
Microbial Inoculants as Agents of Growth Promotion and Abiotic Stress Tolerance in Plants

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Abstract

The use of external chemical inputs such as chemical fertilizers and pesticides undoubtedly resulted in huge increase in agricultural products in the past many decades. Such indiscriminate use of agrochemicals has however resulted in various ecological imbalances and environmental disasters in various parts of the world. The use of plant growth-promoting rhizobacteria (PGPR) as biofertilizers and/or as biocontrol agents to enhance plant growth, increase yield, and suppress diseases in a wide range of agricultural crops is gaining momentum. If PGPR inoculants are to replace agrochemicals in the near future, the search for effective strains must focus on isolation and screening of single or consortium of the bacterial strains that have multiple traits. Moreover, a better result in microbial inoculant development could be achieved by investigating the different modes of actions in disease suppression and plant growth promotion, detection of important genes and traits associated with these, bacterial-host plant interaction, as well as relationships between the bacteria and various environmental factors.

Keywords

Rhizosphere • PGPR • Siderophore • Biocontrol • Growth promotion • Abiotic stress • Antibiosis • BNF • ACC deaminase

2.1 Introduction

The world population, currently estimated around seven billion people, is predicted to increase to around 10 billion in the next 50 years which requires that agricultural productivity be increased within the next few decades to sufficiently feed all these individuals (Glick 2014). A

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typical feature of modern intensive agriculture worldwide is to increase agricultural productivity by the application of external chemical inputs including fertilizers, pesticides, fungicides, and herbicides. It was reported, for instance, that widespread use of chemical fertilizers during the past 50 years has become a major input to supply N and P and had substantially increased food production worldwide (Abd-Alla et al. 2014). This practice is however not sustainable and has several negative impacts both on human health and environmental safety (Franks et al. 2006; Glick 2014). From an environmental perspective, for example, only 30–50 % of applied N fertilizers and 10–45 % of P fertilizers are taken up by crops, and the majority of the remaining nitrogen and phosphorous are lost to the environment through various processes (Adesemoye and Kloepper 2009). Another drawback of the excessive application of chemical pesticides is that it contributes to the development of pest resistance which leads to higher chemical input use (Chavez et al. 2013). Potential alternatives to the use of chemical fertilizers and pesticides are microbial inoculants, environmentally friendly microbial formulations that act as phytostimulants, biofertilizers, and/or microbial biocontrol agents (Olubukola et al. 2012). Thus, nowadays, tremendous effort is being put on research to develop such microbial inoculants which have beneficial plant growth properties in environmentally friendly sustainable agriculture (Barriuso et al. 2008). Such beneficial properties of microbial inoculants could be manifested either by direct promotion of plant growth, by indirectly protecting plants from phytopathogens, or by fortifying certain abiotic stress tolerance in plants that grow in soils with non-optimal environmental factors including extremes of high and low temperature, salinity, drought, acidity, and presence of heavy metals (Kang et al. 2014; Penrose and Glick 2003; Kloepper and Schroth 1978). Microbial inoculants can also play an important role in the formation of soil aggregation which helps stabilize the soil (van Veen et al. 1997).

2.2 Direct Plant Growth Promotion by Microbial Inoculants

For the past several decades, research dedicated to improve crop yield and plant growth with microbial inoculants mainly focused on the symbiotic rhizobia which have been successfully used worldwide for the establishment of the nitrogen-fixing symbiosis with legumes (Reddy 2013; van Veen et al. 1997). These groups of bacteria which generally belong to the alpha proteobacteria are capable of inducing nitrogen-fixing nodules on the roots of several hundreds of leguminous plants (Lorenzo et al. 2000). They are thus involved in direct promotion of plant growth by fulfilling the nitrogen requirement of legumes using a process known as biological nitrogen fixation (BNF) which occurs in the root nodules. On the other hand, there are other groups of soil bacteria living freely in close proximity to the active region of the roots, commonly known as the rhizosphere. In the past, several large areas of arable land in different parts of the world have been inoculated with nonsymbiotic free-living bacteria such as *Azotobacter*, *Azospirillum*, *Bacillus*, *Klebsiella*, and *Pseudomonas* (van Veen et al. 1997). The major mechanisms by which these free-living bacteria promote plant growth include nitrogen fixation, improving plant nutrient uptake, enhancing the growth of the entire root system, and reduction of the membrane potential of the roots (Glick and Bashan 1997).

