	*Biowin-PF*	*Talc*	Bioagri Solutions Pvt. Ltd., Hyderabad, Telangana, India
Pseudomonas fluorescence	Bas Monas	Talc	Basarass Biocon India Pvt. Ltd., Chennai, Tamil Nadu, India
Pseudomonas fluorescence	Gmax Phyton	Liquid	GreenMax AgroTech, Coimbatore, Tamil Nadu, India
Pseudomonas fluorescence	*Pseudomonas fluorescence*	Liquid	Anand Agro Care, Nashik, Maharashtra, India
Pseudomonas fluorescence	*Pseudomonas fluorescence*	Liquid	Universal Bio-Con Pvt. Ltd., Pune, Maharashtra, India
Pseudomonas fluorescence	Rotken	Liquid	Florken Sciences, Nashik, Maharashtra, India
Pseudomonas fluorescence	Alma	Liquid	Raven Biotech Inc., Coimbatore, Tamil Nadu, India
Pseudomonas fluorescence	Florilutions	Liquid	Agrolutions, Bhopal, Madhya Pradesh, India
Pseudomonas fluorescence	Pseudo Q	Liquid	OkBiosystems, Gudiyattam, Tamil Nadu, India

4.9 Conclusions

Biocontrol agents are effective alternative to the chemicals to protect the crops from the infestation of pests and diseases. *Pseudomonas* is an important bioagent in the group of plant growth-promoting rhizobacteria which gives substantial reduction of pest and disease load in the plants, besides triggering the plant growth. Several success reports on the *Pseudomonas* against the biotic stresses of the several agricultural and horticultural crops have been proven worldwide by various authors. Future research should be directed to find out more number of effective strains of *Pseudomonas* against pests, diseases and nematodes. This can be achieved by improving formulation and application techniques to enhance their mode of action to increase their colonization on the root surface. Mechanism of microbial bioagents generally involved with the production of antimicrobial compounds, the competition for nutrients and space and the induction of systemic resistance. Though they exert the mechanism of biocontrol against pests, diseases and nematodes, mode of action of each bioagents significantly varies with other bioagents. Combined

formulation of the bioagents is always better to their individual application as in the combined formulation, the bioagents interact synergistically with each other, and the combined mode of action of these bioagents will provide a strong resistance to the biotic stresses. In such cases, if one bioagent fails, the other one will enhance the resistance mechanism of the plants towards the pest and disease incidence.

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Plant Growth-Promoting Rhizobacteria as Biological Tools for Nutrient Management and Soil Sustainability

5

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Contents

Abstract

Rhizosphere is the unique vicinity which acts as a battlefield for soil microflora and a source of useful metabolites and nutrients for plants. Plant growth-promoting rhizobacteria (PGPR) associate with the roots of various plants to nourish them through direct and indirect mechanisms. Direct mechanisms of PGPR-based plant growth include nutrient acquisition from soil, production of various metabolites like phytohormones and siderophores, etc. Indirect

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mechanisms improve the plant health by controlling various plant pathogens by producing antibiotics, hydrolytic enzymes, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, induction of systemic resistance, etc. In this chapter, the brief introduction about PGPR and their interactions with plant roots are presented followed by a detailed insight into the mechanisms utilized by PGPR in rhizosphere to boost plant growth. In addition, some factors that affect PGPR-plant associations are elaborated. Finally, it has been reviewed that, if wisely utilized, PGPR may serve as future prospects to supplement chemical fertilizers.

Keywords
PGPR · Mechanisms · Rhizosphere · Plant growth · Health improvement

5.1 Introduction

The plant growth-promoting rhizobacteria (PGPR) are agriculturally imperative bacteria presented in the rhizosphere. They are the natural companions of plants making soil nutrients available and suppressing the growth of phytopathogens (Babalola 2010). Kloepper and Schroth (1978) described PGPR as "soil bacteria that after incorporating in to seed colonize plants roots and enhance the plant growth." Soil is enriched with microbes including bacteria, which contribute substantially to its fertility and health (Schoenborn et al. 2004). PGPR enhance plant growth and development by utilizing the variety of mechanisms (Singh et al. 2017). Direct mechanisms make the bound nutrients available to plants by mobilization or solubilization processes, while indirect mechanisms improve plant health by controlling the diseases caused by pathogens (Ahemad and Kibret 2014). These PGPR reside in the rhizosphere, which is a hot area of soil in terms of microbial activity (Walker et al. 2003). Biological control activity of PGPR is a consequence of processes such as competition, antagonism, and siderophore or antibiotic production (Anith et al. 2004).

Rhizosphere provides much information about microbial diversity as the microbes inhabiting the soil utilize variety of carbon and nitrogen sources (Gray and Smith 2005; Jones et al. 2004). Microbes inhabiting the rhizosphere feed on root exudates of plant, and, in turn, these microbes improve the nutrients uptake by plant roots by providing unavailable nutrients from soil (Lugtenberg and Kamilova 2009). Rooting pattern and nutrient supply to plants are affected by the bacterial action leading to change in the nature of molecules secreted by roots. Microbes metabolize a fraction of these organic molecules in order to fulfill their carbon and nitrogen requirements, and consequently, plants utilize some of the microbe-derived molecules for their nourishment (Kang et al. 2010; Sivasakthi et al. 2014). Various

species of *Bacillus*, *Pseudomonas*, *Azospirillum*, *Streptomyces*, *Serratia*, *Enterobacter*, *Azotobacter*, *Arthrobacter*, *Bradyrhizobium*, *Flavobacterium*, *Mesorhizobium*, *Alcaligenes*, *Burkholderia*, *Rhodococcus*, *Klebsiella*, etc. have been documented to promote plant growth and suppress infectious agents (Ahmad et al. 2008; Fernández et al. 2007; Gupta et al. 2017; Shahid et al. 2018).

Moreover, microbial communities of rhizosphere also include various *Actinomycetes* having plant-beneficial role (Bhattacharyya and Jha 2012). Among various rhizosphere species, *Bacillus* and *Pseudomonas* are well characterized for their potential as PGPR (Bottini et al. 2004; Jangu and Sindhu 2011). The utilization of beneficial microbes from soil in order to improve crop production depends on rhizosphere-competent microbes with phytobeneficial potential (Hynes et al. 2008).

This chapter focuses on the PGPR-plant interaction and a brief summary of the basic PGPR-based mechanisms in rhizosphere. Finally, the future prospects of PGPR-based plant growth and health are described in detail in order to draw the logical conclusions.

5.2 Interactions Between PGPR and Plant Roots

The rhizosphere is a battlefield of complex interactions for plants and its microflora with (Raaijmakers et al. 2009). *Azospirillum*, *Rhizobium*, *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Beijerinckia* are among those bacterial species that are well characterized for their rhizospheric interactions (Berg 2000; Chakraborty et al. 2009). All PGPR through antibiosis and competition improve plant health indirectly by stopping growth and activities of soil-inhabiting pathogens (Badri et al. 2009). PGPR have direct beneficial impacts on the health of plants by provoking the systemic resistance against the attack of pathogen or by exposing of plants to PGPR-oriented compounds (Bhattacharyya and Jha 2012). However, in order to examine mechanisms of PGPR-based plant growth, chemistry of rhizosphere, a driving force of microbial attraction, is often ignored. To explore the complex interactions between plant and PGPR at molecular level and to harness their benefits in agriculture, further studies are required. PGPR interact with plants both extracellularly and intracellularly, and huge research insights are required to examine the complexity and outcomes of such interactions.

5.3 PGPR-Based Plant Growth and Health Improvement Mechanisms

PGPR are known to colonize root surfaces (rhizospheric) or inside the tissues of roots (endophytes) (Kumar et al. 2015). A detailed sketch of mechanisms adopted by PGPR for plant nourishment is presented in Fig. 5.1, and various direct and indirect mechanisms are given below.

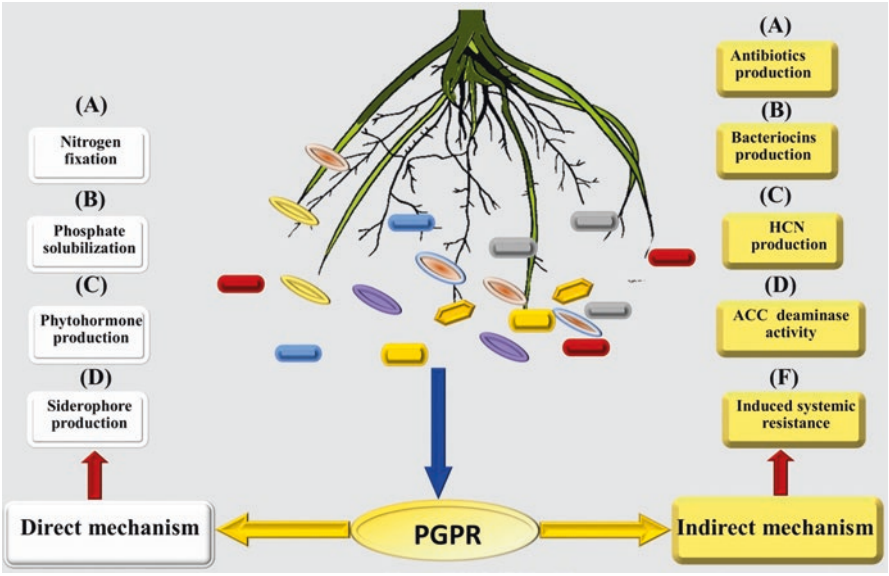

Fig. 5.1 Mechanisms of PGPR-based plant growth and health improvement

5.3.1 Direct Mechanisms

5.3.1.1 Nitrogen Fixation

Nitrogen (N_2) is an essential plant nutrient, and molecular N_2 present in the atmosphere cannot be utilized by plants directly due to the high input of energy for its conversion to plant-available form (Santi et al. 2013). The N_2 is converted into the nitrates and nitrites by fixation process of either symbiotic microbes or free-living diazotrophs (Jackson et al. 2008). The molecular N_2 is utilized by plants by biological nitrogen fixation (BNF), a process of converting N_2 to ammonia (NH_3) by nitrogen-fixing bacteria/rhizobacteria equipped with nitrogenase enzyme system (Ahemad and Kibret 2014).

Although *Rhizobium* and *Bradyrhizobium* are main genera involved in BNF, several *Enterobacteriaceae* family species are also documented as diazotrophs, mainly those isolated from plant rhizosphere. The enteric genera with some representative diazotrophs include *Citrobacter*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, and many unidentified species (Hayat et al. 2010). Moreover, other bacterial species having nitrogen-fixing activity include *Beijerinckia derxii*, *Azotobacter vinelandii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas putida*, *Citrobacter freundii*, and *Pseudomonas fluorescens* (Akram et al. 2017; Kashyap et al. 2017; Kumar et al. 2018).

5.3.1.2 Phosphate Solubilization

Phosphorous (P) is one of the essential nutrients for plants because its shortage restricts crop production up to a dangerous level. Tropical and subtropical soils are

considered P-deficient due to their high acidity (Kamilova et al. 2006). Phosphorus is present in soils in huge amounts both in organic and inorganic form. Thus, in most cases, there is no need of exogenous application of P if already available P pool is made available to plants. Various microbes solubilize inorganic form of phosphates in soil such as rock phosphate (RP). Mineral phosphate-solubilizing bacterial (PSB) species such as *B. megatherium*, *Enterobacter*, *Erwinia*, *O. anthropi* TRS-2, and *Pseudomonas* striata are reported to mobilize the soil-bound phosphates (Chakraborty et al. 2009). They made soil rich in organic acids, thereby making the soil acidic and releasing the bound phosphates. *Azotobacter*, *Beijerinckia*, *Enterobacter*, *Serratia*, *Burkholderia*, *Microbacterium*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Erwinia*, and *Flavobacterium* are among the most important bacterial genera reported for solubilizing soil phosphates (Bhattacharyya and Jha 2012; Shahid et al. 2012, 2015, 2018; Mahmood et al. 2018).

The PSB also improve the plant growth by fixing atmospheric nitrogen, providing the plants with other trace elements like Zn, Fe, etc., or by synthesizing the substances that are important for plant growth (Ahemad 2015).

5.3.1.3 Production of Phytohormone

Five groups of phytohormones are synthesized by PGPR such as auxins, gibberellins, cytokinins, abscisic acid, and ethylene. These phytohormones are very important because they act as communication signals between the plant host and its microflora (Tsavkelova et al. 2006). Auxin, especially indole-3-acetic acid (IAA) production by microbes, has been of great significance and reported a long time ago. It has been stated that ~80% of microbes isolated from rhizosphere of numerous crops synthesize auxins as secondary metabolites.

Consequently, IAA is very essential in plant-rhizobacterial interactions (Spaepen and Vanderleyden 2011). Various rhizobacterial species, such as *Agrobacterium* spp., *Enterobacter* spp., *Azospirillum* spp., *Alcaligenes* spp., *Azotobacter* spp., *Acetobacter* spp., *Rhizobium* spp., *Erwinia* spp., *Herbaspirillum* spp., and *Bradyrhizobium* spp., have been documented as auxin-producing bacteria (Tsavkelova et al. 2006). Many rhizospheric bacteria such as *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Acinetobacter*, *Micrococcus*, *Pseudomonas*, *Agrobacterium*, *Flavobacterium*, *Rhizobium*, *Bacillus*, *Xanthomonas*, *and Clostridium* are known to produce gibberellins (Tsavkelova et al. 2006).

Moreover, rhizobacteria belonging to genera *Azotobacter*, *Arthrobacter*, *Rhizobium*, *Azospirillum*, *Bacillus*, and *Pseudomonas* have been reported for their cytokinin production ability. Thus, PGPR have tremendous ability of phytohormone production. Studies have indicated that IAA, gibberellins, and cytokinins produced by PGPR are beneficial for plants in terms of their nourishment and signal transduction. PGPR also help plants to regulate the endogenous ethylene levels in response to various stresses (Spaepen et al. 2008).

5.3.1.4 Siderophore Production

PGPR secrete low-molecular-weight siderophores with iron-chelating ability, making it very difficult for other microbes to access iron. Siderophores bind the soluble

form of iron from soil to make it available to plants (Chakraborty et al. 2009). Thus, siderophore-Fe complex is up taken by plant roots (Crowley 2006) making the soil environment Fe-deficient for pathogenic fungi (Sharma and Johri 2003). *Pseudomonas fluorescens*, and *Pseudomonas putida* are the best-known siderophore-producing PGPR species. Under Fe-limiting conditions, many pathogens especially fungi are unable to thrive normally (Compant et al. 2005). Siderophores synthesized by *Pseudomonads* have high Fe-chelating ability and, thus, are considered as bio-control agents (Cornelis 2010; Weller et al. 2002).

5.3.2 Indirect Mechanism

The indirect mechanisms are basically the process employed by PGPR to control phytopathogens to make conducive environment for normal plant growth. PGPR are able to control infectious agents by generating growth inhibitors (i.e., antibiotics, antioxidants, and lysis enzymes) or by improving the natural physiological and genetic potential of plants to fight with the pathogens (Paul et al. 2001). Thus, it is possible that rhizobacteria associations trigger some natural mechanisms in plants, a process known as induced systemic resistance (ISR) (Lugtenberg and Kamilova 2009).

Many rhizobacterial species such as *Pseudomonas fluorescens*, *Proteus secrete*, and *Bacillus* have been reported to produce a variety of antifungal molecules under in vitro conditions (Verma et al. 2013). Furthermore, ISR plays a significant role in signalling of jasmonate and ethylene inside the plant cells, and these hormones are involved in the induction of systemic resistance in plants (Glick 2012). The mechanisms involved in controlling plant pathogens by PGPR are competition, fast growth, antibiosis, bacteriocin synthesis, extracellular release of hydrolytic enzymes, and siderophores production (He and Yang 2007).

Some genera of *Serratia*, *Enterobacter*, *Pseudomonas*, *Burkholderia*, *Herbaspirillum*, *Staphylococcus*, *Ochrobactrum*, and *Stenotrophomonas* are known antagonistic species against the plant pathogens (Han et al. 2005; Parke and Gurian-Sherman 2001). PGPR can secrete enzymes to easily disrupt the fungal cells by the lysis of mycelia and hyphae. For instance, fungal cell lysis occurred when extracellular enzymes laminarinase and chitinase were produced by *Pseudomonas stutzeri*. For potential biological control species like *Pseudomonas fluorescens*, fast growth is required in order to control many diseases such as damping-off of sugar beet, root rot of wheat, root rot of pea, and black root rot of tobacco (Sivasakthi et al. 2014). In citrus and avocado plants, some root pathogens are suppressed due to microbial antagonists that are added by organic wastes (Sultana et al. 2006). The mechanisms utilized by antagonist PGPR to lessen the phytopathogenic proliferation are presented in Fig. 5.2.

5.3.2.1 Antibiotics

Antibiotics are low-molecular-weight organic compounds causing direct restriction in metabolism and cell growth of various microbes (Mazhar et al. 2016). The

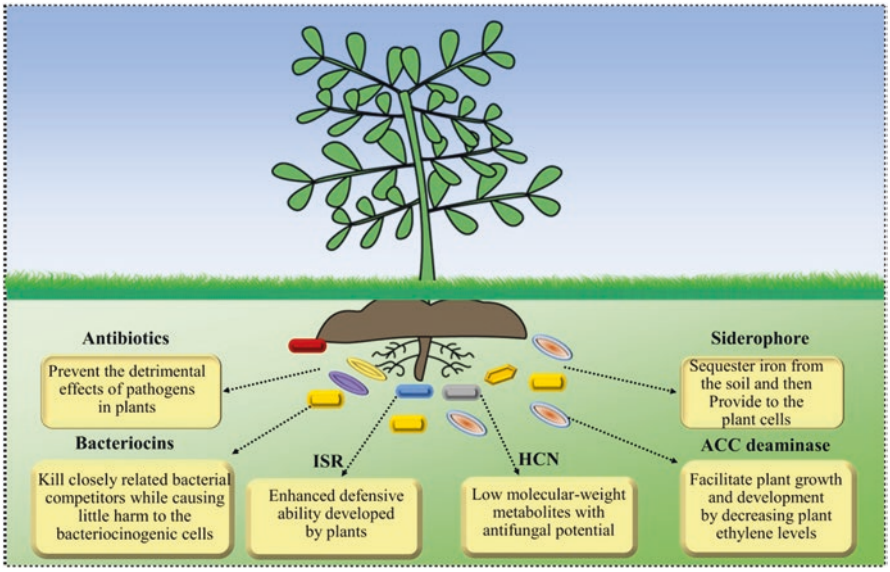

Fig. 5.2 Mechanisms utilized by PGPR to control phytopathogens

synthesis of antibiotics is supposed to be most efficient treatment and antagonistic activity to inhibit the phytopathogens. Various microbes are known to produce these compounds while residing in the rhizosphere zone (Bharti and Tewari 2015; Zhou et al. 2016). PGPR produce some antibiotics, e.g., kanosamine, 2,4-diacetylphloroglucinol (2,4-DAPG), etc. (Martínez-Viveros et al. 2010). The bacterial strain of *P. fluorescens* BL915 is involved in the synthesis of antibiotic known as pyrrolnitrins, which have the ability to inhibit the growth of *Rhizoctonia solani* in cotton. Another antibiotic compound 2,4-DAPG is produced by *Pseudomonads* and is most effectively studied antibiotic for membrane destruction of *Pythium* spp. (de Souza et al. 2003).

Pseudomonads are also known to synthesize phenazine compound with great antibiotic potential against pathogens such as *Gaeumannomyces graminis* and *Fusarium oxysporum* (Beneduzi et al. 2012). Some antibiotic molecules (circulin, polymyxin, and colistin) are produced by *Bacillus* spp. and are widely used to control not only fungal but also bacterial pathogens (Maksimov et al. 2011). Thus, antibiotics play a key role in plant-bacteria interactions in terms of plant biomass and health benefits (Fernando et al. 2005).

5.3.2.2 Bacteriocins

Proteinaceous toxins referred as bacteriocins are produced by PGPR living in a highly competitive environment with plant pathogens. The PGPR is the main group of microbes involved in production of these inhibitors (Riley and Wertz 2002). Bacteriocins have very effective and recognized mechanism to inhibit or reduce the phytopathogenic growth (Beneduzi et al. 2012). Colicin proteins are

most prominent bacteriocins synthesized by *Escherichia coli*. Some other bacteriocins such as megacins, marcescins, cloacins, and pyocins are actively synthesized by *B. megaterium*, *Serratia marcescens*, *Enterobacter cloacae*, and *P. pyogenes*, respectively, and are used in biocontrol experiments (Cascales et al. 2007; Abriouel et al. 2011).

5.3.2.3 Hydrogen Cyanide (HCN)

PGPR produce a low-molecular-weight compound with antifungal potential known as hydrogen cyanide (HCN) (Bashan and De-Bashan 2005). For many metal enzymes, HCN acts as an inhibitor, e.g., cytochrome c oxidases. An enzyme known as HCN synthetase is involved in the synthesis of HCN from glycine (Blumer and Haas 2000). Many bacterial species such as *Alcaligenes*, *Rhizobium*, *Aeromonas*, *Bacillus*, and *Pseudomonas* have the potential to synthesize HCN (Charest et al. 2005; Ahmad et al. 2008; Kumar et al. 2014). The role of HCN in the suppression of root knot and black rot in tomato and tobacco caused by the nematodes *Thielaviopsis basicola* and *Meloidogyne javanica*, respectively, is well established (Siddiqui 2005; Martínez-Viveros et al. 2010). Furthermore, HCN synthesized by *Pseudomonas* spp. in the rhizosphere reduced root proliferation in *Arabidopsis* as a result of the inhibition of an auxin-responsive gene (Rudrappa et al. 2008; Martínez-Viveros et al. 2010).

5.3.2.4 ACC Deaminase Activity

PGPR with inherent potential to synthesize the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase make plants able to tolerate ethylene stress by cleaving its precursor ACC into α-ketobutyrate and ammonia (Glick et al. 2007). Ethylene is a hormone that can induce an abnormal growth in plants leading to the early cell death. The higher ethylene concentrations can be modulated by the onset of the bacterially synthesized ACC deaminase enzyme (Ma et al. 2003).

In many types of soil with high salt and metal concentrations, ACC deaminase-producing bacteria can be inoculated with plant roots in order to make them tolerant to high ethylene concentrations (Saharan and Nehra 2011; Akram et al. 2016; Shahid et al. 2018). ACC deaminase permits decrease the ethylene levels in plants and enhance the nodulation and consequently biomass (Nukui et al. 2000). A wide variety of bacterial isolates that have ACC deaminase activity such as *Achromobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Burkholderia*, *Agrobacterium*, *Pseudomonas*, *Agrobacterium*, *Ralstonia*, *Bacillus*, *Staphylococcus*, and *Rhizobium* have been documented (Blaha et al. 2006; Akram et al. 2016). Thus, different ACC deaminase-producing plant growth-promoting bacteria are being willingly used under stressful conditions.

5.3.2.5 Induced Systemic Resistance (ISR)

Disease suppression may occur by nonpathogenic rhizobacteria that induce a resistance in plants against the deleterious effects of pathogens, a mechanism known as induced systemic resistance (ISR). It is a condition which activates the defense-related genetic and physiological attributes in plants upon the onset of a disease or

adverse environmental conditions (Beneduzi et al. 2012). In plants, ISR is similar to pathogen-induced (SAR) systemic acquired resistance in which inducing bacteria and the challenging pathogen persisted. Thus, induced resistance provides more strength to plants against pathogen (Silveira et al. 2012). It is quite possible that same strain produces resistance against many pathogens in the same plant. Especially, *Pseudomonas* and *Bacillus* spp. are the rhizobacteria mostly examined to activate the ISR (Kloepper et al. 2004; Van Wees et al. 2008). Resistance-inducing and antagonistic rhizobacteria may be collectively valuable in the formulation of new inoculants due to their cumulative effect for biocontrol strategies to efficiently enhance agricultural productivity (Tariq et al. 2017).

5.4 Factors Affecting the PGPR Colonization in the Rhizosphere

There are some biotic and abiotic factors that influenced the PGPR species in rhizosphere. The most efficient bacterial strains are the ones that effectively colonize the roots in the rhizosphere area in order to exert their growth-promoting effects (Prashar et al. 2014). PGPR diversity is influenced by following factors and is represented in Fig. 5.3.

5.4.1 Biotic Factors

Plant is a very important factor in determining bacterial strains that are predominantly near the root zone in terms of its root exudation and chemotaxis. Plant-related

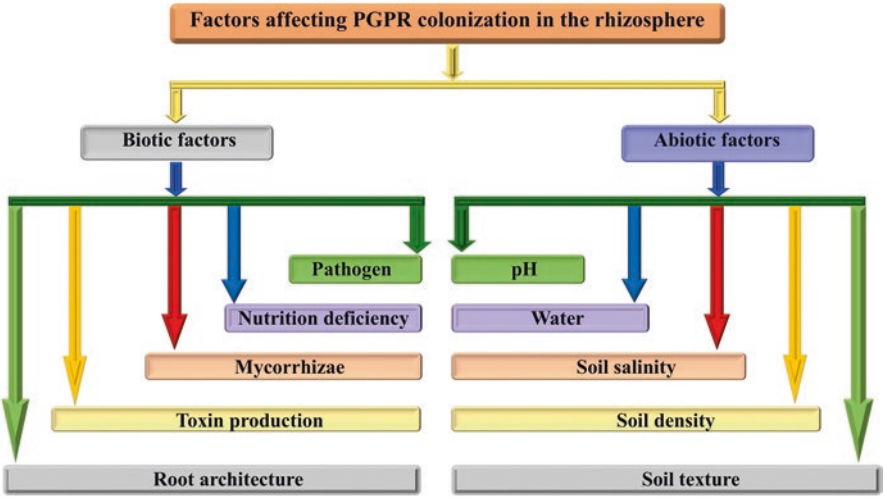

Fig. 5.3 Factor affecting PGPR-based plant growth

characteristics influencing the bacterial colonization include age of the plant, cultivar and root architecture, etc. (Prashar et al. 2014; Santi et al. 2013). Plant age and growth rate are the key decisive factors for bacterial communities in order to colonize the roots and make subsequent establishments in the rhizosphere. Rhizosphere deposits also play an important role to decide which type of bacterial communities would be attracted toward the roots, and these deposits are again linked with plant species, age of plant, root architecture, and type of microbial communities associated with roots (Adesemoye et al. 2008).

However, the metabolic adaptability and functional diversity of PGPR that are well established in rhizosphere are due to many genetic factors possessed by them or due to their interaction with plants itself along with eukaryotic and prokaryotic organisms. The interactions in the rhizosphere and bulk soil are quite different due to the direct influence of roots (Mena-Violante and Olalde-Portugal 2007).

5.4.2 Abiotic Factors

Soil is the medium of plant growth and survival, and it can directly influence bacterial population establishing nearby plant roots. Numerous physical and chemical attributes of soil affect the nutrient availability and physiological and morphological features of newly established PGPR species (Dutta and Podile 2010). Thus, soil texture, nutrient concentration, organic matter, soil pH, temperature, and management practices (residue incorporation, fertilizer, tillage, pesticide, irrigation, and cropping) are the main factors that influence the colonization of bacteria in the rhizosphere (Prashar et al. 2014). Moreover, climatic conditions such as temperature, humidity, wind speed, sunshine, etc. also alter the bacterial population and diversity in the rhizosphere.

5.5 PGPR as Future Prospects

There is a lot to acquire from bacterial associations and existing mechanisms of PGPR-related plant growth due to the poor understanding of complex plant-soil-bacteria interactions. In addition, there exists a great need to explore new mechanisms that plant-associated bacteria adopt to change the plant physiology. During the last few decades, major focus of scientists working on plant-associated bacteria was on *Rhizobium*-based symbiotic nitrogen fixation system. But there is still a lot to learn from no-symbiotic associations of rhizospheric and endophytic bacteria that show diverse patterns of colonization and remarkably alter the growth of host plant (Compant et al. 2005; Husen 2016; Akram et al. 2017). New insights into PGPR-based mechanisms will open new horizons to use state-of-the-art scientific approaches for effective exploitation of PGPR as growth stimulators, modulators, and biocontrol agents (Walsh et al. 2001). For example, in biocontrol activity, identification of role of edaphic factor that increases the antibiotic activity and production is an important factor (Compant et al. 2005).

Similarly, modification of number and diversity of rhizosphere bacteria to effectively utilize raw materials like molasses, sugars, and crop residues can open new insights into the plant-microbe interactions (Gamez et al. 2016; Welbaum et al. 2004). Moreover, less attention is given to organic fraction of soil phosphorus for solubilization/mobilization, and characterization of PGPR strains with inherent potential for organic phosphorus mobilization may reduce the use of chemical phosphatic fertilizers. Similarly, bacterial genetic mechanisms for soil phosphorus solubilization are poorly understood, and attention should be paid to explore new bacterial genetic elements in order to mobilize mineral and organic fractions of soil phosphorus. Recognition of several mechanisms that facilitate the association of bacterial strains with plants and fungi is necessary. Moreover, application of gene transfer techniques like cloning and transformation and genome-editing techniques like CRISPR-Cas9 can be applied to bacterial strains in order to harness their genetic potential (Table 5.1).

Table 5.1 Respective mechanism and representative species involved in PGPR-based plant growth

Chemicals	PGPRs	Beneficial effects	References
Direct mechanism			
Nitrogen fixation	*Azotobacter vinelandii*, *Bacillus*, *Rhizobium*, *Beijerinckia derxii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Pseudomonas putida*	The atmospheric N_2 is converted into plant-utilizable forms and hence improvement in plant development and yield	James et al. (2000), Meunchang et al. (2006)
Phosphate Solubilization	*Pseudomonas* striata, *Enterobacter*, *Erwinia*, *Bacillus megaterium*, *Ochrobactrum anthropi*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Rhizobium*, and *Serratia*	Solubilizing inorganic phosphorus from insoluble compounds and available to the plants	Chakraborty et al. (2009)
Phytohormones production	*Azotobacter*, *Arthrobacter*, *Azospirillum*, *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Flavobacterium*, *Enterobacter*, *Micrococcus*, *Agrobacterium*, *Clostridium*, *Rhizobium*, and *Xanthomonas*	Play an important role as regulators of growth and development of plants	Akhtar and Siddiqui (2009), Bottini et al. (2004), Tsavkelova et al. (2006)
Siderophore production	*Pseudomonas fluorescens*, *Rhodococcus*, *Acinetobacter*, and *Pseudomonas putida*	Solubilize and sequester iron from the soil and then provide it to the plant cells	Chakraborty et al. (2009), Compant et al. (2005)
Indirect mechanism			
Antibiotics	*Pseudomonas* and *Bacillus*	Prevent the detrimental effects of pathogens on plants by production of inhibitory substances	Chaiharn et al. (2009), Verma et al. (2013)

(continued)

Table 5.1 (continued)

Chemicals	PGPRs	Beneficial effects	References
Bacteriocins	*Escherichia coli*, *Acinetobacter*, and *Pseudomonas putida*	Utilized by antagonist to inhibit or reduce the phytopathogenic growth	Beneduzi et al. (2012), Compant et al. (2005)
Hydrogen cyanide (HCN)	*Alcaligenes*, *Rhizobium*, and *Aeromonas*	Play an important role as inhibition of an auxin-responsive gene	Ahmad et al. (2008), Charest et al. (2005), Kumar et al. (2014)
ACC deaminase activity	*Achromobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Burkholderia*, *Agrobacterium*, *Pseudomonas*, *Agrobacterium*, *Ralstonia*, and *Rhizobium*	Plant growth and promotion by decreasing the ethylene level	Blaha et al. (2006), Meunchang et al. (2006)
Induced systematic resistance	*Pseudomonas* and *Bacillus*	Provide more resistance to plants parts against pathogen	Kloepper et al. (2004), Van Wees et al. (2008)

5.6 Conclusion

Plant growth-promoting rhizobacteria (PGPR) are underestimated or ill-studied to explore their potential for plant growth promotion. The main reasons for their non-acceptance at wider level are variability in outcomes, slow action, lack of farmer's counseling, and lack of knowledge about their method of application. Being microbes, their physiological and genetic potential depend on many environmental factors including both biotic and abiotic ones; thus the proper understating of these factors can boost the PGPR-linked agricultural output. In future, the replacement of chemical fertilizers with PGPR-based formulations is a huge challenge for researchers, farmers, industrialists, and policy makers.

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Rhizobacteria-Mediated Root Architectural Improvement: A Hidden Potential for Agricultural Sustainability

6

Sakthivel Ambreetha and Dananjeyan Balachandar

Contents

Abstract

Plant growth-promoting rhizobacteria (PGPR) have been studied over centuries for their role in increasing nutrient uptake, providing various growth hormones and mitigating biotic and abiotic stresses. The production of growth hormones, organic acids and enzymes for nutrient mineralization and solubilization; rhizoremediation of heavy metals; synthesis of osmoprotectants, antioxidants, hydrolytic enzymes and antifungal compounds; quorum quenching; release of siderophores; etc. have been so far linked with plant-beneficial PGPR activities. Apart from these roles, a novel trend in PGPR-mediated plant benefits is struc-

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tural modification of the root system architecture of the associated plants. Root system is the most important organ that satisfies most of the plant needs but unfortunately left unattended in research areas. However, there are certain studies reporting the capability of PGPR to improve or strengthen the plant root system architecture (RSA). Synchronizing the ability of PGPR to improve the root development of field crops is lacking which would be highly useful to help the crops sustain the adverse conditions. In the present review, PGPR-mediated root architectural improvements are linked to plant growth promotion. Various mechanisms adopted by different PGPR for RSA alteration and the resulting multiple benefits to the plant are highlighted and sequentially explained. This article would facilitate detailed understanding on PGPR-mediated RSA studies and pave a platform for further exploration of PGPR related to RSA improvement for achieving sustainable yield of agriculturally important crops.

Keywords
Gene regulation · Hormonal modulation · Rhizobacteria · Root architecture · Sustainability

6.1 Introduction

Plant growth-promoting rhizobacteria (PGPR) refer to beneficial bacterial community dwelling in the rhizospheric region of plant. Upon colonization, they provide macro- and micronutrients from the soil, release growth hormones and induce resistance against biotic and tolerance against abiotic stresses to their plant partner through diversified direct and indirect mechanisms. The proliferation of symbiosis between plant and PGPR also improves the soil aggregation, nutrient carrying capacity and resilience of soil during stress conditions. It is also evident that the healthy plant-microbe interactions could ensure the soil carbon build-up. Apart from these, a new insight to rhizobacterial-based plant improvement is modification of root system architecture (RSA) of a plant. The root topology, spatial arrangement and number and length of root hairs constitute RSA which is crucial for crop anchorage, proficient uptake of water and minerals and efficient microbiotic interactions. PGPR adopt several mechanisms to structurally shape the plant root which mostly becomes highly useful for the growth and sustainability of the plant. Although there are a lot of studies to prove this concept, a holistic approach to explore this idea for plant improvement is still at rest. Very few conceptual papers alone are available for understanding the PGPR-mediated RSA shaping in plant species (Sukumar et al. 2013; Vacheron et al. 2013).

In this review, we discuss about the importance of root architecture in relation to plant growth and fitness; key PGPR as root architecture modifiers; direct role of PGPR in alteration of RSA including rhizobacterial genome-mediated, hormone-mediated and papillae formation; and indirect role of PGPR metabolites in

alteration of plant genetic and hormonal pathways in relation to RSA. The practical difficulties in RSA studies are explained, and the future thrust of microbial-mediated RSA modifications in connection with agricultural sustainability is elaborated.

6.2 Root Architecture and Its Importance

Root system constitutes an important part of any plant and well-studied for its role in anchorage, nutrient and water acquisition, storage (tuber crops) and shelter for rhizospheric microbiota. On this account, root system serves as the heart of the plant without which the entire system would collapse. Root architecture refers to spatial arrangement and distribution of every single component of the root system such as root diameter, number and length of root hairs, undulations of the root axis, root cap and topology, number, length and spatial distribution of primary roots, adventitious roots and lateral roots (Lynch 1995). Root system comprises of several components, some of which are formed at embryo, while others develop postembryonically (Fig. 6.1).

RSA is highly plastic and varies depending on the availability of soil resources, environmental factors, plant type and associated microorganisms. As the water and nutrients are unevenly located in soil, spatial arrangement of root system is most important for effective resource exploitation (Lopez-Bucio et al. 2003). Root

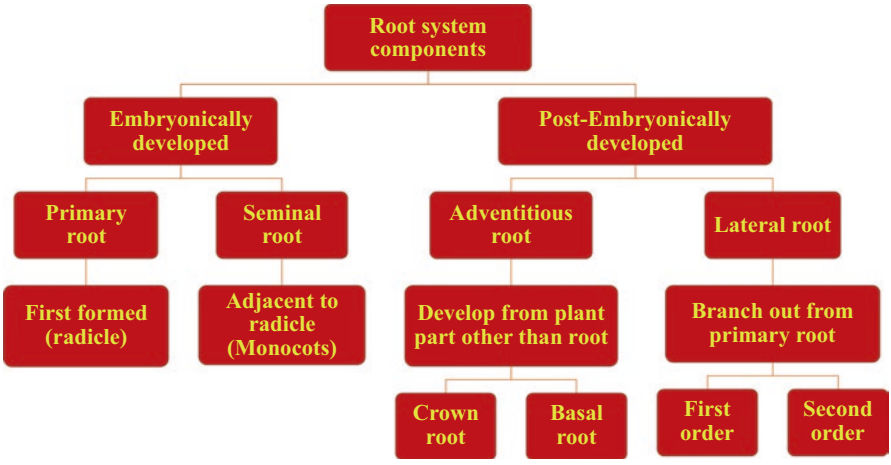

Fig. 6.1 Components of root system and their order of development in a plant: in most of the land plants, radicle of the germinating seed or embryo develops into primary root system. In monocots, seminal roots arise adjacent to the radicle and are responsible for initial absorption of water and nutrients. Both primary and seminal roots develop during seed embryogenesis. For instance, crown or nodal roots develop from the nodes, and basal roots arise from the basal portion of the stem. Lateral roots also develop in later stages but branch out from primary roots. These are first-order lateral roots which give rise to second-order lateral roots and so on

system has a holistic potential to sense and respond to abiotic and biotic stresses in soil and communicate with the aerial plant parts through signalling pathways (Schachtman and Goodger 2008).

Morphology and physiology of the root impact the growth and development of aerial organs through alteration of root to shoot transport of mineral nutrients or organic signalling molecules including hormones and proteins (Ron et al. 2014). For example, whenever plant encounters drought stress, the root communicates to the rest of the plant and reduces the level of endogenous cytokinin production.

This in turn inhibits the shoot growth and enhances root elongation aiding in deeper search for water resource. Endogenous abscisic acid level shoots up on decline in cytokinin which stimulates stomatal closure and prevents transpiration loss (Arkhipova et al. 2007). At the same time, root architecture alters itself according to the type of nutrient demand. Whenever phosphorous deficiency occurs in the soil, plants reduce their primary root length while promoting dense lateral roots and extended root hairs and form clustered bunch of roots to facilitate wide exploration of soil to fetch phosphorous (Niu et al. 2013; Peret et al. 2014). In contrast to this, the plants respond different to nitrogen quest by increasing the primary and lateral root length and ensure deeper resource hunt (Kong et al. 2014). Similarly, plant seeks the help of root architecture to tolerate the water deficit condition. Deep-rooted plants possess extreme tolerance to drought as they percolate the soil to greater depth and uptake the water available in deeper soil zones (Yu et al. 2008; Comas et al. 2013). Root hairs, lateral roots and un-suberized young root tips are directly involved in horizontal and vertical soil exploration for water and mineral uptake (Suzuki et al. 2003).

In the case of tuber crops such as yam, potato and cassava, where the economically important or edible part lies below ground, the root architecture is directly linked to yield potential (Khan et al. 2016). Apart from these, photosynthetic exudates released by the plants get leaked into the soil through the root system, and this contributes to the enrichment of nutrients in the rhizosphere soil (soil under influence of the root). This ultimately attracts millions of microbes, which colonize in and around the root and sometimes travel throughout the plant system, and this is popularly called ‘rhizospheric effect’. The microbes in turn contribute to enhanced plant growth, biotic stress resistance, abiotic stress tolerance and root architecture shaping through various mechanisms. On the whole, the root system architecture delivers endless benefits to the plant system either directly or through recruitment of microbial volunteers.

6.3 Direct Role of PGPR on RSA Modification

PGPR, a sub-group of rhizo-microbiome, isolated and characterized from different crops and regions were reported to modify the root architecture (Vacheron et al. 2013), while no attempts were made to document total rhizo-microbiome effect on RSA modification. Hence, we have summarized the impact of individual PGPR strains on the changes in RSA, rather than whole microbiome approach. Most of the

PGPR adopt certain direct mechanisms to structurally design or shape the root architecture of the associated plants. RSA modulations are generally accomplished by employment of root-inducing (Ri) plasmid, synthesis of growth hormones and triggering certain depositions within root tissues. These RSA modifications serve as one of the important factors helping plants either to withstand stress or to achieve increased productivity, and hence this concept is being agriculturally exploited.

6.3.1 Rhizobacterial Genome-Mediated RSA Modification

Rhizobium rhizogenes is a gram-negative soil-inhabiting bacterium responsible for overproduction of adventitious roots at the site of infection, which was identified for crazy root syndrome (Riker et al. 1930). Phenolic compounds such as acetosyringone released by wounded plants chemotactically attract *R. rhizogenes* (De Cleene and De Ley 1981). The bacteria transfer its Ri plasmid into the plant through horizontal gene transfer facilitated by transfer DNA (t-DNA) (Fig. 6.2c). This bacterial gene on integrating into the plant genome triggers the rapid emergence of hairy roots. These roots are shoot-derived as they arise from hypocotyl region and their similarity with primary root was proved in *Arabidopsis* (Lucas et al. 2011). However, these hairy roots differ from adventitious roots by few characteristics. They possess one additional layer of cortex (Ron et al. 2014), and most interestingly their growth is agravitropic and highly plagiotropic (Veena and Taylor 2007), while the regular adventitious roots are gravitropic and less plagiotropic. However, *Agrobacterium*-induced RSA modifications are highly useful in biotechnological aspects as the hairy roots can grow and proliferate rapidly under in vitro conditions, without exogenous plant growth promoters. They serve as ideal tool for plant-pathogen interaction studies, secondary metabolite production, genetic engineering and bioremediation, which has been already detailed in various review papers (Tepfer et al. 1989; Hu and Du 2006; Georgiev et al. 2007; Veena and Taylor 2007) and is beyond the scope of the present review.

6.3.2 Rhizobacterial Hormone-Mediated RSA Modulation

One of the well-documented strategies of PGPR for RSA improvement is the release of hormones which regulates plant growth. Generally, these hormones are called phytohormones when endogenously produced by the plants. Interestingly, the derivatives of auxins, cytokinins, ethylene, gibberellins and abscisic acid are exogenously supplied by PGPR and notably influence the plant growth in specific manner (Arshad and Frankenberger 1997). Whenever there is a fluctuation in the ratio of growth hormones, the crop faces architectural changes, which can be exploited for shaping the crop desirable to withstand stress and provide sustainable yield. In this aspect, microbially derived plant growth regulators have particularly contributed to RSA modifications (Table 6.1). *Azospirillum*, the

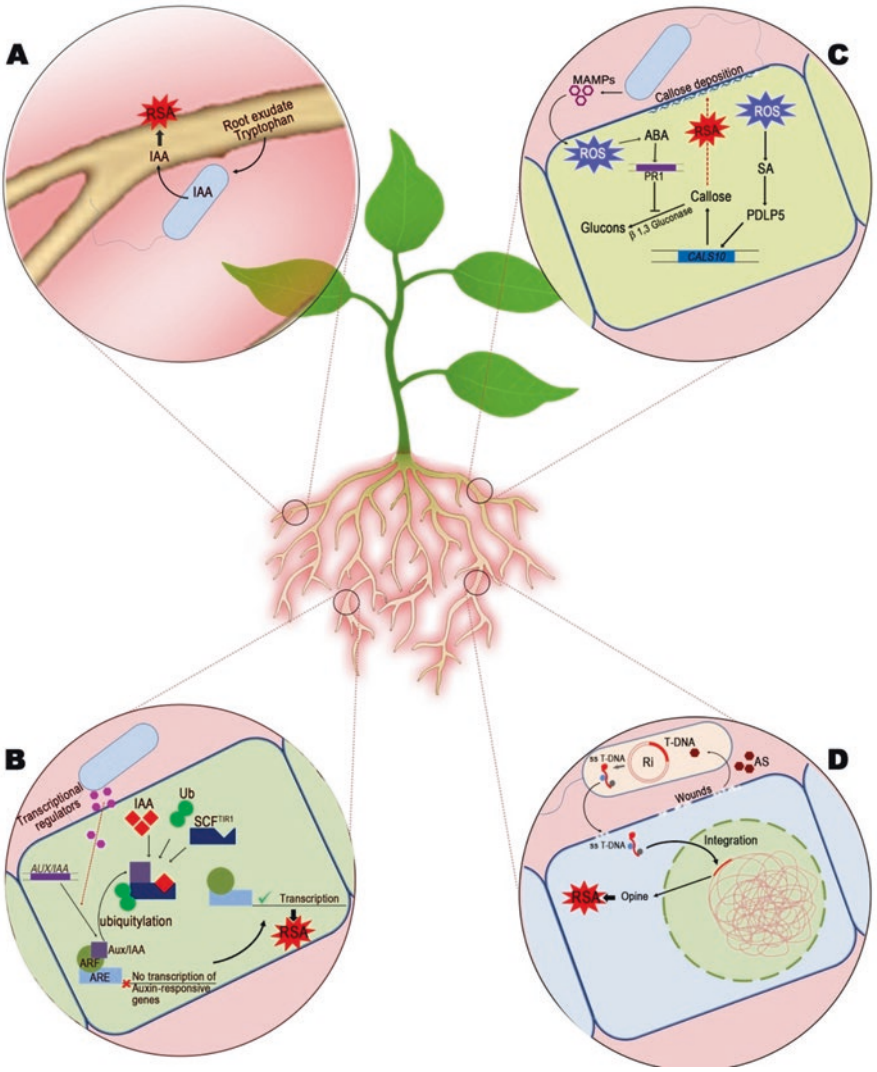

Fig. 6.2 Mechanisms of microbial-mediated root architecture changes in plants. Root architecture of a plant gets altered through four different mechanisms under the influence of rhizobacteria. (**a**) Rhizobacterial hormone-mediated RSA modulation – tryptophan released during root exudation is converted into IAA by soil-dwelling bacteria which is uptaken by the plants. The resulting alteration in root IAA level creates impact over RSA. (**b**) Genetic regulation of RSA-related hormonal pathways of plant by PGPR – auxin-responsive gene of plants contributes to their root architecture shaping. Transcription of these genes is inhibited when their promoter, auxin-responsive element (*ARE*), is blocked due to binding of auxin-responsive factor (ARF) with auxin/indole-3 acetic acid (Aux/IAA). Aux/IAA factor is removed and degraded by ubiquitylation process in the presence of IAA, ubiquitin (Ub) and SCFTIR1 to switch on ARE operon. PGPR release certain unknown compounds which act as transcriptional regulators of *AUX/IAA* which is one among the key genes involved in this process and thereby contributes to plant RSA modulation. (**c**) Rhizobacterial genome-mediated RSA modification – occurrence of wound or damage to the root leads to the

associative symbiotic nitrogen-fixing plant growth-promoting bacteria profusely colonizing the monocot roots, is the first bacterial strain reported to be the root modifier (Barbieri et al. 1986).

