# Potential of PGPR in Agricultural Innovations

#### Haluk Caglar Kaymak

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Abstract Plant growth promoting rhizobacteria (PGPR) are a group of free-living bacteria that colonize the rhizosphere and benefit the root growth in plants. Bacteria of diverse genera such as *Azospirillium*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Pseudomonas*, etc., were identified as PGPR. These PGPR exert a direct effect on plant growth by inducing the production of phytohormones, supplying biologically fixed nitrogen, and increasing the phosphorous uptake by the solubilization of inorganic phosphates. These bacteria affect plant growth by inducer mechanisms that involve suppression of bacterial, fungal, viral, and nematode pathogens. A lot of study showed that inoculation with PGPR resulted in significant yield increases in different crops, rooting of hardwood and semi-hardwood cuttings, increased germination and enhanced emergence of seeds under different conditions, promoted nutrient uptake of roots, total biomass of the plants, increased seed weight, induced early flowering, etc. In this review, the importance of PGPR is discussed for

H.C. Kaymak

Faculty of Agriculture, Department of Horticulture, Atatürk University, 25240 Erzurum, Turkey e-mail: hckaymak@atauni.edu.tr; hckaymak@yahoo.com

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agricultural innovations with special references that utilises direct and indirect plant growth promotion.

# 1 Introduction

The rhizosphere, volume of soil surrounding roots influenced chemically, physically, and biologically by the plant root, is a highly favorable habitat for the reproduction of microorganisms, which exerts a potential impact on plant health and soil fertility (Sorensen 1997; Antoun and Prevost 2005; Podile and Kishore 2006). This environment is relatively rich in nutrients released by the plant roots, and its microbial communities are different from those that are not influenced by the roots (Alexander 1977; Burdman et al. 2000).

In the rhizosphere, very important and intensive interactions occur among the plant, soil, microorganisms, and soil microfauna (Antoun and Prevost 2005). These interactions can significantly influence plant growth and crop yields. In the rhizosphere, bacteria are the most abundant microorganisms. Rhizobacteria are rhizosphere-competent bacteria that aggressively colonize plant roots, could be free-living, parasitic, or saprophytic, and their diversity remains dynamic with a frequent shift in community structure and species abundance (Kunc and Macura 1988). These microbial communities are beneficial for plant growth, yield, and crop quality, and they have been called "plant growth promoting rhizobacteria (PGPR)" (Kloepper and Schroth 1978) including numerous strains of the genera Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Azoarcus, Bacillus, Beijerinckia, Burkholderia, Clostridium, Enterobacter, Erwinia, Flavobacterium, Gluconacetobacter, Klebsiella, Pseudomonas, Serratia, Rhizobium, etc. (Burdman et al. 2000; Sudhakar et al. 2000; Hamaoui et al. 2001; Bertrand et al. 2001; Mirza et al. 2001; Bonaterra et al. 2003; Esitken et al. 2003a; Murphy et al. 2003; Raj et al. 2004; Joo et al. 2004; Esitken et al. 2006; Podile and Kishore 2006; Saleem et al. 2007).

PGPR can be divided into two groups according to their relationship with the plants: symbiotic bacteria and free-living rhizobacteria (Khan 2005). A lot of work have been done to study about the mechanisms and principles of the PGPR–plant relationship, which was widely accepted as the rhizosphere effect (Zhuang et al. 2007). Glick (1995) reported that PGPR function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the environment (Cakmakci et al. 2006; Garcia et al. 2004a, b; Siddiqui and Mahmood 2001), and preventing the plants from diseases (Guo et al. 2004; Jetiyanon and Kloepper 2002; Raj et al. 2003a, b).

In other words, these mentioned bacteria can directly cause plant growth, seed emergence, or improvement in crop yields by producing and secreting plant growth regulators such as auxins, gibberellins (GAs), and cytokinins. They elicit the root metabolic activities, supply biologically fixed nitrogen, and increase the phosphorous uptake by solubilization of inorganic phosphates (Burdman et al. 2000; Podile and Kishore 2006). The near direct effect of PGPR is that these bacteria affect plant growth by indirect mechanisms that involve suppression of bacterial, fungal, viral, and nematode pathogens (Burdman et al. 2000; Kirankumar et al. 2008).

In this review, the importance of PGPR is discussed for agriculture innovations with special reference to their utilization in direct plant growth promotion such as seed emergence, secretion of plant growth regulators, and indirect plant growth promotion such as suppression of pest and disease.

# 2 Direct Plant Growth Promotion

PGPR influence direct growth promotion of plants by fixing atmospheric nitrogen, solubilizing insoluble phosphates, secreting hormones such as IAA, GAs, and Kinetins besides ACC deaminase production, which helps in regulation of ethylene.

# 2.1 Biological Nitrogen Fixation

Nitrogen is a well-known and essential key nutrient for plant growth and development. However, the global nitrogen cycle pollutes groundwater and increases the risk of chemical spills. The production of chemical fertilizers is a highly energyintensive process using large amounts of fossil energy. High-input farming practices achieving high yields have created environmental problems and degradation in natural resources (Şahin et al. 2004). Thus, Figueiredo et al. (2008) reported that during the past couple of decades, the use of PGPR for sustainable and environment friendly agriculture has been increased tremendously in various parts of the world. Increasing and extending the role of bio-fertilizing with PGPR would reduce the need for chemical fertilizers and decrease their adverse environmental effects. Microorganisms are gaining importance in agriculture to promote the circulation of plant nutrients and reduce the need for chemical fertilizers (Şahin et al. 2004; Orhan et al. 2006).

Rhizosphere associated N-fixing bacteria have increasingly been used in nonlegume crop species such as sugar beet, sugar cane, rice, maize, and wheat (Döbereiner 1997; Hecht-Buchholz 1998; Şahin et al. 2004). For example, experiments with *Bacillus* species indicated yield increases in cereals (Belimov et al. 1995; Cakmakci et al. 2001; Öztürk et al. 2003) and maize (Pal 1998).

N-fixation is the first mechanism suggested to promote the growth of plants by *Azospirillum*. The majority of evidence collected during the last 3 decades concerning this mechanism has generated controversy (Bashan et al. 2004). At the same time, *Azospirillum* lead the list of PGPR assessed in worldwide experiments (Burdman et al. 2000; Dobbelaere et al. 2003; Vessey 2003; Lucy et al. 2004; Ramirez and Mellado 2005). *Pseudomonas* and *Bacillus* species (Alam et al. 2001; Cakmakci et al. 2001; Glick et al. 1994; Kokalis-Burelle et al. 2002), and the other PGPR and endophytic

bacteria, such as *Enterobacter*, *Klebsiella*, *Burkholderia*, and *Stenotrophomonas*, have been gaining attention in the recent years, because of their association with important crops and potential to enhance the plant growth (Chelius and Triplett 2000; Sturz et al. 2001; Verma et al. 2001; Dong et al. 2003; Ramirez and Mellado 2005).

Some greenhouse and field experiments have shown repeatedly that the transfer of nitrogen fixed by *Azospirillum* spp. to the plant is not enough (Bashan and Holguin 1997; Kennedy et al. 1997; Kennedy and Chellapillai 1998; Bashan et al. 2004). Yet other studies showed that the bacteria cannot fulfil all of the nitrogen requirements of the plants; nevertheless, it can contribute only significant amounts of nitrogen. For example, seed inoculation of chickpea with *Rhizobium*, N-fixing *Bacillus subtilis* (OSU-142) significantly increased N percentage compared with the control treatment and may substitute costly N fertilizers in chickpea production even in cold highland areas (Elkoca et al. 2008).