2.2.1 Free-Living Plant Growth-Promoting Rhizobacteria (PGPR)

Kloepper and Schroth (1978) first defined the term plant growth-promoting rhizobacteria (PGPR) to describe soil bacteria that colonize the roots of plants and enhance plant growth following inoculation onto seeds. These plant growth-promoting rhizobacteria are mainly present in the region around the roots, the rhizosphere, which is

relatively rich in nutrients as a result of loss of 40 % of the plant photosynthate from the roots (Lynch and Whipps 1991). Apart from the major role of enhancing plant growth, an ideal PGPR must be highly competent in the rhizosphere, must colonize the roots sufficiently, should be compatible with other rhizobacteria, must have broad spectrum of action, should be easily multiplied, and must be safe to the environment (Reddy 2013). A number of rhizosphere bacteria which fulfill the above criteria including members of the genera *Azospirillum*, *Pseudomonas*, *Bacillus*, *Azotobacter*, *Burkholderia*, and *Enterobacter* have been widely reported in the past (Glick and Bashan 1997). However, not all rhizosphere bacterial strains in a given genus or species have beneficial PGPR effect on plants (Penrose and Glick 2003; Glick 2014). It is therefore very essential to conduct reliable screening and selection of PGPR in order to develop efficient microbial inoculants that promote plant growth and yield increase.

2.2.1.1 Siderophore Production

After coining the term PGPR, Kloepper et al. (1980a, b) demonstrated that the best known rhizobacteria with PGPR activities belong to the group of fluorescent *Pseudomonas* species. Direct plant growth promotion by the fluorescent *Pseudomonas* mainly comes from their involvement in improving plant iron nutrition using siderophore secretions. Siderophores are low molecular mass proteins (~400–1500 Da) which have an exceptionally high affinity for iron (Fe^{+3}). Under aerobic condition, most of the iron is only sparingly soluble and therefore not readily available to either bacteria or plants. To overcome this limited supply of iron, PGPR such as *Pseudomonas*, mainly belonging to the fluorescent species, synthesize siderophores (Glick 2012; Neilands 1981; Kloepper et al. 1980a, b). Bacterial siderophores have been demonstrated to have direct benefits to plant growth promotion by acting as a direct source of iron and making it available to plants (Yehunda et al. 1996; Vansuyt et al. 2007). Siderophore-producing microbial inoculants have been shown to have a direct plant growth-promoting effect in various crops in the past. To cite a few examples, inoculation of mung

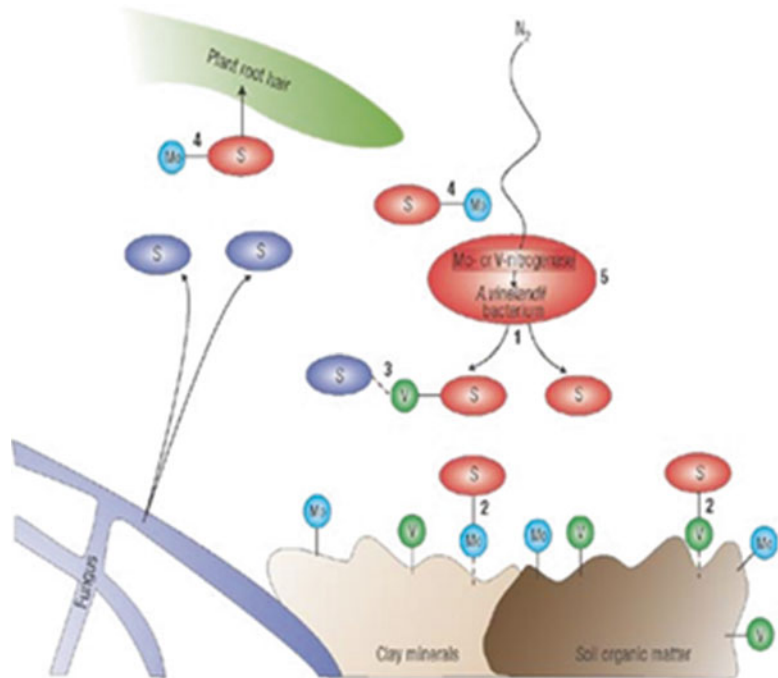
bean with siderophore-producing *Pseudomonas* strain GRP3 under iron-limited condition showed enhanced growth and chlorophyll level (Sharma et al. 2003). In another experiment, inoculation of *Arabidopsis thaliana* with *Pseudomonas fluorescens* resulted in the uptake of the Fe-pyoverdine complex synthesized by the bacteria leading to an increase in the iron level inside the plant tissue and improved plant growth (Vansuyt et al. 2007).