Among the rhizobacterial hormones, indole-3-acetic acid (IAA) is being explored right from the 1990s when scientists believed only auxin derivatives contributed to RSA improvement (Fig. 6.2a). Later on, certain studies revealed the contribution of other growth hormones released by rhizobacteria for RSA modifications (Joo et al. 2004; Arkhipova et al. 2007; Asari et al. 2017). PGPR converts tryptophan present in the root exudates into physiologically active form of auxin, IAA, which at lower concentration improves elongation of primary root and at higher concentration supports lateral root formation (Casimiro et al. 2001).

PGPR synthesized zeatin – a cytokinin compound – improves root exudation and reduces root/shoot ratio, while ethylene released by PGPR stimulates root hair formation at lower concentrations. Abscisic acid and gibberellic acid synthesized by PGPR are least explored for its role in root system modification although few literatures have documented their effects (Gutierrez-Manero et al. 2001; Bottini et al. 2004; Cohen et al. 2008).

6.3.3 PGPR-Induced Papillae in Root Cells Leading to RSA Modification

Upon colonization, PGPR instructs various cellular- and tissue-level structural changes in the root system of the associated plant by inducing depositions or papillae in root cells which mainly constitute callose, phenol and lignin. These depositions enhance the disease-resistant ability of the plant by hardening its cell wall (Schmelzer 2002). Callose is a polysaccharide comprised of glucose molecules with ß-1, 3 linkages, while lignin is a complex organic polymer with cross-linked phenolic compounds. These are usually secreted by the plant cell in response to wounds, infection or pathogen entry.

Fig. 6.2 (continued) release of acetosyringone (AS) which attracts *Rhizobium rhizogenes* towards it and activates the synthesis of single-stranded (ss) T-DNA from bacterial plasmid. ss T-DNA gets transported into the root cell through the wound and integrates into plant chromosome which is facilitated by bacterial virulent factors indicated as red-, blue- and grey-coloured circles. Bacterial T-DNA integrated into plant chromosome initiates opine synthesis which induces root architectural alterations. (**d**) PGPR-induced callose deposition for RSA modification – microbe-associated molecular patterns (MAMPs) released by PGPR induce generation of reactive oxygen species (ROS) within plant cells. ROS generation in turn leads to increase in level of salicylic acid (SA) and abscisic acid (ABA). SA directly triggers callose deposition by regulating the plasmodesmata-located protein 5 (PDLP5)-dependent expression of callose synthase gene (*CALS10*). In contrast, ABA indirectly stimulates higher deposition of callose by blocking the expression of *PR1* and inhibiting the synthesis of callose-degrading enzyme (ß 1,3gluconase). On the whole, PGPR induces callose deposition in root cells which leads to change in RSA

Table 6.1 Role of PGPR-mediated growth hormones in crop-specific RSA modifications

PGPR	Crop	Growth hormone	RSA modification	References
Azospirillum brasilense	*Triticum aestivum*	IAA	Increase in number and length of lateral roots	Barbieri et al. (1986) and Barbieri and Galli (1993)
Bacillus and *Rhizobium*	*Phaseolus vulgaris*	IAA	Promote root growth and nodulation	Srinivasan et al. (1996)
A. Brasilense	*Triticum aestivum*	IAA	Decrease in root length and increase in root hair formation	Dobbelaere et al. (1999)
A. brasilense and *Klebsiella pneumonia*	*Oryza sativa*	IAA	Development of lateral roots and root hairs. Increase in root surface area and root dry matter	El-Khawas and Adachi (1999)
Pseudomonas putida	*Vigna radiata*	IAA	Increase in number of adventitious roots	Patten and Glick (2002)
Unauthenticated PGPR	*Triticum aestivum*	IAA	Root elongation	Khalid et al. (2004)
Unauthenticated PGPR	*Oryza sativa*	IAA	Increase in root length	Ashrafuzzaman et al. (2009)
Bacillus, *Paenibacillus* and *Comamonas*	*Actinidia deliciosa*	IAA	Promote root formation in stem cuttings	Erturk et al. (2010)
A. brasilense	*Arabidopsis thaliana*	IAA	Increase in number of lateral roots and root hairs	Spaepen et al. (2014)
A. brasilense	*Solanum lycopersicum*	IAA and ethylene	Increase in root hair length and root surface	Ribaudo et al. (2006)
Bacillus amyloliquefaciens	*Arabidopsis thaliana*	IAA and cytokinin	Increase in lateral root outgrowth and root hair formation	Asari et al. (2017)
Bacillus sp.	*Lactuca sativa*	Cytokinin	Shorten root length, but increase total root mass	Arkhipova et al. (2007)
Bacillus subtilis	*Platycladus orientalis*	Cytokinin	Reduce root/shoot ratio	Liu et al. (2013)
Bacillus pumilus	*Capsicum annuum*	Gibberellic acid	Increase in root fresh weight	Joo et al. (2004)

Interestingly, colonization of PGPR also induces these depositions in root cell wall and intercellular spaces without causing infection to the host plant (Fig. 6.2d). However, this would aid the plant system to prevent or block the entry of pathogens rather than responding after infection (Ramamoorthy et al. 2001). In some cases, rhizobacteria activate the phenylpropanoid pathway of the plant and induce

Table 6.2 Documented studies on the role of PGPR-mediated root depositions for pathogen resistance

Crop	PGPR	Pathogen(s) resisted	Root deposition	References
Pisum sativum	*Pseudomonas fluorescens*	*Pythium ultimum* and *Fusarium oxysporum* f. sp. *pisi*	Callose	Benhamou et al. (1996a)
Pisum sativum	*Bacillus pumilus*	*Fusarium oxysporum* f. sp. *pisi*	Callose and phenol	Benhamou et al. (1996b)
Lycopersicon esculentum	*Pseudomonas fluorescens*	*F. oxysporum* f. sp. *radicis-lycopersici*	Callose	M'Piga et al. (1997)
Strawberry	*Azospirillum brasilense*	*Colletotrichum acutatum*	Callose and phenol	Tortora et al. (2012)
Piper betle	*Serratia marcescens*	*Phytophthora nicotianae*	Phenol	Lavania et al. (2006)
Lycopersicon esculentum	*Pseudomonas fluorescens*	Spotted wilt virus	Lignin	Kandan et al. (2002)
Gossypium hirsutum	*Bacillus cereus*	*Xanthomonas axonopodis* pv. *malvacearum*	Lignin	Ishida et al. (2008)
Pearl millet	*Bacillus pumilus*	*Sclerospora graminicola*	Lignin	Niranjan Raj et al. (2012)
Cicer arietinum	*Pseudomonas*, *Trichoderma* and *Rhizobium*	*Sclerotium rolfsii*	Lignin	Singh et al. (2013)

overaccumulation of lignin (Kandan et al. 2002). These phenomena are being exploited to provide disease resistance to the plants. Various cases reported for enhanced disease resistance offered to the plants through rhizobacterial-induced root depositions are tabulated (Table 6.2). Considering the PGPR-induced RSA modifications of a plant, this trait has less influence.

These cellular-level modifications were reported to have more significant role for disease resistance, rather than root modification for nutrient and water acquisition. Further research is required to decipher these cellular-level chemical depositions on the root architecture-related benefits.

6.4 Indirect Role of PGPR on RSA Modifications

Apart from the direct genetic element- and hormone-mediated root modifications, PGPR can interfere the hormonal pathways of plant and modulate their hormonal levels and subsequently cause the changes in the root architecture. The interference may be either due to small molecules and enzymes of PGPR or due to direct regulation of those pathway genes by PGPR.

6.4.1 Regulation of RSA-Related Hormonal Pathways of Plant by Metabolites and Enzymes of PGPR

Volatile and non-volatile compounds emitted by PGPR either in the rhizosphere or within the plant roots dictate the hormonal pathways and thereby causing RSA alterations. For instance, *Azospirillum brasilense* and *Pseudomonas fluorescens* produce secondary metabolites such as 2,4-diacetylphloroglucinol (DAPG) and nitric oxide (Bergsma-Vlami et al. 2005) which interfere in auxin-dependent signalling pathway of plants. The former controls the lateral root formation (Creus et al. 2005), while the later induces root branching (Brazelton et al. 2008). There are few studies on coculture of *Arabidopsis* with *Bacillus* demonstrating the effect of bacterial volatiles on hormonal pathways (Ryu et al. 2004; Olah et al. 2005; Ali et al. 2010; Gutierrez-Luna et al. 2010). In all the above-mentioned cases, the root architecture of *Arabidopsis* such as lateral root growth and root hair formation was altered justifying that the impact of volatiles on plant hormonal pathways indirectly influences the RSA. Apart from *Bacillus*, volatile blend of *Pseudomonas fluorescens* WCS417 contains diketopiperazine which interferes with auxin biosynthesis and enhances lateral root and root hair formation in *Arabidopsis* (Zamioudis et al. 2013). *Serratia marcescens* influences multiple signalling pathways including auxin to increase the lateral root growth followed by primary root inhibition in *Arabidopsis* (Shi et al. 2010). A novel compound N-acylethanolamine (NAE) is released by plants as a response to bacterial communication by N-acyl homoserine lactone (AHL). This compound is a mimic of bacterial AHL and has been reported to affect the primary root growth, lateral root formation and root hair development in *Arabidopsis* seedlings (Ortiz-Castro et al. 2009). The PGPR enzyme reported so far to be a RSA modifier is 1-aminocyclopropane 1-carboxylate deaminase (ACCD).

PGPR produce ACCD to downregulate stress ethylene pathway, which cleaves the ethylene precursor ACC released by plant roots. Thus, ACC reuptake and accumulation in roots get reduced, thereby preventing ethylene overproduction during stress. This effect in turn ensures root elongation, which would otherwise get inhibited under abiotic stresses (Glick 2005). ACC deaminase-mediated root growth promotion or modification has been well-documented with *Azospirillum*, *Methylobacterium*, *Bacillus*, *Enterobacter* and *Pseudomonas* (Li et al. 2000; Saleh and Glick 2001; Madhaiyan et al. 2006; Shaharoona et al. 2006; Chinnadurai et al. 2009). There are numerous functionally undetermined compounds released by various PGPR groups that play a vital role in organizing the plant hormonal pathways leading to various architectural changes. A lot of exploration of the total metabolomic profile of PGPR is required to identify the functions of all the volatile and non-volatile compounds exudated by PGPR so as to tune them for the benefit of plants.

6.4.2 Genetic Regulation of RSA-Related Hormonal Pathways of Plant by PGPR

High-throughput investigations involving transcriptomic analysis are recently emerging to detect the role of PGPR on plant RSA. Although several hormonal pathways get altered due to PGPR, auxin is the only hormone extensively analysed at molecular level due to its dominant role in root architecture shaping (Sukumar et al. 2013). It has been demonstrated that coculture with *Bacillus subtilis* increases auxin accumulation in roots. This effect was masked when 1-naphthylphthalamic acid (auxin transport inhibitor) was applied.

Thereby, their experiment proved that this particular PGPR strain upregulates auxin transporter genes leading to transport of auxin from shoot (site of synthesis) to root and this induces RSA alterations (Zhang et al. 2007). The concept of gene regulation by PGPR with respect to auxin was also proved by comparative study with mutants (silencing the genes involved in auxin pathway). Inoculation of wild and IAA transport and signalling mutants of *Arabidopsis* (*AUX1* and *AXR1*) with *Phyllobacterium brassicacearum* led to 50% increase in lateral root length of wild and no effect over that of mutants. This indicates the role of auxin-related genes of plant for PGPR-mediated RSA modifications (Contesto et al. 2010). Later, Zamioudis et al. (2013) used auxin perception and signalling mutants of *Arabidopsis* and evidenced auxin-dependent reduction in lateral root development during *Pseudomonas fluorescens* inoculation. These studies ascertain the fact that once auxin-responsive genes are knocked out in plants, PGPR could not influence its RSA. Hence, it is evident that PGPR influence the RSA by regulating the expression of auxin-related genes in plant.

One step ahead, the bacterial compounds (*Pseudomonas aeruginosa*) responsible for activating auxin-inducible gene expression in *Arabidopsis* were identified to be cyclodipeptides and their derivative, diketopiperazine (Ortiz-Castro et al. 2011). Similarly, DAPG released by *Pseudomonas fluorescens* modified the RSA of wild tomato through regulating auxin pathway genes, which was nullified in auxin-resistant diageotropica mutant tomato (Brazelton et al. 2008). Based on these reports in *Arabidopsis*, we tried to unravel the involvement of auxin signalling genes (*AUX/IAA*) of *Oryza sativa* (rice) for PGPR-mediated RSA changes.

When we traced the time course abundance of six different transcripts (*OsIAA1*, *OsIAA4*, *OsIAA11*, *OsIAA13*, *OsAA14* and *OsIAA23*) of *AUX/IAA* family in *Bacillus altitudinis* (FD48) inoculated rice seedlings, vast variations were found compared to uninoculated rice. Positively modified root architecture with differing expression pattern of auxin signalling genes was noticed in FD48 inoculated rice seedlings indicating that this PGPR strain alters rice RSA by regulating the genes involved in auxin pathway (Ambreetha et al. 2018) (Fig. 6.2b). However, these results are only the inklings and need whole transcriptome analyses to unravel the regulatory systems involved in the auxin-responsive pathway genes so as to use it for the crop productivity under unfavourable conditions. Besides, genetic regulation of other hormonal pathways such as cytokinin and ethylene due to PGPR inoculation is least explored, although they considerably contribute to RSA changes.

6.5 Constrains to Study Microbial-Mediated RSA

Investigation of below ground part of a plant is not as easy as that of visible aboveground parts. Research papers on shoot architecture of the plant are certainly higher than root architecture studies due to practical constrains at laboratory and field level. Field-level experiments deliver real-time results and help to understand the effects under natural varied conditions. On this note, RSA studies would be better if done in open field rather than greenhouse or laboratory level. Unfortunately, investigation of microbial-mediated RSA at field level is very hectic due to one or many of the following reasons.

Root architecture is highly sensitive to abiotic and biotic factors and gets altered erratically under natural conditions. Soil is highly heterogenic and has varying physical and structural properties within a single field. This may affect the uniformity of root architecture even among the crops growing in one field. Moreover, water holding capacity and nutrient distribution of soil may not be uniform at all sites of a field, and root naturally gets directed accordingly. Despite these difficulties, there are certain high-throughput technologies generated for field level study of root architecture.

A group of scientists developed X-ray micro-computed tomography scanning and RooTrak that can non-invasively track the three-dimensional view of moving objects in soil (Mairhofer et al. 2012). Another group of authors innovated combined field imaging and algorithmic approach to assess the root systems under natural condition (Bucksch et al. 2014). However, both technologies have their own pros and cons. Root system is highly plastic and determined by various environmental and genetic factors which cannot be controlled under open-field conditions. Plants get influenced by almost all external factors, and it is highly unpredictable to spot one particular source for root architectural alterations. One can never expect similar number of lateral roots or root length even for two near most plants. Adding to these issues, root architecture analysis at field requires destructive method of sampling unless we afford for high-cost technologies (Trachsel et al. 2011). Another notable factor is diversified groups of soil microorganisms thriving in and around the plants. It is not an easy task to identify which among the million microbes has caused root architectural changes in that plant and is practically tedious to provide sterile soil for the entire field and maintain it throughout the cropping period. These are the major constrains that hinder the researchers from carrying out large-scale field trials regarding microbial-mediated root architecture changes.

Laboratory study provides certain advantages such as maintenance of controlled, microbe-free culturing condition to analyse the effect of particular PGPR over the RSA of a particular plant. Results can be reproduced efficiently due to the absence of impact from external factors. Moreover, easy and non-destructive imaging of RSA is possible by allowing the plants to germinate and grow in gellan gum either in glass box or tubes (Nakamura et al. 2006; Kitomi et al. 2011; Shrestha et al. 2014; Ambreetha et al. 2018) or under hydroponics (Xu et al. 2013) or in rhizobox (Courtois et al. 2013).

The entire architecture of the root can be monitored and imaged all through the experimental period using high-throughput imaging software such as RootScan, RootNav, DART, GiA Roots, IJ Rhizo, Root System Analyser, RootReader2D, RootReader3D and RSML. Despite these advantages, laboratory study possesses certain constrains compared to the field study. First issue is, it is not always possible to artificially provide the naturally occurring form of nutrients to the plant under in vitro culturing. The architecture gets influenced by the shape of the container and nutrient composition of the media used. There are chances for other microbial contamination as plants are grown in nutrient-rich medium, and hence completely sterile environment with controlled light and temperature has to be maintained. However, there is an unresolved question whether all the metabolic and enzymatic activities occurring in the plant will remain similar under laboratory and field condition. Maintenance of huge number of samples as that in field trials is also not possible. Anyhow, regarding microbial-mediated RSA study, it is advisable to go for gnotobiotic investigation to predict the exact mechanism employed by particular organism. Every PGPR differs in its mode of action and impact over plant RSA which can be precisely traced in vitro. Once the concept is proved at laboratory scale, field trials can be done by fertilizing the plants with that particular PGPR in sufficient loads, and RSA changes can be confirmed under natural condition.

In recent years, rhizotron has been in limelight to understand the plant-microbe-soil interactions, which mimic the near field condition, but with all controls as that of in vitro culturing system (Bauke et al. 2017; Atkinson et al. 2019). Rhizotron is a sophisticated device, which observes the root architecture and growth, and its microbiome and physico-chemical changes in the soil in non-destructive way throughout the crop period in real time will help to answer many of the key questions we postulated as above.

6.6 Future Thrust

The study of PGPR-mediated RSA alteration lays a platform to identify the organisms that positively modulate the architecture of agriculturally important crops. Most of the PGPR-influenced RSA modifications have been proved in *Arabidopsis* and not taken to other important crops. A lot of steps have to be crossed to make this concept useful for agricultural productivity. Once a single or consortium of PGPR that effectively improve the root architecture is authenticated, it is possible to structurally shape that crop in such a way to efficiently absorb water and minerals, withstand abiotic stresses and increase the productivity. Right from last century, microbes are used as biofertilizers only to solubilize and transport the minerals from soil to plant or fix it from gaseous form in the atmosphere to plant uptake able form. The development of inoculants that would improve the plant RSA will be a unique approach and serve as a new thrust for crop improvement.

Moreover, scientists are already involved in improving plant RSA and creating resistant varieties through molecular breeding approaches. Compared to those efforts, the use of inoculants would be easy, less time-consuming, environmentally

friendly and cost-effective method for stimulating plant growth and sustainability. An important point to be noted is plant rhizosphere is already rich in microbiota that is naturally recruited by root exudates. In our approach we are only dropping the right PGPR for a plant in the right time so as to help the plant to grow and produce more efficiently. As stated earlier, most of the PGPR render multifarious benefits to the plants and need not be curtailed to RSA improvement alone. PGPR strains can be screened for maximum number of plant benefits and that would be a promising strategy for assuring crop sustainability in the future.

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Role of Rhizobia for Sustainable Agriculture: Lab to Land

7

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Contents

Abstract

Rhizobia is symbiotic diazotrophic soil bacteria infecting the roots of leguminous plants to form root nodules to fix molecular atmospheric nitrogen (N_2) with the aid of nitrogenase enzyme, turning it into a more readily usable form for plants.

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Rhizobia also possess plant growth-promoting (PGP) properties witnessed by a series of molecular dialogue between the plant and the bacteria. Unraveling such mechanisms gave the insight toward multifunctional approach of rhizobia in the rhizosphere of legumes and compatible plants. Bioavailability of nutrients in the soil is enriched by rhizobial action due to metal solubilization and siderophore activity. The combined activity of phytohormones, enzymes, and siderophores contributes toward the growth and development of the concerned plant along with easy nutrient uptake and phytoremediation. Besides, rhizobia aid in biocontrol through antibiosis, parasitism, or competition with different pathogens for essential nutrient uptake. This has made it an important candidate for sustainable agriculture in various economies across the globe.

Keywords
Rhizobia · Sustainable agriculture · Plant growth promoting · Bioavailability

7.1 Introduction

The rapid growth of world population demands more food production to be commensurate with the demands of human consumption. Shortage of arable land has made it difficult to increase cultivated acreage; so efficient management of existing croplands for crop production has become indispensable in the twenty-first century. The use of chemical nitrogenous fertilizers in the twentieth century has promoted crop production by 4–10 times and supported food production over the past 100 years. However, the cost of chemical nitrogenous fertilizers is high for farmers in developing countries, and their production requires a lot of fossil fuel. In addition, the inappropriate or excess application of chemical nitrogenous fertilizers has led to environmental damages of groundwater contamination by nitrates along with air pollution and global warming due to nitrous oxide. Most legume crops, such as soybeans, beans, chickpeas, and groundnuts, and legume forage crops such as alfalfa and clover can fix atmospheric dinitrogen (N_2) by symbiosis with rhizobia, and symbiotic nitrogen fixation via legume-rhizobia symbiosis is the most eco-friendly approach to supplement plants with their nitrogen requirements. Rhizobia are the group of free-living soil bacteria with N-fixing abilities that fix atmospheric nitrogen by establishing a mutual relationship with compatible and leguminous plants (Alice et al. 2017). Rhizobia are a polyphyletic group of *Proteobacteria* with all of the species belonging to the alphaproteobacteria and betaproteobacteria classes. The word *Rhizobium* in particular is derived from a Latin word "rhizo" meaning "root" and "bios" meaning life, term given by Frank (1889). Rhizobia are a diversified group, and in recent years the classification has undergone few changes due to new phylogenetic studies which eventually lead to the new taxa, and consequently there have been many challenges for the nomenclature system. Formerly, *Rhizobium* species were classified into two genera, the genus *Rhizobium* and

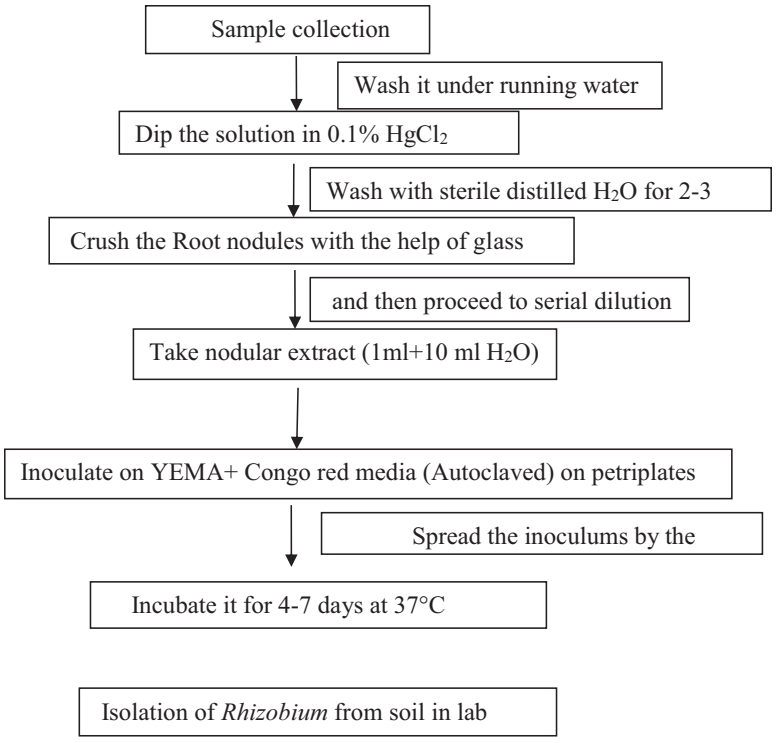
Bradyrhizobium, corresponding to fast-growing strains and slow-growing strains which depend on their growth rate on culture medium and generation time. However, further observations in between the notion of bacterial growth rate and the host range showed a lot of doubt on the validity of the classification. According to the new phylogenetic studies, it has been stated that "few species of Rhizobia moved to new genera" and different methods has been used to establish such updated data like DNA/DNA and DNA/RNA hybridization and serological studies, etc. At present, it includes about 6 rhizobial genera consisting of 28 species like *Rhizobium alamii*, *Rhizobium mesosinicum*, *Rhizobium alkalisoli*, *Rhizobium oryzae*, *Rhizobium phaseoli*, *Rhizobium pisi*, *Rhizobium leucaenae*, etc. (Zakhia et al. 2004). Another report stated the presence of 44 bacterial species and placed them in 12 genera (Sawada et al. 2003). Legume-nodulating bacteria (LNB), an effective colonizer persistent in soil, belong to the proteobacterias with few exceptions which also fall under the group of rhizobia.

In biological nitrogen fixation, dinitrogen is reduced to ammonia through the bacterial enzyme nitrogenase, encoded by the *nif* gene. The leguminous plants provide anaerobic conditions for the effectiveness of the oxygen-sensitive nitrogenase enzyme. Besides symbiosis, rhizobia can facilitate the production of antibiotics, mycolytic enzymes, and siderophore under iron-limiting conditions as well production of hydrogen cyanide (HCN). Rhizobia effectively immunize the plants against different pathogens and confer resistance by enhancing the expression of different genes. They also have upregulation in specific genes leading to enzyme synthesis that enhances the organic phosphate solubilization in soils, also regarded as mineralization of organic phosphorus due to the dead remains of plants and animals containing a larger proportion of phosphorus in them. The lack of disease management strategies faced by researchers of plants that are affected by the different pathogens has become a challenge for the future prospects in agronomy. Rhizobia help as a potent organism in resolving such issues and are able to control soilborne root-infecting fungi in both leguminous and nonleguminous plants (Siddiqui et al. 1998). This has substantially contributed to the agricultural growth and may be impactful to sustainable agricultural practices. The use of plant growth-promoting rhizobacteria (PGPR) is intensively increasing in agriculture and also offers a way to replace chemical fertilizers and other pesticides by various mechanisms. The exact mechanism through which PGPR stimulate plant growth is not clearly established, although several hypotheses have been put forward such as the production of phytohormones (Glick 1995; Bowen and Rovira 1999). Different plant pathologists have applied different strategies for biocontrol practices, but one of the best methods which can be applied is by furnishing the soil with enhanced rhizobial microbiota to provide a frontline defense mechanism against any pathogen attack (Weller 1988). In this regard, an experiment has been conducted on biological control of the *Pythium* sp. by damping off of the pea plant (*Pisum sativum*) and the sugar beet (Bardin et al. 2011). Applications of the particular *Rhizobium* species resulted in the greatest plant growth, pod number, and nodulation (Akhtar and Siddiqui 2010). Recently, leguminous plants have been reported

to be involved in bioremediation by phytoextraction and phytostabilization of already accumulated heavy metals from rhizospheric soil, thanks to *Rhizobium*, isolated from metal contaminated soil, that can adsorb highly toxic heavy metals besides nitrogen fixation (Zheng et al. 2005). It has been demonstrated that *R. leguminosarum* bv. *phaseoli* strains can be specifically used for the phosphate solubilization. However, symbiotic properties of the leguminous plants could be decreased because of high concentrations of heavy metals drastically decreasing the number of rhizospheric rhizobia. For bioremediation purposes, selected plant varieties must be resistant to d-block elements such as iron, zinc, manganese, etc. (Siripornadulsil 2013). The exploitation of rhizobial species for the rehabilitation of contaminated soil and the biochemical and molecular pathways involved in this mechanism provides better novel approaches of bioremediation strategies (Ying et al. 2015). This chapter revolves around the main theme of sustainable agriculture by maintenance of nitrogen in agroecosystem by introducing natural fertilizers at the interest of plant growth and suitable crop management, hence the journey of rhizobia from lab to land for replenishing soil fertility to facilitate crop production. This has further paved ways for exploiting rhizobial mechanisms for future improvement in agro-strategies at the cost of research and development in the growing and powerful economies of the world.

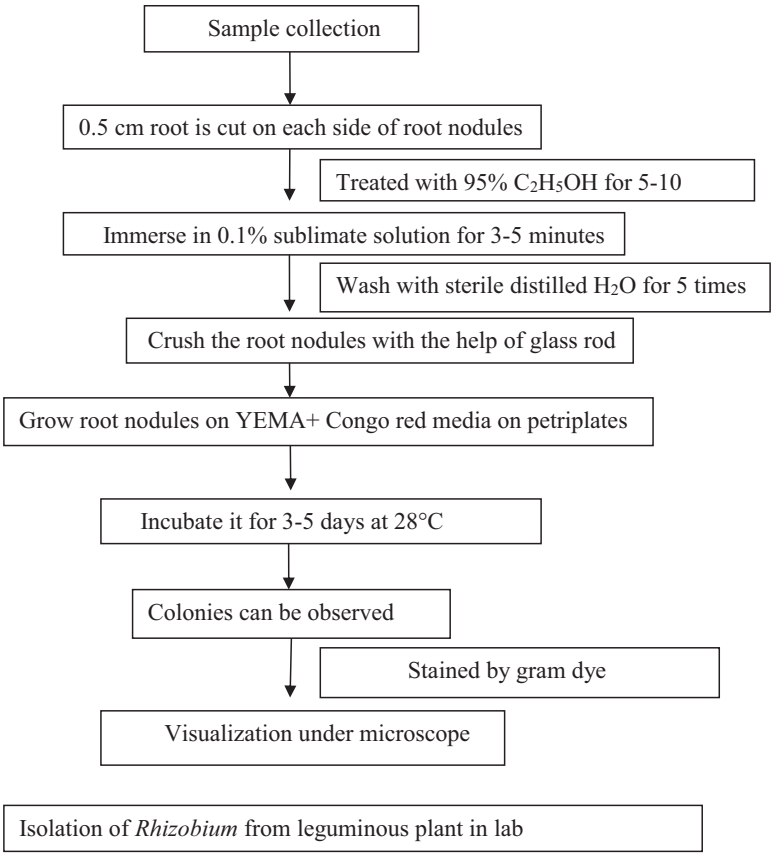
7.2 Isolation of *Rhizobium* from the Soil and Culturing in Lab

Firstly, the nodules were washed under the running tap water in order to remove the anchored soil particles adhered to the nodules. Then the nodules were dipped in 0.1% of $HgCl_2$ solution for 30 s and were washed 2–3 times with sterilized distilled water to remove the traces of toxic $HgCl_2$. Surface-sterilized nodules were transferred into test tube containing 5 ml of sterilized distilled water. These nodules were crushed with the help of rod to obtain a milky suspension. Further yeast extract mannitol agar (YEMA) + Congo red was prepared and heat sterilized by autoclaving. After that, the serial dilution 1 g of nodular extract was taken; it was mixed with 10 ml of sterile distilled water to obtain the nodular extract suspension (i.e., 1 ml of suspension was diluted with 9 ml of distilled H_2O making dilution to 10^{-1}). This step was repeated up to eight to ten times, and then YEMA was put into sterilized petri plates and kept for a while for solidification. The inoculums were spread using spreader, finally incubated for 4–7 days at 37 °C in the incubator.



7.3 Isolation of *Rhizobium* from the Leguminous Plant and Culturing in Lab

Leguminous plant is collected, and the nodules are separated to isolate rhizobia from them. 0.5 cm root is cut on each side of the root nodules and was washed by 95% ethanol for 5–10 min and then immersed in the 0.1% sublimate solution for 3–5 min and then washed with sterile distilled water five times, and finally the root nodules were crushed with the use of forceps. Next, the root nodules were grown on yeast mannitol agar media and Congo red and then incubated at 28 °C for 3–5 days. The colonies appeared and grew apart in petri plates and were then stained by gram dye for visualization under microscope (Gwyn 2006).



7.4 Confirmation of *Rhizobium* by Different Physiological Tests

There are different physiological tests related to *Rhizobium* sp. like salinity and acidity which have been given in Table 7.1.

7.5 Plant Growth-Promoting Characters of *Rhizobium* sp.

7.5.1 Auxin Production

Phytohormones or growth regulators of plants are organic substances synthesized in the specific plant organs that can be translocated to different parts of the plant that can trigger somewhat specific type of responses in the biochemistry, morphology, or physiology of the plant. In plants, auxins play a crucial role in division of cells and its differentiation, elongation, apical dominance, fruit development, and senescence,

Table 7.1 Salinity and acidity test for different strains of *Rhizobium*

Salinity test		Acidity test	
Strains of *Rhizobium*	Inhibitory concentration of NaCl with glycine betaine	Strains of *Rhizobium*	pH
R. melitotis	Positive	*Rhizobium* sp.	4.0–7.0
R. japonicum	Negative	*Azorhizobium*	4.0–7.0
R. trifolii	Negative	*Bradyrhizobium*	4.0–7.0
R. leguminosarum	Negative	*R. tropici*	4.0
Azorhizobium caulinodans	Negative	*R. tropici*	4.25
Rhizobium sullae	Positive	*Bradyrhizobium*	4.25

while specifically the naturally occurring indole-3-acetic acid (IAA) plays a key role in root, leaf, and flower development (Phillips et al. 2011). About 80% of the soil bacteria are only able to produce the IAA, indole-3-butyric acid (IBA), or similar compounds as a product of the tryptophan metabolism. The IAA is a part of signaling and functions as a signaling molecule in microbes because it is reported that IAA influence gene expressions in few microbes. Thus IAA can act as a molecule of signal in plant-microbe interactions. Rhizobia are able to undergo symbiosis with the leguminous plants leading to root nodule formation due to the high abundance of bacteria in the nearby rhizosphere and in the cavity of the roots. These groups of microorganisms are able to secrete different types of hormones, mainly auxin, enhancing the growth of the roots. This is exemplified by the production of high amounts of IAA (99.7% μg/ml) by *Rhizobium* sp. that colonize the root nodules of leguminous plants like *Cajanus cajan*, when grown in basal medium supplied by L-tryptophan. Further studies revealed that the IAA production could be doubled up to 65.3% over control by supplementing a medium with 5 g/l of glucose, 10 μg/ml of $NiCl_2$, and 0.5 g/l of glutamic acid (Zahir et al. 2010).

7.5.2 Mechanism of IAA Biosynthesis by Bacteria

The indole-3-acetic acid biosynthesis in bacteria is either tryptophan-dependent or tryptophan-independent. The pathogenic bacteria such as *Pseudomonas* and *Agrobacterium* produce IAA via the indole-3-acetamide pathways. In *Agrobacterium*, the indole-3-pyruvic acid (IPA) pathway functions in similar ways in both plants and bacteria. Initially, the L-tryptophan is deaminized by an aminotransferase enzyme to IPA. Subsequently, decarboxylase enzyme converts the IPA into indole-3-acetaldehyde (IAAld), which is finally oxidized to IAA by aldehyde oxidase (Fig. 7.1) (Rajagopal 1971; Pollmann et al. 2006).

There is an alternative pathway which exists where tryptophan is directly converted into IAAld by a tryptophan side chain monooxygenase enzyme, which is

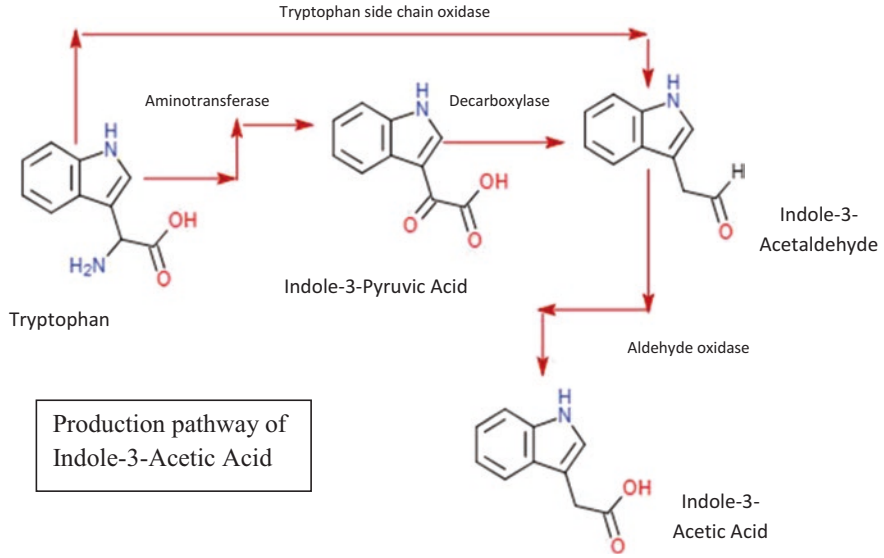


Fig. 7.1 Production pathway of indole-3-pyruvic acid

known as the tryptophan side chain oxidase pathway (Oberhansli et al. 1991). The *ipd*C gene encodes for the indole pyruvate decarboxylase enzyme, which catalyzes the important step in the IPA pathway (Patten and Glick 2002). The indole-3-acetamide (IAM) pathway is seen mainly in phytopathogenic bacteria, although it does occur in photosymbiotic bacteria as well (Kochar et al. 2011). In this pathway, IAA is produced in two step reaction with the tryptophan precursor. Firstly the enzyme is tryptophan 2-monooxygenase, which converts the tryptophan to the IAM intermediate, and the second reaction is further catalyzed by an IAM-specific hydrolase or amidase, which hydrolyzes the IAM to IAA (Fig. 7.2) (Pollmann et al. 2006).

7.5.3 Cytokinin Production

A number of bacteria from plant rhizosphere including PGPR and phytopathogenic bacteria produce cytokinins (Zakhia et al. 2006). Cytokinin is involved in the function of cell division, chloroplast differentiation, and transport of metabolites, also retards senescence of leaf and induces stem morphogenesis in roots, and also controls the functions of organs present on the aboveground. In rhizobia, cytokinin leads to nodule development as essential to initiate the cortical cell division to form root nodule and may also mediate the rhizobial infection in legumes (Frugier et al. 2008). Oldroyd (2007) reported that the production of cytokinin in plants is enhanced by rhizobia through regulation of the different Nod factors pathway, acting as a

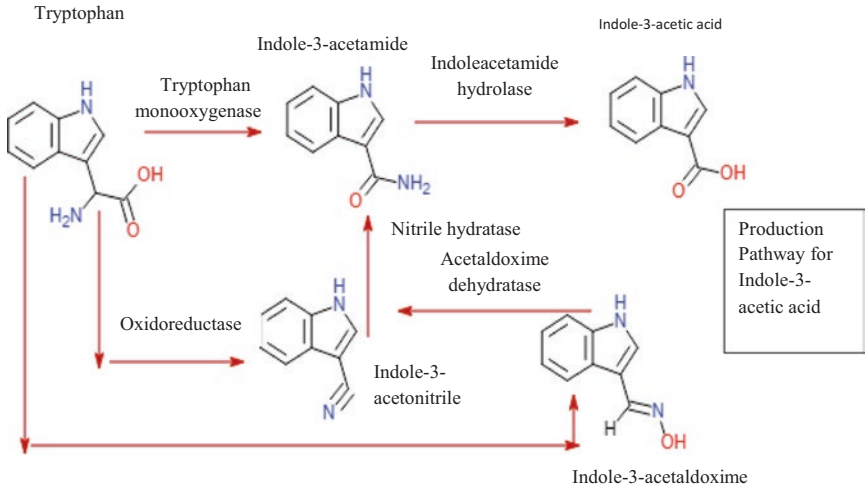


Fig. 7.2 Indole-3-acetamide pathway

mechanism to coordinate in between the epidermal and cortical response during nodulation. The cytokinin produced stimulates cell division in soybean in tissue callus grown in vitro and induces polyploidy during mitosis in the cultured pea root segments.

7.5.4 Ethylene Production

Ethylene, a gaseous hormone endogenously produced by most of the plants, plays a major role in inducing fruit ripening, germination of seed, tissue differentiation, and formation of root and shoot primordium besides flower wilting, lateral bud development, abscission of leaves, and response to both abiotic and biotic stresses in plants (Abeles et al. 1992). Hence to allow the standard growth and development, the production of ethylene in plants tissues is also essential (Safronova et al. 2006). Levels of plant's ethylene are modulated by 1-aminocyclopropane-1-carboxylate (ACC) deaminase and also by the production of the ACC synthase enzyme inhibitor rhizobitoxine. Some rhizobacteria are able to decrease the level of ethylene in plant roots and shoots by cleaving ACC to NH_3 and α-ketobutyrate by the enzymatic action. *Rhizobium leguminosarum*, ACC deaminase-producing bacteria, produces the enzyme to reduce ethylene biosynthesis in plants, which functions by degrading ACC, precursor to ethylene, thereby enhancing root growth (Glick et al. 1998). Experiments suggest that ACC deaminase in *R. leguminosarum* bv. *viciae* enhances the process of nodulation of *Pisum sativum* L. cv.

7.5.5 Mechanism of Nitrogen Fixation in Rhizobia

Rhizobium infects the roots of leguminous and nonleguminous plants like *Parasponia* (of family Cannabaceae) and leads to the formation of nodules. The enzyme system of bacterium furnishes reduced nitrogen as ammonia to the host plant as a constant source resulting in the fulfillment of plant nutritional levels, while the host provides the rhizobia shelter and a homeostatic environment to undergo heterotrophic multiplication by utilizing photosynthates (a carbon source) and micronutrients (Mo, S, Fe, etc.). This exchange mechanism has drawn the attention of socio-microbiologists who study the evolution of mutualistic behavior of rhizobia in trading, diplomacy, and warfare (Alice et al. 2017). It is familiar to us that the free-living rhizobia are unable to fix nitrogen due to a different shape (different from the bacteria found in the root nodules) and require a multitude of mechanisms involving gene regulation and expression of several factors to achieve nodulation.

7.5.5.1 Root Nodule Formation

Rhizobial strains can infect the various species of leguminous plants like pea, beans, soya bean, chickpea, alfalfa, etc. leading to nodulation due to the specificity of genes that determine the compatibility between specific rhizobial strains with the particular leguminous plants. The nodulation is regulated by highly complex chemical signaling in between both the plant and the bacteria.

The interaction between the host plant and free-living rhizobia leads to the release of chemicals by the root cells into the soil, and some of these chemicals encourage the growth of the bacterial population in the area around the roots. Reactions occur in the bacterial cell wall and the root surfaces which are responsible for the mutual recognition and anchorage by the bacteria to root hairs. Flavonoids secreted by the cells of root induces the nod D protein in bacteria which binds to the highly conserved sequence in nod gene promoters termed as the nod box that activates the nod genes. As a result, the nod gene produces nod proteins which consequently activate the different nod factors in the bacteria which induce the nodule formation (Fig. 7.3).

The bacteria produce nod factors, and these stimulate legume roots to be curled up; hence rhizobia invade the root through its hair tips forming infection thread that grows up with the help of root hair cells and penetrates adjoining tissues by branching. As a result bacteria start to multiply within the expanding network of the tubes, and in continuation it produces nod factors which stimulate the root cells to proliferate and eventually form a root nodule. Each root nodule consists of thousands of living *Rhizobium* bacteria, collectively called bacteroids. The portions surrounding the bacteroids are basically structures called as symbiosomes, which contain bacteroids where the nitrogen fixation occurs. Nitrogen fixation by rhizobia occurs following a series of events. Starting from multiplication and colonization at the rhizospheric soil, the attraction between the host and bacteria is chemotactic in nature, induced by the root exudates like different amino acids and sugars leading to attachment of bacteria to epidermal root hair cells (Brewin 1991). Next the characteristic curling of root hairs and invasion of the bacteria to form infection thread by

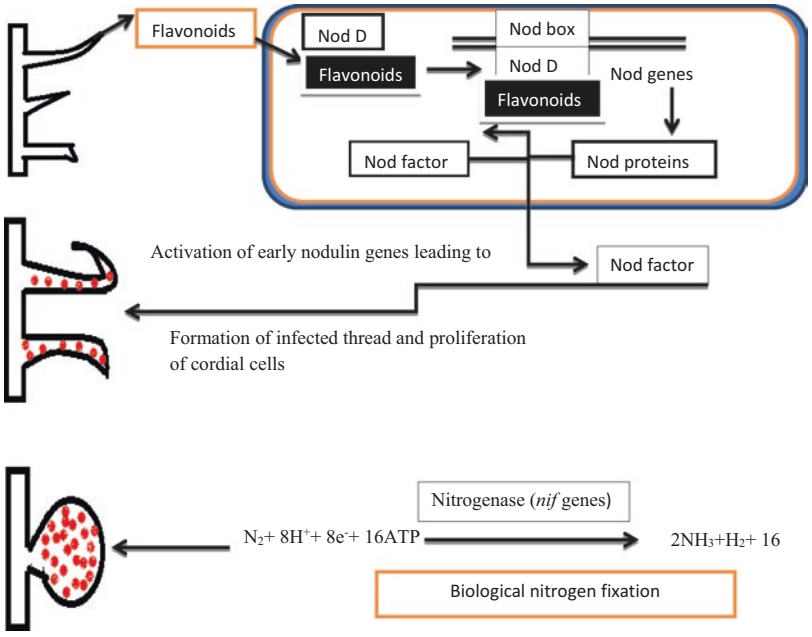


Fig. 7.3 Mechanism of nitrogen fixation in *Rhizobium*

penetration of plasma membrane of root hair cell are observed. Rhizobia reach up to the root cortex, and thus curling occurs due to the specific complex polysaccharides which are present on rhizobia and sensed by lectins. The nodule formation occurs due to the mitogenic agents such as kinetin produced by auxin to promote cell division and extension. Finally bacteria are released from the thread, and they differentiate as the specialized nitrogen-fixing cells in soil. As a result the infection thread gets budded off to form small vesicles containing almost two or more bacteria which stops division and enlarge to get differentiate.