Similarly, N-fixing bacterial strains *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14, and *Bacillus* OSU-142 have great potential, and as formulations, they are used as biofertilizers for better yield and the quality of wheat, sugar beet, and spinach growth (Cakmakci et al. 2007; Cakmakci et al. 2006). The N-fixing *Bacillus* strains and *A. brasilense* sp246 have a potential on plant growth activity of spring wheat and barley cultivation in organic and low-N input agriculture (Öztürk et al. 2003; Canbolat et al. 2006). Inoculation with the *Rhizobium leguminosarum* E11 and *Azotobacter* sp. S8, strain E11 increased root dry weight, root length, and growth in cotton (Hafeez et al. 2004). Significant positive effects on growth, nodule number, and yield of soybean were obtained after inoculation with *Bradyrhizobium* spp strains S62 and S63 (Egamberdiyeva et al. 2004).

Furthermore, inoculation commonly and significantly reduced the required doses of nitrogen fertilization in numerous greenhouse and field experiments in a lot of plant species (Bashan and Levanony 1990; Bashan and Holguin 1997; Bashan et al. 2004).

The strain(s), soil types, climate, and the development of appropriate formulations as well as strategies of field experimentations should be considered for a successful application of PGPR when using as fertilizers.

# 2.2 Solubilization of Phosphates

Phosphorous (P), next to nitrogen, is one of the major and key nutrients limiting plant growth (Kumar and Narula 1999; Sundara et al. 2002; Podile and Kishore 2006). Even in phosphorous rich soil, most of the P is unavailable for the plants, as large amount of soil P is found in its insoluble form. Phosphate solubilizing bacteria (PSB) are common in the rhizosphere and can be used to overcome this problem (Vessey 2003).

PSB secretes organic acids and phosphatases that converts the insoluble phosphates into soluble monobasic and dibasic ions and may also solubilize inorganic phosphate and makes soil phosphorus, which otherwise remain fixed, available to the plants (Kumar and Narula 1999; Whitelaw 2000; Gyaneshwar et al. 2002). In other words, phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms through the process of acidification, chelation, exchange reactions, and production of gluconic acid (Rodriguez et al. 2004; Chung et al. 2005; Hameeda et al. 2008).

PSB are ubiquitous (Gyaneshwar et al. 2002), and *Bacillus, Enterobacter*, *Erwinia*, and *Pseudomonas* spp. are among the most potent strains (Podile and Kishore 2006). PSB is common in rhizospheres of crop plants, and few examples of beneficial association with phosphate solubilizing PGPR and plants include *B. megaterium* (M-3) and chickpea (Elkoca et al. 2008), *B. licheniformis* RC08 and *B. megaterium* RC07, and wheat and spinach (Cakmakci et al. 2007), *Enterobacter agglomerans* and tomato (Kim et al. 1998), *P. chlororaphis*, *P. putida*, and soybean (Cattelan et al. 1999), *Avena sativa* and PGPR strains isolated from the rhizosphere of forage (WenXing et al. 2008), *Serratia marcescens* EB 67, *Pseudomonas* sp. CDB 35, and maize (Hameeda et al. 2008).

In the controlled environment and in the field trials, single and dual N-fixing *B. subtilis* (OSU-142) and P-solubilizing *B. megaterium* (M-3) inoculations significantly increased all the parameters investigated in chickpea (plant height, shoot, root and nodule dry weight, N%, chlorophyll content, pod number, seed yield, total biomass yield, and seed protein content) compared with the control treatment, equal to or higher than N, P, and NP treatments (Elkoca et al. 2008).

In another research, Orhan et al. (2006) reported that plant growth promoting effects of two *Bacillus* strains OSU-142 (N-fixing) and M3 (N-fixing and phosphate solubilizing) were tested alone or in combinations of organically grown primocane fruiting raspberry (cv. Heritage) plants and a significant increase in yield (33.9 and 74.9%), cane length (13.6 and 15.0%), number of cluster per cane (25.4 and 28.7%), and number of berries per cane (25.1 and 36.0%) were observed when compared with that of the control.

Hameeda et al. (2008) reported that plant biomass increased with *Serratia marcescens* EB 67 and *Pseudomonas* sp CDB 35 under both glasshouse and field conditions. And also, seed treatment with EB 67 and CDB 35 increased the grain yield of field-grown maize by 85 and 64% compared with the uninoculated control.

Furthermore, four strains namely, *Arthrobacter aureofaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis*, and *Delftia* sp. are being reported for the first time as PSB after confirming their capacity to solubilize considerable amount of tricalcium phosphate in the medium by secreting organic acids (Chen et al. 2006). Peix et al. (2001) notified that *Mesorhizobium mediterraneum* strain PECA21 was able to mobilize phosphorous efficiently in barley and chickpea when tricalcium phosphate was added to the soil. Also, treating with insoluble phosphates and inoculating with strain PECA21, the phosphorous content, dry matter, nitrogen, potassium, calcium, and magnesium content in both plants were significantly increased.

It was known that natural phosphate rocks have been identified as an alternative for P fertilizers. For example, there are almost 40 million tons of phosphatic rock deposits in India (Rodríguez and Fraga 1999), and this material should provide a cheap source of phosphate fertilizer for crop production (Halder et al. 1990); especially, should be considered in organic production of horticulture and the other crops.

# 2.3 Plant Growth Regulators

Several stages of plant growth and development such as cell elongation, cell division, tissue differentiation, and apical dominance are controlled by the plant hormones, especially auxins and cytokinins. The biosynthesis and the underlying mechanism of auxins and cytokinins action are subjects of intense investigation. Auxins and cytokinins can be synthesized by both the plants and the microorganisms. Although the role of phytohormone biosynthesis by microorganisms is not fully explained, it is stated that direct mechanisms of plant growth by PGPR include production of plant hormones such as auxins, cytokinins, GAs, and lowering of plant ethylene levels (Glick 1995; Costacurta and Vanderleyden 1995; Lucy et al. 2004). A list of examples of plant growth stimulating phytohormones produced by PGPR is given in Table 1.

Auxin, indole-3-acetic acid (IAA), is a quantitatively important phytohormone produced by a member of PGPR, and treatment with auxin-producing rhizobacteria increased the plant growth (Vessey 2003; Erturk et al. 2008). On the one hand, most beneficial bacteria such as *Rhizobium, Bradyrhizobium,* and *Azospirillum* synthesize IAA via the Indole-3-pyruvic acid (IPyA) pathway (Manulis et al. 1991; Costacurta and Vanderleyden 1995; Patten and Glick 1996; Burdman et al. 2000). On the other hand, some pathogenic bacteria such as *Pseudomonas syringae*, *Agrobacterium tumefaciens*, and *Erwinia herbicola* synthesize IAA predominantly via the indole-3-acetamide (IAM) pathway (Dobbelaere et al. 2003).

The role of IAA in the observed plant growth promotion was obtained by attempting to mimic the effect of the bacterium for the root growth by the direct application of IAA on the roots. Inoculation with *Bacillus* RC23, *Paenibacillus polymyxa* RC05, *B. subtilis* OSU142, *Bacillus* RC03, *Comamonas acidovorans* RC41, *B. megaterium* RC01, and *B. simplex* RC19 with tea (*Camellia sinensis*) cuttings enhanced rooting percentages when compared with control because of IAA production of bacteria. Similarly, treatments of hardwood stem cuttings of kiwifruit cv. Hayward, stem cuttings of two rose selections (ERS 14, *Rosa canina*, and ERS 15, *Rosa dumalis*), sour cherry (*Prunus cerasus*) softwood and semi-hardwood cuttings and *Pistacia vera* cuttings with *Agrobacterium rubi* (A1, A16, and A18) and *Bacillus subtilis* OSU142 promoted rooting ratio and increased the numbers of lateral roots (Ercisli et al. 2000; Ercisli et al. 2003; Esitken et al. 2003b; Ercisli et al. 2004; Orhan et al. 2007).