Many plants use microbial siderophores as iron source for growth. Evidence of iron uptake by plants from hydroxamate siderophores produced by *Pseudomonas* spp. has been widely documented (Crowley 2006). Iron (Fe^{+3}) and molybdenum (Mo) are very much required by the free-living and symbiotic nitrogen-fixing diazotrophic bacteria such as *Rhizobium* and *Azospirillum* not only for the electron shuttle reactions but also as a component of the nitrogenase complex. Siderophore production by such types of microorganisms is an added advantage as it helps the bacteria by incorporating the iron and molybdenum into the nitrogenase enzyme complex. Moreover, the bacterial siderophores have higher affinity to these metals than fungal siderophores and compete with the fungal siderophores (Fig. 2.1) (Benjamin and Bruce 2008). *Rhizobium* requires iron to grow in the rhizosphere and for optimum nodulation and development of the *Bacteroides*. This suggests that siderophores are required for effective nitrogen fixation by the symbiotic rhizobia (Tang et al. 1992). In one investigation to select best strains of *Bradyrhizobium japonicum* for high plant yield, inoculation with siderophore-producing strains resulted in higher yield as compared to inoculants that do not produce siderophores (Khandelwal et al. 2002).

2.2.1.2 Indole-3-Acetic Acid (IAA) Secretion

Another very important microbial metabolite involved in direct plant growth promotion by free-living PGPR is indole-3-acetic acid (IAA). Several free-living PGPR such as *Azospirillum* and fluorescent *Pseudomonas* secrete IAA involved in promoting root growth and development (Figueiredo et al. 2010). Apart from their

Fig. 2.1 Bacterial siderophores (*1*) scavenge the metals from unavailable complexes with clay, soil organic matter, or other elements (*2*). The siderophores compete with siderophores produced by fungi for these metals (*3*). The bacterium or plant roots readily take up the siderophore-metal complexes (*4*). Within the bacterium, the metal is incorporated into the enzyme nitrogenase (*5*), to allow the fixation of atmospheric nitrogen (N_2) that would otherwise be unusable to the bacterium (Adapted from: Benjamine and Bruce 2008)



capacity to fix atmospheric nitrogen under microaerophilic conditions, PGPR of the genus *Azospirillum* have long been considered the most important rhizobacteria for improvement of plant growth and crop yield because of their ability to colonize internal tissues of gramineous plants and promote growth by production of the phytohormone indole-3-acetic acid (Bashan et al. 2004; Perrig et al. 2007). Production of this phytohormone by *Azospirillum* species alters the metabolism and morphology of plant roots which result in a better absorption of mineral and water, producing larger and healthier roots (Bashan and de Bahsan 2010). The major outcomes of most inoculations with *Azospirillum* species are therefore changes in plant root architecture, while inoculation also promotes root elongation and development and branching of root hairs (Levanony and Bashan 1989; Okon and Kapulnik 1986). Many important plant microbe interactions are regulated by auxins, IAA being the major type of auxin produced by plants and several free-living PGPR including *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Burkholderia*, and the symbiotic rhizobia (Martinez-Viveros et al. 2010). In general, selection

of PGPR isolates through screening for the production of IAA is one of the strategies in the development of microbial inoculants that stimulate seed germination, accelerate root growth, modify the architecture of the root system, increase root biomass, and ultimately enhance plant growth.

2.2.1.3 Phosphate Solubilization

Although most agricultural soils have large amounts of inorganic and organic phosphates, most of these are immobilized and unavailable to plants. Like Fe, phosphorous (P) is not readily available to plants due to its high reactivity with some metal complexes leading to precipitation or adsorption of 75–90 % of P into soil (Adesemoye and Kloepper 2009). In such soils, correcting P deficiency by applying P fertilizer is quite often unaffordable by most resource-poor farmers in the tropics and subtropics, particularly, in soils characterized by high P-fixing properties (Horst et al. 2001). Several PGPR strains such as *Pseudomonas*, *Bacillus*, *Burkholderia*, *Rhizobium*, and *Flavobacterium* have been reported to have the ability to solubilize such insoluble inorganic phosphate compounds. The use of these phosphate-solubilizing bacteria as

inoculants could increase the P uptake by plants and thus offers the benefit of direct plant growth promotion (Rodriguez and Fraga 1999; Bashan and de Bahsan 2010; Saharan and Nehra 2011).