7.5.5.2 Nitrogenase Enzyme

Nitrogenase is a complex enzyme that plays pivotal role in the conversion of nitrogen gas into ammonia by nitrogen-fixing organisms. It consists of two proteins: a homodimeric reductase Fe protein, the structural subunits of which are encoded by the *nif*H gene, and the other heterotetrameric catalytic component called MoFe protein, encoded by the structural genes *nif*D and *nif*K (Seefeldt et al. 2009; Burgess and Lowe 1996). Transfer of every electron from the Fe protein to the FeMo protein requires two ATP molecules that hydrolyzed to ADP + Pi in the presence of Mg^{2+} ions. The products of the translational process are structural *nif* H,D,K genes required for processing by other *nif* gene products before becoming active. Nitrogenase enzyme contains FeMo-co three metallocomplex which involved in the catalytic site for nitrogen reduction and consist of an organic moiety like Mo Fe7 S9 C-homocitrate (Rubio and Ludden 2008; Hu and Ribbe 2013). There are other two

metalloclusters: one is a (8Fe-7S) which is located in the MoFe protein, and the other is (4Fe-4S) cluster present in the Fe protein and the P cluster. These two metalloclusters are involved in the transfer of an electron from the Fe protein to the FeMo-co catalytic site via the P cluster (Seefeldt et al. 2012). On the basis of biochemical complexity, a large number of *nif* genes are crucial for the assembly of this and act as the function of nitrogenase. The important feature of nitrogenase enzyme is its sensitivity toward oxygen. As a result the protein is damaged by oxygen, while the MoFe protein is relatively insensitive to this. Conformational changes take place in enzyme, and as a result it becomes insensitive toward oxygen. In order to protect this condition, there is presence of oxygen scavenging operating process for which high respiratory activity takes place; as a result enzyme becomes modified to catalytically active form. However, in the free-living organisms and anaerobic nitrogen-fixing bacteria, such type of problems didn't occur. Aerobic bacteria have a variety of various mechanisms for protecting the nitrogenase complex, such as metabolic activity. *Rhizobium* controls the oxygen level in the nodule with leghemoglobin, and this red color, iron-containing protein has a similar function as that of hemoglobin. This provides sufficient oxygen for the metabolic processes of the bacteroids but prevents the accumulation of free oxygen which will destroy the nitrogenase activity.

7.5.6 Siderophore Production

Siderophores are small-sized, high-affinity iron-chelating compounds, secreted by microorganisms as well as plants in the environment which mediates iron transport across cell membranes. Siderophores are the strongest soluble Fe^{3}+-binding agent which is known. When the cellular iron concentration is less than 0.1 μm, the iron-assimilating system gets expressed in all the aerobic and the facultative anaerobic microorganisms (Neilands 1981). Thus it may be concluded that siderophore-producing bacteria can provide plants with iron either directly by improving the iron nutrition or indirectly by inhibiting the growth of pathogens in the rhizosphere which limits availability of rhizospheric iron for plants (Glick 1995). Iron exists in two forms in nature, the divalent (ferrous or Fe^{2}+) or trivalent (ferric or Fe^{3}+) form, determined by the pH and the redox potential of the soil (Bodek et al. 1988). Fe^{3+} ions are reduced into Fe^{2+} ions and released into the cells of rhizobacteria, and this reduction results in the destruction or recycling of siderophores (Rajkumar et al. 2010). It has been reported that *Rhizobium nepotum* isolated from stem nodules of *Aeschynomene indica* produced siderophores, inferred by its growth producing orange to yellow halo in chrome azurol sulfonate (CAS) agar medium (Ghorpade and Gupta 2016). Siderophores form stable complex with the heavy metals such as Al, Cd, Cu, etc. which give advantage to some siderophore-producing bacteria to remove heavy metals from soils and help in iron uptake of plants (Neubauer et al. 2000). Rhizobial species, such as *R. meliloti*, *R. leguminosarum* bv. *viciae*, *R. leguminosarum* bv. *trifolii*, *R. leguminosarum* bv. *phaseoli*, *Sinorhizobium meliloti*, and

Bradyrhizobium sp., were known to produce the siderophores (Antoun et al. 1998; Arora et al. 2001; Carson et al. 2000). *Rhizobium* strain BICC 651, a fast-growing strain, isolated from the root nodules of the chickpea (*Cicer arietinum* L.), produces catechol siderophores in order to respond under iron-deficient conditions (Roy et al. 1994), similar to other catechol siderophore-producing microorganisms. Structurally, the siderophore produced by *Rhizobium* BICC 651 contains 2,3-dihydroxybenzoic acid used as a core compound with two moles of threonine which act as a ligand. *Rhizobium leguminosarum* bv. *viciae* (Carter et al. 2002) and *S. meliloti* 1021 sp. (Lynch et al. 2001) also produce siderophores which are not of catechol type. Catecholate siderophores were isolated from *Rhizobium leguminosarum* bv. *trifolii* (Skorupska et al. 1989), *Rhizobium ciceri* (Roy et al. 1994), *Bradyrhizobium* species (cowpea) (Modi et al. 1985), and *Bradyrhizobium* species (peanut) (Nambiar and Sivaramakrishnan 1987) where the biosynthetic genes have not been investigated and yet to be studied.

7.5.7 Phosphate Solubilization

Nitrogen and phosphorus are only macronutrients for the plant growth that exists in both inorganic and organic forms. The bioavailability of phosphorus in the plants is influenced by the pH, compaction, aeration, moisture, temperature and organic matter of soils and secretion of root exudates. Soil microbes help in the release of phosphorus that is absorbed only in the soluble form like monobasic ($H_2PO_4^-$) and dibasic phosphate (H_2PO_42-) (Bhattacharya and Jha 2012). Phosphorus accounts about 0.2–0.8% of the dry weight, but only 0.1% of this phosphorus is available for plants from the soil (Zhou et al. 1992). In the myriad of essential elements, phosphorus (P) (although abundant in soils in both inorganic and organic forms) is one of the major macronutrients among plant growth-limiting factors, which is solubilized by the strains belonging to the genera *Rhizobium* and is among the most powerful phosphate solubilizers besides *Bacillus* and *Pseudomonas* (Chabot et al. 1998).

Rhizobia species including *R. leguminosarum*, *R. meliloti*, *M. mediterraneum*, *Bradyrhizobium* sp., and *B. japonicum* are the potential phosphate solubilizers (Afzal and Bano 2008; Egamberdiyeva et al. 2004; Rodrigues et al. 2006). These bacteria synthesize organic acids with low molecular weight. For instance, 2-ketogluconic acid having phosphate-solubilizing abilities has been identified in *R. leguminosarum* (Halder et al. 1990) and *R. meliloti* (Halder and Chakrabarty 1993). There had been instances of *M. mediterraneum* usage for the purpose of phosphorus solubilization to enhance the plant growth in the chickpea and barley (Peix et al. 2001). In lettuce and maize, it was demonstrated that *R. leguminosarum* bv. *phaseoli* strains specifically solubilize phosphates as PGPR. This has considerably helped in the sustainable agricultural practices of the abovementioned crops by the phosphate-solubilizing root microbiota comprising of rhizobial population.

7.6 Application of Rhizobia

7.6.1 Biocontrol Agent

Rhizobia improve the plant growth through biocontrol such as antibiosis, parasitism, or competition with pathogens for nutrients by inducing the systemic resistance in between the host plant. Some *Rhizobium* sp. has shown further antimicrobial activities toward fewer species such as *Pseudomonas savastanoi* (Kacem et al. 2009), *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani* f. sp. *phaseoli* (Buonassisi et al. 1986), *Pythium* sp. (Bardin et al. 2004; Huang and Erickson 2007), and *F. solani* (El-Batanony et al. 2007) with the varying degrees of growth inhibition too. Studies on numerous plant-microbe interactions have shown that such antagonistic rhizobacteria could function by producing antimicrobial compounds such as bacteriocin (Rodelas et al. 1998; Joseph et al. 1983) and induce the systemic resistance against plant diseases. Antagonistic activity of rhizobia is mainly attributed to the production of antibiotics, HCN, mycolytic enzymes, and siderophore under iron deficiency.

Thus the use of *Rhizobium* sp. shows beneficial outcomes in modern intensive agricultural practices. *Rhizobium japonicum* have been used as a biocontrol agent for the soybean's root rot disease caused by soilborne *Fusarium solani* and *Macrophomina phaseolina*, respectively. It has been experimentally seen that the filtrate of rhizobial culture causes an inhibition of growth of *Macrophomina phaseolina* on potato dextrose agar medium. Rhizobia have been reported to enhance the expression of plant defense-related genes, effectively showing the immunization of the plants against pathogens. Biocontrol of the root-knot nematode of *M. javanica* was studied on lentil using different PGPR including *Rhizobium* sp. (Siddiqui et al. 2007). Induced systemic resistance can be induced in plants by biopriming plants with PGPRs like rhizobia to allow the plant to combat several pathogenic fungal, bacterial, and viral interactions which may hinder plant growth on prolonged exposure.

7.6.2 Phytoremediation

Resistant rhizobial strains are used for the phytoremediation of metals directly by following processes such as chelation, precipitation, transformation, and accumulation. Microbe-assisted phytoremediation emerges as one of the most effective means through which plants and their associated rhizospheric microbes begin to take up or degrade metals as exemplified by a pot culture study, conducted for examining the inoculation with *R. meliloti* in alfalfa, grown for almost 90 days in an agricultural soil contaminated by weathered polycyclic aromatic hydrocarbons (PAHs). The results suggested that the symbiotic association between the alfalfa and *Rhizobium* can reduce the contamination. However, the legume-rhizobia symbiosis process is

said to be more sensitive to metals (Hao et al. 2014). For example, it was observed experimentally that there was a reduction in the population of *R. leguminosarum* bv. *trifolii* which is able to undergo processes such as symbiosis with white clover (*Trifolium repens* L.) grown in polluted soil with metals (McGrath et al. 1988). The rhizobium-legume interaction has been used to remediate soils contaminated with arsenic and other metals (Pajuelo et al. 2001; Mandal et al. 2008). Commercialized cultivations with legume alfalfa in the world may have potential and effectiveness for the remediation of a number of organic contaminants mainly due to its ability to grow and take up heavy metals in low pH soils (Peralta and Ramon 2002). In further studies, the rhizobial species, isolated from the nodules of green gram (*Bradyrhizobium* sp.), lentil (*Rhizobium* sp.), chickpea (*Mesorhizobium* sp.), and pea (*Rhizobium* sp.), have shown greater tolerance toward one or few metals. Wani and Khan (2013) isolated a strain RL9 possessing not only high tolerance to several heavy metals but also having plant growth-promoting traits, such as production of IAA and siderophores. It was found that lentil plants inoculated with this strain had higher growth and development, chlorophyll content, leghemoglobin, nitrogen content, seed protein, and yield compared to that of other plants grown in the absence of bioinoculant when grown in the presence of Ni^{2+}.

7.6.3 Plant Growth Promotion and Yield of Crops

PGPR are free-living soilborne bacteria that aggressively colonize the plant roots which when applied to seed or crops enhance the growth and yield of plants (Kloepper et al. 1980). Various species of soilborne bacteria in the rhizosphere enhance plant growth by multiplication in soil as well as plant tissue, rhizobia being an eminent example. The investigation of the modes of action of rhizobia is increasing as they are commercially exploited as biofertilizers. These modes of action include only the nitrogen fixation, increasing the nutrient availability in the rhizosphere, stimulating root growth and morphologic development, and also promoting other beneficial plant-microbe symbioses. Biswas (1998) concluded the increment in the N uptake by rice (*Oryza sativa*) plants inoculated by rhizobia. This plant response is significantly because of the effective and potential importance to sustainable agriculture, especially in cropping systems involving rotations of rice and legumes, and they are also found in banana which is produced by inoculation (Mia et al. 2005). The separate application of the L-TRP and *Rhizobium* itself appeared to mitigate the adverse effects caused by the salt stress. However, their combined application produced different as well as huge effects and increases the plant height by (28.2%), a number of nodules per plant (71.4%), biomass (61.2%), yield (65.3%), and N_2 concentration (22.4%) compared with untreated control. The growth promotion may get effected by the higher auxin production in the rhizosphere and improves the uptake of mineral which reduces the adverse effects of salinity stress (Table 7.2).

Table 7.2 Effect of *Rhizobium* strains on various crops for plant growth

Rhizobium sp.	Crop species	Growth condition	Remarks	References
R. tropici co-inoculated with *Paenibacillus polymyxa*	Kidney bean	Greenhouse	Increase nodule number, enhanced plant height as well shoot dry weight	Figueiredo et al. (2008)
R. elti (engineered for enhanced trehalose-6-phosphate synthase)	Kidney bean	Pot studies	Enhanced nodules, nitrogenase activity and biomass production, higher tolerance than wild-type strains	Suárez et al. (2008)
Rhizobium sp. DDSS69	–	In vitro	Induction of 135 and 119 kDa proteins. Variation in the protein profile of stressed and nonstressed cells	Sardesai and Babu (2001)
Rhizobia strains	Lentil	Field study	More nodule formation and increased nodule dry weight and plant biomass	Islam et al. (2013)
Rhizobium RL9	Lentil	Pot experiments	Increased growth, nodulation, chlorophyll, leghemoglobin, nitrogen, seed protein, and seed yield	Wani and Khan (2013)
R. leguminosarum	Maize	Pot experiments	Enhanced plant growth and biomass	Hadi and Bano (2010)
Rhizobium leguminosarum bv. *trifolii*	Rice	Greenhouse and field	Increased grain yield and shoot/root weight	Tran Van et al. (2000)
R. vietnamiensis				
R. leguminosarum	Rice	Pot culture, glasshouse	Increased yield, grain size, and biomass	Hussain et al. (2009).
R. trifolii	Wheat	Pot trials	Increased wheat shoot dry matter and grain yield	Hilali et al. (2001).
R. etli bv. *phaseoli*	Maize	Gnotobiotic	Increased total biomass	Gutierrz-Zamora and Martinez-Romero (2001)
R. trifolii	Maize	Greenhouse, field	Increased yield	Riggs et al. (2001)

7.7 Conclusion and Future Prospects

Environmentally sustainable nitrogen fixation and other important rhizobial functions for increase in crop yield to allow rhizobia from lab to land is the main concept of discussion in this chapter. Rhizobia act as a class of eco-friendly microbiota by renewing levels of rhizospheric nitrogen and supplementing plants

with their required levels of the element in suitable absorbable forms. This is initiated by plant-rhizobia interaction, an important paradigm in plant-microbe signaling which is enabled by the number of genes encoding signals for sequence of plant responses. The dire need of specific gene sequences has led to sequencing of rhizobial genome of *Rhizobium sullae*-type strain IS123^T (phylogenetically found to be closely related to *Rhizobium etli* and *Rhizobium leguminosarum*) to obtain 7,889,576 bp reads. This strain endowed with a rich array of symbiotic genes than other strains is hence focused to compare the genome with other members of *Rhizobiales* (Sablok et al. 2017). Thus several active gene sequences and their functions need to be elucidated for exploiting rhizobial strains for contribution to crop improvement.

Rhizobia can synergistically function with other soil microbiota like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, etc. Studies have been demonstrated in lentils with co-inoculation of *Pseudomonas* sp. with *Rhizobium leguminosarum* under suitable field conditions, and finally increase in grain yield was noted. *Rhizobium* along with other PGPRs like *Pseudomonas fluorescence* has been also used to determine nitrogen and phosphorus content in the grains. Thus several such studies to find enhancement of synergistic action of rhizobia with other strains must be encouraged. Commercialization of PGPR can be achieved by studying several factors like market demand, broad-spectrum action, easy availability, and low capital cost for mass production using fermentation methods, formulation and viability, safety and stability, and longer shelf life. Bioformulation of rhizobia is achieved by designing superior carrier materials with high water holding capacity, biodegradable nature, and nontoxic, chemically uniform material that support bacterial growth. Several ongoing research on rhizosphere biology lead to the need for rhizobacteria with potassium-solubilizing abilities in plants. Potassium which is the third most important macronutrient for plant growth is limited by phytotoxic environment. The use of rhizobia to compensate such loses by rhizo-engineering and transgenic bacteria release to optimize plant growth promotion is another thought-provoking aspect of future research. Thus to conclude, rhizobia can be used as an effective PGPR tool for an alternative to agrochemicals after ensuring ecosystem biosafety of arable lands. The USA, Australia, and several other European economies have hence focused on rhizobial transfer from lab to land for better agricultural management to contribute toward improved crop production.

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8 Plant Growth-Promoting Rhizobacteria: Harnessing Its Potential for Sustainable Plant Disease Management

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Contents

Abstract

The sustainable plant disease management includes the use of beneficial microbes for the effective and sustained production of crop/plants. Numerous species of soil bacteria/rhizobacteria and fungi exist in the rhizosphere of plants which can counteract the pathogenic organisms and stimulate plant growth through direct/indirect mode of action. The plant growth-promoting rhizobacteria (PGPRs),

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viz., *Pseudomonas*, *Bacillus*, and *Streptomyces*, have been well exploited by scientists for the management of plant diseases in economically important agricultural and horticultural crops. In nature, interactions between the pathogenic and beneficial microbes take place which decides the existence of the pathogen in the rhizosphere region. Interaction of PGPR with pathogens in the rhizosphere may lead to an expression of innate immune response of defense genes in the plants which can counter the pathogen infection. This review helps in understanding the dynamics and existence of PGPR in the soil, their role in disease management, and their interaction with the pathogens which explore the possibility of identifying new proteins/genes in host-pathogen interaction. In addition, commercial production of bioagents with the suitable carrier material and delivery system play a major role in managing plant diseases under field conditions. The exploration for PGPR and study of their modes of action are escalating at a rapid pace, as efforts are made to exploit them commercially as bioinoculants.

Keywords
Antibiosis · *Bacillus* sp. · Competition · Induced systemic resistance · Lytic enzymes · *Pseudomonas* sp.

8.1 Introduction

Sustainable agriculture practices involves soil health maintanence, usage of minimal water, and minimize the pollution level in the environment which subsequently increases the food grain production in the country. During the cultivation of crops, biotic stress caused by plant pathogens is a major concern which incurs huge economical loss to the farmers. Various agrochemicals are being utilized by the farmers for the management of the diseases caused by plant pathogens. However, their use is increasingly restricted due to public concerns over toxic residues, development of resistance in the pathogens, and increased expenditure for plant protection. Exploitation of microbe-based management will be an alternative approach to control this disease. In nature, soil harbors numerous beneficial microorganisms with potential genes for governing resistance and promoting plant growth which can be well exploited for managing the plant diseases. The PGPR is currently applied in an extensive array of agri- and horticultural production systems in the form of bioinoculants in a variety of economically significant plants including cereals, millets, pulses, oilseeds, fiber crops, sugar crops, fruits, vegetables, medicinal crops, spices, condiments, ornaments, fodder, and cash crops for augmenting their growth and productivity. Free-living, nonpathogenic, root-colonizing bacteria have been studied for the past century as possible inoculants for increasing plant productivity (Kloepper et al. 1992).

In the last few decades, a large array of bacteria including species of *Alcaligenes*, *Aeromonas*, *Azotobacter*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Acinetobacter*,

Agrobacterium, *Aneurinibacillus*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Gluconacetobacter*, *Gluconobacter*, *Herbaspirillum*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, *Saccharothrix*, *Serratia*, *Thiobacillus*, and *Variovorax* are considered as important PGPR (Dobbelaere et al. 2003; Crepin et al. 2012; Annapurna et al. 2013). These effective rhizobacteria are used in sustainable agriculture as biofertilizers and biocontrol agents (Babalola 2010). Several studies have depicted proteobacteria especially bacteria from family *Pseudomonadaceae* or *Burkholderiaceae* as dominant members of rhizosphere microflora in field conditions (Peiffer et al. 2013).

Rhizobacteria can survive in soil or seed, multiply in the spermosphere in response to seed exudates, get attached to the root surface (Suslow 1980), and later become endophytic by colonizing in root cortex region. They are sporadically dispersed along roots and are distributed in a lognormal pattern in the rhizosphere (Bahme and Schroth 1987). Various PGPR strains screened under laboratory, greenhouse, and field conditions against phytopathogens have been commercialized. The commercially utilized efficient PGPR strains include species of *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Delftia*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, and *Serratia* (Glick 2012). Although various strains of PGPR have been isolated, there is a gap in identification of efficient crop-specific strain with good colonizing ability possessing antagonistic and growth-promoting genes. Also, the type of formulation used for mass multiplication of these biocontrol agents is more important which will help to establish itself in the field for a considerable period of time.

The molecular markers, of late, can be utilized for identification and screening of the efficient strain in a short span of time. Besides, understanding the mode of action of PGPR through genomic and proteomic approaches will help in depicting its role in plant disease management. With this background, this review will address the major PGPR strains utilized in agricultural and horticultural crops for plant disease management, highlight the various mode of action exhibited by these beneficial bacteria against soilborne diseases, and also discuss on the various bioformulations used for the management of plant diseases which will pay a way for sustainable agriculture.

8.2 PGPR in Plant Disease Management

PGPRs are the distinct group of microbes that suppress the deleterious pathogens in crop plants. The genera normally used as biocontrol agents are *Agrobacterium*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Streptomyces*, etc. Among the diversity of PGPR, *Pseudomonas* and *Bacillus* spp. have a wide distribution and are the extensively studied genera for PGPR as a biocontrol. In particular, the soilborne fluorescent pseudomonads have received particular interest due to its excellent root-colonizing abilities and their capacity to produce a wide range of antifungal metabolites (Olivain et al. 2004). These organisms combat the plant disease by competition, enzymatic lysis, production of antibiotics, hydrogen cyanide, siderophores,

induced systemic resistance (ISR), or any other mechanisms. The rhizosphere soil is an active site with complex interactions between the root and the associated PGPR (Sylvia et al. 1998). At this point, the PGPR enhances plant growth and development by direct and/or indirect mechanisms. Direct mechanisms elicit growth promotion by biological nitrogen fixation (BNF), production of hormones such as indole-3-acetic acid (IAA), gibberellic acid (GA_3), cytokinin and phosphate, potassium and zinc solubilization or mobilization (Idris et al. 2008), production of siderophores for sequestering of iron (Fe) from the soil and supply it to the plants and synthesis of hydrogen cyanide, etc. (Keel and Defago 1997). Some strains improve the innate ability to tolerate the stresses like acidity, salinity, drought, etc., besides production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme to lower the ethylene synthesis and synthesis of fungal cell wall lytic enzymes.

Secondly, indirect mechanisms include suppression of harmful/deleterious rhizosphere microbes through induced systemic resistance (ISR), which are normally recognized as having a role in biocontrol (Dobbelaere et al. 2003). Induced systemic resistance is based on the activation of plant defense mechanisms by rhizobacterial strains and is considered natural, eco-friendly, and safe besides providing resistance against a broad spectrum of pathogens (Sticher et al. 1997). The rhizobacteria need to colonize the roots to a sufficient level for induction of resistance in the host. For example, in radish, a minimal number of 10^5 colony-forming units (cfu) per g root of bacteria is required to induce resistance in the host (Raaijmakers et al. 1995). Colonization of plants by biocontrol agents induces cell wall modifications, viz., deposition of callose, pectin, cellulose, and phenolic compounds leading to the formation of a structural barrier at the site of potential attack by phytopathogens (Benhamou et al. 2000). Defense reaction occurs due to accumulation of PR proteins (chitinase, β-1,3-glucanase), phenylalanine ammonia lyase, peroxidase, phenolics, callose, lignin, and phytoalexins (Harish et al. 2009b).

The successful establishment of an introduced PGPR depends on its compatibility/establishment with the crop and also on its interaction with indigenous microflora. An ideal PGPR should be rhizosphere competent, enhance plant growth, be easy to mass multiply, possess broad spectrum of action, have consistent biological control activity, be safe to the environment, and be compatible with other rhizobacteria (Nakkeeran et al. 2005; Barea, 2015). Therefore, identification of a functional PGPR strain possessing the growth-promoting and broad-spectrum biocontrol activity is an ever-challenging one. Utilization of molecular tools to identify the antibiotic biosynthetic genes, quorum quenching/sensing genes, and growth-promoting genes in PGPR will pay way for the selection of efficient microbes in a short span of time (Fig. 8.1). Besides, updating the knowledge on the utilization of PGPR for plant disease management is the need of the day. This review, therefore, will focus on some novel and highly utilized PGPR in disease management with special reference to the genera *Pseudomonas* and *Bacillus*.

Various research groups throughout the world have utilized PGPR strains that were found to be successful in combating the major diseases of field and horticultural crops (Kloepper and Schroth 1978) through direct/indirect mode of action along with plant growth promotion activity (Tables 8.1 and 8.2). The enhancement

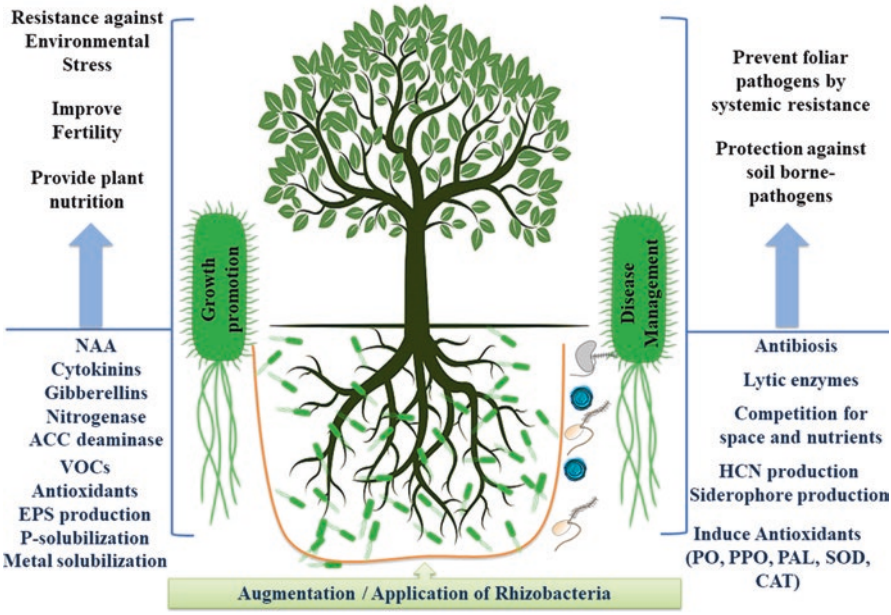

Fig. 8.1 Proposed model for PGPR-mediated plant growth promotion and disease management

of plant growth by PGPR indicates their potential as biofertilizers and biocontrol agents in the field of agriculture (Kloepper and Adesemaye 2009).

8.3 Plant Growth Promotion (PGP) Activities

The studies on the mechanism of growth promotion indicated that PGPR promotes plant growth directly by the production of plant growth regulators (PGR) or indirectly by stimulating nutrient uptake, by producing siderophores or antibiotics to protect plants from soilborne pathogens or deleterious rhizosphere organisms (Kavino et al. 2010). Barea et al. (2005) reported phosphate-solubilizing bacteria (PSB) positive for IAA, GA_3, and cytokinin production. Several isolates of *Pseudomonas* produced auxin or cytokinin and gibberellin.

Fluorescent pseudomonads increased the plant growth of rice and cotton by ~27% and 40%, respectively, when the bacteria were applied to the seed (Sakthivel and Gnanamanickam 1987). Seeds treated with fluorescent pseudomonads resulted in increased number of tillers and grain yield in addition to control of sheath blight disease in rice (Mew and Rosales 1992). An increase in germination of ~30 to 60% in maize by plant growth-promoting strains of *P. aeruginosa* strain 7NSK2 and *P. fluorescens* ANP15 was observed by Hofte et al. (1991). Fluorescent *Pseudomonas* strains improved vegetative sett germination, plant height, cane diameter, brix values, and cane weight in sugarcane (Viswanathan and Samiyappan 1999). Indirect

Table 8.1 Plant growth-promoting rhizobacteria in field crop diseases management

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
Rice	*Magnaporthe grisea*	*Pseudomonas fluorescens*, *Bacillus polymyxa*, *P. fluorescens*	Gnanamanickam and Mew (1992), Vidhyasekaran et al. (1997), and Karpagavalli et al. (2002)
	Pyricularia oryzae	*P. fluorescens*, *Bacillus* sp., *Streptomyces sindeneusis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus pumilus*, *Paenibacillus kribbensis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*	Krishnamurthy and Gnanamanickam (1998), Vidhyasekaran and Muthamilan (1999), Nandakumar et al. (2001), Kanajanamaneesathian et al. (2007), Yang et al. (2009), Zarandi et al. (2009), Guo and Liao (2014), Srivastava et al. (2016), and Rais et al. (2017)
	Rhizoctonia solani	*P. fluorescens*, *B. subtilis*	Rabindran and Vidhyasekaran (1996) and Kumar et al. (2012)
	Sarocladium oryzae	*P. fluorescens*, *P. aeruginosa*	Sakthivel and Gnanamanickam (1987) and Sunish kumar et al. (2005)
	Xanthomonas oryzae pv. *oryzae*	*P. fluorescens*, *P. aeruginosa*, *B. subtilis*, *Lysobacter antibioticus*, *Bacillus lentus*, *Bacillus cereus*, *Bacillus circulans*	Vidhyasekaran et al. (2001). Velusamy and Gnanamanickam (2003), Ji et al. (2008), and Yasmin et al. (2016)
Wheat	*Tilletia laevis*	*P. fluorescens*	McManus et al. (1993)
	Helminthosporium sativum	*P. fluorescens*	Ping et al. (1999)
	Gaeumannomyces graminis var. *tritici*	*P. fluorescens*, *Pseudomonas chlororaphis*	Pierson and Thomashow (1992) and Mazzola et al. (2004)
	Microdochium nivale	*Pseudomonas brassicacearum*	Levenfors et al. (2008)
	Septoria tritici	*P. aeruginosa*	Flaishman et al. (1990)
	Fusarium culmorum	*P. fluorescens*	Khan and Doohan (2009)
	Fusarium graminearum	*Lysobacter enzymogenes*	Jochum et al. (2006)
	Mycosphaerella graminicola	*B. megaterium*	Kildea et al. (2008)
Barley	*Pythium ultimum*	*P. fluorescens*	Gutterson et al. (1986)
	F. culmorum	*P. fluorescens*	Khan and Doohan (2009)
	Pyrenophora teres	*P. fluorescens*	Khan et al. (2010)
Maize	*P. ultimum*, *Pseudomonas arrhenomanes*	*Burkholderia cepacia*	Mao et al. (1998)
	P. ultimum	*P. fluorescens*	Callan et al. (1990)

(continued)

Table 8.1 (continued)

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
	Peronosclerospora sorghi	*B. subtilis*, *P. fluorescens*	Sadoma et al. (2011)
	Fusarium verticillioides	*P. fluorescens*, *B. amyloliquefaciens*	Nayaka et al. (2009) and Pereira et al. (2010)
	F. culmorum	*P. fluorescens*	Khan and Doohan (2009)
	Helminthosporium maydis	*B. subtilis*, *B. cereus*	Lu et al. (2006) and Yun-feng et al. (2012)
	Erwinia carotovora	*Bacillus thuringiensis*	Dong et al. (2004)
	Stenocarpella maydis	*B. subtilis*, *P. fluorescens*, *Pantoea agglomerans*	Petatan-Sagahon et al. (2011)
	R. solani	*B. subtilis*	Muis and Quimiob (2006)
	Fusarium moniliforme	*Bacillus* sp., *Pseudomonas* sp.	Pal et al. (2001)
Sorghum	*P. ultimum*	*P. fluorescens*	Idris et al. (2008)
	Macrophomina phaseolina	*P. chlororaphis*	Das et al. (2008)
	Sclerospora graminicola	*B. pumilus*, *B. subtilis*	Raj et al. (2003)
	Erwinia carotovora subsp. *atroseptica*	*P. chlororaphis*	Das et al. (2008)
Pearl millet	*Sclerospora graminicola*	*P. fluorescens*	Umesha et al. (1998)
Ragi	*P. grisea*	*P. fluorescens*	Vanitha (1998)
Foxtail millet	*M. grisea*	*P. fluorescens*	Karthikeyan and Gnanamanickam (2008)
Pigeon pea	*Macrophomina phaseolina*	*P. fluorescens*	Siddiqui et al. (1998)
	Fusarium udum	*Bacillus licheniformis*	Singh et al. (2002)
Chickpea	*P. ultimum*	*B. pumilus*, *Streptomyces lydicus*, *Streptomyces griseoviridis*	Leisso et al. (2009)
	F. oxysporum f. sp. *ciceri*	*P. aeruginosa*, *Bacillus macerans*, *B. megaterium*	Anjaiah et al. (2003), Landa et al. (2004), and Saikia et al. (2006)
	M. phaseolina	*P. putida*, *P. polymyxa*	Akhtar and Siddiqui (2007)
	Rhizoctonia bataticola	*P. fluorescens*	Ahamad et al. (2000)
Mung bean	*M. phaseolina*	*Burkholderia* sp.	Satya et al. (2011)
Soya bean	*P. ultimum*	*P. putida*	Paulitz (1991)
	Sclerotinia sclerotiorum	*B. subtilis*	Zhang et al. (2011) and Zeng et al. (2012)
	F. oxysporum	*B. subtilis*	Zhang et al. (2009)
	F. graminearum		

(continued)

Table 8.1 (continued)

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
	Soyabean stunt virus	*P. aeruginosa*	Khalimi and Suprapta (2011)
Groundnut	*S. rolfsii*	*P. fluorescens*	Vanitha (1998), and Abd-Allah and El-Didamony (2007)
		B. subtilis	
	Aspergillus niger	*P. aeruginosa*, *Pseudomonas* sp.	Kishore et al. (2005a) and Anjaiah et al. (2006)
		Bacillus sp.	
	Aspergillus flavus	*B. megaterium*	Kong et al. (2010)
	Puccinia arachidis	*P. fluorescens*	Meena et al. (1999)
	M. phaseolina	*P. fluorescens*	Shanmugam et al. (2002)
Sesame	*P. ultimum*	*P. polymyxa*	Ryu et al. (2006)
	M. phaseolina	*P. fluorescens*	Jayashree et al. (2000)
Sunflower	*Plasmopara halstedii*	*B. pumilus*	Nandeeshkumar et al. (2008)
	Sunflower necrosis virus	*Streptomyces fradiae*, *B. licheniformis*	Srinivasan and Mathivanan (2011)
Safflower	*M. phaseolina*	*P. fluorescens*	Prashanthi et al. (2000)
Rapeseed	*S. sclerotiorum*	*B. subtilis*, *P. chlororaphis*	Fernando et al. (2007) and Yang et al. (2009)
		B. amyloliquefaciens	
Cotton	*P. ultimum*	*Enterobacter cloacae*, *Acinetobacter calcoaceticus*, *P. fluorescens*	Nelson (1988), van Dijk and Nelson (1998), and Hagedorn et al. (1990)
	Verticillium dahliae	*Pseudomonas* sp., *Serratia plymuthica*	Erdogan and Benlioglu (2010)
	Thielaviopsis basicola	*Paenibacillus alvei*	Schoina et al. (2011)
	R. solani	*P. fluorescens*, *Pseudomonas cepacia*	Hagedorn et al. (1990), Cartwright et al. (1995), and Ligon et al. (2000)
	X. campestris pv. *malvacearum*	*P. fluorescens*, *B. cereus*	Mondal et al. (2000) and Ishida et al. (2008)
Sugarcane	*Colletotrichum falcatum*	*P. putida*	Viswanathan and Samiyappan (2002)
Sugar beet	*Pythium ultimum* var. *ultimum*	*L. enzymogenes*	Palumbo et al. (2005)
	P. ultimum	*Stenotrophomonas maltophilia*	Dunne et al. (1998)
	R. solani	*P. fluorescens*	Nielsen et al. (1998)
	Cercospora beticola	*B. subtilis*	Collins and Jacobsen (2003)

Table 8.2 Plant growth-promoting rhizobacteria in horticultural crop diseases management

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
Tomato	*P. ultimum*	*P. fluorescens*, *B. subtilis*	Hultberg et al. (2000) and Jayaraj et al. (2005)
	Pythium aphanidermatum	*P. fluorescens*	Ramamoorthy et al. (2001)
	Pythium splendens	*P. aeruginosa*	Buysens et al. (1994)
	Phytophthora infestans	*B. pumilus*, *P. fluorescens*	Yan et al. (2002)
	F. oxysporum f. sp. *lycopersici*	*P. fluorescens*, *S. griseoviridis*	Chin-A-Woeng et al. (1998), Dekkers et al. (2000), Khan and Akram (2000), Minuto et al. (2006), and Omar et al. (2006)
		P. fluorescens, *P. chlororaphis*	
		B. megaterium, *B. cepacia*	
	Alternaria solani	*P. fluorescens*	Geels and Schippers (1983)
	S. rolfsii	*P. fluorescens*, *B. amyloliquefaciens*	Thiribhuvanamala et al. (1999) and Jetiyanon et al. (2003)
	R. solani	*P. fluorescens*	Geels and Schippers (1983) and Szezech and Shoda (2006)
		B. subtilis	
	Ralstonia solanacearum	*P. putida*	Amith et al. (2004)
	X. axonopodis pv. *vesicatoria*	*B. pumilus*	Ji et al. (2006)
	Pseudomonas syringae pv. *tomato*	*P. syringae*, *P. putida*, *P. fluorescens*	Van Peer et al. (1991), Wilson et al. (2002), and Matilla et al. (2010)
	Clavibacter michiganensis subsp. *michiganensis*	*B. subtilis*	Utkhede and Koch (2004)
Potato	*P. ultimum*	*E. cloacae*	Kageyama and Nelson (2003)
	P. infestans	*P. fluorescens*, *S. plymuthica*	Glass et al. (2001) and Slininger et al. (2007)
	Phytophthora erythroseptica	*E. cloacae*, *Enterobacter* sp.	Schisler et al. (2009)
		Pseudomonas sp.	
	R. solani	*P. fluorescens*	Grosch et al. (2005)
	Fusarium sp.	*P. fluorescens*	Al-Mughrabi (2010)
	Verticillium dahliae	*P. fluorescens*	Uppal et al. (2008)
	F. roseum var. *sambucinum*	*B. licheniformis*, *B. cereus*	Sadfi et al. (2002)

(continued)

Table 8.2 (continued)

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
	Gibberella pulicaris	*P. agglomerans*	Schisler et al. (2000)
		P. fluorescens	
	Helminthosporium solani	*B. cereus*, *P. putida*, *Rhodococcus erythropolis*	Martinez et al. (2002)
		Rhodococcus globerulus	
	E. carotovora subsp. *atroseptica*	*P. fluorescens*	Cronin et al. (1997)
	Pectobacterium atrosepticum	*R. erythropolis*	Crepin et al. (2012)
	Streptomyces scabies	*Pseudomonas mosselii*	Singhai et al. (2011)
Carrot	*P. ultimum*	*E. cloacae*	Kageyama and Nelson (2003)
	Alternaria radicina	*B. cepacia*, *B. amyloliquefaciens*	Chen and Wu (1999)
Brinjal	*R. solanacearum*	*P. fluorescens*	Chakravarty and Kalita (2011)
Chillies	*Phytophthora capsici*	*Bacillus* sp.	Jiang et al. (2006)
		S. plymuthica	Kim et al. (2008)
		B. megaterium	Akgül and Mirik (2008)
	Colletotrichum capsici	*P. fluorescens*, *B. subtilis*	Bharathi et al. (2004)
	Colletotrichum sp.	*P. fluorescens*	Hegde and Anahosur (2001)
	Colletotrichum acutatum	*Myxococcus* sp.	Kim and Yun (2011)
	R. solani	*Chromobacterium* sp.	Kim et al. (2008)
	F. oxysporum f. sp. *capsici*	*B. licheniformis*, *P. fluorescens*, *Chryseobacterium balustinum*, *B. subtilis*, *B. amyloliquefaciens*	Domenech et al. (2006)
		P. fluorescens, *B. subtilis*	Sundaramoorthy et al. (2012)
	S. rolfsii	*Streptomyces philanthi*	Boukaew et al. (2011)
Onion	*Botrytis allii*	*B. licheniformis*, *B. amyloliquefaciens*	Lee et al. (2001)
	F. oxysporum	*B. amyloliquefaciens*	
Garlic	*Penicillium hirsutum*	*P. agglomerans*	Kim et al. (2006)
Cassava	*P. aphanidermatum*	*B. pumilus*	Pereira de Melo et al. (2009)
	R. solani		
	S. rolfsii		

(continued)

Table 8.2 (continued)

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
	X. campestris pv. *manihotis*	*B. cereus*, *B. subtilis*, *Pseudomonas* sp.	Amusa and Odunbaku (2007)
Pea	*P. ultimum*	*P. fluorescens*	Naseby et al. (2001)
	Pythium sp.	*P. cepacia*, *P. fluorescens*	Parke et al. (1991)
	P. infestans	*B. pumilus*	Yan et al. (2002)
	Aphanomyces euteiches	*B. mycoides*	Wakelin et al. (2002)
	P. syringae pv. *syringae*	*P. fluorescens*	Seuk et al. (2001)
Beans	*P. splendens*	*P. aeruginosa*	Anjaiah et al. (1998)
	Colletotrichum lindemuthianum	*P. chlororaphis*	Lagopodi (2009)
	Botrytis cinerea	*B. subtilis*	Ongena et al. (2007)
Radish	*F. oxysporum* f. sp. *raphani*	*P. fluorescens*	Leeman et al. (1996)
		P. putida	Scher and Baker (1982)
	P. ultimum	*E. cloacae*	Kageyama and Nelson (2003)
Beetroot	*P. debaryanum*, *P. ultimum*	*P. fluorescens*	Dodd and Stewart (1992)
Cabbage	*P. brassicae*	*Pseudomonas* sp.	Hjort et al. (2010)
Yam	*Botryodiplodia theobromae*	*B. subtilis*	Swain et al. (2008)
	F. moniliforme	*B. subtilis*	Okigbo (2002)
	Penicillium sclerotigenum	*Pseudomonas* sp.	
Lettuce	*R. solani*	*P. fluorescens*	Grosch et al. (2005)
	P. ultimum	*P. fluorescens*	Crawford et al. (1993)
Cauliflower	*F. moniliforme*	*P. fluorescens*	Rajappan and Ramaraj (1999)
Cucumber	*P. ultimum*	*P. fluorescens*	Georgakopoulos et al. (2002)
		E. cloacae	Kageyama and Nelson (2003)
	P. aphanidermatum	*L. enzymogenes*	Folman et al. (2004)
	Fusarium sp.	*P. fluorescens*	Brovko and Brovko (2000)
	F. oxysporum	*P. putida*	Park et al. (1988)
	F. oxysporum f. sp. *cucumerinum*	*P. aeruginosa*	Bradley and Punja (2010)
Cabbage	*X. campestris* pv. *campestris*	*Bacillus velezensis*	Liu et al. (2016)
Carnation	*F. oxysporum* f. sp. *dianthi*	*P. fluorescens*	Van Peer and Schippers (1992)
	P. cinnamomi	*P. fluorescens*	Sorokina et al. (1999)

(continued)

Table 8.2 (continued)

Crop	Pathogen	Plant growth-promoting rhizobacteria	References
Tea	*Exobasidium vexans*	*P. fluorescens*	Saravanakumar et al. (2007b)
Peppermint	*R. solani*	*P. fluorescens*	Kamalakannan et al. (2003)
Mango	*Colletotrichum gloeosporioides*	*P. fluorescens*	Koomen and Jeffris (1993) and Vivekananthan et al. (2004)
	Lasiodiplodia theobromae	*P. fluorescens*, *B. subtilis*	Parthasarathy et al. (2016)
Banana	*BBTV*	*P. fluorescens, Bacillus sp.*	Harish et al. (2008a)
Apricot, peach	*Leucostoma cinctum*	*P. fluorescens*	Rozsnyay et al. (1992)
Apple	*Venturia inaequalis*	*P. fluorescens*	Kucheryava et al. (1999)
Grapevine	*Plasmopara viticola*	*B. subtilis*	Furuya et al. (2011)
Raspberry	*Phytophthora fragariae* var. *rubi*	*Streptomyces* sp.	Valois et al. (1996)
Arabidopsis	*P. syringae* pv. *lachrymans*	*P. putida*	Wei et al. (1996)
	P. aphanidermatum	*Peanibacillus polymyxa*	Timmusk et al. (2009)
	Hyaloperonospora parasitica	*P. fluorescens*	Iavicoli et al. (2003)
Asparagus	*Phytophthora megasperma*	*P. chlororaphis*	Carruthers et al. (1995)
Chrysanthemum	*P. aphanidermatum*, *Pythium dissotocum*	*P. fluorescens*	Liu et al. (2007)
Mushroom	*Pseudomonas tolaasii*	*P. fluorescens*	Bora et al. (2000)
Tobacco	*P. ultimum*	*P. fluorescens*	Howell and Stipanovic (1979)
	Peronospora tabacina	*S. marcescens*	Zhang et al. (2001)

stimulus of plant growth contains a range of mechanisms by which the bacteria protect plants from phytopathogens (Glick 2012). The PGPR strains, viz., *Paenibacillus* sp. *Azospirillum brasilense*, *B. subtilis* subsp. *subtilis*, *B. kururiensis*, and *P. stutzeri*, enhanced biomass production in several trees and nursery saplings (Radhapriya et al. 2018). Also, the application of *Bacillus* spp. in the plant system facilitates plant growth promotion (Gange and Gadhave 2018). The enzyme ACC deaminase secreted by PGPR lowers the plant ethylene levels that are produced during stress conditions and thus directly protects the plant from retardation (Glick 1995). The significance of ACC deaminase gene has been documented in many of the crops which promote plant growth under various conditions (Mayak et al. 2004). Seed bacterization with fluorescent pseudomonads GRC2 resulted in improved seed germination, pod yield, and reduced charcoal rot disease incidence caused by *M. phaseolina* in peanut (Gupta et al. 2002). Similarly, application of *P. fluorescens* Pf1

as seed treatment followed by soil application enhanced the plant growth and has better native rhizobium nodulation and grain yield in legumes (Jayashree et al. 2000). Thus application of PGPR strain promoted the growth of crop by direct and indirect means and thus compensates the loss caused due to pathogens.