In addition, *Azospirillum* is not only capable of nitrogen fixation but also codes for plant growth hormone auxins (Elmerich 1984). Strains of *Azospirillum* showed that production depended on the type of culture media and availability of tryptophan as a precursor. *A. brasilense* Cd produced the highest level of IAA among the

Phytohormones	PGPR	References
Gibberellin	Acetobacter diazotropicus	
	Herbospirillum seropedicae Bastian et al. (1998)	
	Bacillus lichenifirmis	
	Bacillus pumilus	Gutierrez-Manero et al. (2001)
	Bacillus cereus MJ-1	
	Bacillus macroides CJ-29	Joo et al. (2004)
	Bacillus pumilus CJ-69	
IAA	Agrobacterium sp.	
	Alcaligenes piechaudii	Barazani and Friedman (1999)
	Comamonas acidovorans	
	Azospirillum brasilense	Kaushik et al. (2000)
	Aeromonas veronii	
	Enterobacter cloacae	Mehnaz et al. (2001)
	Enterobacter sp.	Mirza et al. (2001)
	Comamonas acidovorans RC41	
	Paenibacillus polymyxa RC05	
	Bacillus RC23	Erturk et al. (2008)
	Bacillus simplex RC19	
	Bacillus RC03	
	Bacillus megaterium RC01	
Cytokinin	Paenibacillus polymyxa	Timmusk et al. (1999)
-	Pseudomonas fluorescens	de Salamone et al. (2001)
	·	Bent et al. (2001)
ACC deaminase	Pseudomonas putida	Mayak et al. (1999)
	Pseudomonas cepacia	Cattelan et al. (1999)
	Enterobacter cloacae	Saleh and Glick (2001)
	Pseudomonas brassicacearum Am3	Belimov et al. (2007)
	Variovorax paradoxus 5C-2	Belimov et al. (2009)
	Pseudomonas putida Biovar B	Rodriguez et al. (2008)
	Pseudomonas putida N21	<b>č</b>
	Pseudomonas aeruginosa N39	Zahir et al. (2009)
	Serratia proteamaculans M35	

 Table 1 Examples of plant growth stimulating phytohormones produced by PGPR

Azospirillum strains tested (El-Khawas and Adachi 1999; Radwan 1998; Bashan et al. 2004).

The isolation and quantification of cytokinins in nonpathogenic soil bacteria in general and diazotrophic bacteria in particular has received a little attention. Cytokinins are a diverse group of labile compounds that are usually presented in small amounts in biological samples and are often difficult to identify and quantify (Dobbelaere et al. 2003).

Cytokinins are produced by bacteria such as *Azospirillum* and *Pseudomonas* spp. (Gaudin et al. 1994). Moreover, a few PGPR strains were reported to produce cytokinins, such as *Rhizobium leguminosarum*, *Paenibacillus polymyxa*, and *Pseudomonas fluorescens* (Noel et al. 1996; Timmusk et al. 1999; de Salamone et al. 2001; Bent et al. 2001; Vessey 2003). These studies sufficiently cloud the production of cytokinins, compared with IAA or GAs, in PGPR. Also, it appears that more

work is necessary before proving for the role of PGPR-produced cytokinins in plant growth promotion.

Also in the case of GAs, the bacterial genetic determinants have not been identified so far. Therefore, no mutants are available to demonstrate the role of this phytohormone in plant growth promotion (Dobbelaere et al. 2003). Also the evidence of GA production by PGPR is rare (Vessey 2003). On the other hand, PGPR such as *R. phaseoli*, *A. lipoferum*, *Azotospirillum brasilense*, *Acetobacter diazotropicus*, *Herbospirillum seropedicae*, *Bacillus licheniformis*, *B. pumilus*, *Bacillus cereus* MJ-1, *Bacillus macroides* CJ-29 were reported to produce GAs (Atzhorn et al. 1988; Bottini et al. 1989; Janzen et al. 1992; Bastian et al. 1998; Gutierrez-Manero et al. 2001; Joo et al. 2004 and Table 1). However, this is not a strong evidence of GA production in a common method of growth promotion by PGPR.

Nevertheless, in recent studies, Gutierrez-Manero et al. (2001) provide an evidence that four different forms of GAs are produced by *B. pumilus* and *Bacillus licheniformis*. Inoculation of alder (*Alnus glutinosa*) with these PGPR could effectively reverse a chemically induced inhibition of stem growth. In addition to this research, Joo et al. (2004) reported that the growth of red pepper plug seedlings was increased by *Bacillus cereus* MJ-1, *B. macroides* CJ-29, and *B. pumilus* CJ-69, though the number of leaves and stem diameter were not significantly changed. The greatest increase is in the height and the root fresh weight of the seedlings was by *B. pumilus*, which could increase the height by 12% and the root fresh weight by 20%.

In the last few years, a new mechanism of plant growth promotion involving ethylene has been proposed (Burdman et al. 2000). Showing that some soil bacteria contain 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Klee et al. 1991) and Glick et al. (1998) put forward the theory that the mode of action of some PGPR was the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that could cleave ACC, the immediate precursor to ethylene in the biosynthetic pathway for ethylene in plants. They submitted that ACC deaminase activity would decrease ethylene production in the roots of host plants and results in root lengthening. In some cases, the growth promotion effects of ACC deaminase-producing PGPR is the best expressed in stress conditions including drought (Zahir et al. 2008) and salt (Nadeem et al. 2007; Zahir et al. 2009) stress.

PGPR (containing ACC deaminase) boost plant growth particularly under stressed conditions by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses such as salinity, drought, waterlogging, temperature, pathogenicity, and contaminants (Saleem et al. 2007). For example, under salinity stress, 1-aminocyclopropane-1-carboxylic acid-deaminase activity of *P. putida* (N21), *P. aeruginosa* (N39) and *Serratia proteamaculans* (M35) might have caused reduction in the synthesis of stress (salt)-induced inhibitory levels of ethylene (Zahir et al. 2009). Similarly, inoculation with *Variovorax paradoxus* 5C-2 improved growth, yield, and water-use efficiency of droughty peas (Belimov et al. 2009). It is reported that inoculation with *P. fluorescens* was found to be more effective in promoting root growth than that with *P. putida* as it caused up to 46% increase in root elongation and up to 94% increase in root weight of pea over the respective uninoculated drought stressed control (Arshad et al. 2008).

In addition to stress factors, recent studies indicated that canola plants inoculated with the *P. putida* strain HS-2 produced an increase in plant biomass (Rodriguez et al. 2008). The ACC-utilizing PGPR *Pseudomonas brassicacearum* strain Am3 increased in-vitro root elongation and root biomass of soil-grown tomato cv. Ailsa Craig at low bacterial concentrations but had negative effects on in-vitro root elongation at higher bacterial concentrations (Belimov et al. 2007).

# 2.4 Effects on Plant Growth

Since the last few decades, the response of agriculturally important crops to inoculation with PGPR was investigated in numerous field and greenhouse experiments carried out in various countries. On the basis of the given data, it was concluded that inoculation with PGPR resulted in significant yield increases in different crops, enhanced rooting of hardwood and semi-hardwood cuttings, seed germination and emergence under different conditions. In other words, they can affect plant growth and yield in a number of ways and enhancement of vegetative and reproductive growth is documented in a range of crops such as cereals or vegetables. Treatments with PGPR increase germination percentage, seedling vigor, emergence, plant stand, root and shoot growth, total biomass of the plants, seed weight, early flowering, grains, fodder and fruit yields, etc., (van Loon et al. 1998; Ramamoorthy et al. 2001). Applications of PGPR in relation to the plant growth in different subjects are described later with recent studies.

#### 2.4.1 Yield and Yield Components

In crop production, there is a continuous demand of increasing crop productivity and quality. There are lot of agricultural practices applied for increasing the yield and the yield components. Recently, one of them is applications of PGPR for increasing yield and environment friendly crop production.