2.2.2 Symbiotic PGPR (Rhizobium-Legume Symbiosis)

The air in the atmosphere is largely 78 % nitrogen gas (N_2), and yet it is ironic that nitrogen (N) has become one of the most limiting nutrients for crop production worldwide (Valentine et al. 2011). This is because atmospheric nitrogen (N_2) is very stable due to the strong triple bond between the two N atoms that require large amount of energy to break. Only few prokaryotic organisms called diazotrophs have the enzymatic machinery to break the strong bond that held the two N atoms. The most effective diazotrophic bacteria, the rhizobia, form a symbiotic interaction with legumes and reduce atmospheric N to a usable form of NH_3 by a process called biological nitrogen fixation (BNF). Symbiotic nitrogen fixation is one of the most important biological processes on the planet which provides the majority of the N requirement in agriculture (Howieson and McInnes 2001). The symbiosis between the root nodule rhizobia and legumes contributes at least 70 million metric tons of fixed nitrogen per year into terrestrial ecosystem which accounts for up to 40 % of the total N fixed on earth (Brockwell et al. 1995; McInnes and Haq 2007).

2.2.2.1 The Symbiotic Process

Nodulation and the associated legume-rhizobium symbiosis are complex processes involving the expression of both bacterial and plant genes which start by the production of a cocktail of phenolic molecules called flavonoids which can passively diffuse across the bacterial membrane (Smith and Wollum II 1989; Wang et al. 2012). As soon as the bacteria perceive the flavonoid signals, it results in the activation of the rhizobial nodulation (*nod*) genes that encode the enzymes required for the synthesis of bacterial Nod factors, a family of lipochitooligosaccharides essential for symbiotic development in most legumes. The Nod factors initiate most of the develop-

mental changes in the legume roots during the early nodulation process such as root hair deformation, membrane depolarization, initiation of cell division in the root cortex, and formation of a meristem and nodule primordium (Abd-Alla et al. 2014).

2.2.2.2 Direct Plant Growth Promotion by Rhizobium Inoculation

Nitrogen fertilizer plays one of the decisive roles in the attainment of high yields from crop plants. Due to this, farmers often apply high amounts of nitrogen fertilizer which is not only very costly but also makes the environment hazardous when used indiscriminately (Abd-Alla et al. 2014). The best alternative to this could be provided by the process of biological nitrogen fixation (BNF) that occurs during the legume-rhizobium symbiotic interaction which plays a critical role in sustainable agriculture by reducing the need for exogenous nitrogen fertilizer (Wang et al. 2012). Therefore, inoculation of legumes with actively nodulating and nitrogen-fixing rhizobia significantly contributes to the N input of many agricultural systems. It provides a source of nitrogen not readily leached and is the most important route for sustainable nitrogen input into agroecosystems (Lindström et al. 2010). It has been experimentally proved that efficient and proper usage of legume inoculation using effective rhizobium inoculants significantly improves crop productivity and soil fertility in a wide range of legume-growing fields (Brockwell and Bottomley 1995).

2.2.2.3 Rhizobium-PGPR Co-Inoculation

Recent exploitation of PGPR co-inoculation with *Rhizobium* constitutes an interesting alternative to improve nitrogen fixation. Nodulation and yield of several legume species including soybean, chickpea, pea, vetch, and clover have been increased as a result of co-inoculation of their respective rhizobium with the diazotrophic *Azospirillum* species. In a related report, co-inoculation of *Bradyrhizobium* and PGPR significantly improved soybean growth and yield as compared to the sole application of *Bradyrhizobium* (Masciarelli et al. 2014). Co-inoculation of rhizobia with the PGPR

Pseudomonas species has also been reported to enhance nodulation and nitrogen fixation by rhizobia (Perez-Montano et al. 2014). Although the mechanism in which the nonrhizobial PGPR is involved is poorly understood, it is believed that the role of the nonrhizobial PGPR such as *Azospirillum* is to increase the competitiveness of the rhizobial strains and to create additional infection sites which can be later occupied by the rhizobia (Antoun and Prevost 2005; Perez-Montano et al. 2014). In addition to their beneficial N₂-fixing activity, rhizobia can improve plant P nutrition by mobilizing organic and inorganic phosphates. Co-inoculation of rhizobia with phosphate-solubilizing bacteria revealed a synergistic effect on symbiotic parameters such as increasing nodule number and plant biomass which resulted in grain yield of legumes (Saharan and Nehra 2011). In another experiment, inoculation of groundnut with a consortium of PRPR comprising *Rhizobium* strain Tt 9 with the PGPR *Bacillus megaterium* var *phosphaticum* resulted in fulfilling about 50 % of the phosphatic fertilizer requirement of the groundnut thereby improving nodulation, plant growth, and yield (Kumar et al. 2011). In general, there is a promising trend of the practice of co-inoculation of rhizobia and PGPR in the development of sustainable agriculture in the future.