8.4 Antibiosis

Antibiotics are mostly deliberated to be low molecular weight organic compounds produced by beneficial microbes and is considered as one of the most important traits of PGPR. Antibiosis shows a vital role in the biocontrol of plant disease which often acts in concert with competition and parasitism. Dennis and Webster (1971) first described the antagonistic properties of *Trichoderma* in terms of antibiotic production which included both nonvolatiles and volatiles. Certain PGPR strains are capable of producing volatile and nonvolatile antibiotics and are important feature for suppression of plant pathogens (Table 8.3). Some of these antibiotic-producing strains were also shown to suppress fungal plant disease in vitro (Whipps 2001).

Several strains of *Pseudomonas* and *Bacillus* spp. have been shown to produce wide array of antibiotics which includes ammonia, butyrolactones, 2–4 diacetylphloroglucinol, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, tropolone, pyocyanin, iturin, surfactin, viscosinamide, zwittermicin A, agrocin 84, as well as several other uncharacterized moieties (Nyfeler and Ackermann 1992; Keel and Defago 1997; Nielsen et al. 1999; Whipps 2001). Burkhead et al. (1994) reported that *P. cepacia* B37W produced pyrrolnitrin antibiotic inhibitory to *Fusarium sambucinum*. Michereff et al. (1994) could correlate the in vitro inhibition of *Pythium* and *Rhizoctonia* by 2,4-diacetylphloroglucinol, an antibiotic produced by *P. fluorescens* PF5 and in vivo control of *C. graminicola*, incitant of sorghum anthracnose. *P. fluorescens* (Trevisan) Migula F113 was shown to control the potato soft rot pathogen, *E. carotovora* subsp. *atroseptica* (van Hall) Dye, by the production of antibiotic 2,4-diacetylphloroglucinol (DAPG) (Cronin et al. 1997). Some evidence was also obtained that siderophore production by *P. fluorescens* F113 may play a role in biocontrol of potato soft rot.

Bacillus cyclic lipopeptides belong to three major families, the iturins (bacillomycins, iturins, and mycosubtilins), the fengycins (plipastatins), and the surfactins (bamylocin A, esperins, lichenysins, pumilacidins, and surfactins) (Jacques 2011). Iturins and fengycins possess antifungal activity against a wide range of phytopathogens, while surfactins are mostly antibacterial (Ongena and Jacques 2008). Bacilysin is a dipeptide composed of an L-alanine and the unusual amino acid L-anticapsin and one of the simplest peptide antibiotics known with antifungal and antibacterial activities. Difficidin and bacilysin from *B. amyloliquefaciens* FZB42 have antibacterial activity against *X. oryzae* in rice (Wu et al. 2015). *B. subtilis* CMB32 produced antifungal lipopeptides which was found to be antagonistic against *C. gloeosporioides* (Kim et al. 2010). Thus antibiotics secreted by the biocontrol agents were found to inhibit the plant pathogens and thus play an important role in disease management.

Table 8.3 Antibiotics produced by PGPR

Target group	PGPR	Antibiotics	References
Oomycetes, fungi	*P. fluorescens*	2,4-diacetylphloroglucinol	Shanahan et al. (1992)
		Phenazine-1-carboxylic acid	Gurusiddaiah et al. (1986)
		Dimer of phenazine-1-carboxylic acid	Sakthivel and Sunish Kumar (2008)
		Pyrrolnitrin	Ligon et al. (2000)
		Pyoluteorin	Keel et al. (1992)
		Mupirocin (pseudomonic acid A)	El-Sayed et al. (2003)
		Rhizoxin analogues	Loper et al. (2008)
		Viscosinamide	Nielsen et al. (1998)
		Tensin	Nielsen et al. (2000)
		Masstolides A	de Bruijn et al. (2007)
	P. aeruginosa	Phenazine-1-carboxamide	Sunish Kumar et al. (2005)
		Pyocyanin	Baron et al. (1997)
	Pseudomonas aureofaciens	Phenazine-1-carboxylic acid	Thomashow et al. (1990)
		Pyrrolnitrin	Elander et al. (1968)
	P. chlororaphis	Phenazine-1-carboxylic acid	Pierson and Thomashow (1992)
		2-hydroxyphenazine	Chin-A-Woeng et al. (1998)
	P. putida	Phenazine-1-carboxylic acid	Pathma et al. (2011)
	P. cepacia	Pyrrolnitrin	Cartwright et al. (1995)
	Pseudomonas pyrrolnitrica	Monodechloro-pyrrolnitrin	Hashimoto and Hattori (1968)
	Pseudomonas borealis	2,3-deepoxy-2,3-didehydro-rhizoxin	Tombolini et al. (1999)
	Pseudomonas spp.	Isopyrrolnitrin	Hashimoto and Hattori (1966a)
		Oxypyrrolnitrin	Hashimoto and Hattori (1966b)
		Amphisin	Sorensen et al. (2001)
		Oomycin A	Kim et al. (2000)
		Cepaciamide A	Howie and Suslow (1991)
		Ecomycins	Jiao et al. (1996)
		2,3-deepoxy-2,3-didehydro-rhizoxin	Miller et al. (1998)
		Butyrolactones	Thrane et al. (2000)
		N-butylbenzene	Gamard et al.(1997)
		Sulphonamide	Kim et al. (2000)

(continued)

Table 8.3 (continued)

Target group	PGPR	Antibiotics	References
	B. amyloliquefaciens	Bacillomycin D	Gu et al. (2017)
	B. cereus	Kanosamine	Milner et al. (1996)
		Zwittermicin A	Silo-Suh et al. (1994)
	B. subtilis	Kanosamine	Vetter et al. (2013)
		Iturin A (cyclopeptide)	Constantinescu (2001)
		Plipastatins A and B	Volpon et al. (2000)
		Fengycins	Zhang and Sun (2018)
Bacteria	*P. fluorescens*	Mupirocin (pseudomonic acid A)	Fuller et al. (1971)
		Azomycin	Shoji et al. (1989)
Virus	*Bacillus* sp.	Karalicin	Lampis et al. (1996)
	B. amyloliquefaciens	Mersacidin	Chatterjee et al. (1992)

8.4.1 Hydrogen Cyanide (HCN) Production

HCN is a volatile, secondary metabolite that overwhelms the growth of microbes and that also disturbs deleteriously the growth and development of plants (Siddiqui et al. 2006). Several studies feature a disease defensive effect to HCN, e.g., in the suppression of "root-knot" and black rot in tomato and tobacco root caused by the nematodes *Meloidogyne javanica* and *Thielaviopsis basicola*, respectively (Voisard et al. 1989).

8.4.2 Siderophore Production

Iron (Fe) is an essential element to virtually all forms of life and plays an important role in different physiological processes such as respiration, photosynthesis, DNA synthesis, and defense against reactive oxygen species. However, its availability is extremely limited by the very low solubility of ferric hydroxide complexes at neutral pH. To survive in such an environment, plant-associated PGPRs have different strategies for obtaining iron from the soil, which includes the synthesis of low molecular weight siderophores, viz., catechols, pyoverdin, and hydroxamate, which are selective ferric ion chelators. These compounds are secreted in response to iron deficiency. Siderophore-producing PGPR can prevent the multiplying of pathogens by repossessing ferric iron in the root zone (Siddiqui 2005). Iron depletion in the rhizosphere does not harm the plants, as the low iron level occurs at microsites of high microbial movement during the establishment of the pathogens.

Plants can utilize various fungal and bacterial siderophores as source of iron, while the total iron levels are too low to pay substantially to plant iron uptake. Plants also use their innate mechanisms to gain iron, dicots via a root membrane reductase protein that converts insoluble Fe^{3+} ion into the more soluble Fe^{2+} ion or in the case of monocots by the production of plant siderophores (Crowley 2006).

Siderophore-secreting microbial strains own iron-regulated outer membrane proteins (IROMPs) on their cell surface that carriage ferric iron complex to the respective cognate membrane; iron thus becomes accessible for metabolic processes (Johri et al. 2003). Siderophore-producing fluorescent pseudomonads are ahead commercial importance as they are harmless, do not prime to biomagnification, and also deliver iron nourishment to the plants, thereby stimulating plant growth (Sayyed et al. 2005). Carrillo-Castaneda et al. (2003) reported encouraging effects on alfalfa plantlet development after the inoculation of siderophore-producing genus such as *Azospirillum*, *Pseudomonas*, and *Rhizobium* grown in iron-starved cultures. The bacterized alfalfa seeds improved their germination as well as the root and stem dry weight. Iron-chelating hydroxamate siderophores of *P. aeruginosa* showed inhibitory action against *R. solani* and *C. gloeosporioides* in chili (Sasirekha and Srividya 2016). Also, inoculation of siderophore-producing rhizobacteria and their consortium increased the growth of wheat plant (Kumar et al. 2018). Nevertheless, as with other PGPR, the growth elevation that occurred may be due to other mechanisms or combinations of one or two mechanisms that rise nutrient availability, subdue pathogens, or upset root growth via hormone production.

8.5 Competitions

Effective colonization and perseverance in the rhizosphere are essential for PGPR to utilize their positive consequence on plants (Elliot and Lynch 1995). Several reports indicate the importance of colonization of the biocontrol agents in rhizosphere and endorhizosphere regions of plant (Forlani et al. 1999). Competition for nutrients, primarily carbon, nitrogen, and iron, might result in biocontrol of soilborne plant pathogens (Benson and Baker 1970). Suppression of damping off of peas by *P. cepacia* showed a significant relationship between population size of the biocontrol agent and the degree of disease suppression (Parke et al. 1991). The bacterial antagonist *P. fluorescens* effectively suppressed the green mold pathogen *P. digitatum* by means of competition and induced systemic resistance on citrus peels (Wang et al. 2018).

Also, suppression of take-all of wheat and *Fusarium* wilt of radish was correlated with the colonization of roots by *Pseudomonas* strains (Bull et al. 1991). Scher et al. (1985) reported that disease suppression by fluorescent pseudomonads depends mainly on its ability to colonize rhizosphere. Introduction of sss gene encoding rhizosphere colonization ability into poor colonizer strain of *P. fluorescens* WCS 307 increased competitive rhizosphere colonization ability in tomato root tip resulting in increased protection against *F. oxysporum* f. sp. *lycopersici* (Dekkers et al. 2000). So, the microbial ability to colonize rhizosphere and their persistence throughout the growing season has become the crucial factor for the selection of effective antagonistic organism. Dekkers et al. (1998b) showed that the gene encoding NADH dehydrogenase I plays an important role in root colonization. Another gene required for efficient colonization is the sss gene, encoding a site-specific

recombinase of the lambda integrase family which helps in adapting cells to rhizosphere conditions (Dekkers et al. 1998a).

8.6 Lytic Enzymes

The antagonistic process relies on the production of hydrolytic enzymes which enhances penetration of the host mycelium and partial degradation of its cell wall via secretion of mycolytic enzymes, viz., chitinases and glucanases. The pathogenic microbes that have shown susceptibility to these hydrolytic enzymes include *B. cinerea*, *F. oxysporum*, *Phytophthora* spp., *P. ultimum*, *R. solani*, and *S. rolfsii* (Glick 2012). The roles of each protein in the enzymatic complex of *Pseudomonas* appear to be different, and enzymes with different or complementary modes of action appear to be required for maximal antifungal effect on different pathogens (Viswanathan and Samiyappan 2002). Minaxi et al. (2012) described that *B. subtilis* solubilized phosphorus, exhibited 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and produced ammonia and indole-3-acetic acid. Various microbes secrete and excrete array of metabolites that can hamper pathogen growth and other activities. Numerous microbes produce and release hydrolytic enzymes that can lyse a wide range of polymers, including chitin, cellulose, hemicellulose, proteins, and nucleic acid (Table 8.4).

Expression and secretion of these hydrolytic enzymes by beneficial microbes can sometimes result in the suppression of plant pathogen activities directly. Several microbes like *B. subtilis*, *B. cereus*, *B. thuringiensis*, and *S. marcescens* have a potential to secrete hydrolytic enzymes for the biocontrol of phytopathogens (Jadhav and Sayyed 2016). Lytic enzymes can reduce different polymeric substances such as chitin, proteins, cellulose, hemicellulose, and DNA (Vivekananthan et al. 2004). Chitinase produced by *S. plymuthica* C48 inhibited spore germination and germ tube elongation in *B. cinerea*, but *S. marcescens* was considered to produce extracellular chitinases which act as antagonists against *S. rolfsii* (Frankowski et al. 2001). It was demonstrated that extracellular chitinase and laminarinase synthesized by *P. stutzeri* lyse mycelia of *F. solani* (Compant et al. 2005).

8.7 Induced Systemic Resistance

The PGPR induces systemic resistance (ISR) through invigorating the physical and mechanical integrity of cell wall as well as altering physiological and biochemical response of host leading to the synthesis of defense molecules against challenge inoculation of plant pathogens. ISR mechanism in plants was imparted by several PGPR determinants, viz., lipopolysaccharides, lipopeptides, salicylic acid, massetolide A, 2,3-butanediol, hexenal, and iron-regulated metabolite Cx (Pal and Gardener 2006). Followed by the interaction of PGPR determinants with plants, several defense reactions occur due to the accumulation of pathogenesis-related (PR) proteins (chitinase and β-1,3-glucanases), peroxidase, polyphenol oxidase,

Table 8.4 Lytic enzymes produced by plant growth-promoting rhizobacteria

Enzymes	Producer	Target pathogen	References
Chitinase	*S. plymuthica*	*B. cinerea*	Frankowski et al. (2001)
		S. sclerotiorum	Kamensky et al. (2003)
	S. marcescens	*S. rolfsii*	Ordentlich et al. (1988)
		Phaeoisariopsis personata	Kishore et al. (2005b)
	S. lydicus	*Pythium* sp.	Mahadevan and Crawford (1997)
	B. cereus	*R. solani*	Chernin et al. (1997)
	Paenibacillus illinoisensis	*R. solani*	Jung et al. (2003)
Endochitinase	*P. fluorescens*	*Tobacco necrosis virus*	Maurhofer et al. (1994)
		F. oxysporum f. sp. *pisi*	Benhamou et al. (1996)
β-1,3-glucanase	*Paenibacillus* sp.	*F. oxysporum* f. sp. *cucumerinum*	Singh et al. (1999)
	P. cepacia	*S. rolfsii*	Fridlender et al. (1993)
	Streptomyces spp.	*P. fragariae* var. *rubi*	Valois et al. (1996)
	Streptomyces sioyaensis	*P. aphanidermatum*	Hong and Meng (2003)
Laminarinase	*Pseudomonas stutzeri*	*F. solani*	Lim et al. (1991)
Proteases	*Stenotrophomonas maltophilia*	*P. ultimum*	Dunne et al. (1998)

phenylalanine ammonia lyase, chalcone synthase, catalase, phenolics, callose, lignin, and phytoalexins. Kloepper et al. (1992) reported that five of six rhizobacteria induced systemic resistance in cucumber which exhibited both external and internal root colonization. Seed treatment of radish with resistance inducing *P. fluorescens* strain WCS374 reduced *Fusarium* wilt in naturally infested field soil upto 50 percent (Leeman et al. 1995).

Chitinolytic enzymes together with β-1,3-glucanases or cellulases are most frequently considered to play a vital role in biocontrol (Chet et al. 1998). The enzymes like chitinases and β-1,3-glucanases lyse the host cell wall and lead to the leakage of protoplasmic contents which are in turn used as a food material for the multiplication of the antagonist. Biological control agents, namely, *P. fluorescens* 89B-27 and *S. marcescens* 90–166, were observed to induce resistance in cucumber against bacterial pathogen *P. syringae* pv. *lachrymans* and fungal pathogens, *F. oxysporum* f. sp. *cucumerinum* and *Colletotrichum orbiculare* (Liu et al. 1995). ISR by PGPR has been achieved in large number of crops including potato (Doke et al. 1987), radish (Leeman et al. 1996), cucumber (Wei et al. 1996), bean (de Meyer and Hofte 1997), tobacco (Troxler et al. 1997), tomato (Duijff et al. 1993), chilli, brinjal (Ramamoorthy et al. 2001), banana (Harish et al. 2009a, b), sugarcane (Viswanathan

and Samiyappan 1999), rice (Harish et al. 2008b), and mango (Parthasarathy et al. 2016) against broad spectrum of pathogens including fungi (Leeman et al. 1995), bacteria (Liu et al. 1995), and viruses (Kandan et al. 2005).

The ISR shares many properties with innate immunity in humans (Lugtenberg and Kamilova 2009). When plants grow, their roots enter quickly into a symbiosis with diverse microbes. This symbiosis may play the role of beneficial (aid in the uptake of water and minerals, such as phosphate, and protection of biotic and abiotic stress) or pathogenic agents in the development of plants (Gnanamanickam 2006). In case of pathogenic bacteria, the immune response of the plant is characterized by the production of salicylic acid, which in revenge induces a set of genes encoding pathogenesis-related proteins in the plant (Gnanamanickam 2006). ISR was observed first with *Pseudomonas* sp. strain WCS417r against *Fusarium* wilt of carnations and by selected rhizobacteria against the fungus *C. orbiculare* in cucumber (Compant et al. 2005). Available reports showed that in rice, seed treatment followed by root dipping and a foliar spray with *P. fluorescens* strains Pf1 and FP7 induces systemic resistance against the sheath blight pathogen, *R. solani* (Jayashree et al. 2000). Thus ISR plays a major role in combating the pathogen during the host-pathogen-biocontrol interaction.

8.8 Formulations of PGPR

Potential PGPR needs to be formulated with suitable carriers for mass multiplication and broad-scale application in fields. Mass multiplication of PGPR in a suitable medium and development of a powder formulation were first carried out in 1980. A dried powder formulation of PGPR is especially important for seed treatment and soil application. Among the various bioformulations, talc- and liquid-based formulations were extensively used in agriculture and horticulture crops for managing diseases (Table 8.5). Although this type of formulation can be produced in large quantity, it may be difficult to store and have a relatively short shelf life, poor quality, and low field performance. Development of bioformulation with short shelf life was possible by using vegetative cells of the antagonists as the active ingredient in the formulations (Kanjanamaneesathian et al. 2007). Various solid formulations, such as floatable granules, floatable pellets, and effervescent fast-disintegrating granules, have been developed for the management of sheath blight disease under controlled conditions (Wiwattanapatapee et al. 2013). These carrier-based formulations help in improving the shelf life, protecting the viability, and easy delivery of the bacterial cells to the targeted sites in the plant system and long-term survival in the soil. Thus formulations with longer shelf life need to be targeted as they can establish in the soil, survive for a considerable period of time, and improve the soil fertility besides protecting from harmful pathogens.

Table 8.5 Different types of formulations from PGPR

Formulation	PGPR	Crop	Disease	References
Talc	*P. fluorescens*	Blue pine	Nursery diseases	Ahangar et al. (2012)
		Chillies	Fruit rot	Bharathi et al. (2004)
		Muskmelon	*Fusarium* wilt	Bora et al. (2004)
		Rice	Sheath blight	Radjacommare et al. (2002)
		Tomato	*TSWV*	Kandan et al. (2005)
		Mung bean	*Macrophomina* root rot	Saravanakumar et al. (2007a)
		Rice	Sheath rot	Saravanakumar et al. (2007b)
		Tea	Blister blight	Saravanakumar et al. (2009)
		Sugarcane	Red rot	Viswanathan and Samiyappan (2002)
		Mango	Anthracnose	Vivekananthan et al. (2004)
		Rice	Sheath blight	Vidhyasekaran and Muthamilan (1999)
Lignite	*P. fluorescens*	Rice	Sheath blight	Vidhyasekaran and Muthamilan (1999)
Peat	*P. fluorescens*	Rice	Sheath blight	Vidhyasekaran and Muthamilan (1999)
	P. chlororaphis, *B. subtilis*	Turmeric	Rhizome rot	Nakkeeran et al. (2004)
Chitin	*B. subtilis*	Groundnut	Crown rot	Manjula and Podile (2001)
		Pigeon pea	*Fusarium* wilt	
Vermiculate	*P. fluorescens*	Rice	Sheath blight	Vidhyasekaran and Muthamilan (1999)
Charcoal	*Bacillus* sp.	Mung bean	Wilt	Pahari et al. (2017)
Wheat bran	*B. subtilis*, *P. putida*	Lettuce, cucumber	Root rot	Amer and Utkhede (2000)
EB™	*P. fluorescens*	Sugar beet	Damping-off	Moenne-Loccoz et al. (1999)
Alginate	*P. fluorescens*	Sugar beet	*Pythium* rot, *Rhizoctonia* rot	Russo et al. (2001)
	Streptomycetes sp.	Tomato	Damping-off	Sabaratnam and Traquair (2002)
Liquid	*P. fluorescens*	Tomato	*Fusarium* wilt	Manikandan et al. (2010)
		Mango	Stem end rot	Parthasarathy et al. (2016)
Water in oil	*Fluorescent pseudomonads* (FP7)	Banana	Anthracnose	Faisal et al. (2014)

8.9 Concluding Remarks and Future Directions

Historically, emphasis in crop science has been placed on the discovery of new disease resistance genes through molecular breeding techniques rather than using the resistance potential already present in plants. The resistance in the plants can be induced by means of beneficial microbes present in the soil rhizosphere. The recent demonstration of the use of biocontrol agents in the laboratory and field situations presents exciting opportunities for the control of plant diseases by multiple mechanisms. Various field experiments with crop plants have shown that eco-friendly approaches using microbial bioagents can lead to long-lasting, broad-spectrum disease control and can be used preventively to bolster general plant health. However, application of bacterial bioformulation in the field at times may exhibit inconsistency in the efficacy due to short shelf life in the environment and their susceptibility to unfavorable environmental conditions. The survival and competitive ability of the microbial strains to be introduced must be improved as very little information is known about the competitiveness of the microbes and factors governing it. In order to harness the potential benefits of bioagents in commercial agriculture, the consistency of their performance must be improved. Development of quality inoculum with increased shelf life and user-friendly formulation are important factors essential for the success of bioinoculant technology. Besides, the molecular mechanisms underlying the host-pathogen-biocontrol interaction should be unraveled through genomic and proteomic approaches to identify the defense genes in the plants. These genes can be exploited for the management of plant diseases. Molecular markers, e.g., reporter gene tagging, PCR, or serological markers, can be used for studying the competence of the inoculated PGPR strains. Once these factors are identified, it may be possible to manipulate them in the field to enhance the stability of their performance. Thus the PGPR possessing the useful biosynthetic genes can be screened through molecular markers and can be exploited for sustainable plant disease management.

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9 Soil Microbial Hotspots and Hot Moments: Management vis-a-vis Soil Biodiversity

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Abstract

All soils are heterogeneous in nature with differentiation in physical, chemical, and biological properties. Heterogeneity in substrate availability creates microbial hotspots and hot moments in soil. Microbial hotspots are microsites in soil with higher microbial activity and respiration rate compared to bulk soil. Microbial hotspot localization may occur around plant root surface (i.e., rhizosphere), on degrading plant roots (detritusphere), plant root or earthworm burrows (biopores), or surface of soil aggregates. In soil, most prevalent hotspots are found in the rhizosphere and on aggregate surfaces but frequently are of mixed origin. Priming effects are limited in microbial hotspots but are of significance of hot moments (short-term microbial hotspots). Residue decomposition induces significant changes in the microbial community. For concept of soil microbial hotspot and hot moment, we extensively reviewed and examined available literature related to management of soil biodiversity. Long-term cropping sequence had also significantly influenced microbial activity in agricultural soils. Increasing biodiversity through improved crop management practices restores positive aboveground-belowground interactions. In these insights, microbial hotspot management should be considered important in soil sustainability and food security.

Keywords

Microbial hotspot · Hot moment · Microbial activity · Soil biodiversity

9.1 Introduction

All soils contain microorganisms, i.e., bacteria, actinomycetes, fungi, protozoa, and nematodes, but the relative amounts differ because of variable soil conditions. Each of these microorganisms contributes to boost soil and plant health through energy and chemical transformations, thereby playing a vital role in biochemical processes or cycles. Microbial activity is the most important indicator of soil health (Kibblewhite et al. 2007) due to its high sensitivity to changes in environmental conditions and land use pattern (Mganga et al. 2015). Microbial activity is affected by microbial community composition (Goberna et al. 2005), the substrate (labile C) availability and energy supply (Schimel and Weintraub 2003) and soil aggregate (Miller and Dick 1995). In the soil system, the most biogeochemical processes are microbially mediated. Thus, nutrient availability through these processes depends upon the functionality of soil microbial diversity. Microorganisms play an

important role in the organic matter decomposition, returning nutrients to plant available forms, humus formation. Humus tends to bind to negatively charged sites of soil particles and thus forms a film and causes aggregation of particles, improving soil structure, aeration, and water retention. In biosphere, owing to the high differentiation of properties and processes, the soil is the most heterogeneous entity. The heterogeneity in the availability of substrate to microorganisms led to the creation of microbial hotspots in the soil. Microbial hotspots are nothing but microsites or smaller volume of soil with a much faster rate of processes compared to average soil conditions. Hotspots have fresh substrate inputs, which supply energy to microorganisms for growth and maintaining their metabolism. Hotspots in soils are characterized by higher enzyme activities, higher microbial respiration, and biomass (Banfield et al. 2017; Hoang et al. 2017).

The short-term microbial hotspots are known as hot moments. Hot moments are a short sequence of events inducing an accelerated rate of processes. Thus, hot moments and hotspots are of dynamic character. The duration of hot moments depends on many factors, i.e., the quantity and quality of substrates, quantity of output, and location of occurrence (Kuzyakov and Blagodatskaya 2015). An amount of labile C and energy drives the microbial activities in all the soils (Blagodatsky et al. 1998). So, the labile C as input not only increases the microbial abundance but also microbial activity in soil.

9.2 Size and Duration of Microbial Hotspots and Hot Moments

Based on the location of labile organic sources as input, the hotspot groups are categorized as follows (Table 9.1). Providing the fact that plants stimulate microbial and enzyme activity in its root zone by releasing labile C and other rhizodeposits, the rhizosphere is one of the most dynamic habitats (Jones et al. 2009; Hinsinger et al. 2009). The detritusphere (soil adjacent to dying and dead roots) may also have the hotspots because of high polymers as well as low molecular weight organic carbon inputs through the dying root (Bastian et al. 2009). In rhizosphere, the plant provides a continuous flow of rhizodeposition during growth period (Kuzyakov and Domanski 2000), whereas dead root decomposition acts as a temporally concentrated C source (Spohn and Kuzyakov 2014). Due to the longer and concentrated source of available labile C from dead roots, it is generally believed that microbial population is higher in detritusphere (Marschner et al. 2012) than rhizosphere (Spohn and Kuzyakov 2014). Tubular soil macropores or voids in soil formed by the activity of roots or pores remaining after root decay (detritusphere), by earthworms (drilosphere), are known as biopores, which are an important microbial hotspot, especially in the subsoil (Hoang et al. 2016). Various processes and C sources contribute to their development simultaneously or continuously.

Root litter addition and earthworm burrows increase microbial biomass (Sanaullah et al. 2011). Biopores affect soil physical conditions especially subsoils by increasing air and water circulation (Kautz 2014). The hotspot development rates

Table 9.1 Properties of hotspot groups in soil (Kuzyakov and Blagodatskaya 2015)

Hotspots	Rhizosphere	Detritusphere	Biopores	Aggregate surface
Origin	Primary biotic; roots	Primary biotic; litter	Secondary biotic; burrowing animals, roots	Secondary (mainly) abiotic; swelling/shrinking
Boundary (mm)	2–10	5–20	1–3	0.1–1
Relative C availability	High	High–medium	Medium–low	Low
C/N ratio	~10	>20	Variable	10–20
Relevance	Whole soil profile	Above mineral soil surface, topsoil	Below A_h/A_p, subsoil	Below A_h/A_p, subsoil
Regularity	Occasional + regular	Regular + occasional	Occasional	Occasional
Duration of hot moments	A day (weeks)	Weeks–months	Days–weeks	Days

are not constant; it varies temporally. The extent and regularity of hot moments generally depend upon (1) regular availability of labile C or the input source and (2) the rates of microbial use of input (Herron et al. 2013). Accordingly, hotspot formation requires variability. Many properties and process rates in soil surrounding hotspots vary by orders of scale within very short distances and periods. The duration of the hotspot is determined by the dynamic nature of C input. Constant availability of inputs or labile carbon source led to the longer existence of hotspots.

9.3 Microbial Activities as a Driver of Hotspot Performance

The microbial hotspots have two to three times greater diversity and microbial biomass over bulk soil (Marschner et al. 2012). However, the dominant part of this total biomass is shared by dormant microbes, while active microbes represent a small share is known to perform a range of biochemical processes. Populations of active microorganism in the root rhizosphere are two to three times more compared to bulk soil (Blagodatskaya et al. 2014). In the detritusphere, a zone where C liberation is taking place for longer and the microbial root competition is fairly weaker compared to rhizosphere, the population and biomass of active microbes reported to 4–20 times greater than that of in bulk soil (Blagodatskaya et al. 2009). Microbial hotspots because of their high density of C substrate and longer hot moments are generally outstanding in terms of active microorganisms' biomass, particularly in a physiologically alert stage (Table 9.2). For example, due to some reason, many researchers reported that the activity of hydrolytic enzymes in rhizosphere zone was 3–5 times greater (Lee et al. 2013), whereas the N_2O emissions from the detritusphere region were 2–9 times more intensive (Blagodatskaya et al. 2010) compared to bulk soil. Therefore, it is very clear that hotspots are not only rich in total microbial biomass, but also the portion of active microbes is also higher in hotspots which are very important for biochemical processes within the soil (Table 9.2).

Table 9.2 Relative changes of PLFA content by activation/deactivation of soil microbial community during hot moments (Kuzyakov and Blagodatskaya 2015)

	Relative changes in PLFA content				
			Bacterial		
Effect of	Total	Fungal	Gram +	Gram −	Source
Hotspots in comparison with bulk soil					
Plant roots (rhizosphere)	↑ 1.5–1.7	↓ 1.1–1.3	↑↓ 1.1	↑ 1.1–1.3	Denef et al. (2009)
Plant growth	↑ 1.7		↑ 1.8	↑ 2	Lu et al. (2004)
Plant species	↑ 1–1.7	↑ 1.4–2	↑ 1–1.2	↑ 1–1.3	Hamer and Makeschin (2009)
Detritusphere	↑ 1.8–5.1	↑ 11–68	↑ 2.1–↓ 1.6		Baldrian et al. (2010)
Detritusphere	↑ 1.1–1.4	↑ 2–2.3	↑ 1.5–↓ 1.3		Marschner et al. (2012)
Detritusphere	↑ 1.2	↑ 2.5–4	↑ 1.1–1.3		Rousk and Bååth (2007)
Activation during hot moments					
Rewetting	↑ 1.4–1.6				McIntyre et al. (2009)
Available nutrients	↑ 2.4	↑ 50	↑ 1.2		Ehlers et al. (2010)
Wheat straw and fertilizer	↑ 1.7	1.1	↑ 1.7	↑ 5.5	Pietri and Brookes (2009)
Barley straw		↑ 4	↑ 1.3		Rousk and Bååth (2007)
Leaf litter	↑ 1.5–4.7				McIntyre et al. (2009)
Sorghum residues	↑ 1.7–2	↑ 2.3–3	↑ 1.5	↑ 2	White and Rice (2009)
Hotspot expiration–end of hot moments					
One-year incubation	↓ 3.5–3.6	↓ 6–10	↓ 2.7–5	↓ 2.9–6	Feng and Simpson (2009)
Soil depth	↓ 3	↓ 3.5	↓ 1.5	↓ 2.7	White and Rice (2009)
Decreasing pH	↓ 2.1	↑ 1.3	↓ 1.7		Djukic et al. (2010)
Grazing		↓ 2	↑ 2		Klumpp et al. (2009)

9.3.1 Microbial Diversity and Community Structure in Hotspots

The microbial hotspots are rich in microbial diversity especially of active microbes over bulk soil (Marschner et al. 2012). The principal reason for higher biomass of microbes in these microbial hotspots is higher substrate or C input availability which stimulates their growth and shapes community structure. Size and shape of the microbial community affected by locality of hotspot and type of substrate availability (Table 9.2).

9.3.2 Microbial Strategies and Competition in Hotspots

Microbial functions within microbial hotspot region are defined by the supremacy of the ecological groups, i.e., r- and K-strategists (Nottingham et al. 2009). However, the phylogenic structure of the microbial community is not directly governed by supremacy as most of the bacterial and fungal phyla are reported to have both r- and K-strategists. The dominant type of strategy within the microbial hotspots can be analyzed by the kinetics and effectiveness of microbial growth. Kinetic parameters of microbial communities suggest that the addition of very minute amounts of labile carbon substrates can be able to activate fast-increasing strategists in the microbial hotspots (Blagodatskaya et al. 2010). Many studies proved that rhizosphere, detritusphere, and biopores are the microbial hotspots with great microbial biomass and activity compared to bulk mineral soil, but a few reports have been found on the aspect of competition between microbes in these hotspot sites. The amount and availability of labile C substrates in detritusphere and rhizosphere are known to define their competition structure. For example, the detritusphere is the preferred site for the competition between microbial species, whereas in rhizosphere this occurs mainly between microbes and plants (Kuzyakov and Xu 2013).

9.3.3 Signal Pathways at Hot Moments

Microorganisms change physiological states (i.e., from active stage to dormant stage and vice versa) on the basis of availability of labile C substrates. So, labile C availability plays an important role in the adaptation of microbes to dynamic environmental conditions in the hotspots. However, some of the physicochemical factors of soil (i.e., moisture and temperature) are also known to play a vital role in the switching of physiological states of soil microbes in the hotspots. Apart from these factors, signaling molecules are also of a prime significant factor in activation/deactivation mechanisms of the microbes. Many studies suggested that transition of active cells to dormancy is mainly governed by quorum sensing, i.e., a phenomenon in which secretion of sensing molecules stirs up the reduction of population density of microbes in subjected hotspots (Gray and Smith 2005).

9.3.4 Instruments for Characterization of Hotspots

The pH changes within microbial hotspot can be reported with gels (Hinsinger et al. 2009) or by placing pH and redox microelectrodes near to the root zone of the plant. Autoradiography and its follow-up imaging are also good means for localization of rhizodeposits and of uptake of nutrients by microbes in the hotspot sites (Rasmussen et al. 2013). However, many times this type of localizations through autoradiography does not necessarily reveal the exact location and pattern of microbial hotspots. These parameters can use to define the microbial activity (CO_2/O_2 changes) of the microbial hotspot. Recently developed soil zymography technology is able to locate

the hotspots by analyzing the activities of various soil enzymes, i.e., protease, amylase, acid and alkaline phosphatases, cellulase, and chitinase (Eickhorst and Tippkotter 2008).

9.4 Ecological Significance of Microbial Hotspot

The higher rates of biochemical processes within the hotspot zone over bulk soil are of special ecological significance as this directly or indirectly associated with substrate availability. Microbial hotspots directly affects decomposition and mineralization of crop residue, amount of rhizodeposits and soil organic matter, microbial populations, and release of nutrients. The hotspots of soil also govern the rates of processes related to C transformation, i.e., microbial immobilization of soil N and other plant nutrients as well as consumption of O_2 and electron acceptors available in the soil and root sites (Rudolph et al. 2013).

9.5 Strategies for Hotspot Management

Rhizospheric and detrituspheric soils are characterized by high concentrations of labile C and hence are hotspots of microbial activity. Furthermore, the microbial community structure modifies with distance from roots or residues. Marschner et al. (2012) reported the higher activities of ß-glucosidase, xylosidase, and phosphatase in the vicinity (1–2 mm) of roots and residue-amended soil at 2 weeks after planting, with usually greater activities in the vicinity of the residue-amended soil over roots (Fig. 9.1).

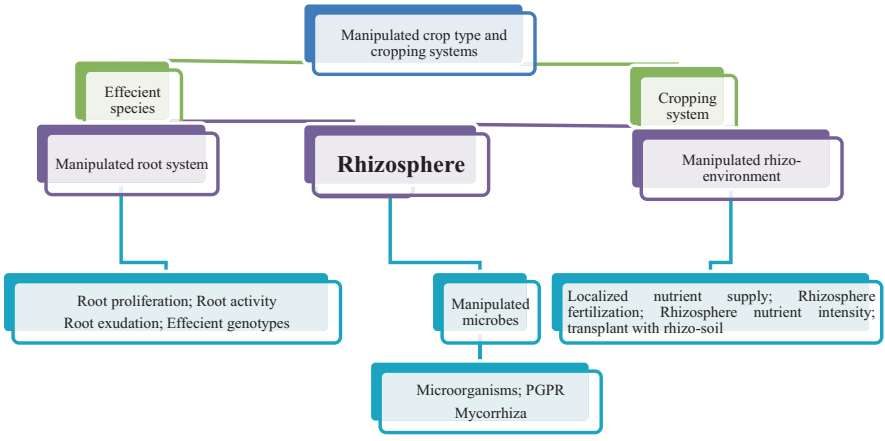

Fig. 9.1 Strategies for hotspot management

9.5.1 Microscale Distribution and Function of Soil Microbes in the Interface Between Rhizosphere and Detritusphere

In general, results of a case study involving samples amended with maize residue show that the degree of microbial structure did not overlap resulting in a U-shaped pattern of activities in the rhizosphere-detritusphere interface. The amount of PLFAs which is directly associated with activity and biomass of soil microbes was nearly 30% more in detritusphere zone over rhizosphere. The major part of reported PLFAs primarily belongs to fungal communities and their activities, and biomass was 5–7 times higher in the roots of plants of residue-amended soil as compared to unamended soil. Plant roots and soils amended with crop residue have the potential to strongly affect the amount of PLFAs especially in the root vicinity zone due to their richness in terms of easily degradable carbon substances. The microscale gradients of various soil enzymes and bacterial and fungal PLFAs within interface and between rhizosphere and detritusphere zones are directly controlled by carbon inputs of the soil. Due to their higher density of carbon, the overall effect of crop residue was larger than that of root compartment.

9.5.2 Role of Organic Layer-Mediated Microbial Hotspots

Soils with high density of organic carbon are treated as organic layer which strongly acts as hotspots for microbes in respect to their activity, abundance, and diversity. A microbial enzymatic activity was found much higher in soils rich in organic carbon compared to mineral soils. In comparison to mineral soil, the organic-rich soils may be a good representative for a hotspot resulted in higher productivity and faster cycling of various essential nutrients in the associated agroecosystem (Lee et al. 2012). Archaeal diversity was found to be greatly affected with changes in soil types, while diversity index of bacteria and fungi did not exhibit any significant change with respect to type of soil. The dissimilarity in microbial abundance and diversity indicated that there was geographical aberration in the microbial community even in Arctic tundra regions with similar temperature conditions. This may be due to historical difference in the development of the soil layer between each tundra region resulting to different evolutionary processes in the microbial populations. The identification of active microbes in context of the spatial heterogeneity of tundra soils, temperature, and moisture conditions is necessary to know the understanding of nutrient cycling in Arctic systems (Lee et al. 2012).

9.5.3 Microbial Community and Their Structures in Residue-Amended Soil

Microbiological processes are playing a great role for the various ecological functions of soils due to their extended role in input and output dynamics of soil organic matter (SOM) content. Organic carbon content of soil which may lose through

erosion or mineralization can be balanced by the incorporation of crop straw (Singh et al. 2015, 2016; Meena et al. 2013, 2015). A comprehensive study of samples (residues, detritusphere, and bulk soil) regarding diversity and structure of different bacterial and fungal communities in terms of PLFAs of soil amended with wheat residue highlighted the existence of a succession of populations following wheat straw incorporation, as proved in recent studies focused on plant residue decay in soil (Ranjard et al. 2003). A Monte Carlo test of samples amended with wheat straw has been executed to assess the significant level of the imbalance between incubation times and has allowed the deduction of magnitude for community that was found in following sequence: bulk soil<detritusphere zone< residue. The variations in bacterial diversity, richness, and community composition residue-incorporated soils at low and high moisture levels show that moisture strongly influences bacterial distribution in residue-incorporated soils (Ranjard et al. 2003).

Overall, these all findings confirmed that soils amended with wheat residue and living roots act as hotspots especially due to their richness in terms of carbon substances which resulted in important community dynamics, particularly in poorly managed soils having substrate for microbial growth. Hence, the view dynamics of community structure seemed to be related to changes in the availability of carbon resources that occur during decomposition. The alterations in the ARISA profile confirmation for the residue zone at the early stages comprised of a strongly increased intensity of various bands which were of slight importance before the wheat straw incorporation (Ranjard et al. 2003).

9.5.4 Changes in Soil Microbial Composition Under Cropping Systems and Tillage Practices

The agriculture practices affect the soil microbial communities in a very complex manner. Therefore proper understanding of these practices is significant for the efficient and proper management of crop production ecosystem. Tillage and crop rotations have been extensively adopted in all agricultural systems, as these practices have the prospective to augment microbial biomass and activity. The soil organic matter (quantity, quality, and its distribution in the soil) is a major factor that strongly affects diversity, biomass, and activity of soil microorganisms as it is the basic food source for soil biota. Conservation agriculture-based practices such as zero tillage and reduced tillage systems are known to reduce land degradation through arresting soil erosion and enhancing SOC which sustains soil health (Hobbs et al. 2008). Usually, the microbial diversity is negatively correlated with intensity of tillage (Yanping-Lei et al. 2017). The impact of soil tillage over microbial parameters of soil mostly determined through climate, location, and below as well as above environmental conditions. The explicit impact of these practices on microbial community composition is yet to be explored.

9.5.5 Chemical Fertilization and Structure and Diversity of Soil Microbes

The soil microbial community structure may be defined as the abundances and relative proportions of important microflora in soil. This is further dependent on the type of the growth medium. Long-term fertilizer applications reported to have diverse effect on soil microbial communities. The effect can vary with the type of fertilizer used, their application doses, soil type, and other factors. Among the inorganic fertilizers, nitrogen (N) improves crop yields, but at the same time, its continuous use for long periods considerably influences the quality and productivity of soil. Yu et al. (2015) noted that combined application of organic-inorganic compound fertilizers resulted in higher cultured bacteria population compared to soil fertilized using chemical fertilizers applied alone. The abundance of soil bacterial communities' shows great difference between different months, viz., May, July, and November. However, irrespective of sampling time, the diversity of soil bacteria and actinomycetes was found maximum with organic-inorganic compound-fertilized soil compared to other soils. Conversely, responses to fertilization management practices were different for fungi, and highest values of fungal diversity were reported in the soil supplied with chemical N fertilizers.

9.5.6 Changes in Soil Microbial Community in Grassland Ecosystems of Temperate Climate

Grassland ecosystems are of great ecological significance due to their extended role in different ecosystem services associated with soil microbial community. The grassland systems of temperate climate are differing in terms of their microbial communities to the tropical grassland, and this variation appears to be ascribed to difference in microbial biomass carbon and metabolic quotient (qCO_2) among them. The soil microbial activity which was accounted by measuring basal respiration may vary with the type of grassland, site interactions, and soil moisture. In improved grasslands the respiration rate was more, and it also reflected as low qCO_2. Respiration in unimproved and semi-improved grasslands was extensively elevated in comparison to improved grasslands. The diversity and biomass of culturable bacteria, namely, pseudomonades, found maximum in the improved grasslands as compared to degraded grasslands. Site distinctiveness of upland grasslands also reported to affect the diversity, biomass, and respiration of microbial communities. Microbial biomass carbon and respiration were significantly greater in the situation due to more rainfall in these grassland ecosystems (Zak et al. 1996). Variation in carbon density due to varying management options affects the size of the microbial biomass carbon; it is consistently higher in the unimproved than in the improved grassland. The soil metabolic quotient (qCO_2) was enhanced when the microbial biomass carbon is operating efficiently. It has been shown that microbial communities from virgin sites have higher qCO_2 than those from operated sites (Anderson and Domsch 1993; Grayston et al. 2001).

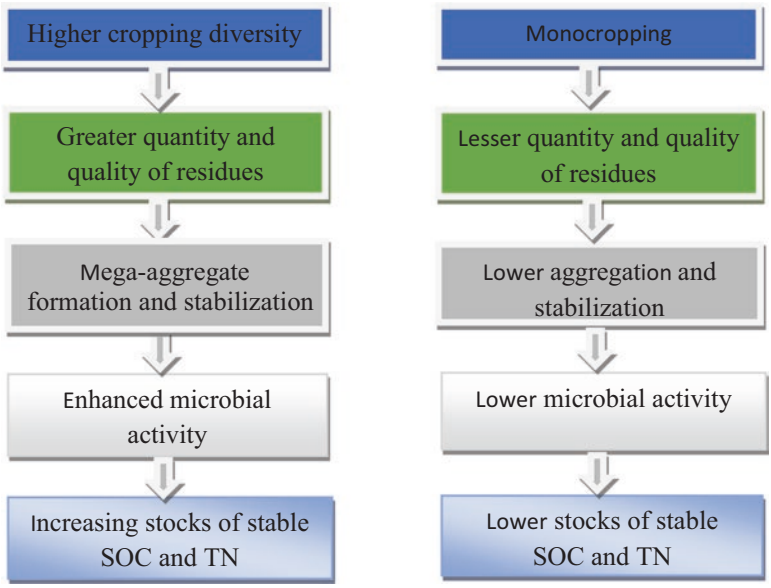

Fig. 9.2 Trajectory of aggregate and SOM formation and stabilization under a high diversity rotation versus monoculture crop

9.5.7 Crop Rotational Diversity

The agricultural intensification that involved continuous adoption of cereal-based rotations without inclusion of legumes led to reductions in crop and soil productivity because of biodiversity loss. Crop rotation that involves legumes may decrease this negative impact by restoring positive above- and belowground interfaces and increase biodiversity. Constructive effect of different rotations on diversity and biomass of aboveground microbes and processes had been observed in natural ecosystems. Increased crop diversity with proper crop rotations may be the results of their favorable impact on physical, chemical, and biological soil properties. The increasing rotational diversity also has positive effects on aggregate formation. So, use of rotational diversity as a feasible management practice for promoting soil sustainability is an appropriate option (Smith et al. 2014). Soil management practice intended to augment soil biological activity and C concentration also increases the stability of mega-aggregates. Study site has the fastest turnover rates and is the most susceptible to changes in management (Tiemann et al. 2015). The highly strong correlation among fungal abundance and that of soil carbon and nitrogen density in the mega-aggregates further supports fungal contributions to soil structural stability (Fig. 9.2).