Floral and foliar applications of PGPR strains *Pseudomonas* BA-8 and *Bacillus* OSU-142 on apple trees significantly increased yield per trunk cross-section area (13.3–118.5%), fruit weight (4.2–7.5%), shoot length (20.8–30.1%), and shoot diameter (9.0–19.8%) in "Starkrimson" and yield per trunk cross-sectional area (TCSA; 14.9%) and fruit weight (6.5–8.7%) in "Granny Smith" compared with the control (Pırlak et al. 2007). Karlıdağ et al. (2007) reported similar results in apple. Thus, *Bacillus* M3 and/or OSU-142 and/or *Microbacterium* FS01 in combination have the potential to increase the yield and growth of apple trees.

In addition, Esitken et al. (2003a, 2005, 2006) and Orhan et al. (2006) reported that *Pseudomonas* BA-8, *Bacillus* OSU-142 and M3 increased the shoot length, crop yield and improved fruit quality of apricot, sweet cherry, and raspberry.

In another research, Cakmakci et al. (2006) suggested that in the greenhouse, inoculations with PGPR increased sugar beet root weight by 2.8–46.7% depending

on the species. Leaf, root, and sugar yield were increased by the bacterial inoculation by 15.5–20.8%, 12.3–16.1%, and 9.8–14.7%, respectively. Effective *Bacillus* species, such as OSU-142, RC07 and M-13, *Paenibacillus polymyxa* RC05, *P. putida* RC06, and *Rhodobacter capsulatus* RC04 may be used in organic and sustainable sugar beet agriculture.

The average weight of tomato fruit per plant treated with *Rhodopseudomonas* sp KL9 strain (82.7 g) was higher than those of others including the uninoculated control. The content of lycopene in the ripe tomato fruit increased by 48.3% with the application of *Rhodopseudomonas* sp. KL9, but *Rhodopseudomonas* sp BL6 did not show any effect on lycopene content although the lycopene content in the cells of *Rhodopseudomonas* sp BL6 were 1.12 mg/g (Lee et al. 2008a).

Dursun et al. (2008) reported that the highest rocket yield, average leaf weight, leaf length, leaf stem diameter, leaf area and root weight were obtained from *Pseudomonas* BA-7 applications when compared with *P. putidae* BA-8, *B. subtilis* OSU-142 and MFD-5, *B. megatorium* M3, *A. rubi* A-1, A-16, and A-18. The highest leaf number (8.23), leaf dry matter (6.70%), and root dry matter (11.85%) were determined in A-18, OSU-142 and MFD-5 applications, respectively, and especially *Burkholderia gladii* BA-7, *Pseudomonas* BA-8, and *Bacillus* OSU-142 have a great potential to increase the parameters of plant growth of rocket.

Although the examples of relations between the yield and PGPR applications can be increased, other recent studies such as de Freitas (2000), Herman et al. (2008), and Yıldırım et al. (2008) clearly demonstrated the potential of PGPR in increasing the plant growth and yield.

#### 2.4.2 Seed Germination and Emergence

Sivritepe and Dourado (1995) reported that priming (osmoconditioning) is one of the physiological methods, which improves seed performance and provides faster and synchronized germination in vegetables. However, bio-priming with different genera, especially PGPR, have a great potential over other priming methods.

Nelson (2004) noted that PGPR were able to exert a beneficial effect upon plant growth such as increase in seed germination rate and percentage. Rodriguez et al. (2001) reported that using *Azospirillum* spp. gave better germination in both tomato and pepper seeds. Also, Vargas et al. (2001) mentioned that *Hafnia alvei* strain P3 increased germination by 36.5% when compared with the control in lettuce and inoculation of the soybean plants either with *Pseudomonas* strain PMZ2 or with *B. japonicum* increased seed emergence (Zaidi 2003). Similarly, Basavaraju et al. (2002) reported that inoculation of *Azotobacter chroococcum* strain C2 significantly increased the germination percentage in radish. The greenhouse inoculation experiment with pepper and maize pointed out that *Azotobacter* sp. strains 17 and 20 promoted pepper germination, while the *Azospirillum* strains 1 and 23 promoted maize germination (Reyes et al. 2008). Although studies were mentioned about the effect of bacterial strains on germination of different vegetable species that were conducted out under optimum conditions, Kaymak et al. (2009) suggested that bio-priming with *A. rubi* strain A16, *Burkholderia gladii* strain BA7, *P. putida* strain BA8,

*B. subtilis* strain BA142, *B. megaterium* strain M3 under saline stress could be useful to obtain higher seed germination percentage in radish.

Also, PGPR can be used under pathogenic factor. Thus, different isolates of plant growth-promoting rhizobacteria (i.e., *B. pumilus* (INR-7), *B. subtilis* (GBO-3), *B. subtilis* (IN937b), *B. pumilus* (SE-34), *Brevibacillus brevis* (IPC-11), *B. pumilus* (T-4), and *B. amyloliquefaciens* (IN937a)) were used for seed treatment to suppress the seedling diseases caused by fungi. Among them, isolates GBO3, IPC-11, and INR-7 increased seed germination and seedling vigour to the greatest extent (Lokesh et al. 2007). Alike, Begum et al. (2003) reported that PGPR, *B. pumilus* (SE-34), *B. pasteurii* (T4), *B. subtilis* (IN937-b), and *B. subtilis* (GBO3) strains reduced the incidence of seed mycoflora, which indirectly enhanced the seed germination percentage and vigour index of the seedlings in okra. In another recent study, de Araujo (2008) reported that the inoculation of seeds with *B. subtilis* is a promising technological alternative for seed treatment owing to the fact that inoculation with *B. subtilis*, formulated with oyster meal, increased emergence in cotton and soybean.

#### 2.4.3 Rooting of Cuttings

There are many physiological and environmental factors that influence root formation, with exogenous treatments on cuttings being particularly important (Couvillon 1998). Growers have attempted to stimulate rooting by applying growth regulators, various chemical substances, etc. However, the use of chemicals can produce environmental problems and increase proportion costs. Ecological problems have raised interest in environmental friendly sustainable agricultural practices (Salantur et al. 2005). Therefore, use of PGPR can overcome such problems associated with environment (Kaymak et al. 2008).

Recent studies showed that bacteria in several genera (*Agrobacterium, Bacillus, Streptomyces, Pseudomonas*, and *Alcaligenes*) induce root formation and growth in stem cuttings (Bassil et al. 1991; Hatta et al. 1996; Rinallo et al. 1999). More recently, PGPR such as *A. rubi* (A1, A16 and A18), *B. subtilis* (OSU142), *Bacillus* (BA16, RC03, RC23), *B. gladii* (BA7), *P. putida* (BA8), *B. megatorium* (M3 and RC01), *Paenibacillus polymyxa* (RC05), *Comamonas acidovorans* RC41, and *B. simplex* RC19 were effectively used for both hardwood and semi-hardwood cuttings to obtain higher rooting percentages in sour cherry (Ercisli et al. 2000; Esitken et al. 2003b), kiwifruit (Ercisli et al. 2003), grapevine (Köse et al. 2003), rose (Ercisli et al. 2004), pistachio (Orhan et al. 2006), tea (*Camellia sinensis* var. *Sinensis*) (Erturk et al. 2008), and mint (*Mentha piperita* L.) (Fig. 1) (Kaymak et al. 2008).

#### 2.4.4 Nutrient Uptake

Living plants require 16 essential elements to survive. Three of 16 elements (carbon, hydrogen, and oxygen) are supplied primarily from air and water. The remaining 13 are normally absorbed by plant roots. Each of these essential elements has at least one specially defined role in plant growth (Swaider et al. 1992; Decateau 2000).