2.3 Microbial Inoculants as Biological Control Agents

Over the past few decades, pathogenic microorganisms that affect plant health have become a major threat to food production and to the stability of the ecosystem worldwide. This has resulted in more and more dependency on agrochemicals by food producers and farmers to protect their crops from potential pathogens (Compant et al. 2005). The increasing use of chemical pesticides in agricultural systems has several drawbacks. First, a large number of resource-poor communities in the developing world cannot afford the high cost of chemical pesticides. Second, chemical pesticides result in the development of pathogen resistance and negatively affect the ecosystem

due to its nontarget environmental impact (Gerhardson 2002). As substitutes for chemical pesticides, the use of bacterial biocontrol agents against a wide variety of phytopathogens especially the root-associated soilborne pathogens has been extensively emphasized. This has led to the isolation and commercialization of numerous microbial inoculants for growth enhancement and as potential antagonists and disease management in various crops (Kakar et al. 2014).

2.3.1 Rhizosphere Competence

Among the major factors in the unsuccessful commercialization of microbial inoculants are the inconsistencies in field trial tests which raised concerns about the perspectives of the practical potentials offered by the microbial metabolites released into the soils (van Veen et al. 1997). For biocontrol agents to be effective once introduced into the soil, they should have a strong rhizosphere competence so that they colonize the root effectively and survive along with growing plant roots over a long period of time in the presence of indigenous microflora (Weller 1988; Lugtenberg and Deckers 1999). When introducing microbial inoculant strain into the soil, it is necessary that the strain should be inoculated at a density many times higher than the indigenous population. Additional approaches include using repeated inoculation as well as utilization of antibiotic-resistant bacteria simultaneously with antibiotic (Nautiyal 1997). Being an important first step in the interaction of an introduced microbial strain with plant roots, it is better to determine if the bacteria really have efficient root colonization capacity. Using molecular techniques such as the green fluorescent protein (gfp), it is possible to monitor the location of individual rhizobacteria on the root using confocal scanning microscopy (Bloemberg et al. 2000; Bloemberg and Lugtenberg 2001). Figure 2.2 represents one such study using confocal scanning laser microscopy to monitor the colonization of tomato root by a strain of *Bacillus simplex* KSB1F-3 in a glasshouse study (Hassen and Labuschagne 2010).

2.3.2 Antibiosis

Control of phytopathogens by applying chemical pesticides has resulted in the development of resistance to individual chemical controls over time, demanding a constant development of new pesticides. Moreover, there is a growing concern over environmental contamination (Martinez-Viveros et al. 2010). Microbial inoculants which are involved in indirect plant growth promotion are characterized by protecting plants from attack by phytopathogens. One of the mechanisms used by biocontrol PGPR to prevent plants from pathogen attack is by the production of antibiotics, low molecular weight compounds produced by microorganisms. Antibiosis plays an important role in disease suppression by PGPR and is often thought to act in concert with competition and parasitism (Reddy 2013). It is one of the most powerful and widely studied biocontrol mechanisms for combating phytopathogens.

Several different types of antibiotics produced by microbial inoculants with strong PGPR functions have been shown to be effective against a wide range of fungal pathogens. Over the past many years, contemporary *Pseudomonas* biocontrol research revealed the production of four

classes of antibiotics by different strains of fluorescent pseudomonads: phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin (Prn), and pyoluteorin (Plt) (Weller 2007; Thomashow and Weller 1988). From a practical point of view, a PGPR strain of *Pseudomonas fluorescens* CHAO that produce DAPG sufficiently suppressed take-all of wheat and black root rot of tobacco. In addition, this strain produces Plt, Prn, IAA, and the siderophores pyochelin and pseudobactin (a pyoverdinin siderophore) due to which it is considered as a PGPR strain with the highest biocontrol and growth-promoting potential (Weller 2007; Weller et al. 2012). Parallel to the discovery of such antibiotic-producing strains, several genes and traits have so far been detected. For example, the gene *phzF* detected in *Pseudomonas chlororaphis* can be used as a marker for the capacity of a PGPR to produce the antibiotic phenazine-1-carboxylic acid (PCA), a class of the broad-spectrum antibiotic suppressive to take-all and *Rhizoctonia* root rot and *Fusarium* wilt. Similarly, *phlD* is used as a key marker in the biosynthesis of 2,4-DAPG by *Pseudomonas* species (Wang et al. 2014). Other *Pseudomonas fluorescens* strains with potential biocontrol traits