9.6 Conclusions and Future Perspective

Soils are one of the most heterogeneous units of the biosphere. Soils also have enormously high isolation of property and processes within nano- to macroscales. This chapter analyzed the properties, occurrence, and management of hotspot in soil. Long-term integrated fertilization enhances soil microbial diversity. With high availability of labile C and energy, the most important hotspots are the rhizosphere and detritusphere. In warmer climate, the concentrated release of C in root-detritusphere zone leads to broader hotspot area and distribution in the root-detritusphere than in the rhizosphere. Although areas occupied by hotspots are very small (1–5%) compared to bulk soil, but this is compensated by very high process rate in hotspots (up to two times higher). The effect of bacterial competition on straw decomposition accounts for the strong influence of moisture on bacterial community structure. Interactions between rotational and microbial diversity have a positive influence on functional relationships of biodiversity in agroecosystems. Thus, microbial hotspot management is important in soil sustainability and food security.

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Surfactin: An Emerging Biocontrol Tool for Agriculture Sustainability

10

Fauzia Yusuf Hafeez, Zakira Naureen, and Ambrin Sarwar

Contents

Abstract

The agricultural productivity is a serious concern to meet the ever-increasing demands of growing human population all over the world. Thus, to attain sustainable agriculture, without harming the environment, use of different green compounds is a prerequisite. This chapter highlights the use of surfactins as a biocontrol agent, which is an eco-friendly and cost-effective approach for managing plant diseases. Biosurfactants especially surfactin produced by *Bacillus* and *Pseudomonas* species can serve as green surfactants, and they exhibit wide biocontrol activity. Surfactins are eco-friendly and less toxic and thereby have several widespread applications in food, agriculture, cosmetics, and pharmaceutical industries. Several rhizosphere and plant-associated microbes capable of

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producing surfactin play vital role in motility, signaling, and biofilm formation, indicating that biosurfactants (surfactins) direct plant-microbe interaction. In agriculture, surfactins can be used against plant pathogens as biocontrol agents and for increase in the bioavailability of nutrient for beneficial plant-associated microbes. Particularly, antifungal activity of surfactins and their role in surface colonization by pathogens and beneficial bacteria results in biocontrol activity. Therefore, exploring surfactins from bacterial isolates for applications in agriculture warrants a detailed research.

Keywords
Surfactins · Sustainable agriculture · Plant pathogens · Surfactin production · IS

10.1 Introduction

Excessive use of chemical pesticides in current decade has not only led to contamination of land and water resources but also caused ecological imbalance of soil microbes, thus promoting the development of resistant pathogenic strains posing tremendous threat to human health and environment. The continuous health and environmental hazards incited a persistent quest of alternative biocontrol agents such as biosurfactants which can be used for preservation of food and dairy products and agricultural applications (Mandal et al. 2013).

Biosurfactants are amphipathic molecules considered as multifunctional materials of the twenty-first century due to their numerous properties of practical utility in industry, environment, and agriculture (Santos et al. 2016). These are produced by a variety of microorganisms specifically the members of the *Pseudomonas* and *Bacillus* species (Kloepper et al. 2004). Three *Bacillus*-derived lipopeptide families, i.e., surfactin, iturin, and fengycin, hold special significance in this context for their efficacy against different pathogens of economically important plants (Ongena and Jacques 2008), viz., direct inhibition of phytopathogens and induced systemic resistance (ISR).

Surfactin produced by strains of *B. subtilis* is the main representative of lipopeptides with anionic properties. Surfactin is named due to its excellent surface active properties, and it is one of the most important biosurfactants of microbial origin with strong emulsification properties. Surfactin discovered by Arima et al. (1968) was identified as macrolide lipopeptide through molecular characterization by Kakinuma et al. (1969). It is amphiphilic compound having tendency to exist in both hydrophobic and hydrophilic environments (Ishigami et al. 1995). It exhibits significant membrane dynamics and surface-interface characteristics leading to excellent applications in biotechnology-based processes, environmental pollution management, and pharmaceutical industry (Nitschke and Costa 2007; Abdel-Mawgoud et al. 2008; Banat et al. 2010; Cao et al. 2010; Mulligan 2009).

Surfactin is a cyclic lipoheptapeptide (Glu-Leu-Leu-Val-Asp-Leu-Leu) having LLDLLDL (chiral sequence) interweaved hydroxy fatty acid having chain length of C-12 to C-16 carbon to form a cyclic lactone ring structure (Seydlova et al. 2011).

Depending on the size of lipids and amino acid arrangement, the surfactin type may vary (Korenblum et al. 2012). Amino acids are positioned at 2, 3, 4, 6, and 7, whereas the Glu and Asp residues are positioned at 1 and 5, respectively. Generally, isoforms of surfactin do exist with numerous peptidic variants having difference in length of aliphatic chain (Tang et al. 2007). The configuration of β-hydroxy fatty acids and amino acids in the surfactin is dependent on the bacterial producer strain and nature of culture conditions (Seydlova et al. 2011). The function and release of biosurfactant(s) are linked with uptake of hydrocarbons, so hydrocarbon-degrading microorganisms mostly synthesize them (Banat et al. 2010).

10.2 Production of Surfactin

Natural surfactins are less toxic than the synthetic ones with an added advantage of biodegradability. Surfactin production by various strains of *Bacillus subtilis* is manifested by Srf A operon, sfp, and com A (Rongswang et al. 2002). The srf A operon encodes a protein which forms a surfactin synthetase (non-ribosomal peptide synthetase) (Cosmina et al. 1993). Whereas, com A encrypts a srf A gene transcription activator (Roggiani and Dubnaum 1993), and sfp is srf A activation enzyme which encodes 4/-phosphopantetheinyl transferase (Nakano et al. 1992; Lambalot et al. 1996) which catalyzes the conversion of the inactive proteins of surfactin synthetase to active forms (Pfeifer et al. 2001).

Abiotic factors such as oxygen availability, variation in temperature, and growth medium constituents which influence the growth and activity of microbial cells also influence the surfactin production (Cameotra and Makkar 1998). Biosurfactin production by bacterial strains is dependent on availability and absence of many micronutrients. For instance, limitation of iron stimulates biosurfactant production in *Pseudomonas fluorescens* (Persson et al., 1990a, b) and *P. aeruginosa* (Guerra-Santos et al., 1984, 1986). Whereas, addition of manganese and iron salts leads to the production of biosurfactants in both *Rhodococcus* sp. and *B. subtilis* (Cameotra and Makkar 1998). pH plays a crucial role in biosurfactin production. For instance, sophorolipid production in *T. bombicola* is stimulated by the pH of medium (Guerra-Santos et al. 1984). *Pseudomonas* sp. produces maximum quantity of rhamnolipids at pH ranging 6.0–6.5, while further increase in pH 7.0 results in significant reduction of rhamnolipid synthesis (Guerra-Santos et al. 1984). *Bacillus* spp. grown in optimal pH for production of surfactin yields about 0.1 g/liter of surfactin (Fig. 10.1).

The major concern in marketing of surfactins is higher production cost which cannot be compared with chemical surfactants. Different approaches have been practiced to make it economical, such as the optimization of fermentation environment, downstream processing, usage of inexpensive and waste substrates, and the growth of efficient surfactin-producing strains (Banat et al. 2010). Studies report surfactin production using Pharma media or semisynthetic medium (Al-Ajlani et al. 2007) composed of 2% glucose as the carbon (C) source and 5 g L^{-1} of L-glutamic acid as the nitrogen (N) source and trace metals (Nakano et al. 1992),

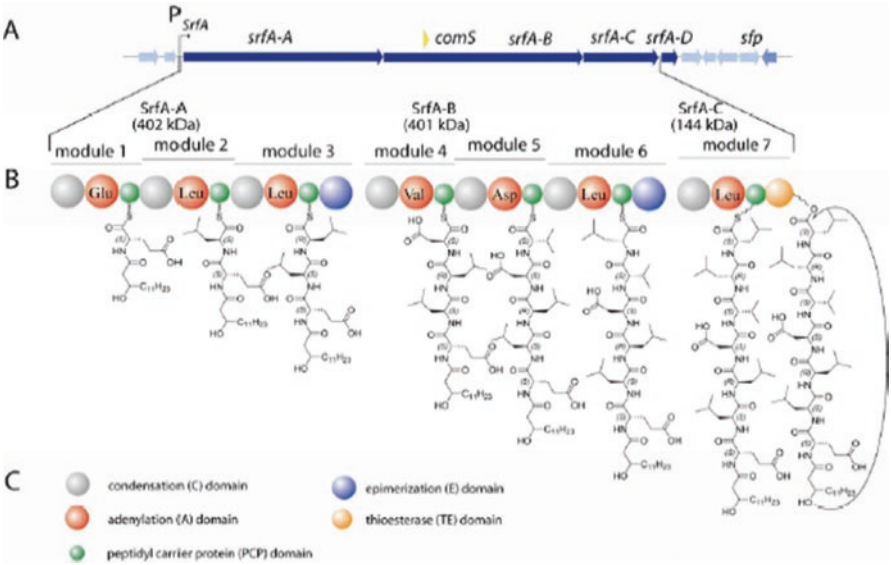

Fig. 10.1 The surfactin biosynthetic assembly line (Adopted from Chiocchini 2006). (**a**) The surfactin biosynthetic gene cluster of *Bacillus subtilis* encodes for the non-ribosomal protein template for the synthesis of the lipoheptapetide surfactin. (**b**) This biosynthetic complex consists of three surfactin synthetases, SrfA-A, SrfA-B, and SrfA-C, consisting of seven distinct modules, each responsible for recognition, activation, and loading of a single amino acid substrate. Two epimerization domains are found in modules 3 and 6, converting L-Leu into the stereoisomer D-Leu, respectively. The cyclization and release of the final heptapeptide as macrolactone are catalyzed by the TE domain. (**c**) In different colors the single domains are represented

complemented with yeast extract (0.1%). Mainly, the requirement of mineral was beneficial for the surfactin production (Wei et al. 2007). Cheap and renewable sources for surfactin production have been derived from agro-industrial crops such as sugar beet, soybean, potato, cassava (Noah et al. 2005), wheat, maize, sorghum, and rice. Vegetable oils, dairy wastes, and distillery fresh apple juice result in higher yields of surfactin, e.g., 2000 mg L^{-1} and 3500 mg L^{-1}(Mulligan and Gibbs 1993; Ponte et al. 2009). Purification, concentration, and recovery play a significant role in the overall cost of surfactin production (Mukherjee et al. 2006). Solvent extraction, acidic precipitation, and purification through chromatographic techniques, e.g., TLC, HPLC, and LCMS, are the main procedures for surfactin isolation. Numerous advanced techniques for surfactin purification and recovery have been developed, together with diverse nanofiltration and ultrafiltration combinations through polymeric membranes (Shaligram and Singhal 2010). Significantly higher yield of surfactin purification and recovery was achieved, showing tremendous potential for industrial applications (Isa et al. 2007; Chen et al. 2008; Juang et al. 2008; Shaligram and Singhal 2010).

10.3 Applications of Surfactin in Agriculture

Although biosurfactants have received much attention at present because of their potentials in bioremediation such as degradation of pesticides, hydrocarbons, and soil augmentation, they hold promise for application in agriculture because of their capability to enhance biocontrol potential of microbial strain, elimination of biofilm-forming pathogens in animal and poultry feed (Cheng et al. 2018), eradication of weeds, inhibition of aflatoxin production, antagonism of phytopathogens (Nihorimbere et al. 2011), and induction of systemic resistance (Desoignies et al. 2013). In addition biosurfactants are used for wetting, dispersion, suspension, and emulsification of pesticides and fertilizers and increasing the bioavailability of nutrients for promoting growth of beneficial microorganisms in soil (Sachdev and Cameotra 2013).

10.4 Biocontrol of Plant Pathogens

One of the most important properties of biosurfactants which makes them potentially useful in agriculture is their capability of interacting with membrane lipids. Surfactin being a biosurfactant displays its biocontrol activity by attaching to the lipid bilayers because of its cation-binding capability. Furthermore, it solubilizes membrane either by acting as detergent or modification of membrane permeability. Surfactin enters in lipid bilayer via hydrophobic interactions; thus both thickness of membrane and arrangement of hydrocarbon chains are affected (Bernheim and Avigad 1970; Maget-Dana and Ptak 1995).When surfactin penetrates in membrane it results in the dehydration of polar head groups of phospholipids, disturbing the packing of lipids and lipid bilayer stability. These changes in structure lead to membrane instability, hence rupturing the target pathogenic microorganism (Carrillo et al. 2003).

Surfactin and its isoforms exhibit strong antibacterial, antifungal, antiviral, and antimycoplasma activities (Mulligan 2005; Haddad et al. 2009) which can be utilized to eliminate phytopathogens and feed contaminants. Surfactin produced by marine *Bacillus circulans* (Das et al., 2008) and *B. subtilis* R14 strain (Fernandes et al., 2007) was found to be antagonistic against multidrug-resistant bacterial strains of *Alcaligenes faecalis*, *E. coli*, *P. aeruginosa*, and methicillin-resistant *S. aureus*. The minimum bactericidal concentrations (MBC) and minimum inhibition concentrations (MIC) used were less than that of the conventional antibiotics tested at the same time (Das et al. 2008). Studies using surfactin-negative mutants of *Bacillus subtilis* indicated that surfactin is major role-player in combating bacterial fruit blotch disease in melon (Fan et al. 2017).

Surfactin deserves special attention due to high biodegradability, eco-friendly behavior, low toxicity, and significant antifungal activity against various phytopathogens (Yu et al. 2002; Athukorala et al. 2009). Surfactin purified from *Bacillus* sp. showed efficient antagonistic activity against *Fusarium oxysporum*, *F. moniliforme*,

F. solani, and *Trichoderma* spp. (Sarwar et al. 2018). Surfactin extracted from *Bacillus licheniformis* BC98 was found to antagonize *Magnaporthe grisea* in in vitro assays (Tendulkar et al. 2007).

10.5 Antibiofilm and Anti-adhesion Properties

Biofilm formation and surface adhesion are important mechanisms for survival of bacteria in the environment. Bacterial population is capable of surviving in extreme conditions of the environment by biofilm formation (Morikawa 2006). In addition to antiviral and antimicrobial activities, surfactins are proven inhibitors of microbial adhesion and biofilm formation (Mireles et al. 2001). Surfactin from strain of *Bacillus circulans* exhibited anti-adhesion activity against different species of bacteria such as *Escherichia coli*, *Salmonella enterica*, *Salmonella typhimurium*, and *Proteus mirabilis* (Das et al., 2008). For instance, biofilm formation in two selected pathogenic strains of *E. coli* and *S. aureus* was reduced to 90% and 97%, respectively, on polystyrene due to anti-adhesion activity of surfactin (Rivardo et al. 2009). Anionic nature of surfactins could lead to electrostatic repulsion between molecules of surfactin and the bacteria adsorbed onto the surface of polystyrene. Thus, it looks that surfactin has potential as an anti-adhesive compound that can be used to defend the surfaces from microbial contamination (Zeraik and Nitschke 2010). In additional studies, synergistic interactions were observed between silver and surfactin, acting as active antibiofilm agents. Solubility of metal is increased due to negative charge of surfactin and could therefore enable the exopolymeric substance penetration in encapsulated biofilm (Rivardo et al. 2010). This outcome confirms the potential of surfactin as anti-adhesive compound that can prevent microbial contamination in food and feed.

10.6 Induction of Systemic Resistance

Besides having antagonistic and cytotoxic activities surfactin has been reported as a powerful inducer of systemic resistance in crop plants such as tomato, tobacco, bean, and beet against various phytopathogens (Ongena and Jacques 2008; Jourdan et al. 2009; Le Mire et al. 2018). For instance, application of surfactin at micromolar concentrations led to the elicitation of early defense responses, induction of defense-related enzymes phenylalanine ammonia lyase (PAL) and lipoxygenase (LOX), and the production of the plant defense hormone salicylic acid in tobacco cell suspensions (Jourdan et al. 2009). However, there are only few reports on role of surfactins as elicitors of induced resistance in major monocotyledonous plants (Balmer et al. 2013). In a recent study, surfactin has been investigated as a potential elicitor of ISR by activating both salicylic acid- and jasmonic acid-dependent defense response in winter wheat against the *Septoria tritici* blotch (STB) disease causative agent

Zymoseptoria tritici (Le Mire et al., 2018). However more studies are required to elucidate the role and exact mode of action of surfactins as ISR elicitors in various crop plants.

10.7 Inhibition of Aflatoxin Production

Aflatoxins are a group of polyketide-derived furanocoumarins which are the most toxic and carcinogenic compounds among the known mycotoxins produced by various *Aspergillus* species *A. flavus* and *A. parasiticus* (Farzaneh et al. 2016). Aflatoxins are primarily produced in agricultural commodities like corn, cotton seeds, and peanuts in the field and during storage. Consumption of agricultural products contaminated with aflatoxins can cause mycotoxicosis in humans and farm animals (Mukherjee et al. 2006). In this context control of aflatoxin producing fungi is one of the most important strategies in the prevention of mycotoxin contamination in food crops. Biosurfactants can be metabolites of choice in this scenario because of their known antifungal properties. These can kill fungi by increasing membrane permeability and inhibition of spore germination. For instance, surfactin can cause irreversible pore formation in fungal membrane when used at a higher concentration, thus resulting in increase in permeability of the membrane leading to the rupturing of membrane and bursting of cells of *A. flavus* in pistachio nuts (Farzaneh et al. 2016).

10.8 Improvement in Root Colonization and Growth of PGPR

Root colonization is one of the most important attributes of plant growth-promoting rhizobacteria (PGPR). It is very important for the PGPR to colonize plant root in order to provide beneficial effects to the plants. The association of PGPR with plant roots is governed by several factors such as microbial motility, biofilm formation, and secretion of root exudates by plants and quorum-sensing molecules such as acyl-homoserine lactone (AHL). In addition to this, biosurfactants have been reported to enhance the motility, signaling, and biofilm formation of PGPRs indicating their importance in promoting efficient root colonization and consequent improvement in plant health afforded by PGPRs. Besides that biosurfactants increase the bioavailability of nutrients in soil by providing wettability and proper distribution of chemical fertilizers in soil (Sachdev and Cameotra 2013). This not only enhances the growth of beneficial microorganisms in soil but also promotes plant growth, thus playing an essential role in sustainable agricultural practices. Surfactin also supports colonization of surface through biofilm formation and improving acquisition of nutrients through surface wetting and emulsification properties. Like other biosurfactants, surfactins have been reported to enhance plant root colonization capability of *Bacillus subtilis* strains in wheat (Le Mire et al. 2018) and *Bacillus amyloliquefaciens* in *Arabidopsis thaliana* (Dietel et al., 2013).

10.9 Conclusion and Future Perspectives

Surfactins have significant applications in sustainable agriculture, but the use of environment-friendly surfactins is rare. Role of surfactins in biological control is under intensive investigations nowadays. As mentioned above surfactin applications could be useful in food preservation, antibiosis, and plant disease suppression. Surfactin appears to be a promising biocontrol agent in agriculture practices and could be used to develop eco-friendly biopesticides for replacing harmful chemical pesticides. The higher occurrence of surfactins and surfactin producing rhizobacteria is strong evidence for its effective role in sustainable agriculture. In literature, *Bacillus* and *Pseudomonas* species are main surfactin producers demonstrating that only two genera are studied up till now. Some modern approaches, for example, functional metagenomics, can help in the discovery of surfactins. Therefore, we can conclude that a collective contribution by researchers from different fields, for instance, microbiology, biochemistry, molecular biology, environmental sciences, and computational biology, is requisite.

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Molecular Approaches to Study Plant Growth-Promoting Rhizobacteria (PGPRs)

11

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Contents

Abstract

The plant growth-promoting rhizobacteria (PGPRs) are very important for the ecosystem, so there is a need to study their diversity and functions. The yield and growth of different plants and crop varieties have been improved with various PGPR strains. The specific agricultural and environmental issues are addressed by applying specialized strains of the PGPRs. The development of efficient new techniques to identify PGPRs is greatly needed. The new techniques are mostly

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based on molecular level to study PGPRs which may reduce the time of detections from days or weeks to hours. These techniques are extremely specific, selective, and reliable to detect PGPRs. The link between function and structure of the PGPRs can be identified with these methods, and efficiency of using PGPRs in improving different crop varieties and plants is increased. In this chapter, different molecular approaches available to study PGPRs will be discussed.

Keywords
Plant growth-promoting rhizobacteria · Biotechnology · Genomics, soil microorganisms · Biosensors

11.1 Introduction

The soil zone around the plant roots, having high microbial activity, characterized by the countless range of dynamic and complex biological, physical, and chemical interactions is called as rhizosphere. In the rhizosphere, microbes have a significant role in the plant nutrient cycle and organic matter transformation. A wide range of bacterial/rhizobacterial species develop interactions with host plants for improving plant nutrition, growth, and suppression of disease (Pii et al. 2015). So, many studies have been done for isolating PGPRs and for determining their activities in the rhizospheric soil (Verma et al. 2018a).

The PGPRs have gained importance because of their simple mode of action, low cost, and easy access. The PGPRs can be applied on plants, and they always exert a positive impact on their growth (Verma et al. 2018b). The biological nitrogen fixation (Porte et al. 2017), production of phytohormones (Park et al. 2017), phytopathogen control (Pérez-De-Luque et al. 2017), and K, Zn, and phosphate solubilization (Lagos et al. 2015) are some main features of PGPRs. As phytostimulators, phytopathogen biocontrol agent, rhizo-mediators, and biofertilizers, the PGPRs can be used as alternatives/substitutes for agrochemicals. Despite of the fact that PGPRs are eco-friendly, their mass-level application is limited in agriculture due to their less efficiency (Babalola 2010) under field conditions.

About the distribution, communication, diversity, and competence of PGPRs, limited information is available, but the interest of using them in increasing crop production has gained importance worldwide (Lagos et al. 2015). Thousands of different fungal, archaeal, viral, and bacterial taxa are available in the rhizosphere; however, only 1% of the soil microbes have been cultured actually (Elsas and Boersma 2011). The characterization, composition, and functions of PGPRs in higher resolution are possible by Illumina and Roche 454 sequencing platforms which are next-generation sequencing tools. The genomes of unculturable PGPRs and their novel products can be identified with metagenomics approaches (Lagos et al. 2015). At the specific root zone, the microbial population and its role in the plant growth, health, and nutrient uptake can be determined. By using protein (metaproteomics) and RNA (metatranscriptomics), soil microbial functionality can be inferred (Bastida et al. 2009).

Current information about the role of PGPRs in plant growth has been discussed in this chapter. The use of advanced molecular biology and biotechnology techniques to manipulate plant microbe interaction is also described here with some future prospects.

11.2 Applications of PGPRs in Agriculture

After application of the PGPRs in plants, morphological and biochemical alterations occur that lead to increase in abiotic stress tolerance, and this phenomenon is called induced systemic tolerance (IST) (Etesami and Maheshwari 2018). The plant growth is badly affected by several factors called stress. Production of reactive oxygen species (ROS), for example, H_2O_2, OH radicals, and superoxide, is high due to stress conditions. Oxidative stress affects plant growth via oxidizing membrane lipid, nucleic acid, pigments, and proteins (Gouda et al. 2018). Through different processes, PGPRs enhance plant growth and help to fight abiotic stresses like ACC deaminase formation and slow down the formation of harmful ethylene, changes in the content of plant hormone, introduction of enzymes against ROS (antioxidative enzymes), development/progress in absorbance of necessary nutrients, extracellular polymeric substances (EPS) formation, increased production of elements necessary for growth, etc. (Etesami and Maheshwari 2018). For example, insoluble nickel is released by some PGPRs, capable of accumulating in plants (Ahemad and Kibret 2014) and adding resistant genes against abiotic stresses (Etesami and Maheshwari 2018). The PGPRs that are needed to be commercialized must have the capability of competing with other microbes for increasing growth of plants, heat tolerance, resistance for UV radiation and combating with reacting agents (Ortega et al. 2017).

PGPRs have ability to produce antibiotics against microbes which are harmful to plants. The propagation of lethal plant microbes is controlled by kanosamine, zwittermicin A, and xanthobaccine synthesis via *Bacillus*, *Stenotrophomonas*, and *Streptomyces*, respectively (Gupta et al. 2015). For the establishment of PGPRs, there are multiple choices. Nanoencapsulation technology helps to save and permit more precise discharge of PGPRs. The trials which alter the genetic material increase the functions and formation of PGPRs (Ortega et al. 2017). Production and growth of crops is increased by reutilizing nutrients present in soil. It is performed through rhizobacteria which are essential to improve richness of soil (Nehra and Choudhary 2015). Development and growth of plant is regulated by PGPRS. Phytohormones synthesized by rhizobacteria are cytokinin, auxins, ethylene, and gibberellins; these substances have positive effects on root development by increasing the root hairs, number of lateral roots, and absorbance of water and essential elements (Gupta et al. 2015).

Plants synthesize vitamins for better development. During unfavorable conditions, production of vitamins decreases, while the microbial strains help to promote their growth. Different vitamins produce by *Bacillus* species (pantothenic acid,

riboflavin, biotin, and thiamine) can be taken up by roots (Shameer and Prasad 2018). Plant growth and formation of important nutrients is mainly depending on nitrogen. The efficient PGPR *Rhizobium* sp., a cluster of bacteria which is present in soil mainly near the origin of leguminous plants, helps to resolve the atmospheric nitrogen (Kumar et al. 2015). *Pseudomonas putida* in maize increases the weight of seed, height of plant, area, number of kernel per cob, and dry weight of shoot (Ahemad and Kibret 2014).

PGPRs are commonly used as biofertilizers which decrease the requirements of biochemical manure, drop antagonistic ecological effects, and enhance soil efficiency status. Various rhizobacteria containing luminous *Pseudomonas* discharge different types of antifungal particles during in vitro state. The bacteria isolated from rhizosphere are very efficient to control pathogenic fungi. The nodule region and rhizosphere of tea (*Camellia sinensis*), favorable environment for PGPR straining characterized by *Proteus*, *Pseudomonas*, and *Bacillus*, suppress the pathogenesis of *Fusarium oxysporum* in plants. Inhibition of plant microorganisms, which are found in soil, via high-affinity iron-chelating molecule (produced by fungi and bacteria, transport iron molecule across the cell membrane) was detected, and the mutant-type rinsing was highly active in repressing infection contrast to non-siderophore manufacturing mutants (Kumar et al. 2015). Alginate is usually used for bacterial cell encapsulation. It is produced by microbes like *Pseudomonas* and *Azotobacter*. The alginate bead has catalytic capability and keeps the cell viable for long time. Alginate beads capture the abundant microbes and help to protect them from biotic stress (Nehra and Choudhary 2015). When temperatures of soil decrease, fungal microorganisms are very virulent.

In the field, biocontrol PGPRs which have the ability to bear cold environment are probably more active. Additionally, in freezing areas and where winter is prolonged, PGPRs should be very potent to face the environment either it may be cooler or warmer. Scientist described that PGPRs including psychrophilic and psychrotrophic pathogens produced anti-freezing substances in soil, which accumulated in close proximity to the cell in order to protect the bacteria (Shameer and Prasad 2018).

11.3 Techniques to Study PGPRs

The PGPRs can be studied by different techniques such as phenotypic and molecular which help to identify and characterize them. It is very difficult to get the complete information by using phenotypic approaches, so it is suggested to use methods for better understanding of functions of PGPRs (Table 11.1).

11.3.1 Phenotypic Techniques

The physiological, biochemical, and morphological properties of PGPRs define their phenotypic characteristics. Conventional phenotypic trials consider the microscopic

Table 11.1 Advantages and disadvantages of different approaches used for PGPR detection

Techniques	Analyses	Advantages	Disadvantages	References
Phenotypic techniques	Microbial respiration, gram staining, biochemical tests (e.g., biolog plates and VITEK cards), phospholipid fatty acid analysis (PLFA), and ATP level assay	Short time period is required for performing all these analyses	Any change in environmental conditions can change the phenotypic characteristics, and reproducibility is very difficult	Modi and Jacob (2017)
Molecular techniques	Ribosomal RNA sequencing, real-time PCR, finger printing (denaturing gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SCCP), and terminal restriction fragment length polymorphism (TRFLP)), biosensors (immunosensors), BioMEMS, proteomics, and DNA microarray	Rapid, sensitive, specific, and identify accurate homology	Live material is required as prerequisite, complex analysis and absence of regulatory factors during in vitro analyses	Lagos et al. (2015)
Recent molecular techniques	Metabolomics, metagenomics (Roche 454 pyrosequencing platform and Illumina sequencer), metatranscriptomics, and metaproteomics	High-throughput and highly sensitive techniques	Highly expensive techniques and sometimes give false-positive results	Verma et al. (2018a)

appearance of bacterial cells (endospore, shape, inclusion bodies, and flagella), colony morphology (form, color, and dimension), vulnerability of microbes against antimicrobial agents, the microbial growth range in different conditions (temperature and pH), and different properties of microbes on various growth substrates. Even the Gram reaction is a valuable diagnostic tool to analyze the composition of the cell wall. According to the bacterial strain being studied, many other tests can be performed (Rodríguez-Díaz et al. 2008). The short versions of conventional biochemical assessments (such as biolog plates, API kits, and VITEK cards) are available to perform taxonomical studies and are mostly composed of dehydrated agents. The reaction is started after the addition of standardized inoculum.

As the percentage of culturable PGPRs is too low, non-culturable techniques like phospholipid fatty acid analysis (PFLA) may also be used (Stazi et al. 2015). Maybe it is not possible to detect the specific strains or species by this method, but the variations in concentration of fatty acids can depict the difference between various microbial groups. Hence, there are some limitations of using these technologies as described below: the reproducibility of results, based on phenotypic examination in different laboratories while working on PGPRs, is a big challenge. The nature of the microbes is another disadvantage of using phenotypic approaches. Therefore, it is

necessary to compare the results obtained with the results of similar type of experiments performed on closely related microbes.

11.3.2 Molecular Techniques to Study PGPRs

The functions and diversity of PGPRs, studied through a wide range of valuable molecular tools, have gained importance (Hill et al. 2000). But the partialities of all molecular techniques must be measured and estimated before their application on rhizobacteria. The general scheme of analysis of PGPRs is given in Fig. 11.1.

11.3.2.1 Quantitative PCR (qPCR)

The specific genes and their level of expression can be detected and quantified either from DNA or RNA samples that are obtained from different environments by a widely used molecular technique known as quantitative PCR (qPCR). It is a quite sensitive technique. In the rhizosphere samples, the concentration of RNA or DNA may be very low for correct quantification and detection. The primer specificity and efficiency of amplification are some limitations that are responsible for the false results in real-time PCR (Marschner et al. 2011). However, the distribution of a particular type of PGPRs and their genes in bulk soils and rhizosphere can be studied by this technique (Elsas and Boersma 2011). The quantification and detection of functional genes, responsible for various processes in rhizosphere like biocontrol of phytopathogen and nutrient cycling, can be easily determined by real-time PCR.

The *Pseudomonas* species are used as model organisms to study plant-microbe interaction in rhizosphere to identify gene expression. Some bacterial genes from

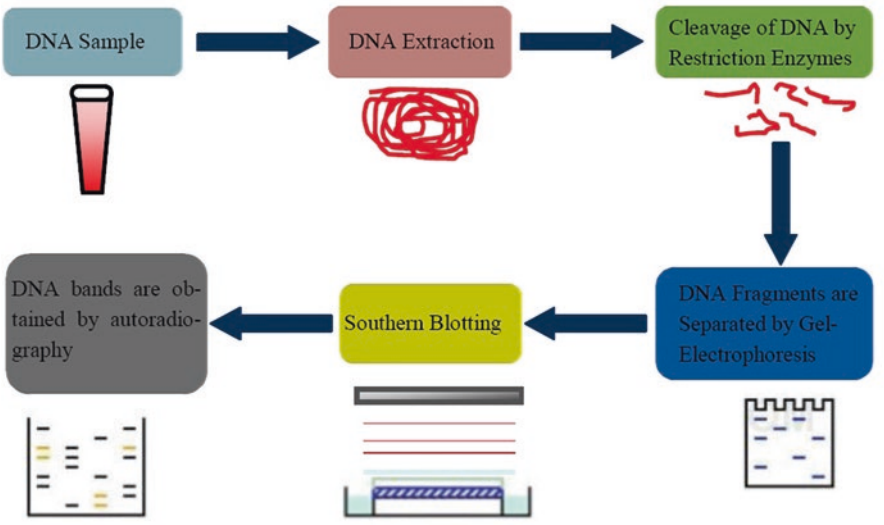

Fig. 11.1 The general diagrammatic analysis of PGPRs

the *Pseudomonas* species were introduced in rhizosphere in response to the root exudates (Barret et al. 2011). These genes are responsible for controlling cell motility, signal transduction, and metabolism and perform some unknown functions too. An example is 2,4-diacetylphloroglucinol (2,4-DAPG) genes that can characterize and quantify the genotypes of *Pseudomonas* glow in the rhizosphere (Mavrodi et al. 2007).

11.3.2.2 Fingerprinting

A molecular technique that is used for the identification purposes, based on base pair pattern of individual strain, is called fingerprinting. In the rhizosphere, many colonies of PGPRs are present that can be premeditated by various PCR-based fingerprinting practices, like denaturing gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SCCP), and terminal restriction fragment length polymorphism (TRFLP) (Berlec 2012). Both active and inactive types of rhizobacteria can be studied by these techniques but only when they are present dominantly among different bacterial colonies. As compared to DGGE, the TRFLP is less effective for the assessment of diversity in bacterial colonies, but TRFLP is good for comparing many samples at the same time. The amplification of 16S-rRNA by PCR provides the base for fingerprinting techniques, but the gene copy number of 16S-rRNA is different in various PGPRs, ranging from 1 to 15, and it depends upon their life strategies. For example, the taxa having less copy number of 16S-rRNA can easily survive in the environments that have low nutrient value (Lagos et al. 2015).

11.3.2.3 Microarray

High-throughput analysis of the complete set of genes that are arranged in a definite pattern and used for genetic analysis is known as microarray. The microbial activity in the rhizosphere can be studied by transcriptome profile analysis by microarray and give information about gene expression responsible for synthesis of these signals (Wu et al. 2011). It is a sensitive and rapid technique to detect the PGPRs by high-throughput means. It is used for the analysis of multiple samples simultaneously by hybridizing multiple fragments of DNA on a single chip of microarray (Lee et al. 2008). The nucleic acids, such as cDNA, oligonucleotides, and genomic DNA, are immobilized on nylon membrane or glass slides to develop a well-ordered two-dimensional matrix for microarray analysis. By using robotic micro-deposition of (0.5–2 kb) cDNAs that are amplified by PCR or by combinational chemistry, DNA is synthesized on the glass surface in situ.

Enzymatically or fluorescently labeled DNA probe is hybridized with the library of microarray and can be detected by enzyme-mediated detection system or fluorescence scanning. Though it is very expensive, it is used due to better resolution that is up to the strain level. It can identify diverse sequences from a complex DNA mixture simultaneously, and it can also compare many samples in one go. Hence, there are some limitations such as nonspecific binding, matrix-associated inhibitors, cross-hybridization, and sample size which are needed to be addressed (Stazi et al. 2015). The *Pseudomonas aeruginosa* is a gram-negative bacterium that is present

everywhere and is analyzed by its transcript profiles via microarray for detecting its motility (Tremblay and Déziel 2010).

The results represented that most of the colonies showed downregulation of the genes linked with virulence and upregulation of the genes concerned for the energy metabolism. The study of *Azospirillum brasilense* transcriptome demonstrated that indole-3-acetic acid (auxin) is a signaling molecule which disturbs cell surface protein and accumulated transport proteins (van Puyvelde et al. 2011). However, the microarray technology depends on the known genes of bacterial species. So, microarray cannot provide efficient information about functions of unknown genes of bacteria and distribution in the environment. The analysis of transcriptome profile by microarray of strain BH72 which belongs to *Azoarcus* species at the time when it is exposed to root exudates secreted by rice showed that 2.0% and 2.4% of genes are downregulated and upregulated, respectively (Shidore et al. 2012). A schematic chart of sample analysis using microarray technique is discussed (Fig. 11.2).

11.3.2.4 Bio-microelectromechanical Systems (BioMEMS)

The microscale or nanoscale fabrication is involved in the designing of BioMEMS.

These are used to identify the separation, growth, purification, manipulation, and immobilization of single and multiple bacterial cells, toxins, and other biomolecules secreted by the PGPRs. It is also used to detect soil pollution (Stazi et al. 2015).

11.3.2.5 Biosensors

The bacterial cells that have a reporter gene, typically a fluorescent marker-like green fluorescent protein (GFP), are defined as biosensors (Sørensen et al. 2009). The colonization of bacteria and activity can be detected at single-cell level by confocal microscopy and epifluorescence microscopy. The rhizosphere colonization can be monitored and localized successfully by introducing GFP-tagged plasmids in *Enterobacter cowanii* strain PRF116, *Pseudomonas putida* strain PRD16, and some endophytic bacteria (Götz et al. 2006). The investigation on *P. putida* strain W619 tagged with GFP showed that it is not involved in promoting growth (Weyens et al. 2012).

But, there are problems like reporter genes that are present in very limited number, sample preparation can alter the performance of biosensor, and detection can be limited due to high background fluorescence (Marschner et al. 2011). The immunosensor (immune response-based biosensor) is also an important type of biosensors. The immune response is induced by many lipopolysaccharides that are present on the surface of PGPRs like *Pseudomonas* species.

The PGPRs are analyzed by using this property via ELISA and an immunosensor which is based on the antigen-antibody interaction, known as piezoelectric biosensor (Agrawal et al. 2012). The step of washing or adding detergents can be eliminated, and the analysis can be done faster by quartz crystal microbalance (QSM). It works on the principle of coating the sensor surface with specific antibody and allowing the sensor to contact with bacterial suspension.

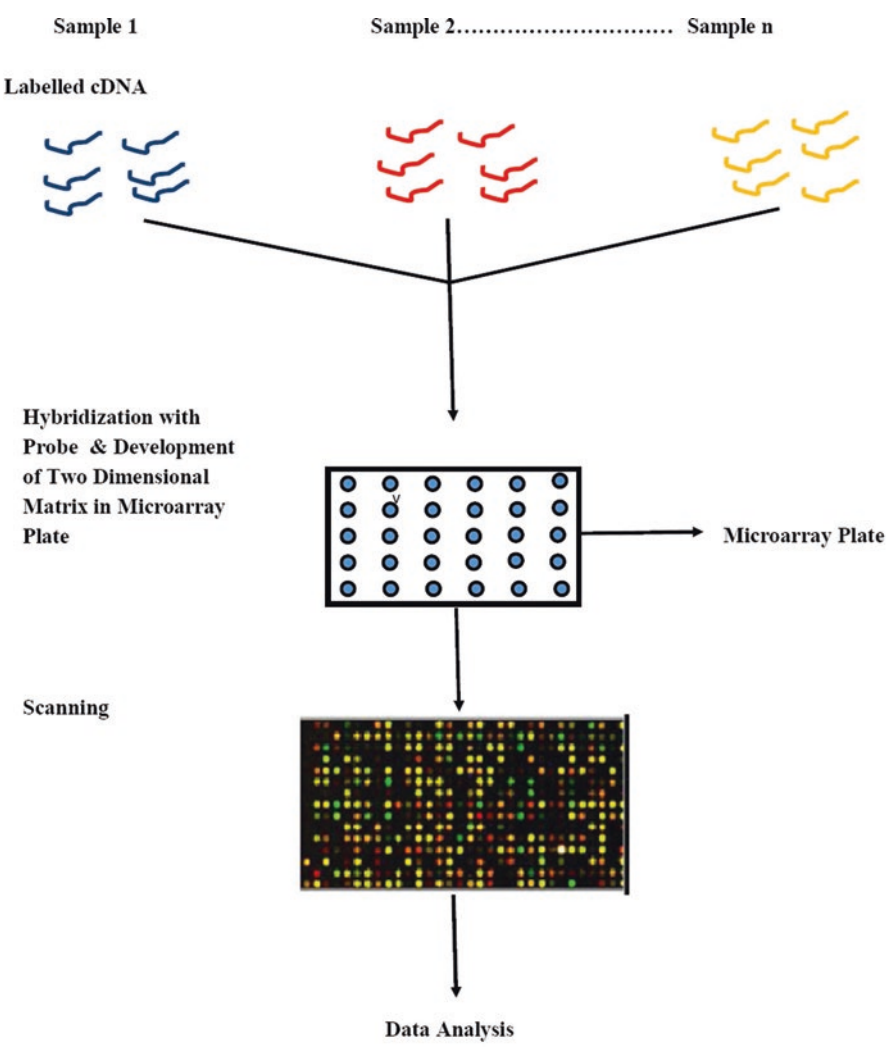

Fig. 11.2 A schematic chart of sample analysis through microarray technique

The bacteria present in the suspension bind with the sensor with high specificity. As a result, the mass of the sensor is increased, and the resonance frequency of the bacteria is decreased due to decreasing bacterial population in the culture. Most of the biosensors are designed to reuse multiple times without losing their activity because of the high prices of piezoelectric crystals. The immunosensors are very efficient systems that have multiple applications in the fields of clinical diagnostics, environmental monitoring, biotechnology, and food industry. The efficiency of QCM biosensor is enhanced by using silver nanoparticles (Choudhary et al. 2010).

It is also reported that the antigen-antibody binding sensors are more efficient and versatile as compared to nucleotide-binding-based sensors. The identification and detection of PGPRs by antibodies need a reporting system that can calculate the ratio of microbe-antibody binding, amplify the reaction, and transduce it in the signals (Stazi et al. 2015).

11.3.2.6 Proteomics

The total protein complement present in a cell is called its proteome that can be used to evaluate gene expression, determine the location of the protein, and identify the posttranslational modifications, and it is collectively called proteomics. But extraction of intracellular proteins from the soil to detect PGPRs is not an easy task due to several reasons; (1) proteins can be degraded by proteolytic enzymes, (2) tightly adhered with soil minerals, (3) combined with humic acids, and (4) form soil colloids. These all situations may cause hindrance in the analysis (Arenella et al. 2014). As a result of microbial activity, only 4% of the total soil nitrogen is produced from intracellular protein, but when protein is stabilized with surface reactive particles it enhanced up to 30–50% (Nannipieri 2014). At the time of sampling, characterization of intracellular proteins gives information about the functions of microbes in the rhizosphere, while past microbial events can be detected by the characterization of extracellular stabilized protein.

The proteins that are responsible for different metabolic functions like energy production and carbohydrate metabolism, amino acid and lipid biosynthesis, membrane transport, and signal transduction are mostly found in the forest soils and rhizosphere (Lin et al. 2013). Particularly, different bacterial proteome analyses are carried by two-dimensional difference gel electrophoresis (DIGE), mass spectrometry (MS), and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) for measuring the expression of genes that are involved in plant pathogen detection, identification of endosymbiotic interactions, and determination of nitrogen-fixing bacteria in leguminous plants.

11.3.3 Recent Molecular Techniques to Study PGPRs

Now, it is inferred that the improvement of previous approaches and development of new techniques like metabolomics, metagenomics, metatranscriptomics, and metaproteomics are more precise assessment of behavior and composition of PGPRs as compared to old molecular techniques (Table 11.2). These advancements raise questions regarding the function and role of microbial communities (Hirsch et al. 2010).

11.3.3.1 Metabolomics

The study of total metabolite content that is found in the sample obtained from a specific region is called metabolomics. It is not limited only to metabolic profiling; it also includes the study about the identification of metabolites to completely understand the metabolites produced by PGPRs and their quantification to detect its

Table 11.2 A comparison between recent molecular tools being used in the soil microbiology

Technique	Metaproteomics	Metagenomics	Metatranscriptomics	Metabolomics
Target	Protein	DNA	RNA	Metabolites
Utilizations	Characterization of gene function	Biodiversity studies	Biodiversity	Metabolic profiling
	Metabolic analysis	Quantification of genes	Expression analysis	To study plant growth
Drawbacks	Lower percentage of nitrogen, i.e., only 4% is present in the intracellular proteins	DNA is obtained from both liable and non-liable cells	RNA's liability	Complex networks
	Nitrogen is not found to be involved in sampling but has a role in expression	It considers only the prevalent members of the soil community	Interference with the humic compounds of the soil	Interdependence of metabolites
	Adsorption on the soil minerals and humic colloids is observed			

Adopted from Wilmes et al. (2015), Nesme et al. (2016), and Verma et al. (2018a)

abundance. The difference among metabolites can also be determined by metabolomics. In root exudates, many metabolites are present that can be identified by this analysis. Many PGPRs are present near the plant roots which help plants in performing better functions. This technique helps in determining the functions of the rhizospheric microbiome.

Many compounds, secreted by the plants and PGPRs in the rhizosphere, can be detected and identified by the metabolic engineering in plants. It also gives the chance to critically analyze the function of each individual compound in plants and rhizosphere (Dam and Bouwmeester 2016). The great diversity of the metabolites in various species can more complicate the metabolomic analysis, especially in plants that produce approximately 100,000–200,000 metabolites (Oksman-Caldentey and Inze 2004).

It is technical to choose either comprehensive global metabolic profiling or targeted metabolite analyses to analyze primary and secondary metabolism simultaneously. A minimum number of amino acids, carbohydrates, vitamins, organic acids, lipids, and other compounds like alkaloids, terpenoids, glucosinolates, and phenylpropanoids along with the secondary metabolites should be included in a comprehensive metabolic profile that may vary according to the plant and rhizobacteria being studied. Therefore, it is concluded that interactions between the plants and hosts are not only the point of interest in metabolomics studies, but also the great diversity of chemical classes makes it more confusing. Moreover, different technologies are required to make a comprehensive report of changes in each metabolite level according to the plant and microbe interaction.