Fig. 1 Effect of inoculation with PGPR (Agrobacterium rubi A16, Burkholderia gladii BA7, Peseudomonas putida BA8, Bacillus subtilis OSU142, and Bacillus megatorium M3) on root formation of mint cuttings

PGPR have been promised as a component in approaching for maintaining adequate plant nutrition and reducing the negative environmental effects of fertilizers. PGPR might increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing the accumulation of nitrates and phosphates in agricultural soils (Yang et al. 2009). It is known that phosphorous and nitrogen is the major and key nutrients limiting plant growth and important macronutrient required for plant growth (Kumar and Narula 1999; Sundara et al. 2002; Podile and Kishore 2006).

Additionally, some PGPR promote root development (Mantelin and Touraine 2004) by the production of phytohormones such as indole acetic acid (Kloepper et al. 2007). Given that root tips and root surfaces are sites of nutrient uptake, it is likely that one mechanism by which PGPR lead to increased nutrient uptake is via stimulation of root development (Yang et al. 2009). It has also been suggested that PGPR increase uptake of mineral ions via stimulation of the proton pump ATPase (Mantelin and Touraine 2004), although experimental evidence for this is lacking (Yang et al. 2009).

Several studies can be given about the relations with PGPR and enhancement of nutrient uptake. For example, Naveed et al. (2008) notified that PGPR application significantly enhanced N, P, and K uptakes. The *Pseudomonas fluorescens* biotype G (N-3) was found to be the best in increasing the grain yield of maize and nutrient uptake. In addition, the inoculation process with *Azospirillum* and *Bacillus* spp. showed positive response in enhancing higher accumulation of nitrogen, phosphorus, and potassium in the plant tissues, enhanced root dry weight and top growth of the oil palm seedlings under field nursery conditions (Amir et al. 2005).

In other recent study, Dursun et al. (2008) reported that *Burkholderia gladii* BA-7, *P putidita* BA-8, *B. subtilis* OSU-142 and MFD-5, *B. megaterium* M3, *A. rubi* A-1, A-16, and A-18 applications increased mineral contents particularly N, K, P, Zn, Fe, Mn, Na, Ca, and Mg in rocket leaves when compared with the control.

In a study aimed at assessment of effects of foliar application of bacteria *Bacillus* OSU-142, *Burkholderia* OSU-7, and *Pseudomonas* BA-8 on yield and growth of apricot, it was stated that application of bacteria resulted in an increase of N, P, K, Ca, and Mg contents of leaves (Esitken et al. 2005). In a similar study, Esitken et al. (2003a) suggested that N, P, K, Ca, and Mg contents of leaves were higher on OSU 142-treated trees than on the untreated control and OSU 142 has the potential to increase the yield of apricot trees.

Therefore, PGPR contributed significantly to the reducing nutrient build up in the soil. Several studies are underway that will further define the utility of PGPR in nutrient management strategies aimed at reducing fertilizer application rates and nutrient runoff from agricultural sources (Yang et al. 2009; Kumar et al. 2009).

# **3** Indirect Plant Growth Promotion

Induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism, production of metabolites suppressive to deleterious rhizobacteria are some of the mechanism that indirectly benefit plant growth.

# 3.1 Induced Systemic Resistance

More recently, biological control has been considered as an alternative strategy to manage soil-borne plant diseases. Available literature revealed positive effects of specific strains of rhizobacteria on growth of many plant species in soils in which more or less defined pathogens cause substantial losses. For this reason, several rhizobacteria have extensively been used as biological agents to control many soil-borne plant pathogens (Jeun et al. 2004; Dell'Amico et al. 2005; Rajkumar et al. 2005).

A strain, *P. fluorescens* WCS417, active against *Fusarium oxysporum* f. sp. *dianthi* was tested on carnation and results showed that bacteria, while remaining confined to the plant root system, were still protective when the pathogen was slash-inoculated into the stem (Van Peer et al. 1991). This protective effect had to be plant-mediated because in this case the rhizobacteria and the pathogenic fungus were never found to contact each other on the plant (Van Loon and Bakker 2006). Several strains of PGPR, which applied to roots of cucumber, and the leaves were subsequently challenged inoculation with the anthracnose fungus *Colletotrichum orbiculare* (Gang et al. 1991). The phenomenon was called ISR. (Van Loon et al. 1998; Vallad and Goodman 2004; Van Loon and Bakker 2006; Choudhary et al. 2007)

It is thought that the inducing rhizobacteria in the plant roots produce signal, which spreads systemically within the plant and increases the defensive capacity of the distant tissues from the subsequent infection by the pathogens. ISR thus extended the protective action of PGPR from their antagonistic activity against soil-borne pathogens in the rhizosphere to a defense-stimulating effect above the surface of the ground tissues against foliar pathogens (Van Loon and Bakker 2006).

ISR appears phenotypically similar to SAR, which is the phenomenon that once a plant has been infected by a pathogen and been able to effectively resist it, it has become more resistant to subsequent challenge inoculation by the same and other pathogens and, in some instances, even insects (Sticher et al. 1997; Van Loon et al. 1998; Van Loon and Bakker 2006). SAR occurs in distal plant parts following localized infection by a necrotizing pathogen. It is controlled by a signaling pathway that depends upon the accumulation of salicylic acid and the regulatory protein NPR1. In contrast, ISR is induced by selected strains of nonpathogenic PGPR. ISR functions independent from SA, but requires NPR1 and is regulated by jasmonic acid and ethylene (Walters and Heil 2007).

To reduce crop loss, pesticides are generally used. They are cost-effective and thus have become an integral part of modern agriculture. Environmental and human health-related concerns associated with use of hazardous chemicals have necessitated the search for eco-friendly alternatives. Such approaches must enhance and sustain agricultural productivity and at the same time be safe from environmental and health perspectives (Raj et al. 2003a).

Therefore, for economic reasons biological crop protectants can only seldom compete with highly effective chemicals. However, ISR is only one of the mechanisms that may be mobilized to counteract plant pathogens in an environmentally friendly and durable way. Integrating ISR-triggering PGPR into disease management programs in conjunction with other strategies will be a worthwhile approach to explore (Van Loon and Bakker 2006).

# 3.2 Suppression of Plant Diseases, Insects, and Nematodes by PGPR

Biocontrol is the process by which a pathogenic organism is maintained at low inoculum density or controlled or eradicated by beneficial organisms. Several microorganisms such as PGPR and insects present in the natural environment serve as potential biocontrol agents.

# 3.2.1 Bacterial Plant Diseases

The bacteria associated with plants exist as epiphytes, endophytes, and pathogens. Phytopathogens are comparatively few in both type and number, and all bacterial phytopathogens described to date fall within the domain Bacteria, formerly known as the *Eubacteria*. Bacterial phytopathogens that possesses a cell wall can be

subdivided into Gram-positive (*Clavibacter*, *Curtobacterium*, *Rathayibacter*, and *Streptomyces*) and Gram-negative (*Acidovorax*, *Agrobacterium*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Ralstonia*, and *Xanthomonas*) (Saddler 2002).

Bacterial soft rot of vegetables; blackleg of potato; fire blight of pome fruits; angular leaf spot or black arm, of cotton; bacterial blights of bean, lack rot of crucifers, southern bacterial wilt, bacterial wilt of cucurbits, ring rot of potato, bacterial canker of tomato, crown gall, hairy root, and cane gall, and common scab of potato are the more common bacterial diseases (Walker 1957; Waller et al. 2002).

Several cultural practises such as crop rotation, mixed cropping and intercropping, selection of cultivar, tillage, planting time, fertilization and irrigation, or highly effective chemical substances affect some diseases in different ways depending on the form of their application (Termorshuizen 2002). Recently, many microorganisms are increasingly used as inoculants for biocontrol (Romero et al. 2003; Chinnasamy 2005; Aliye et al. 2008; Xue et al. 2009). PGPR are nonpathogenic, environmental-friendly, cheaper to produce and easy to handle, and may create long-lasting effects (Chinnasamy 2005).