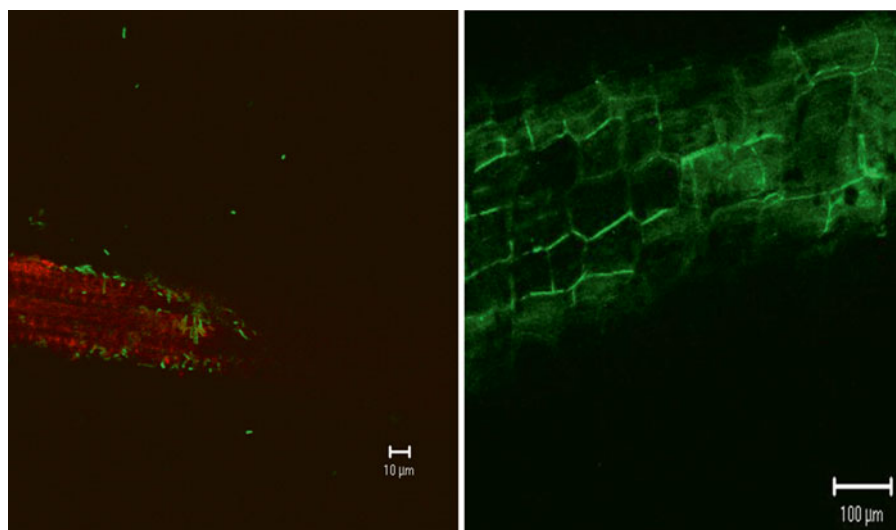


Fig. 2.2 Root colonization of *gfp*-tagged *Bacillus simplex* KBS1F-3 after inoculation of 2-week-old tomato seedlings with the tagged bacterial suspension (left). Plants

treated with the wild-type strain show no fluorescence (right) and the green color of the root is due to auto fluorescence (Adapted from Hassen and Labuschagne 2010)

produce the antibiotics pyrrolnitrin and pyoluteorin encoded by the genes *prnD* and *pltC* and are highly active against *Pythium* and *Rhizoctonia* species (Loper et al. 2007; Glick and Bashan 1997).

2.3.3 Siderophore Production

Production of siderophores (pyoverdinin and pseudobactin) by PGPR inoculants was identified as a new mechanism of biological control. Biocontrol strains of PGPR produce siderophores that have high affinity for iron so that fungal pathogens are unable to survive in the rhizosphere of the host plant due to lack of iron. Therefore, the major mode of action of siderophores as biocontrol agents is limiting the amount of iron available to the pathogens for growth (Kloepper et al. 1980a, b; Glick 2012). Production of a large amount of siderophores by *Pseudomonas* spp. in pure culture results in sequestering of all available iron leading to suppression of fungal pathogens. Previous field trial researches revealed that there are several direct evidences for the suppression of fungal pathogens in different crops by bacterial siderophores. *Pseudomonas fluorescens* WCS 358 is one of such potential examples of PGPR that inhibit *Fusarium* wilt of radish due to its siderophore mediated iron competition (Leeman et al. 1996a).

Siderophore production by certain *Pseudomonas* spp. also has a secondary effect by triggering systemic acquired resistance (SAR). To cite an example, the siderophore pseudobactin produced by strain WCS374 induced SAR to *Fusarium* wilt in radish (Leeman et al. 1996b). Biocontrol of wilt disease, damping off of cotton caused by *Pythium ultimum*, and *Pythium* root rot of wheat by siderophore-producing fluorescent pseudomonades are also very good examples of the role of siderophores in biocontrol of fungal pathogens. The rationale behind the effectiveness of bacterial siderophores against fungal pathogens which may also produce certain types of siderophores is that bacterial siderophores have higher affinity for iron than fungal siderophores due to which biocontrol PGPR outcompete fungal

pathogens for the available iron in the rhizosphere (Glick and Bashan 1997) (Fig. 2.3).