Nowadays, most of the plant microbiologists are considering metabolomics with transcriptomics and genomics approaches to study the plant pathology. To study the rhizospheric bacteria, metabolomics is a post-genomic technique. Different techniques like nuclear magnetic resonance (NMR), chromatography, mass spectrometry (MS), and spectroscopy are used to identify, estimate, and report the abundance of metabolites at the specific time. The RNA-based or enzyme-based approaches are not enough to investigate the effects of metabolites in the system.

A comprehensive biochemical status report is generated by metabolomics that is free from abovementioned pitfalls. Various processes along with bioinformatics and data visualization methods are used to develop a metabolic profile and to identify the roles of metabolites in the rhizosphere. To obtain a complete record of the metabolites present in an organism is known as metabolic profiling. All metabolites in a sample can be detected by fingerprinting regardless of their identification. The techniques like spectroscopy, electro-spray ionization mass spectrometry (ESI-MS), and NMR are used to screen differential metabolites. So, prior to going for expensive metabolic profiling, it provides a very cheap initial approach (Verma et al. 2018a). The MS approaches coupled with liquid chromatography help in identification of metabolite from an extract. The metabolites from a sample have been detected before the identification and selection of the sample and control via MS profiling along with computer-assisted inductive approaches (Goodacre et al. 2004).

11.3.3.2 Metagenomics

The study of the complete genomic DNA of a cell in a specific location is called metagenomics. The different ecological functions of all species of PGPRs are needed to be understood before evaluating the distribution, activity, and richness of PGPRs in the rhizosphere and bulk soils. It is revealed by the meta-genome study of soil by the (internal transcribed spacer 1) ITS1 region and 16S-rRNA genes through second-generation sequencing or next-generation sequencing that 1 g of soil has 33,346 bacterial and archaeal operational taxonomic units (OTUs) (Mendes et al. 2011) and 3320 fungal OTUs (Schmidt et al. 2013). The rhizosphere and bulk soils can be examined by both Illumina and Roche 454 platforms.

The actinobacteria, acidobacteria proteobacteria, and bacteroidetes are major taxa that are found in the oak rhizosphere and bulk soil while studying bacterial diversity by Roche 454 pyrosequencing platform (Uroz et al. 2010). By the characterization of composition of the bacterial populations in the micro-sites of *Lolium perenne*, it is identified that actinobacteria, acidobacteria, and proteobacteria are present there in abundance (Lagos et al. 2014). Similarly, in the study of bacterial diversity in an apple rhizosphere by Illumina sequencer, it was found that actinobacteria, proteobacteria, gemmatimonadetes, acidobacteria, and bacteroidetes were present (Sun et al. 2014).

11.3.3.3 Metatranscriptomics

The characterization of the complete mRNA of all cells present in an organism is called metatranscriptomics that provides information about metabolic processes of the microbial communities. The novel genes and their functions in rhizosphere and bulk soil can be identified by the metatranscriptomics analysis, and it can also correlate the metabolic activities of these novel genes. But metatranscriptomic approaches cannot be used widely in the rhizospheric soils because mRNA is very unstable and is very difficult to extract from the complex ecosystems. Some other important methodological challenges are short half-life of the mRNA molecule and its difficulty to separate it from other RNA molecules like rRNA, miRNA, and tRNA and interference of humic acids. Furthermore, the majority of experiments of rhizosphere and bulk soils are focused only on bacteria which means there is a need for further studies to gather more information about molecular ecology of other microbes which are present in microbiome of the rhizosphere like archaea, microalgae, protozoa, and fungi (Lagos et al. 2015).

In 2007, a project known as genomic encyclopedia of bacteria and archaea (GEBA project) was initiated to characterize the bacterial phylogeny and to better understand the microbial genomes. This project is initiated by the collaboration of the German Collection of Microorganisms and Cell Cultures, US Department of Energy (USDOE), Institute of California Davis USA, and Joint Genome Institute. The sequencing of 200 bacterial genomes has been done up till now. Some other projects associated with GEBA project are GEBA-RNB (root-nodulating bacteria), GEBA-MDM (microbial dark matter), and GEBA-type strain. The identification of the unique functions of protein families is the main purpose of GEBA-type strain

project, while GEBA-RNB project is based on the symbiotic bacterial sequenced strains (100 strains), which provides information about the interaction between roots and bacteria, nitrogen fixation, and endosymbiotic relationships.

On the other hand, the objective of GEBA-MDM project is to find the novel bacteria and archaea that are not found in GEBA project already, by using single-cell genomic approach. The evolutionary studies of bacterial and archaeal genomes and phylogenetic analysis of these strains are improved by this project (Rinke et al. 2013). A third-generation sequencing tool is Pacific biosciences single-molecule real-time (PacBio-SMRT) sequencer (Niedringhaus et al. 2011). It can sequence the strain Mg1 which belongs to *Streptomyces* species that has the ability of degrading another bacteria, *Bacillus subtilis* (Hoefler et al. 2013). It is also a good tool for the analysis of long sequencing reads of 16S-rRNA obtained from the environmental samples.

11.3.3.4 Metaproteomics

It provides information about the soil microbes and their roles like bioremediation or degradation processes and biogeochemical processes (Bastida et al. 2012). The proteogenomics is a significant tool to study the ecology, evolution, and physiology of PGPRs, their populations, and consortia in many environments to link particular PGPR species with its specific function (VerBerkmoes et al. 2009). This approach is significant due to its property of combining metagenomics and metaproteomics to provide authentication of results generated by metagenomics studies with the help of protein data. Hence, it is elementary to understand that the databases made to identify soil protein interactions are still incomplete. Nonetheless, many metaproteomics experiments have discovered the diversity of proteins which are expressed due to the plant-microbe interaction. The results obtained from this experiment showed that ratoon sugarcane made important changes in the soil such as catabolic diversity, enzyme activities, and level of expression of soil proteins generated from the microbes and plants. This experiment also revealed the fact that 24.77% of proteins which are present in soil are obtained from bacteria, and the majority of the microbial proteins with upregulated expression are involved in signal transduction and membrane transport (Lin et al. 2013).

A same type of experiment is performed on the herb *Rehmannia glutinosa*, showing that the identified proteins of plants and microbes are responsible for amino acid metabolism, response to stress, and energy metabolism (Wu et al. 2011). But, as compared to the previous study, it shows less percentage of proteins that are generated by bacteria for signal transduction. Furthermore, in the rhizospheric soil of *Lactuca sativa*, high amounts of proteins are present that are responsible for energy metabolism, response to stress, and virulence determination (Moretti et al. 2012).

11.4 Conclusions

The plant growth-promoting rhizobacteria (PGPRs) are the biological entities to boost up the soil properties which directly or indirectly help to enhance plant growth and development. There are lots of conventional methods to study PGPRs, but the modern procedures are getting more and more popularity due to various reasons. The modern molecular and biotechnological methods are more robust, accurate, and sensitive. They include simple PCR to qPCR, sequencing to next-generation sequencing, and genomics to metagenomics. This chapter reviewed the most advanced procedures to study PGPRs with more efficiency in limited time.

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Impact of Land Uses on Microbial Biomass C, N, and P and Microbial Populations in Indian Himalaya

12

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Abstract

Changes in land use affect microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP), and microbial populations, important parameters to sustain ecological systems. MBC, MBN, MBP, and microbial populations vary markedly among land uses and with conversion of one land use to another. A literature survey suggests that forest lands have favourable influence on MBC, MBN, MBP, and microbial populations, followed by agroforestry, silvipasture, and agriculture, respectively. Microbial quotients also vary with land use and have strong positive correlations with MBC, MBN, and MBP. It is well established that population pressure is causing the conversion of forest lands to non-forest purposes. This conversion of forest land to agriculture and other uses affects the quantity of MBC, MBN, MBP, and microbial populations. The ratio of MBC to soil organic carbon (SOC), MBN to total N, and MBP to total P varies significantly. The soil microbial biomass (SMB) is lively and active in regulating the transformation of soil organic matter (SOM). These activities are vital for the cycling of nutrients in the soil. In general, the increased level of microbial biomass in the soil is beneficial and a decreased level is seen as harmful, provided it should improve or decrease the functional biology of the soil. Interpretation of soil microbial biomass parameters is very difficult. Here, we have tried to explain the importance of microbial biomass, its role and measurement, including examples from Indian Himalaya.

Keywords

Agroforestry · Grasslands · Himalaya · Land uses · Microbial biomass · Silvipastoral

12.1 Introduction

The microbial biomass is an important component of the soil ecosystem, a major reservoir of terrestrial carbon (C) (Heimann and Reichstein 2008), and through decomposition of organic matter is involved in climate change (Davidson and Janssens 2006). The flora, fauna, soil microorganisms, and climate have complex interactions that regulate climate change (Bardgett et al. 2008). Microbial biomass carbon (MBC) responds faster to climate change than does the bulk organic matter of the soil. Microbial biomass along elevation gradients with different land uses has not been analyzed and is inadequately understood. Soil microbial biomass C, nitrogen (N), phosphorus (P), and microbial population dynamics along an elevation gradient are also paramount in soil fertility and quality (Mganga et al. 2016). As per the findings of Margesin et al. (2009), an increasing trend was reported for a gram-negative bacterial population along an elevation gradient. A higher diversity of efficient microorganisms was observed at higher elevations in South Korea (Singh et al. 2014). Microbial biomass C and N increase linearly along the elevation gradient (Pabst et al. 2013; Huang et al. 2014; Lin and Chiu 2015).

Measuring microbial biomass (MB) to quantify microorganisms as a whole unit in the soil (Jenkinson and Ladd 1981; Powlson 1994; Stockdale and Brookes 2006) became necessary because it is very difficult to study the microbial soil population components that cannot be cultured (Gonzalez-Quinones et al. 2011). According to Jenkinson and Ladd (1981), the microbial biomass in the soil is a live constituent of the soil organic matter (SOM), which excludes plant roots and soil animals larger than 5000 μm^3. Sustainable land use development is only possible by knowing how different land uses affect the biological functions of the soil. Hence, it is paramount to interpret soil quality in the form of biological indices from the quantity of microbial biomass in the soil. The present article aims to evaluate different land uses, that is, agriculture, agroforestry, silvipastoral, grassland, and forests, for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), and the microbial population (Yadav et al. 2018).

12.1.1 Soil Microbial Biomass and Soil Quality

Soil qualities of different land uses are determined by microbial biomass, enzymatic activities, and the structure and functions of the microbes (Vallejo et al. 2010). Soil nutrients and microbial biomass are strongly influenced by the types of land use and their management, especially in the tropics. Conversion of land use from forest to agriculture significantly reduces the quantity of nutrients and microbial biomass C, N, P, and microbial populations in the soil (Sharma et al. 2004; Becker et al. 2007; Pabst et al. 2013). On an average, C stock decreased approximately 25% to 30% through conversion of forest to agro-ecosystems (Don et al. 2011). Changes in the basic soil properties, that is, content and distribution, also include microbial changes in the soil. Conversion of grassland to an agricultural ecosystem has led to a decrease of approximately 16% microbial biomass in the soil of northeast India (Singh and Yadava 2006). In comparison to organic matter, microbial biomass in the soil is more responsive to land uses (Kumar et al. 2017).

These efficient microorganisms have a vital function in the biogeochemical cycles of terrestrial ecosystems (Paul and Clark 1996) and biomass returns in different ecosystems (Solaiman 2007). Microbial biomass, the bacteria and fungi in the soil, is a indicator of the living fraction of SOM, at 1% to 5% (Jenkinson and Ladd 1981; Anderson and Domsch 1989), and is considered as a labile pool for available soil nutrients to plants (Sun et al. 2010). Soil organic matter and microbial biomass are together responsible for the maintenance of soil quality. Thus, it is very important to examine the effect of land use and elevation gradient on microbial biomass C, N, and P and microbial populations in the soil (Rawat et al. 2016).

A soil quality indicator should meet the following five criteria (Doran and Zeiss 2000):

1. Sensitivity to management variation
2. Good correlations with soil functions
3. Useful to clarify ecosystem processes
4. Can be understood and used by land managers
5. Easy and economical to measure

12.1.2 Soil Microbial Biomass (SMB) Supports Plant Output (Gonzalez-Quinones et al. 2011)

- Supports and anchors plant growth via stabilization of soil aggregates through the fungal network and bacterial exudates
- Suppresses crop disease through infection of roots by mycorrhizal fungi
- Provides and buffers physicochemical environment for plant growth by decomposition of organic materials and humus formation
- Controls water availability for plant growth in the form of plant–mycorrhizal associations
- Controls nutrient availability for plant growth through mineralization, N-fixation, and phosphorus-, potassium-, and Zn-solubilization

12.1.3 Resiliency to Adverse Environment (Gonzalez-Quinones et al. 2011)

- Aids resiliency to ecological alterations and management effects via horizontal gene transfer
- Buffers flows of water and decreases off-site effects through hydrophobic compound synthesis and biofilm formation
- Acts as buffers to nutrient losses and shrinking off-site effects via nitrification
- For pollution impacts, acts as buffers through pesticides and other xenobiotic degradation
- For greenhouse gases, may act as source/sink via denitrification and oxidation of methane
- Aids in self-maintenance of aboveground and belowground ecosystems through cycling of carbon and immobilization

Globally, it has been recommended that the microbial biomass of the soil should be used as an indicator of soil quality (Insam 2001; Schloter et al. 2003; Bending et al. 2004; Winding et al. 2005). It is being proposed that the microbial biomass of the soil could act as a C-cycling indicator but not as indicating plant productivity (Carter et al. 1999). Microbial biomass measurement and interpretation approach in the soil are not universal in consideration of the objectives, land uses, environment, and management practices (Yadav and Sidhu 2016).

12.2 Measuring Soil Microbial Biomass

12.2.1 Soil Sampling

There is large heterogeneity in microbial distribution and abundance in the soil of various farms, even sometimes in the same field. More samples from the same field are collected to increase the homogeneity of the soil samples and can be analyzed

either separately or as a composite sample by mixing well. It is being recommended that for heterogeneous sites the number of replicates should be increased on a per hectare basis to maintain homogeneity. When a large area should be sampled, then the increasing number of samples adds to the cost of sample analysis (Patil 2002). In such a situation a composite sample can be made by pooling some soil samples, but not a large number of multiple samples.

12.2.2 When to Take Soil Samples

It is well known that the microbial biomass of the soil, in both number and activity, varies not only with different seasons but also within seasons (Devi et al. 2014; Bhuyan et al. 2013). Variability in the microbial biomass of the soil is associated with fluctuation in soil moisture content from moist and dry spells (Ladd et al. 1994; Murphy et al. 1998b), available labile C input at the time of active plant growth (Murphy et al. 2007; Gonzalez-Quinones et al. 2009), and changes in management practices (Sharma et al. 2004). Frequency of sampling should be increased (Murphy et al. 2007), or a homogeneous period of weather conditions and management practices should be identified for the collection of soil samples. The period after the withdrawal of the monsoons and up to March–April is suitable for stable moisture content and weather aspects more than at any other period of the year.

12.2.3 Whether Soil Samples Can Be Stored

Soil microbial biomass is likely to decline during storage of samples because of organic carbon (OC) substrate depletion (Bloem et al. 2005), which is a greater problem for stored wet soil compared to dry soil. It is a general recommendation that storage of soil samples be avoided, but when it is necessary, samples can be stored at 2–4 °C in the dark up to 3 months; samples from freezing (3 months) areas can be stored for a period of 6 months at 18 to −22 °C (Wollum 1994; OECD 1995). Soil microbial biomass from different land uses is affected variably by storage of soil at field moisture. The soil sampling strategy should be planned in advance to reduce the time that samples are stored before analysis.

12.2.4 Soil Microbial Biomass Estimation

Several methods are available for analysis of microbial biomass in the soil. MBC can be analyzed by the fumigation–extraction method (Vance et al. 1987). For microbial biomass C and N analyses, two subsamples of 10 g of each field moist soil sample are weighed into a 250 ml beaker. The second subsample is fumigated with 30 ml ethanol-free chloroform for 24 h in vacuum desiccators at room temperature. Soluble C from fumigated and nonfumigated samples is extracted with 25 ml 0.5 M K_2SO_4 by shaking on an orbital shaker for 1 h. Without chloroform fumigation, C

content in K_2SO_4 extracts from each soil sample is accepted as WOC (Blagodatskaya et al. 2009). After determining solute organic C in fumigated and nonfumigated extracts, a k_{EC} factor of 0.45 is used to convert microbial C flush (difference between extractable C from fumigated and nonfumigated samples) into MBC (Vance et al. 1987).

For microbial biomass N analysis, the fumigated and nonfumigated samples are digested (Anderson and Ingram 1993); nitrogen is analyzed by the modified Kjeldahl method. Microbial biomass N is calculated from the equation of $MBN = E_N \times 1.46$, where E_N is the difference between the amount of extractable N from the fumigated and nonfumigated soils (Brookes et al. 1985). The extracted P is determined by the chlorostannous-reduced molybdophosphoric blue color method (Jackson 1967). The correction for chloroform-released P that is absorbed by the soil during extraction is made by adding a known quantity of P during extraction and then correcting for its recovery. Microbial biomass P was calculated by the formula $E_P \times 2.5$, where E_P is the difference between the amount of extractable P from the fumigated and nonfumigated soils (Brookes et al. 1982).

For microbial analysis, samples are sealed in containers and the microbial population is estimated within 24 h of sampling. The samples are serially diluted with distilled water up to 10^6 dilutions, and a 100-µl aliquot is pour-plated in selective media (Nutrient Agar for bacteria; Rose Bengal Agar for fungi; Ken Knights and Munaier's Agar for actinomycetes). The petri plates are incubated at optimum temperature (28 ± 1 °C for bacteria; 30 ± 1 °C for fungi and actinomycetes) in triplicate, and the appearing microbial colonies were counted (3 days for bacteria; 5 days for fungi; 7 days for actinomycetes) after incubation and expressed as total culturable colony-forming units (CFUs)/g dry weight of soil sample. For actinomycetes, streptomycin and cycloheximide are also added to inhibit the growth of bacteria and fungi at the final concentration (Yang and Yang 2001).

12.3 Role of Landowners and Government

The policy of the government influences decisions of the stakeholders (landowners) related to soil quality apprehension. Educational institutes can take a vital role in understanding the functioning of microbial biomass as just now the government of India has launched the soil health card scheme. There should be coordination between land owners and soil scientists; the scientist should be able to understand the concerns of landowners related to outcomes of their farm. Closer links to management practices are needed if SMB measurements are to be of clear and practical use to farmers (Kelly et al. 2009) and support improved soil management (Sojka et al. 2003).

12.4 Microbial Indices

Dalal (1998) and Anderson (2003) have recommended that the application of easy indices (ratios of biochemical attributes) may offer easily interpretable indicators to prevail in interpreting great difference in microbial biomass values of the soil. The important microbial indices that are frequently used besides MB measurements in soil are these (Gonzalez-Quinones et al. 2011): (1) the rate of microbial carbon dioxide (CO_2) evolution per amount of SMB-C (qCO_2), termed the metabolic quotient; and (2) the amount of SMB-C per unit soil organic C (C_{mic}:C_{org} ratio), termed the microbial quotient.

12.5 Soil Microbial Biomass

The SMB is an important source for soil carbon and nutrients, which potentially influence the retention of organic C and N within the SOM. Therefore, it is essential to understand the mechanism of microbial abundance, turnover, and carbon and nutrient sequestration (Xu et al. 2013). The microbial biomass is to be measured as a driving factor of soil organic materials and a labile pool for plant nutrients (Jenkinson and Ladd 1981). Hence, diminution of the soil microbial biomass could lead to decreased rates of nutrient cycling and decreased magnitude of the nutrient pool. The microbial community of soils is influenced by a wide variety of factors such as physical, chemical, and biological, which include soil type and texture (Buyer et al. 2002; Ulrich and Becker 2006), aggregate size (Schutter and Dick 2002), moisture (Williams and Rice 2007), pH (Fierer and Jackson 2006), temperature (Yang et al. 2010), and soil depth (Haripal and Sahoo 2014). The SMB also depends on various agricultural management factors such as tillage operation (Cookson et al. 2008), fertilizer (Grayston et al. 2004), organic amendments (Saison et al. 2006), crop rotation, and land use system (Xu et al. 2013). The microbial community is also influenced by season, climatic factors, and type of vegetation (Haripal and Sahoo 2014). However, the relationship between soil elements and soil microbial biomass and nutrient concentrations, especially at the natural habitat level, is not well understood (Cleveland and Liptzin 2007; Hartman 20112011). The concentrations of major plant nutrients C, N, and P in soils and soil microbial biomass vary by orders of magnitude in different habitats (Figs. 12.1 and 12.2).

12.5.1 Microbial Biomass C

In the literature survey we found that soil MBC varied significantly with respect to different soil types and soil depths. The average amount of MBC at 0–10 cm depth ranged from ~105 to 513 μg g^{-1} soil and ~81–364 μg g^{-1} soil in the 10–20 cm soil layer (Haripal and Sahoo 2014). Soil MBC also varied seasonally in one research study: it was observed that during the months of August–October, MBC was maximum at ~240 μg g^{-1} but was drastically reduced during December to January, 85 μg

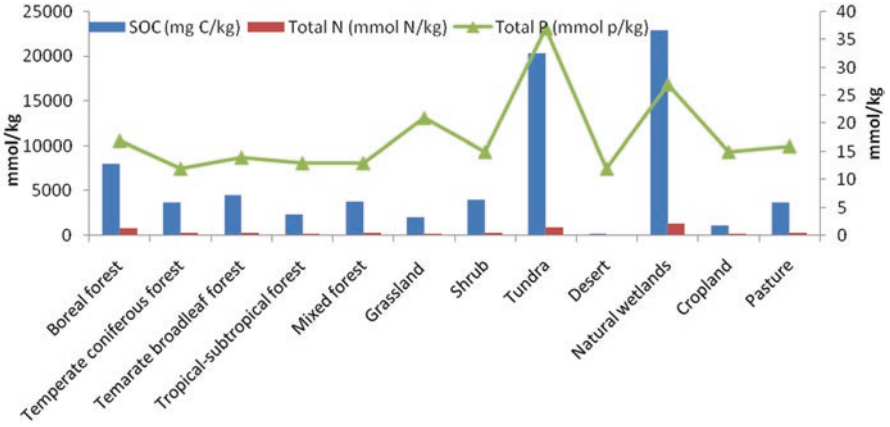

Fig. 12.1 Variation of soil organic carbon, total nitrogen, and phosphorus content in different habitats. (From Xu et al. 2013)

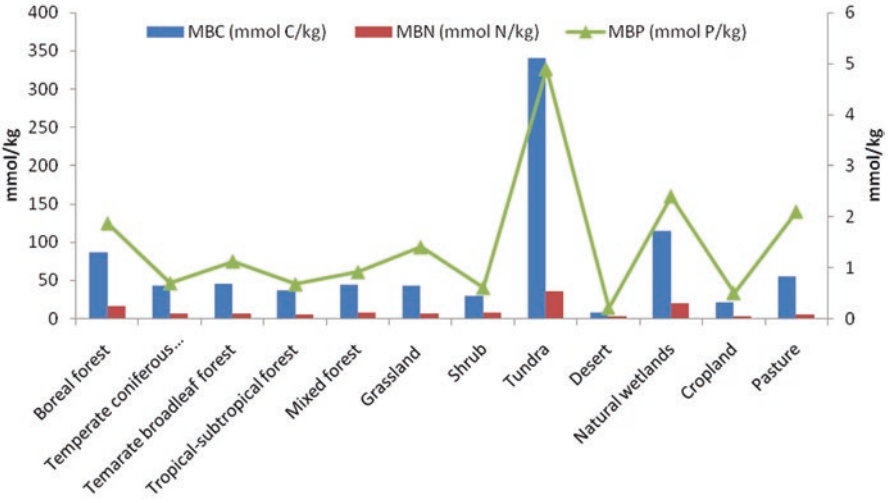

Fig. 12.2 Variation of soil microbial biomass carbon, nitrogen, and phosphorus content in different habitat. (From Xu et al. 2013)

g^{-1} in the study area, Sambalpur district of Odisha, India (Haripal and Sahoo 2014). MBC ranged from ~219 to 864 μg g^{-1} for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), ~215–297 μg/g at 0–10 cm depth, 62–78 μg/g at 10–20 cm depth, and 29.6–35.3 μg/g at 20–30 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), 213–238 μg g^{-1} at 0–15 cm depth and 199–215 μg g^{-1} at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), 457–1000 μg g^{-1} at 0–20 cm depth and 307–692 μg g^{-1} at 21–40 cm depth for different fruit tree orchards

and control (without fruit trees) in Northwestern Himalaya (Debnath et al. 2015), 265–576 μg g^{-1} at 0–15 cm depth, 225–535 μg g^{-1} at 15–30 cm depth, 278–501 μg g^{-1} at 30–45 cm depth, and 198–497 μg g^{-1} at 45–60 cm depth for different land uses (Pal et al. 2013), and 318–520 μg g^{-1} at 0–30 cm depth for various land uses in Central Himalaya (Yadav et al. 2018). MBC of cultivated soil also varies with long-term fertility management: MBC in rice fields in different years starting from the 2nd, 4th, 6th, and 11th to 15th year were 104.5, 155.3, 274.2, 398.9, and 515.8 μg g^{-1}, respectively (Haripal and Sahoo 2014). These data suggests that MBC changes with type of land use and also with depth, generally decreasing with increasing soil depth. MBC was the maximum in temperate natural forest and declined to a minimum in the wasteland subtropical in Sikkim Himalaya (Tables 12.1 and 12.4).

12.5.2 Microbial Biomass N

As for MBC, MBN is also strongly influenced by the type of habitat. For example, in one study in different habitats, from forest to grasslands, desert, croplands, and pasture land, MBN varied significantly, the value ranging from ~35 to 2.3 mmol N kg^{-1} (Xu et al. 2013). Long-term management could also affect MBN content in the soil system. In a study conducted in a rice field, taking samples from 5 different years, starting from 2nd to 4th, 6th, 11th, and 15th year duration, it was observed that the MBN value (Fig. 12.3) was significantly increased with increasing duration (Haripal and Sahoo 2014). The data showed that the MBN varied from ~30 to 142 μg g^{-1} at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), ~32 to 38.6 μg g^{-1} at 0–10 cm depth, 11–13.3 μg g^{-1} at 10–20 cm depth, and 4.3–5 μg g^{-1} at 20–30 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), 19.9–21.5 μg g^{-1} at 0–15 cm depth and 15.4–19.2 μg g^{-1} at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), 47.2–82.3 μg g^{-1} at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006), 180–320 μg g^{-1} at 0–20 cm depth and 101–156 μg g^{-1} at 21–40 cm depth for different fruit tree orchards and control (without fruit trees) in Northwestern Himalaya (Debnath et al. 2015), 20.9–31.2 μg g^{-1} at 0–15 cm depth, 16.9–28.9 μg g^{-1} at 15–30 cm depth, 14.5–23.7 μg g^{-1} at 30–45 cm depth, 8.9–25.5 μg g^{-1} at 45–60 cm depth for different land uses (Pal et al. 2013), and 42.4–66.7 μg g^{-1} at 0–30 cm depth for various land uses in Central Himalaya (Yadav et al. 2018).

12.5.3 Microbial Biomass P

As per the findings of various studies, across different land uses MBP ranged from ~12 to 43 μg g^{-1} at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), ~16 to 18 μg g^{-1} at 0–10 cm depth, 46–5.6 μg g^{-1} at 10–20 cm depth, and 2.33 μg g^{-1} at 20–30 cm depth for

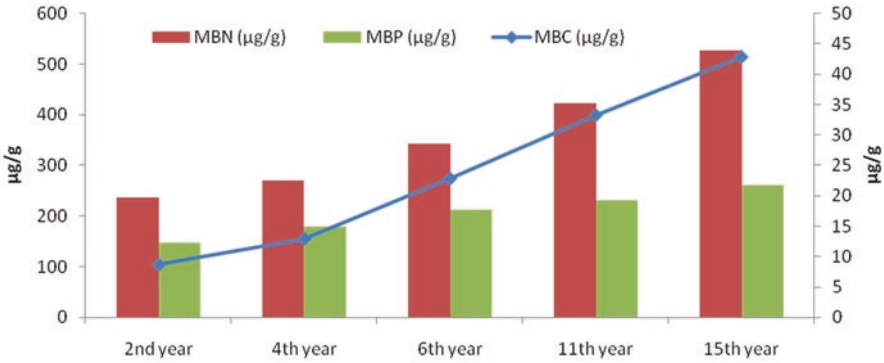

Fig. 12.3 Microbial biomass C, N, and P content in different age series of rice fields in Sambalpur district of Odisha, India. (From Kamala Haripal and Sunada Sahoo 2014)

grasslands and cropland in eastern Himalaya (Singh and Yadava 2006), 9.2–13.5 µg g^{-1} at 0–15 cm depth and 7.2–9.6 µg g^{-1} at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), 28.2–37.3 µg g^{-1} at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006), 48–92 µg g^{-1} at 0–20 cm depth and 37–67 µg g^{-1} at 21–40 cm depth for different fruit tree orchards and control (without fruit trees) in Northwestern Himalaya (Debnath et al. 2015), 2.6–6.5 µg g^{-1} at 0–15 cm depth, 2.5–4.1 µg g^{-1} at 15–30 cm depth, 2.4–4.2 µg g^{-1} at 30–45 cm depth, 2.2–4.0 µg g^{-1} at 45–60 cm depth for different land uses (Pal et al. 2013), and 42.4–66.7 µg g^{-1} at 0–30 cm depth for various land uses in Central Himalaya (Yadav et al. 2018) (Tables 12.1, 12.2, 12.3, and 12.4).

12.5.4 Microbial Quotients

Across land uses, MBC:MBN was recorded in the range of 6–11 at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), 6.8–6.9 at 0–10 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), 8.8–11.9 at 0–15 cm depth and 10–13.9 at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), 10.1–11.3 at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006), 2.6–4.6 µg g^{-1} at 0–20 cm depth and 3.2–5.2 at 21–40 cm depth for different fruit tree orchards and control (without fruit trees) in Northwestern Himalaya (Debnath et al. 2015), and 8.4–10.6 at 0–30 cm depth for various land uses in Central Himalaya (Yadav et al. 2018) (Tables 12.1, 12.2, 12.3, and 12.4). Microbial quotient also varied by duration of year in the same rice crop, and the values of MBC:MBN of different year series varied from 11.7 to 5.3 at the surface soil (Haripal and Sahoo 2014).

Table 12.1 Microbial C, N, and P in soil of different land use/cover types at 0–15 cm soil layer in Sikkim Himalaya (Sharma et al. 2004)

Land use/cover	MBC ($\mu g\ g^{-1}$)	MBN ($\mu g\ g^{-1}$)	MBP ($\mu g\ g^{-1}$)	MBC:MBN	MBC:MBP	MBC:MBN:MBP	Microbial C to OC %	Microbial N to total N %	Microbial P to organic P %
Temperate natural forest dense	864 ± 57	142 ± 17	43 ± 4	6	20	20:3:1	2.7	4.9	7.8
Temperate natural forest open	712 ± 96	96 ± 10	31 ± 2	7.4	23	23:3:1	2.7	3.9	6.8
Subtropical natural forest open	764 ± 36	71 ± 8	31 ± 2	10.8	25	25:2:1	5.2	3	6.5
Cardamom based agroforestry	583 ± 59	63 ± 9	22 ± 1	9.2	27	27:3:1	2.6	2.7	3.7
Mandarin based agroforestry	471 ± 49	48 ± 6	18 ± 1	9.8	26	26:3:1	3.3	2.5	3.1
Open cropped area temperate	390 ± 39	38 ± 5	16 ± 1	10.3	24	24:2:1	1.8	1.9	2.7
Open cropped area subtropical	291 ± 25	34 ± 5	16 ± 1	8.6	18	18:2:1	2.2	1.7	2.9
Wasteland area temperate	259 ± 33	33 ± 4	13 ± 0.7	7.8	20	20:3:1	1.5	1.7	3.7
Wasteland area subtropical	219 ± 34	30 ± 4	12 ± 0.8	7.3	18	18:3:1	1.9	1.9	3.3

Table 12.2 Microbial C, N and P in soil of different land use/cover types at varying depths (cm) in Eastern Himalaya

Land use/cover	MBC (μg g^{-1})	MBN (μg g^{-1})	MBP (μg g^{-1})	MBC:MBN	MBC:MBP	MBN:MBP	Microbial C to OC%	Microbial N to total N%	Microbial P to organic P%	References
0–10 cm										
Grassland	297.00	38.67	18.00	6.8 ± 0.2	14 ± 0.6	2.18 ± 0.04	2.11 ± 0.12	2.44 ± 0.10	10.15 ± 0.43	Singh and Yadava (2006)
Cropland	254.00	32.00	15.66	6.9 ± 0.3	15 ± 0.7	2.13 ± 0.06	2.02 ± 0.11	2.46 ± 0.08	9.57 ± 0.41	
10–20 cm soil layer										
Grassland	78.00	13.33	5.66	–	–	–	–	–	–	Singh and Yadava (2006)
Cropland	62.00	11.00	4.66	–	–	–	–	–	–	
20–30 cm soil layer										
Grassland	35.33	5.00	2.33	–	–	–	–	–	–	Singh and Yadava (2006)
Cropland	29.67	4.33	2.33	–	–	–	–	–	–	
0–15 cm soil layer										
Soybean agro-ecosystem	228 ± 18	21.5 ± 2.1	9.7 ± 1.5	10.6 ± 8	23.4 ± 12	2.2 ± 1.41	1.46 ± 0.03	0.68 ± 0.14	2.71 ± 0.10	Bhuyan et al. (2013)
Millet agro-ecosystem	213 ± 2.2	23.5 ± 2.3	12.2 ± 1.6	9.0 ± 0.98	17 ± 1.3	1.9 ± 1.40	1.32 ± 0.02	0.85 ± 0.14	4.83 ± 0.14	
Maize agro-ecosystem	234 ± 1.9	26.5 ± 0.6	13.5 ± 1.7	8.8 ± 3.1	17.3 ± 1	1.9 ± 0.35	1.13 ± 0.07	0.46 ± 0.03	4.00 ± 0.02	
Vegetables agro-ecosystem	238 ± 21	19.9 ± 1.3	9.2 ± 1.2	11.9 ± 6	25.6 ± 17	2.1 ± 1.07	3.37 ± 0.01	1.23 ± 0.01	2.64 ± 0.03	
15–30 cm soil layer										

Soybean agro-ecosystem	207 ± 16	16.3 ± 1.4	7.2 ± 0.96	12.7 ± 11	28.5 ± 16	2.2 ± 1.50	1.4 ± 0.44	0.59 ± 0.14	1.62 ± 0.08	Bhuyan et al. (2013)
Millet agro-ecosystem	199 ± 2	19.2 ± 1.1	9.4 ± 1.2	10 ± 1.7	21 ± 1.6	2.0 ± 0.93	1.26 ± 0.24	0.54 ± 0.18	5.32 ± 0.12	
Maize agro-ecosystem	221 ± 3	19.2 ± 0.61	9.6 ± 1.6	11.5 ± 5	22.8 ± 2	1.9 ± 0.37	1.14 ± 0.03	0.35 ± 0.02	3.32 ± 0.03	
Vegetables agro-ecosystem	215 ± 24	15.4 ± 0.65	7.2 ± 0.77	13.9 ± 7	29.5 ± 31	2.1 ± 0.84	3.37 ± 0.01	0.90 ± 0.02	2.38 ± 0.06	
0–10 cm soil layer										
Oak forest stand (unexposed to sun)	832	82.3	37.3	10.1	22.2	–	2.23	1.73	5.9	Devi and Yadava (2006)
Oak forest stand (exposed to sun)	534	47.2	28.2	11.3	18.9	–	1.46	1.1	5.5	

Source: Singh and Yadava (2006), Bhuyan et al. (2013), and Devi and Yadava (2006)

Table 12.3 Microbial C, N and P in soil of different land-use/cover types at varying depths (cm) in Northwestern Himalaya

Land use/ cover	MBC (μg g^{-1})	MBN (μg g^{-1})	MBP (μg g^{-1})	MBC:MBN	MBC:MBP	MBN:MBP	References
0–20 cm soil layer							
Control	457	180	48	2.61	9.94	3.9	Debnath et al. (2015)
Apricot orchard	852	242	63	3.55	13.78	3.86	
Plum orchard	1000	320	51	3.27	20.8	6.42	
Peach orchard	928	278	92	3.46	10.24	3.12	
Cherry orchard	825	181	56	4.64	15.23	3.27	
21–40 cm soil layer							
Control	307	101	37	3.24	8.42	2.81	Debnath et al. (2015)
Apricot orchard	592	138	47	4.5	15.67	3.27	
Plum orchard	692	156	42	4.53	19.26	4.55	
Peach orchard	684	142	67	5.12	10.63	2.14	
Cherry orchard	513	104	46	5.22	11.23	2.34	
0–15 cm soil layer							
Forest	576	31.24	6.55	–	–	–	Pal et al. (2013)
Grassland	487	28.76	5.24	–	–	–	
Horticulture	435	30.01	4.87	–	–	–	
Agriculture	324	24.34	3.21	–	–	–	
Wasteland	265	20.98	2.65	–	–	–	
15–30 cm soil layer							
Forest	535	28.97	4.08	–	–	–	Pal et al. (2013)
Grassland	401	23.34	4.00	–	–	–	
Horticulture	398	18.96	3.79	–	–	–	
Agriculture	301	19.78	3.02	–	–	–	
Wasteland	225	16.99	2.54	–	–	–	
30–45 cm soil layer							
Forest	501	23.76	4.27	–	–	–	Pal et al. (2013)
Grassland	376	21.56	3.01	–	–	–	
Horticulture	302	18.65	2.98	–	–	–	
Agriculture	301	14.56	2.87	–	–	–	
Wasteland	278	15.34	2.41	–	–	–	
45–60 cm soil layer							
Forest	497	25.54	4.01	–	–	–	Pal et al. (2013)
Grassland	324	19.01	2.99	–	–	–	
Horticulture	298	15.45	3.21	–	–	–	
Agriculture	225	10.05	2.21	–	–	–	
Wasteland	198	8.98	2.21	–	–	–	

Source: Debnath et al. (2015) and Pal et al. (2013)

Table 12.4 Microbial C, N, P and microbial population in soil of different land use/cover types at 0–30 cm depth in Central Himalaya (Yadav et al. 2018)

Land use/cover	MBC (μg g^{-1})	MBN (μg g^{-1})	MBC:MBN	Microbial C to OC %	Microbial population		
					Bacteria[a]	Fungi[b]	Actinomycetes[c]
Agrisilviculture	520.79 ± 106.3	55.07 ± 12.7	9.3 ± 0.06	3.17 ± 0.23	82.83 ± 11.86	9.40 ± 3.69	45.47 ± 6.38
Agrihorticulture	522.27 ± 106.9	55.53 ± 11.9	9.37 ± 0.04	3.30 ± 0.27	77.83 ± 11.89	11.43 ± 3.82	50.53 ± 6.37
Ahortisilviculture	545.8 ± 107.3	56.93 ± 12.2	9.59 ± 0.05	3.24 ± 0.32	85.77 ± 11.90	14.33 ± 3.68	41.03 ± 9.43
Asilvihorticulture	539.05 ± 115.4	56.00 ± 12.7	9.64 ± 0.06	3.27 ± 0.28	80.87 ± 11.86	12.40 ± 3.73	47.47 ± 6.38
Chirpine silvipasture	432.6 ± 95.9	42.47 ± 10.0	10.23 ± 0.05	2.60 ± 0.35	86.33 ± 8.95	15.40 ± 4.24	61.40 ± 5.51
Mixed silvipasture	450.58 ± 105.2	43.40 ± 11.8	10.37 ± 0.03	2.51 ± 0.39	95.40 ± 8.94	20.47 ± 4.32	70.27 ± 5.72
Banz oak siivipasture	698.45 ± 57.9	66.75 ± 9.3	10.65 ± 0.03	3.37 ± 0.21	111.5 ± 6.10	22.58 ± 3.15	79.67 ± 2.90
Grassland	318.59 ± 83.8	46.90 ± 13.85	8.44 ± 0.09	1.93 ± 0.29	72.83 ± 11.83	8.37 ± 3.86	40.37 ± 6.17

Source: Yadav et al. (2018)
Note: Values after ± sign are standard deviations
[a]Number of colonies × 10^5
[b]Number of colonies × 10^4
[c]Number of colonies × 10^2

As shown in Tables 12.1, 12.2, 12.3, and 12.4, MBC:MBP ranged from 18 to 27 at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), 14–15 at 0–10 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), ~17 to 26 at 0–15 cm depth, and ~21 to 30 at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), ~19 to 22 at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006), and 10–21 at 0–20 cm depth and ~8 to 19 at 21–40 cm depth for different fruit tree orchards and control (without fruit trees) in Northwestern Himalaya (Debnath et al. 2015). MBC:MBN:MBP ranged from 18:2:1 to 27:3:1 at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004).

The range of microbial C to OC% in various land uses was 1.5 to 3.3 at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), 2–2.1 at 0–10 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), 1.1–3.3 at 0–15 cm depth and 1.1–3.3 at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), 1.4–2.2 at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006), and from 1.9 to 3.37 at 0–30 cm depth for various land uses in Central Himalaya (Yadav et al. 2018). The decline of microbial biomass in lower layers is attributed to the lesser availability of SOC (Tables 12.1, 12.2, 12.3, and 12.4).

Tables 12.1 through 12.4 show the range of recorded microbial N to total N% to be 1.7–4.9 μg g^{-1} at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), 2.44–2.46 at 0–10 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), 0.46–1.23 at 0–15 cm depth, and 0.35–0.90 at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya (Bhuyan et al. 2013), and 1.1–1.7 at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006).

Among various land uses, microbial P to organic P% ranged from 3.1 to 7.8 at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), from 9.5 to 10.1 at 0–10 cm depth for grassland and cropland in eastern Himalaya (Singh and Yadava 2006), from 2.6 to 4.8 at 0–15 cm depth and from 1.62 to 5.32 at 15–30 cm depth for various crop-based agro-ecosystems in eastern Himalaya, respectively (Bhuyan et al. 2013), and from 5.5 to 5.9 at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006) (Tables 12.1, 12.2, 12.3, and 12.4).

12.5.5 Seasonal Changes in MBC, MBN, and MBP

According to the findings of a research study by Kamala Haripal and Sunada Sahoo in 2014, microbial biomass carbon varies with seasonal changes (Fig. 12.3). MBC was maximum during the month of August and minimum in December at three different soil depths. In another study, both MBC and MBN content were high (Yang et al. 2010) during the summer season followed by spring and autumn (Fig. 12.4).

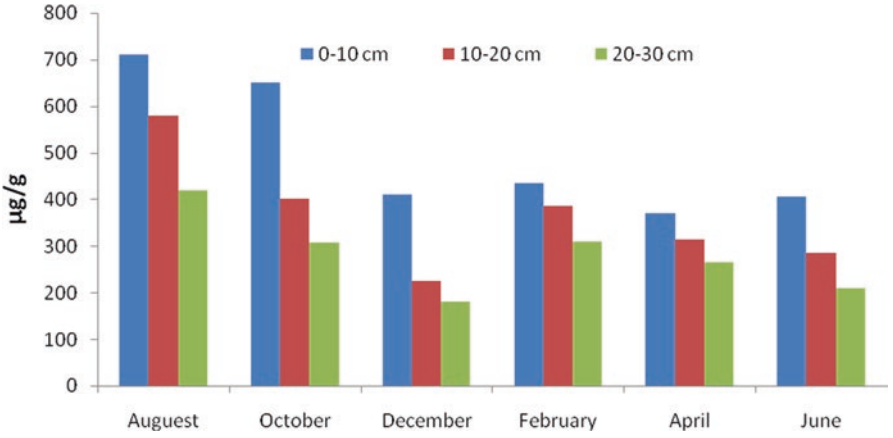

Fig. 12.4 Seasonal variation of MBC at different soil depth in long-term experiment of 15 years in rice field in Sambalpur district of Odisha, India. (From Haripal and Sahoo 2014)

Across land uses, MBC ranged from 170 to 882, 131 to 671, and 355 to 1039 µg g^{-1} at 0–15 cm depth for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), from 284 to 326, 216 to 255, and 262 to 310 µg/g at 0–10 cm depth, from 51 to 60 and 66 to 81 µg/g at 10–20 cm depth, from 35 to 38, 23 to 32, and 31 to 36 µg g^{-1} at 20–30 cm depth for grasslands and croplands in eastern Himalaya (Singh and Yadava 2006), and from 392 to 738, 740 to 1182, and 382 to 465 µg g^{-1} at 0–10 cm depth for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006). Maximum MBC was recorded during the winter season in soybean, vegetable, and millet by atomic emission spectrometry (AES), whereas in the maize agro-ecosystem, it was found during the autumn season. Minimum values were recorded during the rainy season in all the sites (Table 12.5). In land uses, MBN range was 24–156, 20–74, and 44–194 µg g^{-1} at 0–15 cm depth, respectively, for different agroforestry, cropped, and wasteland areas in Sikkim Himalaya (Sharma et al. 2004), 41–48, 26–31, and 29–37 µg g^{-1} at 0–10 cm depth, 15–17, 8–10, and 10–13 µg g^{-1} at 10–20 cm depth, and 6, 3–4, and 4–5 µg g^{-1} at 20–30 cm depth for grassland and cropland in eastern Himalaya (Singh and Yadava 2006), and 38–70, 54–99, and 44–69 µg g^{-1} at 0–10 cm depth for two different oak forest stand in eastern Himalaya (Devi and Yadava 2006).

According to Table 12.5, MBP range was 11–46, 10–29, and 14–54 µg g^{-1} at 0–15 cm depth for different agroforestry, cropped, and wasteland areas, respectively, in Sikkim Himalaya (Sharma et al. 2004); 21–21, 12–14, and 15–19 µg g^{-1} at 0–10 cm depth, and 7–8, 3–4, and 4–5 µg g^{-1} at 10–20 cm depth, and 3, 2, and 2 µg g^{-1} at 20–30 cm depth for grasslands and croplands, respectively. in eastern Himalaya (Singh and Yadava 2006); and 23–33, 36–44, and 21–31 µg g^{-1} at 0–10 cm depth, respectively, for two different oak forest stands in eastern Himalaya (Devi and Yadava 2006), with different land uses.