For instance, tomato is prone to a number of bacterial diseases, among which bacterial canker disease caused by *Clavibacter michiganensis* ssp. *michiganensis* is one of the most important diseases and nearly 100% crop loss can occur (Boudyach et al. 2001; Umesha 2006). Utkhede and Koch (2004) reported that treatments with *B. subtilis* (Quadra 136 and 137) and *Trichoderma harzianum* (R), *Rhodosporidium diobovatum* (S33), applied as a spray at 0.3, 0.6, 10 g<sup>-1</sup>, have the ability to prevent the incidence of bacterial canker of tomato plants caused by *C. michiganensis* subsp. *michiganensis* under greenhouse conditions. Similarly, tomato seeds were treated with PGPR strains *B. subtilis* GBO3, *B. amyloliquefaciens* IN937a and *Brevibacillus brevis* IPC11 were recorded for maximum disease protection for bacterial canker under greenhouse conditions (Girish and Umesha 2005). Recent studies about the relations with bacterial diseases and PGPR are given in Table 2.

#### 3.2.2 Fungal Plant Diseases

Fungal pathogens found on plants can be classified in different taxonomic groups. A few fungal pathogens such as rusts, powdery and downy mildews are obligate parasites. However, most of the plant pathogens are necrotrophs, killing plant tissues for their nutrition (Waller and Cannon 2002).

Exclusion or eradication of a disease from production areas, highly effective chemical substances or biological control of plant diseases have been suggested to protect the plants from fungal pathogens. Recently, PGPRs are increasingly and extensively used in biological control of fungal plant diseases (Altindag et al. 2006; Lourenco et al. 2006; Saravanakumar et al. 2007; Akgul and Mirik 2008; Sang et al. 2008; Dutta et al. 2008).

For example, apricot is the most important fruit crop grown in Anatolia, with approximately 600,000 tons of fruit produced annually, and Turkey dominates

Phytopathogens	Species	PGPR	References
Pseudomonas syringae pv. lachrymans	Cucumber	Pseudomonas putida 89B-27 Serratia marcescens 90–166	Liu et al. (1995)
Pseudomonas syringae pv. glycinea	Soy bean	Pseudomonas sp. Erwinia herbicola	May et al. (1996)
Xanthomonas albilineans	Sugar cane	Pentoena dispersa	Zhang and Birch (1997)
Erwinia amylovora	Apple	Erwinia herbicola C9-1 Pseudomonas fluorescens A506	Pusey (1997)
		Single-strain treatments and three-way mixture of <i>Bacillus pumilus</i> INR7, <i>Curtobacterium flaccumfaciens</i> ME1 and <i>Bacillus subtilis</i> GB03	Raupach and Kloepper (1998, 2000)
Ralstonia solanacearum	Tomato	Bacillus subtilis B2G	Lemessa and Zeller (2007)
		Pseudomonas sp. (APF1)	
		Acinetobacter sp. (Xa6) Enterobacter sp. (Xy3)	Xue et al. (2009)
Clavibacter michiganensis subsp. michiganensis		Azospirillum brasilense Sp7	Romero et al. (2003)
Xanthomonas campestris pv. vesicatoria		Azospirillum sp. (BNM-65)	
Ralstonia solanacearum	Eucalyptus	Pseudomonas fluorescens WCS417r Pseudomonas putida WCS358r	Ran et al. (2005)
	Potato	Bacillus subtilis PFMRI Paenibacillus macerans BS-DFS and PF9	Aliye et al. (2008)
Xanthomonas axonopodis pv. malvacearum	Cotton	Bacillus cereus MT5-5, MT5-6, L2-1 Achromobacter xylosoxidans L2-2, Brevibacterium sp. MT5-11	Ishida et al. (2008)

Table 2 Examples of suppression of bacterial diseases by PGPR in different plant species

apricot production in the world (Ercisli 2009). Therewithal, brown rot caused by *Moniliana laxa* Ehr. is one of the most destructive diseases of apricot in Turkey. This pathogen is able to destroy the whole annual crop in the phase of blossom, although it can kill shoots up to 30 cm beyond the initial blossom infection, and management of brown rot in Turkey is in general carried out by fungicide application (Gulcan et al. 1999). Altindag et al. (2006) suggested that *Burkholdria gladii* OSU 7 has the potential to be used as biopesticide for effective management of brown rot disease on apricot.

Similarly, pepper (*Capsicum annum* L.) is one of the most important market vegetables grown worldwide, but the yield and quality of marketable peppers are frequently limited by Phytophthora blight. The incidence of this disease has

continued to increase production areas since the pathogen can infect roots, crowns, and even foliar parts of pepper plants through splashing rains or overhead irrigation waters (Ristaino and Johnston 1999; Hausbeck and Lamour 2004). Control of this disease has usually depended on chemical and cultural measures such as use of phenylamide fungicides or metalaxyl as well as crop rotation, soil amendments, use of protective mulches and water management (Matheron and Porchas 2000; Hausbeck and Lamour 2004). In a recent study, Sang et al. (2008) reported that *Pseudomonas corrugata* (CCR04 and CCR80), *Chryseobacterium indologenes* (ISE14), and *Flavobacterium* sp. (GSE09) showed consistently good control efficacy against *Phytophthora capsici*. Also, these strains could be applied by either drench or root-dip treatment as alternatives to agricultural chemicals to control Phytophthora blight of pepper. In another recent study, Akgul and Mirik (2008) also reported that *Bacillus megaterium* strains could be used for biocontrol of *Phytophthora capsici*.

The combination of *Pseudomonas* strains Pf1, TDK1, and PY15 was more effective in reducing sheath rot (*Sarocladium oryzae*) disease in rice plants compared with individual strains under glasshouse and field conditions (Saravanakumar et al. 2009).

Hernandez-Rodriguez et al. (2008) obtained that *Burkholderia* sp. MBf21, MBp1, MBf15, and *P. fluorescens* MPp4 stood out for their plant growth stimulation in maize and for the biological control exerted on *Fusarium verticillioides* M1. The strains *Burkholderia* sp. MBf21 and MBf15 showed the best results in disease suppression, which was achieved up to 80%.

The combined use of PGPR (*Bacillus cereus* strain BS 03 and a *Pseudomonas aeruginosa* strain RRLJ 04) and rhizobia (strain RH 2) were recommended for induction of systemic resistance against fusarial wilt (*Fusarium udum*) in pigeon pea (Dutta et al. 2008). Recent studies and more examples about the suppression of fungal diseases by PGPR are given in Table 3.

#### 3.2.3 Viral Plant Diseases

Viruses are obligate parasites of submicroscopic size, with one dimension smaller than 200 nm. Virus particles, or virions, consist of segments of double or single-stranded RNA or DNA encased in protein structures, in some cases with lipid and additional substances (Waller 2002). So far at least 700 plant viruses has been discovered, many of which cause catastrophic diseases and have wide host ranges. They have been classified into three families and 32 groups (Martelli 1992; Waller 2002).