2.3.4 Induced Systemic Resistance (ISR)

Plant growth-promoting rhizobacteria can trigger an induced systemic resistance (ISR) in plants which is phenotypically similar to the systemic acquired resistance (SAR) that occurs when plants activate their defense mechanism in response to infection by phytopathogens (Glick 2012). Unlike SAR, induced systemic resistance does not cause visible symptom on the host plant, but is effective against different types of pathogens (Compant et al. 2005). ISR by rhizobacteria was first demonstrated using *Pseudomonas* spp. and other gram-negative bacteria. However, a few effective cases of induced systemic resistance and promotion of plant growth have also been reported for the gram-positive *Bacillus* spp. Strains of the species *Bacillus subtilis*, *B. pumilus*, and *B. amyloliquefaciens* elicited significant reductions in the incidence of various diseases on greenhouse and field trials on tomato, sugar beet, watermelon, tobacco, and cucumber (Kloepper et al. 2004).

2.4 Abiotic Stress Tolerance in Plants by Microbial Inoculants

Many agricultural crops worldwide are exposed to several abiotic stresses such as extremely high or low temperature, salinity, drought, acidic soils, and metal toxicity. Depending on the type of crop, such abiotic stresses result in yield losses between 50 and 82 % (Kang et al. 2014). In response to such abiotic stress, plants undergo a variety of metabolic and physiological responses and typically stimulate the synthesis of 1-aminocyclopropane 1-carboxylic acid (ACC), which is a precursor to the synthesis of ethylene. Ethylene in turn helps to induce multiple physiological changes in the plants at molecular level (Saleem et al. 2007; Sharma et al. 2013). The