Table 12.5 Seasonal variation in soil microbial biomass C, N, and P in different land use systems at varying depths in Sikkim and Eastern Himalaya

Land use/cover	MBC (μg g^{-1})			MBN (μg g^{-1})			MBP (μg g^{-1})			References
	S	R	W	S	R	W	S	R	W	
Sikkim Himalaya	0–15 cm soil layer									
Temperate natural forest dense	882 ± 146	671 ± 119	1039 ± 112	156 ± 28	74 ± 16	194 ± 25	46 ± 10	29 ± 4	54 ± 8	Sharma et al. (2004)
Temperate natural forest open	724 ± 22	524 ± 84	886 ± 148	94 ± 14	64 ± 14	130 ± 26	31 ± 5	24 ± 5	38 ± 9	
Subtropical natural forest open	761 ± 136	641 ± 88	889 ± 186	73 ± 8	41 ± 8	100 ± 13	32 ± 5	26 ± 5	36 ± 6	
Cardamom-based agroforestry system	576 ± 76	384 ± 94	790 ± 214	61 ± 11	36 ± 11	91 ± 22	21 ± 3	19 ± 3	25 ± 4	
Mandarin-based agroforestry system	469 ± 109	313 ± 103	631 ± 162	41 ± 8	31 ± 3	72 ± 18	19 ± 2	16 ± 5	20 ± 3	
Open cropped area temperate	377 ± 69	263 ± 82	529 ± 137	32 ± 7	24 ± 4	57 ± 11	16 ± 3	14 ± 2	19 ± 7	
Open cropped area subtropical	292 ± 98	222 ± 27	360 ± 104	29 ± 11	22 ± 6	52 ± 14	15 ± 2	15 ± 7	18 ± 5	
Wasteland area temperate	237 ± 83	166 ± 57	374 ± 76	28 ± 8	21 ± 2	50 ± 10	12 ± 2	11 ± 2	15 ± 2	
Wasteland area subtropical	170 ± 63	131 ± 31	355 ± 64	24 ± 3	20 ± 5	44 ± 7	11 ± 3	10 ± 2	14 ± 2	
Eastern Himalaya	0–10 cm soil layer									
Grassland	326 ± 19.8	255 ± 12.48	310 ± 14.05	48 ± 5.84	31 ± 2.79	37 ± 1.45	21 ± 2.53	14 ± 1.69	19 ± 1.34	Singh and Yadava (2006)
Cropland	284 ± 8.7	216 ± 12.58	262 ± 11.19	41 ± 4.45	26 ± 1.94	29 ± 1.51	20 ± 2.23	12 ± 1.69	15 ± 1.58	
	10–20 cm soil layer									

Grassland	93 ± 5.9	60 ± 5.02	81 ± 1.30	17 ± 1.69	10 ± 1.29	13 ± 1.49	8 ± 0.53	4 ± 0.68	5 ± 0.82	Singh and Yadava (2006)
Cropland	69 ± 2.8	51 ± 4.56	66 ± 1.28	15 ± 0.98	8 ± 1.30	10 ± 0.91	7 ± 0.38	3 ± 0.68	4 ± 0.64	
	20–30 cm soil layer									
Grassland	38 ± 4.1	32 ± 2.19	36 ± 0.63	6 ± 0.67	4 ± 0.55	5 ± 0.09	3 ± 0.39	2 ± 0.27	2 ± 0.21	Singh and Yadava (2006)
Cropland	35 ± 4.0	23 ± 2.63	31 ± 1.22	6 ± 0.54	3 ± 0.65	4 ± 0.57	3 ± 0.81	2 ± 0.30	2 ± 0.29	
Eastern Himalaya	0–10 cm soil layer									
Oak forest stand (not exposed to sun)	738.32	1182.6	465.1	70.51	99.98	69.02	33.88	44.25	31.25	Devi and Yadava (2006)
Oak forest stand (exposed to sun)	392.92	740.93	382.58	38.04	54.5	44.95	23.46	36.00	21.19	

Source: Sharma et al. (2004), Singh and Yadava (2006), and Devi and Yadava (2006)
S summer, *R* rainy, *W* winter

12.6 Conclusions and Future Prospective

The literature suggests that conversion of land from one usage to another may have either detrimental or positive effects on microbial biomass and soil organic matter. Soil C, N, and P may decrease immediately following such changes, but soil reserves may also recover after a relatively short period of time if management practices are adopted. Destruction of soil organic matter likely results in a decline in productivity. In the Indian Himalayan, microbial biomass is directly related to plant biomass and is very sensitive to changes in land use and land cover as it decreases remarkably after such alterations. Therefore, afforestation of agricultural land is advisable. Furthermore, the loss of organic matter and productivity might be counteracted by strengthening agroforestry systems and crop residue management.

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Potassium-Solubilizing Bacteria (KSB): A Microbial Tool for K-Solubility, Cycling, and Availability to Plants

13

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Contents

Abstract

The potassium (K) requirement of crops is fulfilled solely from the soil solution form. About 98% of the K is fixed in the soil system whereas only 2% is readily available to plants. Many of the efficient microbes have a key role in solubilizing the unavailable form of K to stimulate crop yield. The lack of good-quality K-mineral has hindered the manufacturing of K-fertilizers in India; hence, the entire quantity of K-fertilizers is imported. This situation warrants a call for alternative means and technology to cater to the growing need of K requirements of crops and restore soil fertility. This book chapter will be helpful to display the indigenous sources of potassium as a substitute for costly imported K-fertilizers

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in time of need while discussing the concept of solubilization of native K-minerals. In this chapter, the main emphasis is a brief introduction of the significant scenario of potassium, past research work carried out in India and abroad, and summarizing the role of the latter of significant importance to soil scientists, agricultural microbiologists, and students interested in the area of soil microbiology who may work on the microbial consortium for solubilization of K to enhance crop production.

Keywords
K-solubilizing · Agricultural soils · Crop productivity

13.1 Introduction

Proper feeding of our rapidly growing population is a challenge because heavy industrialization and urbanization have led to shrinkage of agricultural areas and a food production crisis. The intensive cropping system requires a greater amount of fertilization. Hence, nutrient deficiency is inevitable unless the levels of soil fertility are measured (Bahadur et al. 2015). It is well established that the application of fertilizers increases crops yields, but there is a continuous imbalance in fertilization that deteriorates soil health and creates groundwater pollution as well as decreasing crop production. Among the many nutrients required by plants, potassium is notable, and has sufficiently large reserves as minerals in certain soils. However, plants are dependent on potassium solution uptake, and potassium in its absorbable form is greatly limited in the soil environment. The amount of K required by plants is much greater than that of any soil-supplied nutrient excluding nitrogen. However, plants directly cannot use the mineral K unless it is available in soil solution. Many of the bacteria and rhizobacteria have the capacity to solubilize or mineralize the fixed form of mineral K into soil solution: these bacteria are called potassium-solubilizing bacteria (Zeng et al. 2012). Their importance in agriculture through symbiosis with crop plants, and as biological control agents against several plant pathogens through different plant growth-promoting (PGP) activities and cycling of nutrients, is known. Use of these efficient microbes in agriculture to increase the yield of crops without harming the environment as well as maintaining soil quality and health is the ultimate goal (Archana et al. 2013).

Soil microbes are important candidates for sustenance of soil health as they perform several functions including dissolution of K-minerals (Maurya et al. 2014) and several other processes that aid in soil structure improvement, increased plant growth, and processes related to the supply of K to plants. Continuous imbalanced use of agrochemicals to increase crop yield may result in groundwater pollution and deterioration of soil nutrients, thus causing depletion of crop yield (Sheng and He 2006).

There is a crucial requirement to turn back to nature with agents such as the use of beneficial microbes to promote sustainable agriculture. K^+ is a crucial cation that is present in plant tissues and fulfills a wide range of physiological and biochemical functions in plants (Zhang and Kong 2014). In most cultivated crop plants, the quantity of K absorbed by the plants ranks second only after nitrogen (Meena et al. 2014). When K^+ is dissolved into soil solution, it is adsorbed on/in clay and organic colloids but can also be part of some more complex chemical compounds (Zandonadi et al. 2010). Potassium is involved in enzyme activation, photosynthesis, and protein synthesis.

The issue of potassium in the soil for sustainable management was partially ignored during the past two decades when the focus was on the potential environmental impact of application of phosphorus and nitrogen (Saha et al. 2016). However, in recent years, awareness among farmers regarding the importance of potassium in crop production is increasing in several parts of the world. Indian soils show K deficiency because available soil K levels have dropped from rapid agricultural development without restoring the soil, and hence K-fertilizers are used again and again as per crop needs (Prajapati 2016). Most Indian soils are deficient in both available and unavailable forms of potassium; being one of the most important macronutrients for productivity, potassium has become a yield-limiting factor in crop production (Rajawat et al. 2016). The availability of K in the soil for plant uptake is dependent on many factors including the forms and level of K, such as solution, exchangeable and nonexchangeable forms, and varying degrees of weathering of such K-minerals as biotite, muscovite, and feldspar (Sparks and Huang 1985). However, there is no reserve source of good-quality potassium-bearing minerals in India for commercial K-fertilizer production; hence, the entire required amount of K-fertilizers is imported in the forms muriate of potash (KCl) and sulfate of potash (K_2SO_4). The minerals are a reservoir of nutrients in the soil (Uroz et al. 2007), and India is very lucky to hold the world's largest reserves of sheet mica and produce the most in the world. The use of waste mica (WM) with efficient K-solubilizers may fulfill the requirement of plant K. Feldspar and mica are the major sources of inorganic K in soils. The Geological Survey of India (GSI) has stated that Koderma, a district in the state of Jharkhand (India), is the world's largest mica reserve and has approximately 95% of India's mica, including Jharkhand (60%), Andhra Pradesh (25%), and Rajasthan (10%). However, other areas such as Maharashtra, Madhya Pradesh, West Bengal, Karnataka, Kerala, Tamil Nadu, Orissa, Haryana, and Himachal Pradesh, account for only 1% of the mica production of India (Singh et al. 2018).

Among the efficient bacteria/rhizobacteria, *Bacillus mucilaginosus* (Basak and Biswas 2008) and *Bacillus edaphicus* NBT (Sheng 2005) are reported as K-solubilizers for the cotton crop. Rock mineral products are solubilized very slowly to the available form of K. Ground rock acts as a slow releaser of K-fertilizer in different conditions (Prajapati and Modi 2014). Imbalanced applications of chemical fertilizers show negative environmental influence and also increase cultivation cost. Thus, judicious application of potassium-solubilizing bacteria (KSB) has been considered as an eco-friendly approach (Rajawat et al. 2012). Many

efficient microbes are able to solubilize the fixed form of K by direct and indirect mechanisms such as acidolysis, production of organic acids, chelation, complexolysis, and ion-exchange reactions (Meena et al. 2013). These transformations have been a subject of study for a long time and are still a matter of curiosity. Efficient use of KSB as inoculants for sustainable agriculture may allow replacement of chemical fertilizer.

13.2 Potassium (K) and Its Importance

The requirements of plants for potassium (K) are greater than those for any other soil-supplied nutrient, except nitrogen (Table 13.1). All crops require K, especially plants with a high carbohydrate content such as banana and potatoes. K functions in the opening and closing of the stomata because of its presence in the guard cells of leaves of plants and also in drought tolerance and regulation of the cell membrane (Verma et al. 2015). Indian soils having a range of 5–300% total K (Mengel and Kirkby 1987) have four forms of K (nonexchangeable, solution, exchangeable, and structural). K is a major essential macronutrient for the growth and development of plants, essential in all cell metabolic processes. Consequently, K deficiencies can become a problem because K is easily decreased in soils. Applications of efficient microbes have a major function in agriculture by converting the unavailable forms of a nutrient to available forms.

Table 13.1 Requirements of potassium (K) in soil system

Soil	Location	Crop	K (kg ha^{-1})
Typic chromuserts	Rahuri, Maharashtra	Gram	273
Black soil	Jabalpur	Gram	577
Calcareous soil	Bihar	Gram	143
Acid Alfisol	Kangra (HP)	Maize	164
Black soil	Jabalpur	Maize	300
Calcareous soil	Bihar	Maize	136
Acid Alfisol	Kangra (HP)	Rice	296
Black soil	Jabalpur (MP)	Rice	380
Black soil	Guntur, Andhra Pradesh	Rice	258
New alluvial soil	Kalyani, West Bengal	Rice	195
Calcareous soil	Bihar	Rice	221
Acid Alfisol	Kangra (HP)	Wheat	166
Alluvial	IARI, New Delhi	Wheat	283
Typic chromuserts	Rahuri, Maharashtra	Wheat	225
Black soil	Jabalpur	Wheat	379
Calcareous soil	Bihar	Wheat	163
Old alluvial soil	Kalyani, West Bengal	Wheat	448

13.3 Availability in Soil

Most (90–98%) of the K is found in the fixed form; another form of K, which is nonexchangeable (~10%), is predominantly present in the interlayer K of nonexpanded forms as ilite and lattice in K-feldspars (Fig. 13.1). Common soil potassium-bearing minerals, in the order of availability of their potassium to plants, are biotite, muscovite, orthoclase, and microcline (Sparks 1987; Huang and Longo 1992).

Meena et al. (2015b) studied the release of K from biotite and muscovite with four K-solubilizers at 7, 14, and 21 days of incubation. The K-solubilization capacity of various isolates showed a significant change in muscovite and biotite powder. The soluble K-contents in all isolated treatments were significantly higher than control.

13.4 K-Solubilization

Many researchers have studied solubilization/mobilization/mineralization worldwide. Meena et al. (2015a) isolated K-solubilizers from soil after primary and secondary screening: efficient strains were characterized up to genus level. They concluded that application-efficient K-solubilizers enhance K availability in agricultural soils. P- and K-solubilizers not only can activate the insoluble phosphate and potassium mineral but can also change that into available P and K (Wu et al. 2005). Thus, the quality of crops can be improved, the effect on the environment can be decreased, the physicochemical properties improved, and the cost of production

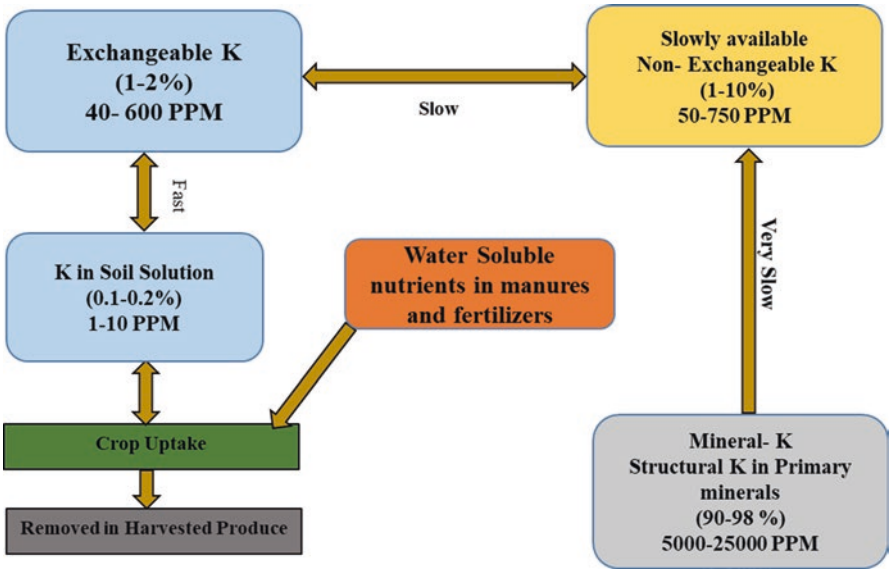

Fig. 13.1 Potassium dynamic in soil system

reduced. Rate of K release from the K-bearing illitic minerals in soil is a much slower process compared to plant K uptake rate, especially at the vital growth stages, thereby affecting plant growth (Rawlings 2002; Diep and Hieu 2013; Maurya et al. 2014).

13.5 K-Solubilizing Mechanisms

Production of different organic acids such as oxalic, tartaric, gluconic, 2-ketoglutonic, acetic, malic, and succinic acid (Maurya et al. 2014) enhance chelation (Meena et al. 2015a) and acidolysis (Styriakova et al. 2003; Meena et al. 2015b). Experimental results showed that production of organic acids enhances K availability in agricultural soils. Another report showed that the K-solubilization could be attributed to excreting various organic acids that involved either directly dissolved rock K or chelated silicon ions to bring potassium into solution form (Prajapati et al. 2012). pH of the culture solution did not significantly change during incubation, indicating that acids were not excreted in significant quantity. Thus, dissolution of K- mineral is not pH dependent as in case of P-solubilization there are many mechanisms of K-solubilization production of polysaccharides (Table 13.2). These polysaccharides contain some free carboxylic groups that cause chelation, and this caused the disintegration of K-minerals.

Table 13.2 Potassium-solubilizing microbes (KSMs) produce various organic acids in different strains, which help in solubilization of insoluble potassium to soluble potassium

Organism	Predominant acid produced	References
Penicillium frequentans, Cladosporium	Oxalic, citric, gluconic acids	Argelis et al. (1993)
Paenibacillus mucilaginosus	Tartaric, citric, oxalic	Liu et al. (2012) and Hu et al. (2006)
Aspergillus niger, Penicillium sp.	Citric, glycolic, sucinnic	Sperber (1958)
Bacillus megaterium, *Pseudomonas* sp., *Bacillus subtilis*	Lactic, malic, oxalic, lactic	Taha et al. (1969)
Bacillus megaterium, Citrobacter freundii	Citric, gluconic	Taha et al. (1969)
Arthrobacter sp., *Bacillus* sp., *B. firmus*	Lactic, citric	Bajpai and Sundara (1971)
Aspergillus fumigatus, Aspergillus candidus	Oxalic, tartaric, citric, oxalic	Banik and Dey (1982)
Pseudomonas aeruginosa	Acetate, citrate, oxalate	Sheng et al. (2003) and Badar et al. (2006)
Bacillus mucilaginosus	Oxalate, citrate	Sheng and He (2006)

13.6 Conclusion and Future Prospects

Efficient application of K-solubilizers along with waste mica (muscovite and biotite) could be a viable strategy to solubilize the insoluble form of K to sustain crop and soil health. Further long-term field studies are needed to observe the effect of the new fertilization method and tested to promise a large-scale field application.

The judicious use of K-solubilizers is providing an alternative eco-friendly tool to substitute for chemical fertilizer. These efficient K-solubilizers will be identified from the many rhizospheric soils and advocated for use in croplands among farmers based on field testing on many crops. By using these alternatives, farmers can solubilize the K present in their own agricultural soil and save as much as 25–40% of their K-fertilizer requirement and expenses. Overall, it may be said that although with many effective advantages, the commercial propagation of potassium solubilizers and their preservation and transportation to a farmer's fields for crop production is a challenge yet to be fulfilled.

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ACC Deaminase-Producing Bacteria: A Key Player in Alleviating Abiotic Stresses in Plants

14

Swapnil Sapre, Iti Gontia-Mishra, and Sharad Tiwari

Contents

Abstract

Plants are subjected to many abiotic stresses in the environment. These abiotic stresses may be aggravated in the coming future due to global climate change. Almost all the environmental stress causes the production of ethylene in plants, which is detrimental to plant survival. Therefore, managing ethylene generation in plants is becoming as an attractive strategy to increase crop yields. 1-Aminocyclopropane-1-carboxylic acid is a precursor for production of ethylene

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in plants. The plant growth-promoting rhizobacteria that possess 1-aminocyclopropane-1-carboxylic acid deaminase activity are known to modulate plant growth under extreme environmental conditions by lowering ethylene concentrations in plants; hence, they can be termed as 'stress modulator'. Ethylene is also known to reduce the nodule formation in various legumes prevailing under abiotic stress. 1-Aminocyclopropane-1-carboxylic acid deaminase-producing rhizobial strains can intensively promote nodulation in legumes under stress conditions. Another approach for combating abiotic stress in plants is through the incorporation of *acdS* gene from bacteria to crop plants. The recent molecular biology tools (metagenomics, transcriptomics, proteomics and next-generation sequencing) have been implied to reveal the diversity and application of potential 1-aminocyclopropane-1-carboxylic acid deaminase-producing plant growth-promoting rhizobacteria under various environmental conditions. These rhizobacteria have shown a vital interplay in conferring resistance and adaptation of plants to various abiotic stresses and have immense potential in organic farming and sustainable agriculture.

Keywords
PGPR · ACC deaminase · Abiotic stress · Ethylene

14.1 Introduction

Plants require optimum environmental condition for their proper growth and development. Since plants are static, they have to face several adverse environment conditions (heat, cold, salinity, drought, flooding, etc.). The intensity of these stresses might be more in the coming future due to global climate change. Due to the outcome of these different environmental stresses, plant growth is eventually lower and results in yield loss. Almost all the environmental stress leads to ethylene production in plants, which has detrimental effect on plant growth under such conditions. Therefore, managing ethylene generation in plants is becoming as an attractive strategy to increase crop yields. The major challenges confronted during breeding and genetic engineering of plants to overcome abiotic stress are because of the complexity of stress-responsive pathways. Besides, these interventions are tedious and time-consuming and have limited success rate.

Recently, it has been very effectively demonstrated by many researchers that use/inoculation of plant growth-promoting rhizobacteria (PGPR) has improved plant growth and productivity under several environmental stresses (Bharti et al. 2016). PGPR enhances plant growth and productivity by a wide array of mechanisms like solubilization of inorganic nutrients (P, Zn, K), production of phytohormones, modulation of stress ethylene and stimulation of root growth (Gontia-Mishra et al. 2017a). The PGPRs that contain 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity can modulate plant growth under extreme environmental

conditions by altering ethylene concentrations in plants; hence, they can be termed as stress modulator (Kang et al. 2010).

The chapter summarizes the importance of ACCD-PGPR (ACC deaminase-producing PGPR) in sustainable management of abiotic stresses. The first section of the chapter illustrates the detrimental effect of abiotic stresses which leads to ethylene generation on crop plants. The later part tabulates the role of ACC deaminase enzyme in lowering consequences of stress ethylene in plants. The third section emphasizes that ACCD-PGPR can influence the development of nodules in legume plant prevailing in the stressful conditions. The last section demonstrates the influence of novel molecular biology technologies in better use of ACCD-PGPR for sustainable agriculture.

14.2 Role of Ethylene in Regulation of Plant Processes and Stress Responses

Ethylene is the smallest and simplest form of plant hormone produced by plants and regulates various important plant processes (Abeles et al. 1992). These processes include seed germination, fruit ripening, senescence, development of root hair and nodules, root elongation, etc. (Johnson and Ecker 1998). Another mechanism which generates ethylene is induction of a wide array of abiotic and biotic stresses encompassing exposure to temperature extremes, salt, drought, flooding, heavy metals, organic and inorganic chemicals, nematodes and phytopathogens (Gontia-Mishra et al. 2014). Hence the ethylene produced during such stress conditions is regarded as 'stress ethylene' (Glick 2014). This kind of ethylene initiates the transcription and further expression of genes resulting in plant senescence.

The ethylene biosynthesis in plants follow a simple regime where methionine is transformed to S-adenosyl methionine (SAM) by enzyme SAM synthetase, which is subsequently used as substrate by ACC synthase to generate 1-aminocyclopropane-1-carboxylic acid (ACC). Thus, the ACC produced in the above step act as precursor for production of ethylene by the action of ACC oxidase (Wang et al. 2002).

14.3 PGPR with Special Context to ACC Deaminase Production

The enzyme ACC deaminase was originally characterized by Honma and Shimomura (1978), and sooner its importance was recognized in plant growth promotion (Glick et al. 1998). The role of PGPR possessing ACC deaminase activity in combating the effect of stress ethylene is very well studied. The bacterial ACC deaminase catalyses the cleavage of ACC to ammonia and α-ketobutyrate, leading to lower ethylene concentration in stressed plants (Jacobson et al. 1994; Glick et al. 1999). When ACC deaminase-containing PGPRs are present on the roots of a stressed plant, they act as a reservoir for ACC, retarding the ethylene levels in plants and increasing their root growth (Glick et al. 1998). Hence, plants

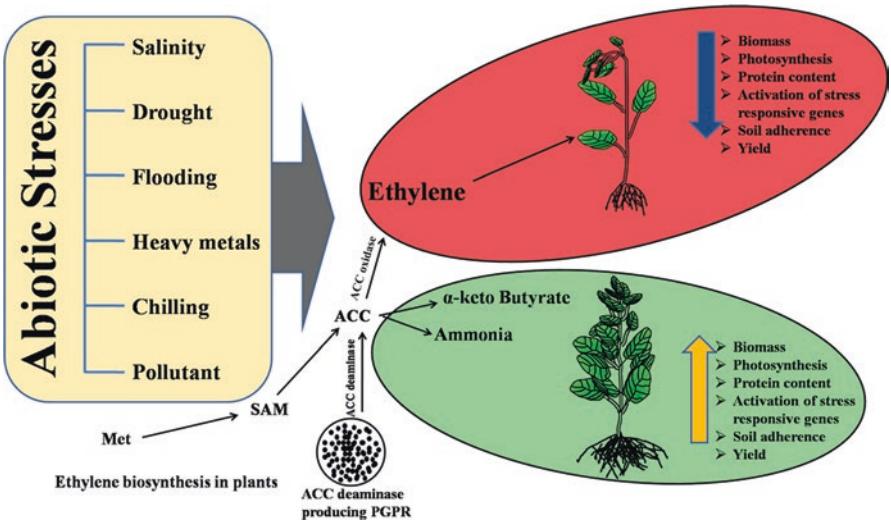

Fig. 14.1 Diverse abiotic stresses and function of ACC deaminase-producing PGPR in plant growth promotion using various mechanisms

inoculated with ACC deaminase-containing PGPR can better tolerate various stress conditions due to their extensive root growth. The by-product of ACC hydrolysis to ammonia and α-ketobutyrate are utilized by PGPRs as nutrients for their growth. How PGPR containing ACC deaminase activity helps in managing the levels of ethylene due to abiotic stress and enhances plant growth and development is shown in Fig. 14.1. The ACC deaminase activity is widespread in bacteria including *Alphaproteobacteria* (Ma et al. 2003a), *Betaproteobacteria* (Gontia-Mishra et al. 2017b) and *Gammaproteobacteria* (Gontia-Mishra et al. 2017a), *Actinobacteria* (Siddikee et al. 2010; Gontia et al. 2011; Jha et al. 2012), *Firmicutes* (Siddikee et al. 2010; Timmusk et al. 2011), *Bacteroidetes* and *Flavobacterium* (Marques et al. 2010; Mesa et al. 2015).

14.4 Abiotic Stresses in Plant: Managing Stress Response Through the Inoculation of ACC Deaminase-Producing PGPR

Plants face multiple types of abiotic stresses in the environment. ACCD-PGPR protects the plant from the adverse effect of environmental stressors such as salinity, water deficit, waterlogging, high temperature, metal toxicity and organic pollutants by lowering the activity of stress ethylene (Glick 2014). The use of ACCD-PGPR for alleviating various abiotic stresses and their positive influence on host plant is shown in Table 14.1.

Table 14.1 The role of PGPR having ACC deaminase activity in mitigating different kinds of abiotic stresses in plants

PGPR strain	Source	Crop	Effect on plants	References
Drought				
Bacillus pumilus and *Bacillus firmus*	Rhizosphere of *Solanum tuberosum*	*S. tuberosum*	Enhanced mRNA expression levels of the various ROS scavenging enzymes and higher proline content in tubers induced by PGPR-treated plants contributed to increased plant tolerance to salt, drought and heavy metal (zinc) stresses	Gururani et al. (2013)
Bacillus safensis and *Ochrobactrum pseudogregnonense*	Rhizosphere of *Triticum aestivum* and *Imperata cylindrica*	*T. aestivum*	Enhanced antioxidant responses were evident as elevated activities of antioxidant enzymes as well as increased accumulation of antioxidants such as carotenoids and ascorbate	Chakraborty et al. (2013)
Bacillus sp. and *Pseudomonas* sp.	Rhizosphere of *Cicer arietinum*	*C. arietinum*	Improved germination, root and shoot length and fresh weight of chickpea seedlings	Sharma et al. (2013)
Pseudomonas aeruginosa	Rhizosphere of *Vigna radiata*	*V. radiata*	Accelerating the accumulation of inherent levels of antioxidant enzymes, cell osmolytes, and consistently expediting the up-regulation of stress responsive genes in PGPR-treated plants under water stress conditions	Sarma and Saikia (2014)
Bacillus thuringiensis and *Paenibacillus polymyxa*	*Pinus ponderosa* and *Oryza sativa*	*T. aestivum*	Greater plant biomass and fivefold higher survivorship under severe drought; reduced emissions of stress volatiles	Timmusk et al. (2014)
Achromobacter xylosoxidans, *Pseudomonas oryzihabitans* and *Variovorax paradoxus*	Rhizoplane of *Pisum sativum* and *Brassica juncea*	*S. tuberosum*	Increased root biomass and tuber yield	Belimov et al. (2015)
Pseudomonas putida	Desert regions of Rajasthan	C. *arietinum* L.	Altered various physical, physiological and biochemical parameters as well as modulating differential expression of stress-responsive genes	Tiwari et al. (2016)

(continued)

Table 14.1 (continued)

PGPR strain	Source	Crop	Effect on plants	References
Pseudomonas simiae		*Vigna radiata*	Mutant AU-M4 and wild-type strain AU-inoculated plants exhibited superior tolerance against drought stress, as shown by their enhanced plant biomass (fresh weight), higher water content, higher proline accumulation and lower osmotic stress injury	Kumari et al. (2016)
Klebsiella sp., *Enterobacter ludwigii* and *Flavobacterium* sp.	Rhizosphere of *T. aestivum*	*T. aestivum*	Affected various growth parameters, water status, membrane integrity, osmolyte accumulation and stress-responsive gene expressions, which were positively altered by PGPR inoculation in wheat under drought	Gontia-Mishra et al. (2016a)
Enterobacter cloacae and *Citrobacter* sp.	Rhizosphere of *T. aestivum*	*T. aestivum*	Bio-inoculants showed growth enhancement of wheat seedlings under salinity and drought stress	Gontia-Mishra et al. (2017a)
Mitsuaria sp. and *Burkholderia* sp.	*Arabidopsis thaliana*	*A. thaliana* and *Zea mays*	Bio-inoculation reduced evapotranspiration and changed the plant proline and malondialdehyde and phytohormone contents under drought stress	Huang et al. (2017)
Salt				
Brachybacterium saurashtrense and *Pseudomonas* sp.	Root of *Salicornia brachiata*	*S. brachiata*	Significant plant growth-promoting activities were observed in *Salicornia* in salt stress conditions	Jha et al. (2012)
Agrobacterium tumefaciens, *Zhinguelliuella* sp., *Brachybacterium saurashtrense*, *Vibrio* sp., *Brevibacterium casei* and *Haererohalobacter* sp.	Root of *Salicornia brachiata*	*Arachis hypogaea*	Improvement of biochemical, physiological and ion balance in salinity	Shukla et al. (2012)

Bacillus aryabhattai, Brevibacterium epidermidis and *Micrococcus yunnanensis*	Rhizosphere of halophytic plants	*Capsicum annum*	Growth promotion in inoculated red pepper plants under inhibitory levels of salt stress is due to ACC deaminase activity	Siddikee et al. (2012)
Bacillus amyloliquefaciens	Alkaline soil	*Oryza sativa*	Confers salt tolerance in rice by modulating differential transcription	Nautiyal et al. (2013)
Pseudomonas fluorescens and *P. migulae*	Rhizosphere of *Solanum lycopersicum*	*S. lycopersicum*	Higher fresh and dry biomass, higher chlorophyll contents and a greater number of flowers and buds	Ali et al. (2014)
Bacillus pumilus, *Halomonas desiderata* and *Exiguobacterium oxidotolerans*	Rhizosphere of grass family	*Mentha arvensis*	Improved foliar nutrient uptake and enhanced antioxidant machinery	Bharti et al. (2014)
B. flexus, Isoptericola dokdonensis, Arthrobacter soli and *Streptomyces pactum*	Inner tissues of *Limonium sinense*	*L. sinense*	Stimulated the growth of the host plant but also influenced the flavonoids accumulation	Qin et al. (2014)
Dietzia natronolimnaea	–	*Triticum aestivum*	Enhanced gene expression of various antioxidant enzymes and higher proline content in PGPR-inoculated wheat plants contributed to increased tolerance to salinity stress	Bharti et al. (2016)
Pseudomonas frederiksbergensis	Soil	*Capsicum annuum*	Salt stress resistance in the bacterized plants was evident from the improved antioxidant activity in leaf tissues and the decreased hydrogen ion concentration	Chatterjee et al. (2017)
Enterobacter sp.	Soil	*Oryza sativa*	Reduced the antioxidant enzymes and stress-induced ethylene in inoculated plants	Sarkar et al. (2018)
Klebsiella sp.	Rhizosphere of *T. aestivum*	*Avena sativa*	Inoculation of PGPR strain enhanced plant growth under salt stress condition. Positively modulated the expression profile of *rbcL* and *WRKY1* genes	Sapre et al. (2018)

(continued)

Table 14.1 (continued)

PGPR strain	Source	Crop	Effect on plants	References
Waterlogging/flooding				
Achromobacter xylosoxidans, *Serratia ureilytica*, *Herbaspirillum seropedicae* and *Ochrobactrum rhizosphaerae*	Rhizosphere of *Ocimum*	*Ocimum sanctum*	Reduced ethylene generation and enhanced herb production	Barnawal et al. (2012)
Pseudomonas putida	Soil	Cucumber	*P. putida* UW4 significantly released the inhibition of hypoxic stresses on cucumber plants biomass	Li et al. (2013)
Temperature (chilling/heat)				
Burkholderia phytofirmans	Onion roots	*Vitis vinifera*	Bacterial inoculation stimulates grapevine root growth and plant biomass and improves its ability to withstand cold stress	Barka et al. (2006)
Burkholderia phytofirmans	Onion roots	*Vitis vinifera*	Biopriming with bacteria reduces levels of stress-related metabolites	Theocharis et al. (2012)
Pseudomonas frederiksbergensis, *Pseudomonas vancouverensis*	Soil	*S. lycopersicum*	Bio-inoculation improved seed germination and plant growth under chilling stress	Subramanian et al. (2016)
Klebsiella sp.	*Sorghum bicolor*	*Triticum aestivum*	*Klebsiella* sp. SBP-8 protects the plants against adverse effects of salt and heat stress; reduce stress-induced ethylene and regulation of ion transporters	Singh et al. (2015)
Heavy metal				
Arsenic (As)				
Acinetobacter sp.	As-contaminated soil	*Cicer arietinum*	Significantly increases plant growth and yield of the chickpea plant	Srivastava and Singh (2014)
Pseudomonas grimonti and *P. taiwanensis*	Abandoned field soil	*Zea mays*	Reduced stress ethylene emission	Shagol et al. (2014)

Brevundimonas diminuta	Rhizosphere of *Oryza sativa*	*Oryza sativa*	Significantly restored the hampered root epidermal and cortical cell growth of rice plant and root hair elimination	Singh et al. (2016)
Zinc (Zn)				
Burkholderia sp.	Heavy metal-contaminated paddy field	*Sedum alfredii*	The total shoot and root uptake of Cd, Pb and Zn in *S. alfredii* inoculated with bacteria increased greatly, produced more biomass and removed more metals from soil	Guo et al. (2011)
Phyllobacterium myrsinacearum	Rhizosphere of *Sedum plumbizincicola*	*S. plumbizincicola*	Significantly increased *S. plumbizincicola* growth and organ metal concentrations (Zn, Cd and Pb)	Maa et al. (2013)
Enterobacter intermedius	Metal contaminated soil	*Sinapis alba*	Promote plant growth and enhance Zn, Cd and Cu uptake by shoot and root	Płociniczak et al. (2014)
Pseudomonas aeruginosa	Agricultural field irrigated with industrial effluents	*T. aestivum*	Enhanced antioxidative enzyme activities and the contents of non-enzymatic components	Islam et al. (2014a)
Proteus mirabilis	Agricultural field irrigated with industrial effluents	*Z. mays*	Inoculation increased plant growth-promoting activities and avoidance of cumulative damage upon exposure to Zn	Islam et al. (2014b)
Rhodococcus erythropolis, *Achromobacter* sp. and *Microbacterium* sp.	Metal-contaminated soil	*Trifolium repens*	Enhanced clover biomass in the presence of Zn and Cd	Pereira et al. (2015)
Cadmium (Cd)				
Serratia nematodiphila, *Enterobacter aerogenes*, *Enterobacter* sp. and *Acinetobacter* sp.	Roots of *Solanum nigrum*	*Solanum nigrum*	Enhanced plant growth and Cd uptake by root, leaf and stem	Chen et al. (2010)
Serratia marcescens, *Arthrobacter* sp., *Flavobacterium* sp. and *Chryseobacterium* sp.	Roots of *Solanum nigrum*	*S. nigrum*	Increased dry weights and Cd accumulation	Luo et al. (2011)

(continued)

Table 14.1 (continued)

PGPR strain	Source	Crop	Effect on plants	References
Micrococcus sp. and *Klebsiella* sp.	–	*Helianthus annuus*	The highest Cd accumulation in the whole plant was observed	Prapagdee et al. (2013)
Bradyrhizobium sp.	Nodules of *Glycine max*	*Glycine max* and *Lolium multiflorum*	Alleviating Cd toxicity and enhances plant biomass	Guo and Chi (2014)
Pseudomonas putida	–	*Eruca sativa*	Higher levels of shoot length, root length, whole fresh plant, dry weight and chlorophyll contents with an increase in Cd uptake for *E. sativa* plants	Kamran et al. (2015)
Cu				
Pseudomonas sp.	Rhizosphere soil of *Zea mays*	*Z. mays* and *Helianthus annuus*	Enhanced shoot and root dry mass with root elongation and accumulation of Cu	Yang et al. (2013)
Nickel (Ni)				
Bacillus safensis and *Micrococcus roseus*	Soil collected from lead and zinc mine	*H. annuus*, *Amaranthus retroflexus* and *Medicago sativa*		Motesharezadeh and Savaghebi-Firoozabadi (2011)
Lead (Pb)				
Acinetobacter sp. and *Bacillus* sp.	Rhizosphere of *Commelina communis*	*B. napus*	Enhanced biomass and Pb content	Zhang et al. (2011)
Enterobacter sp. and *Klebsiella* sp.	Rhizosphere soils of *Polygonum pubescens*	*Brassica napus*	Significantly higher dry weights, concentrations and uptakes of Cd, Pb, Zn in both above-ground and root tissues	Jing et al. (2014)
Enterobacter sp., *Serratia* sp. and *Klebsiella* sp.	Rhizosphere of plants growing in mining residues	*Helianthus annuus*	Promoted the growth of plant under Cu, As, Pb	Carlos et al. (2016)

Chromium (Cr)				
Pseudomonas fluorescens and *Bacillus thuringiensis*	Rhizosphere of *Zea mays*	*T. aestivum*	Significantly increased the accumulation of Cr in root and shoots with enhancement of plant growth	Shahzadi et al. (2013)
Mercury (Hg)				
Enterobacter ludwigii (HG 2) and *Klebsiella pneumoniae*	Rhizosphere of *Alternanthera sessilis* and *Cyperus esculentus*	*T. aestivum*	Bio-inoculation significantly registered better growth promotion of wheat seedlings under metal stress	Gontia-Mishra et al. (2016b)
Pollutants				
Bacillus circulans, *Enterobacter intermedius* and *Staphylococcus carnosus*	Rhizosphere of *Z. mays* and pepper	*Z. mays*	Exhibited significant vegetative growth	Ajuzieogu et al. (2015)
Acinetobacter sp.	Soil	*A. sativa*	Decreased the MDA and free proline contents, indicating that PGPR and AMF could make the plants more tolerant to harmful hydrocarbon contaminants	Xun et al. (2015)
Pseudomonas aeruginosa and *Serratia marcescens*	Rhizosphere of *Echinochloa*	*A. sativa*	Enhanced plant growth	Liu et al. (2015)
Burkholderia sp.	Soil	–	Plant growth promotion and reduced phytotoxicity of phenol	Chen et al. (2017)

14.4.1 Salinity

Salinity is an important environmental stress that severely affects plant productivity worldwide. It disrupts photosynthesis and increases photorespiration, altering the normal ion balance in plant cells (Miller et al. 2010). The main effect of salt on plant growth compasses nutrient imbalance by rendering proper nutrient uptake and/or transport to the shoot causing ion deficiencies (Munns and Tester 2008). The ACCD-PGPRs have been broadly used to combat salinity stress in many crops including tomato, groundnut, etc. (Mayak et al. 2004; Saravanakumar and Samiyappan 2007).

14.4.2 Drought

Insufficient availability of water, i.e. drought, is another important stress which adversely affects the plant growth and yield. This stress affects several physiological and biochemical functions of plants like decreased water potential and turgor loss and stomatal closure and disturbs membrane and protein structure (Kaushal and Wani 2016). Overall the drought stress retards plant growth leading to yield losses, and hence there is need to resort on strategies for better plant growth under this stress. Several authors have reported the utilization of ACCD-PGPR for ameliorating water deficit stress in crops such as chickpea (Tiwari et al. 2016), wheat (Gontia-Mishra et al. 2016a) and *Lavandula dentata* (Armad et al. 2016).

14.4.3 Flooding/Waterlogging

In the present scenario, change in climate drastically affects the availability of water leading to drought or flooding/waterlogging in some areas (Loreti et al. 2016). Flooding can perturb many physiological processes of plants such as respiration in roots, making the environment anoxic (low or no O_2), affecting the yield of terrestrial crops worldwide (Sairam et al. 2009; Striker 2012). During flooding condition, ethylene is generated in quite high amounts inside the plant tissue due to the increased activity of ACC synthase in the waterlogged roots (Gontia et al. 2014). The ethylene produced hardly escapes from the plant tissue due to flooding, leading to various stress-related responses in plants (Loreti et al. 2016). PGPRs with ACC deaminase activity are capable of rerouting ACC from the ethylene biosynthesis pathway in the root of host plants, hence causing low ethylene production. This strategy has been applied by few researchers to reduce the flooding/waterlogging stress in many plants (Barnawal et al. 2012; Li et al. 2013).

14.4.4 Temperature (Chilling and Heat)

Extreme temperatures either low or high cause substantial yield loss in crop plants. Plants mostly adjust their cellular metabolism which is disrupted due to rise or fall in temperatures (Yadav 2010). A variation in temperature leads to drastic change in

the structure of membranes, catalytic properties, function of enzymes and transport of nutrients (Subramanian et al. 2016). The chilling temperature is termed as low temperature between 0 and 15 °C, which accounts to yield loss in several tropical and subtropical crops. Cold stress usually causes low germination, retarded growth of seedlings, chlorosis of leaves and reduced tillering (Yadav 2010). In horticultural crops, chilling induces surface lesions, discoloration due to reduced chlorophyll content and accelerated senescence. Similar to other environmental stresses, chilling also triggers ethylene generation which hampers the plant growth.

There are few reports on the use of ACCD-PGPR for alleviation of chilling stress in grapevine and tomato (Theocharis et al. 2012; Subramanian et al. 2016). Recently, psychrotolerant PGPRs (*Flavobacterium* sp., *Pseudomonas frederiksbergensis* and *Sphingomonas faeni*) with no ACC deaminase activity were transformed with a plasmid pRKACC harbouring the *acdS* gene from *Pseudomonas putida* UW4. These transformed PGPRs which overexpressed *acdS* gene were examined to determine their role in mitigating chilling stress in tomato and foxtail and finger millets (Subramanian et al. 2015; Srinivasan et al. 2017).

14.4.5 Heavy Metals

The contamination of agriculture soil by heavy metals is of utmost environmental concern worldwide. Some of the heavy metals such as zinc, copper, cobalt, etc. are required by the plant in trace amounts, but high concentrations of these essential elements are deleterious to plant growth. The heavy metals and metalloids include lead, zinc, cadmium, selenium, chromium, cobalt, copper, nickel, mercury and arsenic. Since in plant system the roots are essentially involved in uptake of nutrients and metals, the high concentration of heavy metals in soils induces the generation of stress ethylene which in turn inhibits root growth (Saleem et al. 2007). There are a large number of reports on inoculation of ACCD-PGPR for improvement of plant growth under metal stress (Burd et al. 1998; Chen et al. 2010; Plociniczak et al. 2014). Plants are usually utilized to remediate/metabolize the toxic compounds (heavy metals) into less toxic intermediates in contaminated soil; this process is termed as phytoremediation (Glick 2010).

Phytoremediation is an effectual and comparatively inexpensive and eco-friendly method for clearing out the contaminated soil (Arshad et al. 2007). The application of ACCD-PGPR supports phytoremediation by enhancing the root development under metal stress which further increases the uptake of toxic metals. The use of ACCD-PGPR for promoting plant uptake of metals has been very well reviewed by Glick (2010) and Arshad et al. (2007).

14.4.6 Organic Pollutants

Polycyclic aromatic hydrocarbons (PAHs), herbicides and pesticides are anthropogenic sources of pollutants that can contaminate the soil (Van Oosten and Maggio 2015). The organic pollutants retard plant development via unknown mechanisms; this is the outcome of stress ethylene generation. The use of plants alone for

remediation confronts many limitations. The large number of soil microorganisms has the property of degrading organic pollutants in the environment including refrigerants and organic solvents. The ACCD-PGPRs have shown persistent result in augmenting plant development under the existence of organic pollutants (Reed and Glick 2005; Xun et al. 2015).

These PGPRs can also support the associated plants in phytoremediation by biotransformation of toxic elements. It is a known fact that ACCD-PGPR contributes largely in root elongation and growth which can account for better phytoremediation of organic compounds by the host plants.