Some chemicals are used to produce virus-free plant material because they inhibit virus replication in agricultural crops. However, there are no therapeutic agents or viricides that can be applied to plants to control virus diseases. Consequently, control measures are based mainly on avoiding infection by using host plant resistance or disrupting the epidemic cycle of the disease. The use of

Phytopathogens	Species	PGPR	References
Rhizoctonia solani sclerotia	Cyclamen	Serratia marcescens B2	Someya et al. (2000)
Fusarium oxysporum f. sp. cyclaminis			
Fusarium oxysporium	Soybean	Pseudomonas PMZ2 Bradyrhizobium japonicum	Zaidi (2003)
Sclerospora graminicola	Pearl millet	Bacillus pumilus INR7 and SE34 Bacillus subtilis GB03	Raj et al. (2003b)
		Pseudomonas fluorescens UOM SAR 14	Raj et al. (2004)
Cronartium quercuum f.sp. fusiforme	pine	Bacillus pumilus SE34 and T4	Enebak and Carey (2004)
Puccinia psidii	Eucalyptus	Pseudomonas aeruginosa FL2 Pseudomonas sp. MF4	Teixeira et al. (2005)
Didymella bryoniae	Muskmelon	Pseudomonas fluorescens	Sudisha et al. (2006)
Pythium ultimum, Pythium debaryanum, Rhizoctonia solani, Fusarium oxysporum, Phytophthora capsici, Botrytis cinerea, Botrytis allii, Cladosporium fulvum, Aspergillus sp.	Sesame (in vitro)	Paenibacillus polymyxa E681	Ryu et al. (2006)
Exobasidium vexans	Tea	Pseudomonas fluorescens Pf1	Saravanakumar et al. (2007)
Fusarium spp. Didymella bryoniae Myrothecium spp.	Watermelon	Bacillus subtilis GBO-3 and Brevibacillus brevis IPC-11 Bacillus pumilus SE34 and T4	Lokesh et al. (2007)
Fusarium oxysporum f. sp. lycopersici	Tomato	Paenibacillus lentimorbus GBR158	Son et al. (2008)
Phytopthora capsici	Red pepper	Bacillus subtilis R33 and R13	Lee et al. (2008b)
Phytopthora capsici Fusarium oxysporum L sp. lycopersici		Paenibacillus polymyxa GBR-462 Azospirillum brasilense Bacillus subtilis	Kim et al. (2009) Abo-Elyousr and Mohamed (2009)
Rhizoctonia solani	Wheat	Azotobacter sp. WPR-51	(2009) Fatima et al. (2009)

Table 3 Examples of suppression of fungal diseases by PGPR in different plant species

genetically resistant cultivars provides effective control of many viral diseases. Mechanisms of resistance vary, some are explained to effects on vectors, whereas others may inhibit viral replication (Waller 2002).

Kirankumar et al. (2008) reported that *Pseudomonas* B-25 was highly efficient in promoting growth, fruit yield, and nutrient uptake of tomato in the presence of tobacco mosaic virus (TMV) pathogen, and the incidence of pathogenesis was markedly less after PGPR treatment. Similarly, biological control using PGPR

protected tomato plants against cucumber mosaic virus (CMV) under greenhouse and to a limited extent in the field conditions (Sikora and Murphy 2005). In another research, *P. fluorescens* strains were investigated for biocontrol efficacy against tomato spotted wilt virus (TSWV) in tomato. Virus concentration value clearly showed a reduction in viral antigen concentration in *P. fluorescens*-treated tomato plants corresponding to reduced disease ratings. All the *P. fluorescens*-treated tomato plants also showed enhanced growth and yield compared with control plants. Hence, it was suggested that PGPR could play a major role in reducing TSWV and increasing yield in tomato plants (Kandan et al. 2005). Banana bunchy top disease (BBTD) caused by Banana bunchy top virus (BBTV) is the most serious virus disease of banana plantations world wide. *P. fluorescens* Pf1 and CHA0 were significantly effective in reducing BBTV under field conditions, recording 33.33% infection with 60% reduction over control (Harish et al. 2008).

In a greenhouse experiment, *P. fluorescens* FB11 and *Rhizobium leguminosarum* FBG05 were tested alone and in combination as seed inoculants to induce systemic resistance in faba bean against bean yellow mosaic potyvirus (BYMV). The results demonstrated that BYMV challenged plants emerged from *Pseudomonas* inoculated seeds not only showed a pronounced and significant reduction in percent disease incidence but also a significant reduction in virus concentration in the challenged plants, compared with the nonbacterized seeds. *Rhizobium* alone also showed a significant reduction in both in percent disease incidence and in viral concentration value, but the reduction was less pronounced than that resulting from *Pseudomonas* inoculation (Elbadry et al. 2006).

In a recent study, the PGPR combinations (combinations included *B. subtilis* GB03 and IN937b, *B. pumilus* SE34, INR7 and T4, *B. amyloliquefaciens* IN937a) formulated with chitosan were referred to as biopreparations. The result indicated that treatment of tomato plants with biopreparations resulted in significant enhancement of plant growth and protection against infection by CMV (Murphy et al. 2003). Zehnder et al. (2000) reported that CMV symptom development was significantly reduced on PGPR-treated (*B. pumilus* SE34, *Kluyvera cryocrescens* IN114, *B. amyloliquefaciens* IN937a, and *B. subtilus* IN937b) plants compared with control, but the percentage of infected plants and tomato yields were not significantly different among treatments, suggested that PGPR-mediated induced resistance against CMV infection following mechanical inoculation into tomato can be maintained under field conditions.

Tomato plants treated with PGPR (*B. amyloliquefaciens* 937a, *B. subtills* 937b, and *B. pumilus* SE34), applied as an industrially formulated seed treatment, a spore preparation mixed with potting medium (referred to as powder), or a combined seed-powder treatment, were evaluated under field conditions for induced resistance to tomato mottle virus (ToMoV), resulted in reduced ToMoV incidence and disease severity. In some cases, a corresponding increase in fruit yield was observed. The use of PGPR could become a component of an integrated program for management of this virus in tomato (Murphy et al. 2000)

It was known that there are no highly effective chemical substances that can be applied to plants to control viral disease of agricultural or horticultural crops. For exclusion or eradication of a viral disease from production areas, highly effective chemical substances cannot be suggested; however, biological control with PGPR may be suggested to protect these areas or plants from viral pathogens. Nevertheless, it is recommended that more work must be conducted because of the complexity and variability of virus diseases.

#### 3.2.4 Nematodes

Plant–parasitic nematodes cause serious crop losses in production areas,e.g., yield loss of tomato due to root-knot nematodes (*Meloidogyne* spp.) ranges from 39.7 to 46.0% in India (Reddy 1985), and are among the most important agricultural pests (Koenning et al. 1999; Siddiqui and Akhtar 2008). The control of nematodes is difficult because nematodes mostly inhabit the soil and generally attack and settle around or inside the roots of the plants. During the last few decades, plant disease control has been based largely on the use of chemicals (Siddiqui et al. 2001). Although chemical nematicides are effective, easy to apply, and show rapid effects, they have begun to be withdrawn from the market in some developed countries owing to concerns about public health and environmental safety (Schneider et al. 2003; Nico et al. 2004). The search for novel, environmentally friendly alternatives with which to manage plant–parasitic nematode populations has, therefore, become increasingly important (Tian et al. 2007).

Biological control using microbial antagonists is one potential alternative to chemical nematicides (Burkett-Cadena et al. 2008). PGPR can also be used for the biological control of plant parasitic nematodes. Among the biological control agents that have been assessed are *B*. spp. and *Pseudomonas* spp. dominant populations in the rhizosphere that are able to antogonize nematodes (Tian et al. 2007).

Recently, rhizobacteria-mediated ISR in plants has been shown to be active against nematode pests. Plant growth-promoting rhizobacteria can bring about ISR by strengthening the physical and mechanical resistance of the cell wall of plants. They also change the physiological and biochemical ability of the host to promote the synthesis of defence chemicals against the challenge pathogen (van Loon et al. 1998; Ramamoorthy et al. 2001; Tian et al. 2007). Siddiqui and Shaukat (2004) concluded that fluorescent Pseudomonads ISR against root-knot nematode via a signal transduction pathway, which is independent of SA accumulation in roots.

In other words, PGPR may suppress pests and pathogens of plants and promote plant growth. For example, *P. aeruginosa* and *B. subtilis* exhibited nematicidal activity by killing the second stage larvae of *Meloidogyne javanica* to a varying degree. Especially, *B. subtilis* significantly suppressed root-knot infection and nematode population densities under greenhouse and field conditions and thereby enhanced plant growth and yield in mungbean (Siddiqui et al. 2001).

In a different example, *P. putida* promoted tomato growth in nematode-infected and nematode-free plants but growth promotion was higher in the infected ones. *P. putida* was better in reducing galling and nematode multiplication than arbuscular mycorrhizal fungus (Siddiqui and Akhtar 2008).

In another recent study, Li et al. (2005) reported that *Brevibacillus brevis* and *B. subtilis* exhibited strong nematicidal activity by killing the second stage larvae of *Meloidogyne javanica* to varying degrees in the greenhouse. The toxic principles of bacterium *B. subtilis* B7 that showed the highest juvenile mortality were partially characterized.

The influence of *P. fluorescens* as the treatment on the seed germination, migration, and penetration of *Meloidogyne incognita* in aubergine was evaluated under laboratory conditions. The results revealed that *P. fluorescens* promoted germination (87.5%) and was effective in reducing root penetration by *M. incognita* and the number of gall formation was also controlled by 70.3% (Inam-ul-Haq et al. 2003).

Rhizobacteria reported to show antagonistic effects against nematodes include members of different genera are given in Table 4.

#### 3.2.5 Insects

Next to phytochemical insecticides, biocontrol agents of microbial origin play a role in pest management (Gandhi et al. 2006). Among the biocontrol agents, the strains of PGPR, *P. fluorescens* is the promising one (Commarea et al. 2002). They activate systemic resistance (Raupach and Kloepper 1998) by inducing plants' latent defense mechanisms and to control insect pests (Zehnder et al. 1997; Commarea et al. 2002) in addition to exerting beneficial effect on plant growth promotion (Gandhi et al. 2006).

Herman et al. (2008) notified that there are several examples of plants treated with PGPR, which showed a decrease in insect herbivory. Zehnder et al. (1997) used PGPR to reduce feeding by the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber. Boughton et al. (2006) reported that plants treated with defence elicitors caused the green peach aphid, *Myzus persicae*, to significantly decrease in their population growth when compared with that of the control plants. Similarly, Herman et al. (2008) notified that *B. subtilis* and *B. amyloliquefaciens* could be useful in *Myzus persicae* management program, for pepper plants grown in locations with consistently high aphid pressure. Additionally, white clover and *Medicago* plants grown in the presence of a *Pseudomonas*-like PGPR were better able to resist the effects of blue-green aphids (Kempster et al. 2002).

The talc-based formulation of two *P. fluorescens* PF1, FP7 and its mixture were tested against leaffolder in rice. The application of talc-formulation through seed, root, soil, and foliar spray significantly reduced leaffolder incidence both under greenhouse and field conditions. The mixture of two strains performed better than the individual strains. Additionally, *Pseudomonas* treated leaves altered the feeding behavior of leaffolder larvae and reduced larval and pupal weight, increased larval mortality and incidence of malformed adults under in vitro conditions. An increased population of natural enemies of leaffolder and predatory spider was noticed in *Pseudomonas* treated plots under field conditions, which yielded 12–21% more rice (Commarea et al. 2002). PGPR belonging to *Pseudomonas* spp. are being exploited

Nematodes	Species	PGPR	References
Meloidogyne	Lettuce and	Pseudomonas sp. W34	Hoffmann-Hergarten
incognita	tomato	Bacillus cereus S18	et al. (1998)
Globodera	Potato	Agrobacterium radiobacter G12A	Hackenberg et al. (1999)
pallida		Rhizobium etli G12	Reitz et al. (2000)
Meloidogyne incognita	Tomato and banana	Bacillus cereus, B. subtilis, Pseudomonas fluorescens, Pseudomonas chlororaphis Burkholderia cepacia	Jonathan et al. (2000)
	Bell pepper	Burkholderia cepacia Bc-2 Burkholderia cepacia Bc-F	Meyer et al. (2001)
Meloidogyne javanica	Tomato	Pseudomonas aeruginosa IE-6S(+) Pseudomonas fluorescens CHA0	Siddiqui and Shaukat (2002)
·		·	Siddiqui and Shaukat (2003)
		Pseudomonas aeruginosa strain 7NSK2	Siddiqui and Shaukat (2004)
		Pseudomonas fluorescens CHA0	
Globodera rostochiensis	Potato	Pseudomonas oryzihabitans	Andreoglou et al. (2003)
Meloidogyne javanica	Lentil	Pseudomonas putida, P. alcaligenes, Paenibacillus polymyxa, Bacillus pumilus	Siddiqui et al. (2007)
Meloidogyne incognita	Tomato and soybean	Pseudomonas fluorescens CHA0	Siddiqui et al. (2005)
0	Tomato	Rhizobium etli G12	Reimann et al. (2008)
		Bacillus amyloliquefaciens FZB42	Burkett-Cadena et al. (2008)
	Chickpea	Pseudomonas alcaligenes	Akhtar and Siddiqui
		Bacillus pumilus	(2008)
Meloidogyne	Chickpea	Pseudomonas putida 3604	Siddiqui and Akhtar
javanica		Pseudomonas alcaligenes 493	(2009a)
Meloidogyne incognita	Tomato	Bacillus subtilis, Paenibacillus polymyxa	Siddiqui and Akhtar (2009b)
		Burkholderia cepacia	
	Chickpea	Pseudomonas putida	Akhtar and Siddiqui
		Pseudomonas alcaligenes	(2009)

Table 4 Reported PGPR show antagonistic effects against nematodes

commercially for plant protection to induce ISR against various pests and diseases. The performance of PGPR has been successful against certain pathogens, insect, and nematode pests under field conditions (Ramamoorthy et al. 2001). Murphy et al. (2000) studied the effects of PGPR treatment on whitefly nymphs number in field trials in Florida. They recorded significantly lower numbers of whitefly nymphs on PGPR-treated plants compared with the untreated tomato.

The metabolic pathways associated with insect-active secondary plant metabolites may be affected by induction of SAR or ISR, which could in turn effect changes in plant concentrations of insect feeding stimulants. Because induction of SAR and ISR involves different metabolic pathways, it is not unexpected that plants treated with PGPR or other elicitors will vary in their suitability as insect host plants (Stout et al. 2002).

Consequently, it can be said that PGPR would be of great potential, especially to conserve natural enemies and to avoid potential problems encountered when some insecticides fail to control populations that have developed resistance (Wang et al. 2002).

# **4** Conclusions and Future Prospects

Since Kloepper and Schroth (1978) reported that microbial communities that exert benefit for plant growth have been called PGPR, there has been an increasing effort in advancing bacterial inoculants such as *Azotobacter*, *Azoarcus*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Gluconacetobacter*, *Klebsiella*, *Pseudomonas*, *Serratia*, *Rhizobium*, etc., for plant growth promotion in agriculture. Significant advances in the explanation of the mechanisms involved in plant growth promotion have been made, especially when using molecular biology approaches (Dobbelaere and Okon 2003). Mechanisms involved in plant growth promotion of phytohormones, suppression of diseases, rooting of cuttings, increase germination and emergence of seeds under different conditions, promoted nutrient uptake of roots, total biomass of the plants, induce early flowering, increase in yield, etc.

Different PGPR have been examined under controlled and field conditions, and generally plant growth promotion such as yield increases in different crops, reduction of fertilizer and pesticides have been clearly demonstrated. The scientific basis of PGPR should continue to be investigated, tested, and explored for better and effective use of strains in the future, and free exchange of PGPR strains between researchers and countries (Podile and Kishore 2006) may help this. There is good possibility that the commercial mix of PGPR for various aims such as improved crop yield or suppression of pests and disease developed will be used extensively in the production of different crops in sustainable and environment friendly agriculture.

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