14.5 Role of ACCD-PGPR in Nodulation Under Stress Conditions

Symbiotic nitrogen fixation is essential for sustainable agriculture. The roots of legumes generally coexist with nitrogen-fixing bacteria especially rhizobium, which aid in the development of nodules. The development and growth of nodules respond to various extreme environmental stress and plant hormones (abscisic acid, auxin, cytokinin and ethylene). Ethylene production is generally induced due to stress conditions, which is accumulated in plant tissues (Nascimento et al. 2012a). Ethylene is also known to adversely modulate the nodule formation in various legume plants (Gage 2004) such as *Medicago sativa* (Peters and Crist-Estes 1989), *Pisum sativum* and *Trifolium repens* (Lee and LaRue 1992). It reduces the nodule numbers as well as notably drops the levels of fixed nitrogen (Guinel 2015). The ACC deaminase activity is noted in many rhizobium species such as *Sinorhizobium meliloti*, *Rhizobium leguminosarum* and *Mesorhizobium loti* (Duan et al. 2009).

It is a very well-accepted fact that ACC deaminase-producing rhizobial bacteria can nodulate the roots of associated legumes intensively, under stress and non-stress conditions (Ma et al. 2003b). It was known that native rhizobium species have a lower ACC deaminase activity in comparison with free-living bacteria. Hence, there is ample literature demonstrating the reduced level of stress ethylene in pulse crops inoculated with ACCD-PGPR (Duan et al. 2009; Shaharoona et al. 2011). It was documented by Shaharoona et al. (2006) that co-inoculation of *Pseudomonas putida* (ACC deaminase-producing bacteria) with *Bradyrhizobium japonicum* in mung bean enhanced the nodulation. Similarly, in another study, the co-inoculation of ACCD-PGPR (*Serratia proteamaculans* and *Citrobacter koseri*) with *Mesorhizobium ciceri* increased the nodulation in chickpea (Shahzad et al. 2010).

These were examples of increased nodulation in legume crops by co-inoculation with ACC deaminase-producing bacteria under non-stress condition, but there are other experiments in which the co-inoculation with ACC deaminase-producing bacteria have proved better nodulation in legumes under various environmental stress. It was noted that the inoculation of *Pseudomonas syringae* bacteria having ACC deaminase activity along with *Rhizobium phaseoli* promoted better seedling growth and improved nodulation capacity in mung bean under saline condition (Ahmad et al. 2011). In an experiment, the co-inoculation of ACCD-PGPR (*Bacillus subtilis* LDR2) with rhizobial bacteria (*Ensifer*

meliloti) enhanced nodulation and root growth of fenugreek under drought stress (Barnawal et al. 2013). In a similar study, the application of ACCD-PGPR *Arthrobacter protophormiae* was reported to stimulate plant growth through improved colonization of *Rhizobium leguminosarum* resulting in a better nodulation in *Pisum sativum* under salinity stress (Barnawal et al. 2014). In another instance, the inoculation of rhizobacteria *Pseudomonas* sp. having ACC deaminase activity, along with *Mesorhizobium* sp. and *M. metallidurans*, increased the nodule weight of *Lotus corniculatus* growing in metal-contaminated soil (Soussou et al. 2017). In another strategy, gene for ACC deaminase (*acdS*) was overexpressed in rhizobial bacteria which lack ACC deaminase activity, proving a practical method to improve their nodulation in legumes.

The earliest report states that the heterogeneous production of *acdS* gene from *Rhizobium leguminosarum* into *Sinorhizobium meliloti* and the inoculation of transformed *S. meliloti* in alfa-alfa enhanced nodulation by 40% in comparison with inoculation with non-transformed *S. meliloti* (Ma et al. 2004). Additionally, *Mesorhizobium loti* MAFF303099 which was genetically modified to continuously express ACC deaminase stimulated better nodule development in Lotus plants (Conforte et al. 2010). Similarly, *Mesorhizobium ciceri* strain LMS-1which was genetically engineered to express the *acdS* gene of *Pseudomonas putida* UW4, when used as inoculants in chickpea, demonstrated improvement in nodulation and overall plant growth under salt-affected soil (Nascimento et al. 2012a, b; Brígido et al. 2013). A congruent report of the inoculation of transformed Sinorhizobium meliloti (overexpressing ACC deaminase gene from Rhizobium leguminosarum) in Medicago lupulina under copper stress demonstrated an affirmative effect by better adaptability and increased the nodulation in plants (Kong et al. 2015). Hence, it could be concluded that the ACCD-PGPRs, either in co-inoculation experiment or using transformed *Rhizobium* strain expressing *acdS* gene, are capable to improve the symbiotic association of the bacteria under normal and stress conditions. The different strategies to improve nodulation in legumes under stress condition via the use of ACC deaminase-possessing rhizobial strains are presented in Fig. 14.2.

Ethylene produced due to abiotic stress reduces nodulation in leguminous plants. The application of either ACC deaminase-producing rhizobium strain or co-inoculation of rhizobium strain (non ACC deaminase producing) with ACCD-PGPR (ACC deaminase-producing PGPR) improves nodulation in legumes under abiotic stress as well as non-stress condition. Rhizobium strain transformed with *acdS* gene exerts similar effect on nodulation in legumes under abiotic stress condition. Figure 14.2 summarizes different approaches used to enhance nodulation in legumes under adverse environmental condition incorporating ACCD-PGPR.

14.6 Development of Transgenic Plants Overexpressing *acdS* Gene

Transgenic plants, which exhibit new or improved phenotypes, are engineered by the overexpression and/or introduction of genes from other microbes like bacteria. Over the years, various genetically modified plants were developed overexpressing

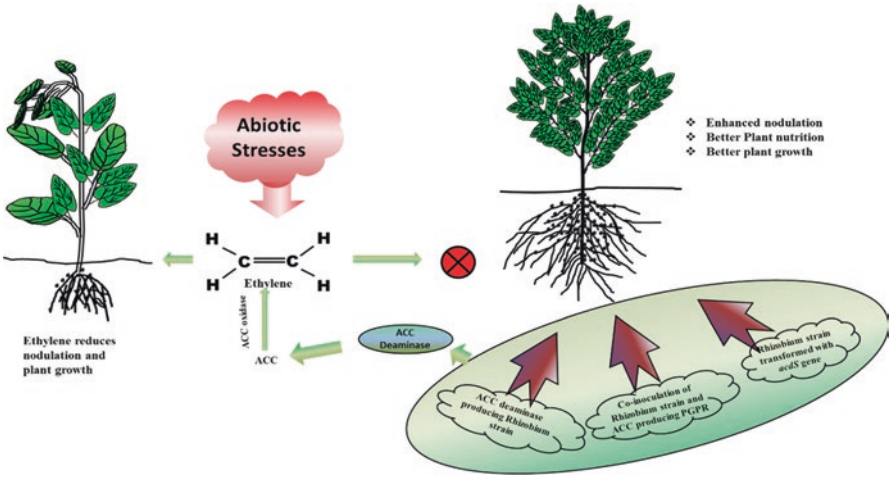

Fig. 14.2 Effect of ACC deaminase-producing PGPR on nodulation of leguminous plants under abiotic stress condition

acdS gene from bacteria and reported to lower the ethylene concentration in plants as well as enhanced the tolerance to various kinds of abiotic stresses (reviewed by Gontia-Mishra et al. 2014).

The transgenic tomato, canola and tobacco transformed to express *acdS* gene from bacteria were able to survive better in soil contaminated with various heavy metals such as Ni, Co, Cu, As, Pb and Zn (Grichko et al. 2000; Nie et al. 2002; Stearns et al. 2005; Zhang et al. 2008). Similarly, transgenic canola and tomato expressing *acdS* genes (from *Enterobacter cloacae* and *Pseudomonas putida* UW4) demonstrated remarkable tolerance to flooding (Grichko and Glick 2001; Farwell et al. 2007). In another report, a transgenic canola plant overexpressing *acdS* gene from *Pseudomonas* has demonstrated improved salt tolerance (Sergeeva et al. 2006). In a recent study, transgenic *Arabidopsis* plants were created overexpressing *acdS* gene from *P. fluorescens*. The transgenic plants showed better performance in overall growth parameters than the non-transformed plants under ethylene and salt stress (Kim et al. 2014).

Additionally, transgenic *Arabidopsis* plants were developed which expressed *acdS* gene from *Trichoderma asperellum* ACCC30536 and showed better performance under salt stress. These transgenic plants had improved root development and reduced reactive oxygen species (ROS) level in terms of antioxidant enzymes in comparison with non-transformed plants under stress condition (Zhang et al. 2015). Similarly, transgenic *Camelina sativa* (oil seed crop) was developed which expressed *acdS* gene from *P. putida* UW4 under the control of either the CaMV 35S promoter or the root-specific rolD promoter. The transgenic *C. sativa* lines had enhanced growth parameters, yield and oil content in contrast to wild-type plants under salinity (Heydarian et al. 2016). It could be noted from the above studies that transgenic plants expressing the *acdS* gene have facilitated plant growth,

especially root growth under various abiotic stresses, making this strategy fit for use in reducing the effect of different stresses on the crop plants.

14.7 Omics-Based Approaches in PGPR Research

Various ecological niches have to be intensively screened to unveil the microbial diversity with extensive potential. With advent of new techniques in molecular biology such as use of qPCR can be utilized to detect efficient ACCD-PGPR species residing in soil, rhizosphere or endophytes. Another upcoming approach, i.e. metagenomic analysis of particular soil or plant tissue, is utilized to unravel the microbial diversity through culture-independent method. This method depends on the isolation of genome of a specific habitat which is subsequently cloned and analysed to disclose the ecology and functions of uncultivable microbial community. Combined approach of sequence-based as well as function-based examination of metagenomic libraries can lead to mine novel genes for PGPR activities from uncultivable community will append enormously to our knowledge of their functionality in plant growth promotion (Nikolic et al. 2011).

The novel gene obtained through the metagenomic studies can be exploited to design and develop PGPRs with improved performance (Leveau 2007). Thus an amalgamation of sophisticated culture-independent molecular approaches along with culture-based microbiological applications should be applied to get genetically diverse PGPR to perform efficiently in agriculture. With the advent in genome sequencing using various next-generation sequencing (NGS) techniques, the data on complete genome sequence of bacteria has increased. The information obtained from complete genome data is extensively utilized to derive interesting findings. Recently, many agriculturally important PGPRs such as *Pseudomonas aeruginosa* PGPR2 (Illakkiam et al. 2014), Bacillus sp. strain PTS-394 (Qiao et al. 2014), Bacillus amyloliquefaciens subsp. plantarum UCMB5113 (Niazi et al. 2014) and Methylobacterium oryzae (Kwak et al. 2014) have been completely sequenced.

The genome sequencing of PGPR has opened up a new venture and opportunities to deduce genes for different metabolic pathways and interaction with other molecules to initiate plant growth promotion. In this context, *Pseudomonas* spp. UW4 (showing ACC deaminase activity) have been sequenced which provided insights to various mechanism utilized by the bacterium to promote plant growth. The whole-genome sequencing revealed the presence of genes actively participating in plant growth promotion such as indole-3-acetic acid (IAA) and acetoin biosynthesis, ACC deaminase and siderophore production and phosphate solubilization (Duan et al. 2013). The genome analyses of these important PGPRs will definitely endow an elementary basis for future studies towards understanding the functionality of these PGPR and plant-beneficial microbe interactions as well as to improve the agricultural yields.

The physiological and biochemical data from plant-microbe interaction probably ignore the plethora of influence on each other, thus motivating the utilization of recent functional approaches. Many of the mechanisms underlying plant-microbe

dialogue in the rhizosphere are poorly understood. Multi-omics approaches encompassing genomics, transcriptomics, proteomics and metabolomics integrated studies on plant-microbe interaction and their external environment provide voluminous information to generate a better depiction of cells' inside (Meena et al. 2017). Transcriptomics and proteomics approaches are being indulged to elucidate mechanistic insights of the interaction of plant-microbe under stress conditions (Cheng et al. 2010). A microarray-based study of plant-microbe interaction amid rice and *Azospirillum* sp. revealed modulation of 16% of total rice genes (Drogue et al. 2014).

Additionally, halotolerant PGPR having ACCD activity positively stimulated the transcription profile of some antioxidant genes in peanut seedlings under salinity stress (Sharma et al. 2016). Proteomics is an upcoming strategy to divulge the expressions of whole proteins in plant-microbe interactions (Parray et al. 2016). In a study, the interaction of ACCD-PGPR *Pseudomonas putida* UW4 with cucumber plant under waterlogging condition was examined using two-dimensional difference in-gel electrophoresis (DIGE) to detect regulation of proteins (Li et al. 2013). Similarly, the influence of *Pseudomonas putida* on the proteomic profiles of canola was investigated facing salinity stress (Cheng et al. 2012).

14.8 Concluding Remarks and a Look Forward

Environmental stresses are becoming a major problem which claim productivity losses in agriculture. The use of PGPR in agriculture is attaining ample attention to mitigate various environmental stresses. In this respect, ACCD-PGPR has the selective advantage over other PGPRs as they protect the plants from the effect of stress ethylene under abiotic stress conditions. In the conclusion, it is recommended to extend the number as well as diversity of ACCD-PGPR from diverse ecological niches and investigate their role in alleviating stress in crop plants. Still there is a lacuna in our discernment of plant-microbe interaction. With the introduction of several -omics techniques, the new insights for better perception of plant-microbe interactions can be bridged. These advances definitely have application in agriculture to enhance the crop productivity under stress conditions. PGPRs have shown a vital interplay in conferring resistance and adaptation of plants to different abiotic stresses and have immense potential in resolving future food security issues. In the past decade, the use of ACCD-PGPR to alleviate stress has been implied to a limited extent. Hence, utilization of ACCD-PGPR either alone or in co-inoculation with other effective PGPRs needs extensive implementation for sustainable agriculture. Another vital approach in the above line could be the employment of transformed PGPR with *acdS* gene and transgenic plants overexpressing *acdS* gene from microbial origin in agriculture to combat productivity losses due to abiotic stress. The transcriptomic study reveals the interaction of crops with PGPR which may provide the insights on how these PGPRs stimulate host machinery to adapt in extreme environmental conditions. Simultaneously, future research must be focused to increase the number of potential ACCD-PGPR strains to be used as stress modulators.

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Sustainability of Crop Production by PGPR Under Abiotic Stress Conditions

15

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Contents

Abstract

Rhizobacteria, which live around or on plant root surfaces, improve plants directly or indirectly by their plant growth-promoting abilities. Various studies about plant growth-promoting rhizobacteria (PGPR) show enhanced plant growth, development, and productivity under stressed and nonstressed conditions. PGPR produce some plant growth regulators, 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, siderophores, and release organic acids. PGPR also have N-fixing and phosphate- (P), potassium- (K), and zinc- (Zn) solubilization abilities and increase the acquisition of nitrogen and phosphorus. PGPR produces ACC-deaminase and reduces the ethylene concentration in plant tissues when the plants are exposed to stress. PGPR may also reduce the occurrence of

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secondary soil salinity by reducing fertilizer use. Thus, rhizobacteria can ensure sustainable agricultural production through these mechanisms in environmental stress factors.

Keywords
Beneficial bacteria · Abiotic stress · Environment · Agriculture

15.1 Introduction

Agriculture meets our food demands, our food security, and provides employment. Further, agriculture contributes to the national economy in many countries where people earn their living with agriculture. However, 38% of the suitable agriculture lands of the world has been degraded by agricultural practices. Also, abiotic stress factors have negatively affected many suitable agricultural areas (Reddy 2014). In the past century, increasing water scarcity, drought, soil and water salinity, and environmental pollution have led to significant crop losses worldwide. Nowadays, population problems and declining utilizable agricultural areas have become a threat affecting sustainable agriculture production globally (Shahbaz and Ashraf 2013). Also, various adverse environmental conditions such as soil and water salinity, drought, heavy metal toxicity, extreme temperatures, and flood affect the production and cultivation systems of agricultural crops. Because of the decrease in available agricultural lands, good management practice of the remaining agricultural land is requisite to maintain production, obtain economic growth, protect biodiversity, and meet the increasing food demands. In this context, sustainable agricultural land has been protected and maintained with good agricultural practices and organic farming methods in the past decades. Beneficial microorganisms (bacteria, mycorrhizae, insects, etc.) are part of these sustainable agriculture practices. Bacteria provide benefits by their capabilities for such actions as N_2-fixing, phosphate solubilization, and production of siderophores, indole acetic acid (IAA), cytokinins, and 1-aminocyclopropane-1-carboxylate (ACC)-deaminase. This chapter highlights the potential of beneficial bacteria for our agro-ecosystems and sustainability of lands.

15.2 Plant Abiotic Stress Factors, Damage, and Plant Response

15.2.1 Salinity Stress

Salinity is the main environmental stress factor that reduces agricultural area and crop yield and quality. Drought stress also triggers soil salinity (Yamaguchi and Blumwald 2005); 20% of the Earth's agriculture areas (~45 million ha) is salt affected and degraded. The soil salinity rate is estimated as 30% of the global

agricultural lands (Shrivastava and Kumar 2015). Salinization occurs in two ways: the primary type is from natural occurrences and the secondary type results from human activity for agricultural production. Primary salinity areas such as salt lakes, salt pans, salt marshes, and salt flats occur in soils and waters.

Secondary salinity results from human activities, development of land for agriculture, and the resultant agricultural practices, especially the use of more synthetic fertilizers (Bharti et al. 2013). Salinity decreased the tillers, leaf area, and yield of monocotyledon plant species and decreased the branch number and leaf area of dicotyledonous plant species (Flowers and Colmer 2008). Plants that have salt stress protection ability are classified as two types: halophytes and glycophytes. Although halophyte plants can survive under saline condition, 99% of glycophyte plants are killed by high saline concentrations (Eynard et al. 2005; Munns and Tester 2008). Plant response to salinity stress can be divided into two stages. The first stage is called osmotic response, in which plants accumulate Na^+ and Cl^- ions, which are not at toxic levels so plant growth is not inhibited. In this period, plants have reduced leaf development and area, shoot development speed, lateral bud growth, flowering, and crop production (Fricke and Peters 2002; Rahnama et al. 2011). In addition to decreased growth, plants close the stomata to prevent water loss (Fricke 2004). Stomata closure results in decreased carbon fixation and assimilation in leaves because CO_2 uptake is restricted (Lenis et al. 2011; Munns and Tester 2008). Therefore, photosynthetic activity is reduced by stomatal closure, and light energy accumulates in the leaf tissues. Light energy in excess of plant need is transferred to oxygen acceptors, and then reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$) are produced (Møller et al. 2007). The higher ROS concentration damages cell walls and results in cell death (Møller et al. 2007). The next stage is ionic stress, in which Na^+ and Cl^- ions accumulate to toxic levels in leaf tissues. The excess Na^+ and Cl^- limit K^+ uptake, and insufficiency of K reduces protein synthesis. The higher Na^+ concentration raises the Na^+/K^+ ratio, decreases the availability of K^+, and disrupts enzymatic processes (Bhandal and Malik 1988). In addition to Na^+ accumulation, when Cl^- ions are excessively accumulated in the leaf, acquisition of NO_3^- and NH_4^+ is reduced. The reduced NO_3^- and NH_4^+ levels cause decreased nitrate reductase activity in plant tissue (Tuteja 2007).

15.2.2 Drought Stress

Drought stress, which has appeared almost all over the world, has affected more people worldwide in the past 40 years than any other natural hazard. Global climate change is intensifying drought on the Earth's surface and also increasing drought severity and duration. Drought is a complicated natural phenomenon with different intensity levels, periods, land coverage, and effects. Intensive drought periods cause severe socioeconomic and environmental problems, including degradation of natural resources, migration, famines, and low economic success. Agriculture, which absorbs about 80% of all drought effects in itself, is among the first of the sectors

that are hit and affected by drought, which has poly-effects on crop yield, food safety, and rural life (Anonymous 2018). Drought stress triggers interacting metabolic activities, including blocking of antioxidant enzyme activity and producing ROS in metabolic pathways (Binzel and Reuveni 1994; Tsugane et al. 1999). The ROS species have a significant role in the life cycle of plants.

The reaction of plants to drought is generally described by physiologists as escape, avoidance, and tolerance (Levitt 1980). To escape from drought stress effects, plants accelerate flowering and produce seed to complete their life cycle before drought stress severity reaches a critical point. Avoidance is a mechanism of limiting transpiration or promoting water uptake. In avoidance, water loss and tissue dehydration do not appear in plant tissues. To restrict water loss, stomatal closure, increased root volume, and decreasing leaf area are significant avoidance strategies. Thus, plant tissues keep a high water potential. Plant tolerance to drought stress is defined as the ability to cope with decreased tissue water potential (Verslues et al. 2006). This ability allows the plants to function, or at least survive, under stress conditions. There are differences in genotype tolerance to drought, arising from genotypic flexibility.

15.2.3 Heavy Metals Stress

In the periodic table, the 53 elements including iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) that are essential to plants are defined as heavy metal elements, heavy metals (HMs), whose density is more than 5 g cm^{-3} (Hollemann et al. 1985). However, many essential elements have reactive features and cause toxicity in plants if they are accumulated at a higher level than is required. The toxic metals such as cadmium (Cd), lead (Pb), chromium (Cr), and mercury (Hg) have ionic properties similar to those of Fe, Cu, Zn, and Mn, so they can enter plants with transporters. Thus, these metals cause toxicity to plants when present at elevated levels (Kohzadi et al. 2019). When the HM elements reach a toxic level, they cause damage and changes in plant physiology and function. HM toxicity promotes ROS, which exert oxidative damage to plant cells and in plant metabolism. The oxidative stress triggered via HMs includes disruption of the main metabolic processes such as photosynthesis, electron transport, antioxidant enzyme activity, and metabolites. In addition, the accumulation of the ROS increases lipid peroxidation, which causes degradation of the cell membranes.

Moreover, ROS production is always considered detrimental to plant cells. The plant antioxidant defense mechanism regulates the ROS mechanism under abiotic stress condition. Some antioxidant enzymes such as catalase (EC 1.11.1.6), superoxide dismutase (EC 1.15.1.1), peroxidase (EC 1.11.1.7), glutathione peroxidase (1.11.1.9), glutathione reductase (EC 1.8.1.7) (CAT, SOD, POD, GPX, GR), and nonenzymatic antioxidants such as ascorbic acid, glutathione, α-tocopherol, and carotenoid, scavenge the harmful effects of ROS (Hirt and Shinozaki 2003).

15.2.4 High- and Low-Temperature Stress

High temperatures cause heat stress as a global agricultural factor. High temperature negatively affects morphological development, biochemical properties, and the physiological aspects of plant species (Zandalinas et al. 2018). The damages of high temperature appear as a sunburned leaf, branch, and stem, leaf senescence, and abscission. In addition, plant and root growth are inhibited by high temperature (Vollenweider and Günthardt-Goerg 2005). Thus, plant growth and production may be reduced with drastic economic results. Under high temperatures, plants accumulate metabolites such as antioxidants, osmoprotectants, and heat-shock proteins (Bokszczanin et al. 2013). The ROS act as signaling molecules, and antioxidant enzymes are produced against ROS (Bohnert et al. 2006). Low temperature damages plants in two ways, such as chilling injury and freezing injury.

Generally, chilling injury appears in tropical and subtropical region plants. These plants show marked physiological dysfunction when they are exposed to a low temperature below 12 °C (Lyons 1973); freezing injury occurs below 0 °C temperatures. The plant cells are damaged by intracellular and extracellular freezing. The freezing occurring in the cell could be avoided by plants; however, plant cells are subjected to dehydration stress when the water freezes in extracellular spaces (Yamada et al. 2002). The plant cell membranes are mechanically injured by solid ice, which is formed when the extracellular water freezes (Steponkus et al. 1993). In agricultural areas where subzero winter temperatures occur, plant tolerance to survive under freezing conditions is important (Levitt 1980).

Although exactly how plants continue living and survive under freezing temperatures is unknown, freezing alters the membrane cryostability of plants. To avoid freezing injury, plants increase membrane cryostability, which may be associated with changes in the membrane in other cellular components surrounding the plasma membrane. The general thought is that the hydrophilic molecules such as sugar, enzymes, and dehydrins induce changes of lipids and membrane proteins to increase the cryostability of the plasma membrane (Lee et al. 2014; Strimbeck et al. 2015).

15.2.5 Flood Stress

The last abiotic stress factor for agricultural production to be discussed is a flood. In general, this stress appears in rice production areas, and each year, one-fourth of the rice lands in the world are inundated by unpredictable flash floods that appear a few times a year nowadays (Mackill et al. 2012). The plants need oxygen (O_2) to surviving but severe flooding reduces O_2 availability in soil. Aerobic respiration can be restricted by lower O_2 levels. Flooding also causes ethylene to accumulate and increases CO_2 concentration, depending on the light conditions of submerged plant organs. Flooded agricultural areas can suffer from light intensity, thus decreasing photosynthetic activity (Bailey-Serres and Voesenek 2008). Plant species show some adaptive features to survive under low O_2, such as changing of the

petiole:internode elongation ratio, cellular modifications, development of lateral and adventitious roots, and formation of arenchyma tissue.

15.3 Reactive Oxygen Species

Reactive oxygen species (ROS) are produced under various abiotic environmental stress conditions such as salinity, drought, low and high temperatures, HMs, nutrient deficiency, and UV radiation. ROS are highly toxic and reactive, causing damage to proteins, lipids, carbohydrates, and DNA, which results in oxidative stress (Gill and Tuteja 2010). ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$), are produced in chloroplasts, mitochondria, endoplasmic reticulum (ER), plasma membranes, peroxisomes, apoplast, and cell walls under both normal and stress conditions (Sharma et al. 2012). To manage ROS, plant species have two defense mechanisms. The first mechanism is producing enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), guaiacol peroxidase (GOPX), and glutathione *S*-transferase (GST). The other mechanism is producing nonenzymatic antioxidants including ascorbic acid (AsA), glutathione (GSH), tocopherols, flavonoids, and proline (Dar et al. 2017; Gill et al. 2011). Some antioxidant enzymes such as SOD, CAT, and POD eliminate ROS, whereas others such as GPX eliminate internal lipid peroxidation products. Glutathione *S*-transferase has a significant role in eliminating toxic secondary oxidation radicals (Timofeyev and Steinberg 2006).

15.4 Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) are so named because they inhabit around or on root surfaces in the rhizosphere (Ahmad et al. 2008). These bacteria affect the plant life cycle both directly and indirectly. PGPR directly improve plant growth by the acquisition of essential minerals such as nitrogen and phosphorus. Also, they contribute to producing plant hormones such as auxins, cytokinins, and gibberellins. In addition, they produce the siderophore, an iron chelator and 1-amin ocyclopropane-1-carboxylate (ACC)-deaminase, which reduces ethylene concentration in plant tissues. Results from many studies about PGPR in both normal growth conditions and abiotic/biotic stress conditions showed that PGPR increased plant growth, productivity, and health in various plant species and cultivars.

15.5 Using PGPRs in Abiotic Stress Factors

The major cause of the decline in annual crops is the combined effects of abiotic stress factors. Farmers use aquifer-based irrigation intensively for crop production around the world, but this practice causes water scarcity and threatens the long-term

sustainability of agricultural areas. Excessive water use by the flood irrigation system in arid and semi-arid lands results in salinization, which dramatically decreases crop yields. Extreme temperature levels are another main stress factor that limits plant growth and productivity: temperature is changed by global warming and climate change conditions. Intensive farm practices degrade soil structure and directly reduce agricultural production (Sharpley et al. 2015).

In fact, better management of agricultural areas can increase production efficiency. There is no doubt that developing new resistant cultivars against salinity, drought, low and high temperatures, poor soil nutrient composition, and other abiotic stress factors is necessary to meet the future food demand, although to reduce the negative effects of salinity, drought, low and high temperatures, floods, and highly alkaline soil, the cultural practices are so expensive and sometimes insufficient.

In addition, even though resistant plant cultivar breeding takes a long time period and labor, it may not be the solution for all abiotic stresses in all plant cultivars. Currently, efficient PGPR applications are used to alleviate and eliminate these stress factors. These bacteria are classified into two groups of bacterial mechanisms. The first group has the ability to increase germination of seed and yield. The second group affects plant growth positively by indirect means such as biocontrol. PGPR promotes plant growth with nitrogen fixation, potassium and phosphate solubilization, and siderophore production. The PGPR also produce auxins, cytokinins, and gibberellins and synthesizes ACC-deaminase to control ethylene under stress conditions.

The atmosphere includes approximately 78% nitrogen (N), a crucial nutrient for plant productivity. However, atmospheric N is an N_2 form, which is not usable by plants. For plants to utilize atmospheric nitrogen, it is converted into suitable forms by nitrogen-fixing microbes using the nitrogenase enzyme system in the soil (Kim and Rees 1994). The N_2-fixing microbes are classified as (a) symbiotic N_2-fixing bacteria, including *Rhizobiaceae* family bacteria that live symbiotically with Leguminosae family plants (e.g., *Rhizobia*) and nonleguminous trees (e.g., *Frankia*), and (b) nonsymbiotic N_2-fixing forms (free-living, associated, and endophytes) such as *Cyanobacteria*, *Azospirillum*, and *Azotobacter* (Bhattacharyya and Jha 2012). *Rhizobia* is the microbe most used by plants for N_2-fixing (Table 15.1).

Phosphorus is the second most significant element after nitrogen for plants and is also plentiful in soils (Ignatowicz 2017). Although phosphorus is found in abundant

Table 15.1 Some N_2-fixing bacteria strains

Plant growth-promoting rhizobacteria (PGPR) strains	References
Azospirillum spp.	Tien et al. (1979)
Azotobacter spp.	Jack et al. (1953)
Azolla spp.	Arora and Singh (2003)
Cyanobacteria spp.	Meeks (1998)
Gluconacetobacter diazotrophicus	Urquiaga et al. (1992)
Rhizobia spp.	Zahran (1999)

quantities in the soil, the available form of P is generally low for plants (Bhattacharyya and Jha 2012). This low available P content causes P deficiency in plants. To overcome P deficiency, frequent applications of synthetic phosphorus are used. However, synthetic fertilizers are less well absorbed by plants and part is rapidly converted into an insoluble form in soil (McKenzie and Roberts 1990). Applying phosphate fertilizers regularly not only has a higher cost but has also undesirable environmental effects. In this context, microbes (Table 15.2) with the ability of P-solubilizing are promising for plant fertilizers in soil having poor P content (Khan et al. 2007).

The rhizosphere includes macro- and microelements and has maximum microbial activity (Burdman et al. 2000). Iron, a crucial element for the plant life cycle, is the fourth most abundant element in the Earth's crust. Most iron is not in available forms for plant species. To acquire iron from the soil, iron is reduced from Fe^{+3} to Fe^{+2}. Iron reduction occurs in soil in such ways as soil acidification through proton extrusion, iron chelation through secretion of complexing molecules such as siderophores, phenolics, and carboxylic acids, and reduction through secretion of compounds having reducing properties or reductase activity.

PGPR release siderophores that are low molecular weight, high-affinity Fe^{+3} chelators, to increase the availability of the iron for plant roots. Rhizobacteria such as *Azotobacter*, *Bacillus*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* reduce Fe^{+3} to Fe^{+2}, an available form for plant nutrition (Table 15.3).

Table 15.2 Phosphate-solubilizing bacteria (PSB) strains

PGPR strains	Some reports up to 2018
Azotobacter spp.	Kumar and Narula (1999)
Bacillus spp.	Banerjee et al. (2010), Ipek et al. (2014), and Sharma et al. (2017)
Beijerinckia spp.	Bhattacharyya and Jha (2012)
Burkholderia spp.	Song et al. (2008)
Enterobacter spp.	Bhattacharyya and Jha (2012)
Erwinia spp.	Bhattacharyya and Jha (2012)
Pseudomonas spp.	Premono et al. (1996) and Sharma et al. (2017)
Rhizobium spp.	Rudresh et al. (2005)
Serratia spp.	Bhattacharyya and Jha (2012)

Table 15.3 Siderophore-producing bacteria strains

PGPR strains	Some reports up to 2018
Azotobacter spp.	Wani et al. (2007b)
Acinetobacter spp.	Ahmad et al. (2008)
Bacillus sp. PSB10	Wani and Khan (2010)
Bravibacterium spp.	Noordman et al. (2006)
Enterobacter asburiae	Ahemad and Khan (2010)
Klebsiella spp.	Ahemad and Khan (2011)
Pseudomanas jessenii	Rajkumar et al. (2008)
Rhizobium spp.	Wani et al. (2007a)

Table 15.4 Plant growth regulator-producing bacteria strains

Plant hormones	PGPR strains	Some reports up to 2018
Indole-3-acetic acid	*Aeromonas veronii, Agrobacterium* spp., *Alcaligenes piechaudii, Azospirillum brasilense, Rhizobium leguminosarum*	Azzam et al. (2012), Sharma et al. (2016), Barazani and Friedman (1999), Molina et al. (2018), and Camerini et al. (2008)
Cytokinins	*Agrobacterium rubi* A18, *Bacillus megaterium* M3, *Paenibacillus polymyxa, Pseudomonas fluorescens*	Esitken et al. (2003, 2010), Neris et al. (2017), and Großkinsky et al. (2016)
Zeatin and ethylene	*Azospirillum* spp.	Perrig et al. (2007)
Gibberellic acid	*Azospirillum lipoferum, Bacillus* spp.	Fulchieri et al. (1993) and Ipek et al. (2014)
Abscisic acid	*Azospirillum brasilense*	Perrig et al. (2007)
ACC-deaminase	*Bacillus pumilus, Burkholderia cepacia, Enterobacter* spp., *Pseudomonas fluorescens, Pseudomonas* spp.	Ali et al. (2017), Vial et al. (2011), Shaharoona et al. (2008), and Poonguzhali et al. (2008)

PGPR that produce plant growth regulators are important in the protection of plant species (Table 15.4). Most rhizobacteria can produce auxins and thus affect auxin level in plant roots; therefore, they increase plant growth significantly. The root system is generally affected by auxins. This relationship affects increasing size, weight, and the number of branch and root surfaces that contact with the soil. Root development increases plant ability to absorb nutrient elements (Gutierrez Manero et al. 1996).

The gibberellins have a similar effect as do auxins and cytokinins on plant growth. They are produced in the meristematic tissue of shoot and root and increase shoot elongation. Spray application of PGPR promotes shoot elongation (Esitken et al. 2006; Pirlak et al. 2007). The plants are held in rest status by abscisic acid (ABA) to survive under harsh or stress conditions.

ABA takes part in control of dormancy, seed germination, root growth, and guard cell action, and provides a significant response to salinity and cold. ABA inhibits root elongation because it can increase the plant root ability to take water from the soil. The ABA level increases in plants under drought or salinity stress conditions, and it promotes closing the stomata to prevent water loss. Because ABA is produced in terminal buds and roots, when the PGPR is applied by spraying and irrigation to plants, plants can respond to unsuitable environmental conditions (Table 15.5).

Ethylene is the gaseous formation of the plant hormone, synthesized under biotic and abiotic stress conditions such as water deficiency, saline soil/water, flooding, heavy metals, and pathogenicity. Ethylene negatively affects plant root growth and consequently plant growth totally. PGPR produce the ACC-deaminase enzyme, a vital enzyme that decreases and inhibits ethylene biosynthesis in the plant. When ethylene level increases in plants, the bacterial enzyme ACC-deaminase converts

ACC, a precursor of ethylene, to ammonia and α-ketobutyrate (Ali and Kim 2018). The bacterial strains *Bacillus pumilus*, *Burkholderia cepacia*, *Enterobacter* spp., and *Pseudomonas* spp. produce ACC-deaminase (Table 15.4).

Table 15.5 shows some selected new references about the PGPR mechanism in various plant species, especially field crops. In Table 15.5, references show PGPR treatments for salinity and drought were more studied than other stress factors. Under stress conditions, almost all bacterial species or strains used produce ACC-deaminase and IAA production (Table 15.5). It was determined that PGPR with ACC-deaminase activity highly prevents the production of ethylene, which causes shoot and root elongation to decrease in the plant under stress condition, and plants continued to grow and develop at decreasing ethylene levels. Also, IAA production of PGPR can promote more root growth under stress condition and better plant growth could be obtained.

It is known that PGPR have the ability of N_2-fixing, phosphate solubilization, and increasing availability of Fe, Zn, and Mn, increasing plant nutrition under stress conditions (Aras et al. 2018; Esitken et al. 2010; Ipek et al. 2014, 2018; Seymen et al. 2015). In addition, PGPR increases antioxidant enzyme activity and organic acid content and decreases the negative effects of ROS in apple and pear in high calcareous soil conditions (Aras et al. 2018; İpek et al. 2017).

PGPR treatments on different plants improved morphological features, physiological parameters, biochemical properties, and plant nutrition. In these studies, plant height, shoot length, shoot diameter, root length, root number, fresh and dry plant weight, fresh and dry root weight, trunk diameter, leaf area, fruit yield, and germination rates were promoted by PGPR applications (Abd El-Daim et al. 2014; Delshadi et al. 2017; Hou et al. 2018; Hussain et al. 2018). In addition to morphological parameters, physiological and biochemical features and plant nutrition have been studied. Chlorophyll content, antioxidant enzyme activity, nonenzymatic antioxidant content, proline content, protein content, membrane permeability, stomatal conductance, photosynthetic activity, amino acid content, organic acid content, plant growth regulators, and leaf relative water content (LRWC) have been affected positively by PGPR treatments (Hussain et al. 2018; Kakar et al. 2016; Kumar et al. 2016).

The researchers reported that PGPR applications decreased the use of fertilizers such as N, P, and K under stressed and nonstressed conditions (Aras et al. 2018; Arıkan and Pırlak 2017; Esitken et al. 2010; İpek et al. 2017). Decreasing fertilizer use significantly reduces the possibility of secondary salinity occurrence. Thus, using PGPR could prevent the formation of salinity in the soil.

15.6 Conclusions and Future Prospects

Abiotic stress conditions are widespread and damaging in almost all agricultural crops. Most agricultural crops such as fruits, vegetables, grapes, and other dicot species are generally considered sensitive to abiotic stress conditions. There are some ways to cope with these stress factors such as using tolerant species, varieties,

Table 15.5 References about PGPR mechanisms under abiotic stress conditions

Bacteria species and/or strains	Plant species	Abiotic stress	Bacteria mechanism	Some reports up to 2018
Pseudomonas fluorescens	Pistacia	Salinity	ACC-deaminase activity, phosphate solubilization, siderophore production, IAA production	Azarmi et al. (2015)
Bacillus subtilis, Pseudomonas putida, P. fluorescens	Faba bean	Salinity	–	Metwali et al. (2015)
Dietzia natronolimnaea STR 1	Wheat	Salinity	–	Bharti et al. (2016)
Agrobacterium tumefaciens, Klebsiella spp., *Ochrobactrum anthropi, Pseudomonas stutzeri*	Peanut	Salinity	ACC-deaminase activity, phosphate solubilization, nitrogen fixing, IAA production, CAT activity	Sharma et al. (2016)
Serratia marcescens CDP-13	Wheat	Salinity	ACC-deaminase activity, phosphate solubilization, siderophore production, IAA production, nitrogen fixing, ammonia production	Singh and Jha (2016)
Bacillus subtilis EY2, *Bacillus atrophaeus* EY6, *Bacillus spharicus* EY30, *Staphylococcus kloosii* EY37, *Kocuria erythromyxa* EY43	Sweet cherry	Salinity	Phosphate solubilization, CAT activity, nitrogen fixing	Arıkan and Pırlak (2017)
Pseudomonas fluorescens FY37	Canola	Salinity	ACC-7deaminase activity	Bazyar et al. (2017)
Klebsiella sp. SBP-8	Wheat	Salinity	ACC-deaminase activity, phosphate solubilization, siderophore production, IAA production, gibberellic acid production, ammonia production, chitinase activity	Singh and Jha (2017)

(continued)

Table 15.5 (continued)

Bacteria species and/or strains	Plant species	Abiotic stress	Bacteria mechanism	Some reports up to 2018
Bacillus pumilus, Exiguobacterium sp. AM25	Tomato	Salinity	IAA production	Ali et al. (2017)
Enterobacter sp.	Rice	Salinity	ACC-deaminase activity	Sarkar et al. (2018a)
Novosphingobium sp.	*Citrus macrophylla*	Salinity	–	Vives-Peris et al. (2018)
Pseudomanas putida				
Rhodopseudomonas palustris G5	Cucumber	Salinity	Phosphate solubilization, potassium dissolving, siderophore production, IAA production, aminolevulinic acid (ALA) production	Ge and Zhang (2019)
Pseudomonas koreensis	Sunflower	Drought	ACC deaminase activity, siderophore production, IAA production, chitinase activity	Macleod et al. (2015)
Bacillus thuringiensis	Soybean	Drought	Nitrogen fixing	Prudent et al. (2015)
Bradyrhzobium japonicum				
Citrobacter freundii	Tomato	Drought	ACC deaminase activity, phosphate solubilization, siderophore production, IAA production, chitinase activity	Ullah et al. (2016)
Bacillus amyloliquefaciens	Rice	Drought	Phosphate solubilization, siderophore production, IAA production	Kakar et al. (2016)
Brevibacillus laterosporus				
Pseudomonas putida	Chickpea	Drought	ACC-deaminase activity, phosphate solubilization, siderophore production, IAA production	Kumar et al. (2016)
Bacillus amyloliquefaciens				

(continued)

Table 15.5 (continued)

Bacteria species and/or strains	Plant species	Abiotic stress	Bacteria mechanism	Some reports up to 2018
Rhizobium phaseoli	Mungbean	Drought	–	Kumari and Chakraborty (2017)
Pseudomonas agglomerans	*Avena sativa*	Drought	–	Delshadi et al. (2017)
Pseudomonas putida				
Azospirillum lipoferum	Wheat	Drought	–	Kanwal et al. (2017)
Pseudomonas putida FBKV2	Maize	Drought	–	Skz et al. (2018)
Enterobacter aerogenes S-10, *B. thuringiensis* S-26, *Streptococcus pluranimalium* S-29, *P. stutzeri* S-80, *B. amyloliquefaciens* S-134, *B. pumilus* S-137, *B. simplex* D-1, *B. thuringiensis* D-2, *B. muralis* D-5, *B. simplex* D-11	Wheat	Drought	IAA production	Raheem et al. (2018)
Bacillus subtilis, Bacillus thuringiensis, Bacillus megaterium	Chickpea	Drought	Phosphate solubilization, CAT activity	Khan et al. (2018)
Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis	Soybean	Drought	Grow in media reduced water activity	Martins et al. (2018)
Bacillus methylotrophicus	Cucumber	Drought	–	Hou et al. (2018)
Rhizobium phaseoli	Chickpea	Drought	Phosphate solubilization, siderophore production, IAA production, CAT activity, peroxidase activity, organic production	Hussain et al. (2018)
Pseudomanas putida	*Elsholtzia splendens*	Heavy metal	IAA production	Xu et al. (2015)
Pleum phleoides, Trifolium repens	*Brassica oxyrrhina*	Heavy metal	ACC deaminase activity, IAA production,	Ma et al. (2016)
Azotobacter	*Lepidium sativum*	Heavy metal	–	Sobariu et al. (2017)

(continued)

Table 15.5 (continued)

Bacteria species and/or strains	Plant species	Abiotic stress	Bacteria mechanism	Some reports up to 2018
Bacillus sp., *Stenotrophomonas* sp.	Radish	Heavy metal	ACC deaminase activity, siderophore production, IAA production, CAT activity, EPS production	Akhtar et al. (2018)
Azotobacter chroococcum	Maize	Heavy metal	ACC deaminase activity, siderophore production, IAA production, ammonia production, HCN production	Rizvi and Khan (2018)
Enterobacter sp.	Rice	Heavy metal	Phosphate solubilization, nitrogen fixing, IAA production, HCN production	Mitra et al. (2018)
Azospirillum brasilense, Bacillus amyloliquefaciens	Wheat	High temperature	–	Abd El-Daim et al. (2014)
Bacillus subtilis	Okra	High temperature	–	Mathiba et al. (2017)
Bacillus safensis, Ochrobactrum pseudogrignonense	Wheat	High temperature	–	Sarkar et al. (2018b)
Bacillus sp., *Serratia* sp.	Pigeon pea	High temperature	Siderophore production, IAA production, flavonoid production	Modi and Khanna (2018)
Bacillus aryabhattai, Bacillus siamensis	Chinese cabbage	High temperature	–	Yoo and Sang (2018)
Bradyrhzobium japonicum	Soybean	Low temperature	–	Zhang and Smith (1994)
Rhizobium leguminosarum	Lentil	Low temperature	–	Lee (2009)
Serretia nematodiphila	Pepper	Low temperature	–	Kang et al. (2015)
Bacillus sp.	Raspberry	Low temperature	–	Belyaev et al. (2017)

(continued)

Table 15.5 (continued)

Bacteria species and/or strains	Plant species	Abiotic stress	Bacteria mechanism	Some reports up to 2018
Pseudomonas spp.	Wheat	Low temperature	Siderophore production, IAA production, HCN production	Yarzábal et al. (2018)
Achromobacter xylosoxidans, Serratia ureilytica, Herbaspirillum seropedicae, Ochrobactrum rhizosphaerae	*Ocimum sanctum*	Waterlogging	ACC deaminase activity, phosphate solubilization, siderophore production, IAA production, nitrate reduction	Barnawal et al. (2012)
Pseudomonas putida	Cucumber	Hypoxic stress	ACC deaminase activity	Li et al. (2013)
Rhizobium sp.	Wetland rice, *Sesbania cannabina*	Flood stress		Mitra et al. (2016)
Klebsiella variicola	Soybean	Flood stress	IAA production	Kim et al. (2017)

and rootstocks. In addition, some agricultural practices such as using drip irrigation, chemical fertilization, greenhouses, agricultural machinery, and drainage systems. However, these applications could not succeed in all stress condition and their costs are rather high. On the other hand, using more chemical fertilizers causes environmental concerns. In this regard, sustainable agricultural techniques and biofertilization could be a solution, just as PGPR can appear to decrease sensitivity to these stress conditions. PGPR can increase plant tolerance by producing some plant growth regulators, ACC-deaminase, siderophores, and releasing organic acids. In current studies about PGPR applications, root inoculation significantly affected plants to tolerate abiotic stress conditions. In the future, the PGPR mechanism should be studied on abiotic stress conditions.

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