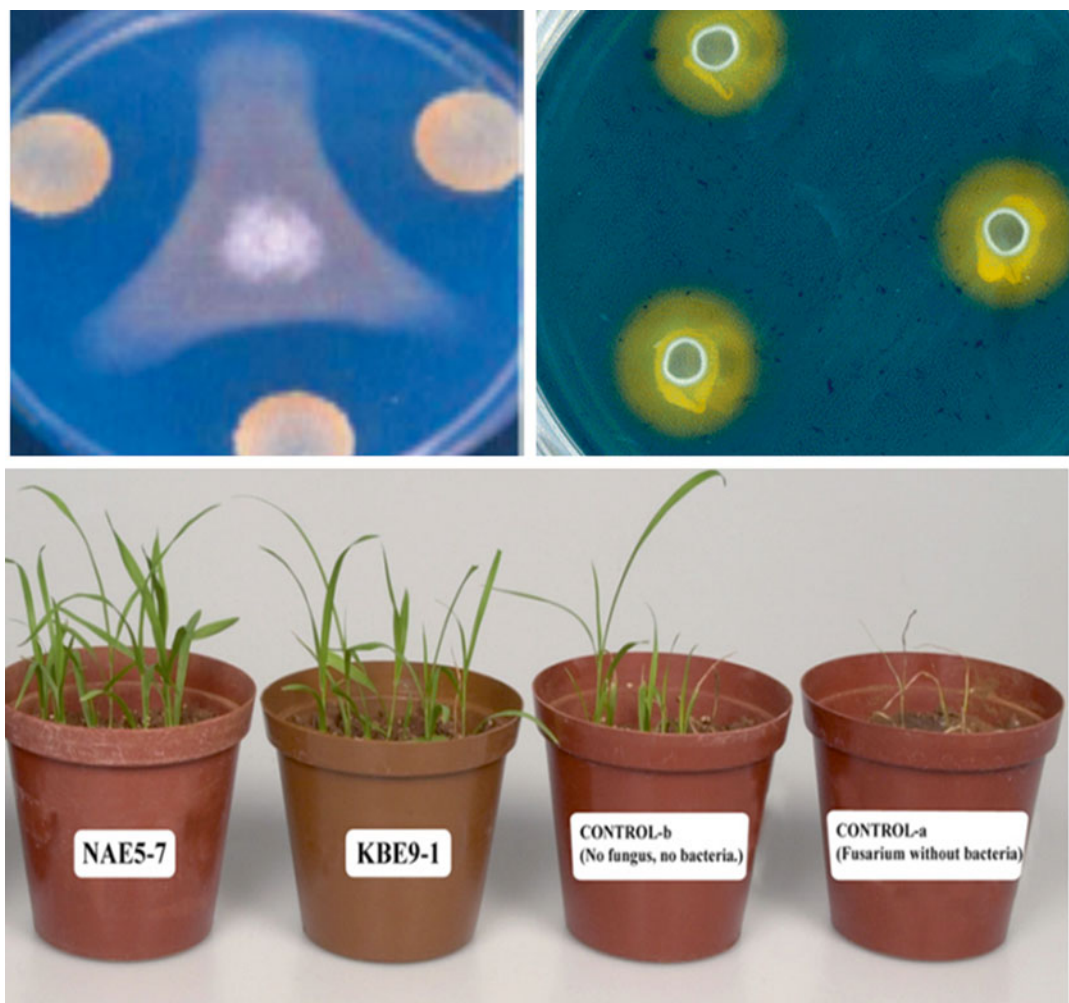


Fig. 2.3 *In vitro* antibiosis activity against *Fusarium oxysporum* (top left) and production of siderophore on CAS agar medium (top right) by some PGPR strains from sorghum rhizosphere. Glasshouse inhibition of *Fusarium oxysporum* root rot in sorghum after inoculation with

rhizobacterial strains NAE5-7 and KBE9-1 (bottom). Control plants inoculated only with the pathogen and without the rhizobacteria are all infected and dead (bottom right) Source: Idris et al. (2007)

stress ethylene can trigger a senescence response in the plant leading to leaf or fruit abscission, disease development, prevention of enzyme and antibiotic and synthesis, and ultimately inhibition of growth (Glick and Bashan 1997).

2.4.1 ACC-Deaminase Activity

Although ethylene is required by many plants in the course of their growth, to break seed dormancy, high level of ethylene following germina-

tion is inhibitory for root elongation. A number of PGPR strains are able to produce the enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase, a pyridoxal 5' phosphate (PLP)-dependent enzyme that cleaves the plant ethylene precursor ACC into ammonia and α -ketobutyrate thereby lowering the level of ethylene and the associated stress in plants (Penrose and Glick 2003; Blaha et al. 2006). ACC deaminase is produced by plant growth-promoting bacteria to effectively protect plants against a wide range of abiotic stresses such as drought, salinity, heat,

flooding or water logging, and heavy metal stress. Rhizobacteria belonging to the genera *Pseudomonas*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, and *Kluyvera* have so far been documented to have ACC deaminase activity (Saleem et al. 2007; Blaha et al. 2006).

Salinity stress inhibits plant growth as a result of inhibition of seed germination, seedling growth, vigor, and flowering due to the accumulation of stress ethylene. ACC deaminase-positive PGPR reduce the level of stress ethylene and confer salinity tolerance in these plants (Gontia-Mishra et al. 2014). Flooding is also another important abiotic stress that affects many plants as a result of lack of oxygen (anoxia). This results in various symptoms as a result of large quantities of ethylene and leads to yield reductions. Treatments of such plants abiotically stressed by flooding using ACC deaminase-positive PGPR strains could alleviate the stress (Barnawal et al. 2012). Drought stress affects plant-water relations both at cellular and whole plant level limiting crop productivity in most dry regions of the world. Selection and development of inoculants with drought-tolerant ACC deaminase-containing rhizobacteria could be the best strategy to protect plants growing in arid areas.

With the threat of the so-called global warming, heat stress is another threat to world agriculture as extremely high temperature results in hormonal imbalances in plants affecting their growth. ACC deaminase activity by the plant growth-promoting rhizobacteria *Burkholderia phytofirmans* helped potato plants to maintain normal growth under heat stress. In the other extreme of temperature stress, a psycho-tolerant ACC deaminase bacterium strain of *Pseudomonas putida* UW4 promoted canola growth at low temperature under salt stress (Saleem et al. 2007). In general, bacteria that express ACC deaminase activity are capable of lowering a wide range of abiotic stresses in plants. The *acdS* gene coding for the enzyme ACC deaminase can be a very useful candidate for the development of a microbial inoculants that can be used in the management of abiotic stress in plants (Ali et al. 2014). Apart from this role, there are several suggestions that the passion of *acdS* gene and the associated ACC

deaminase activity by PGPR strains facilitates bacterial competitiveness and persistence in the rhizosphere (Glick 2014).

2.4.2 Other Stress Tolerance Traits

Certain PGPR such as *Pseudomonas* produce exopolysaccharides (EPS) which not only protect the bacteria from water stress, but they also play a vital role in the formation and stabilization of soil aggregates, regulation of plant nutrients, and water flow across plant roots through biofilm formation (Grover et al. 2011). Generally, salinity stress causes an imbalance in the ion flux in side plants, but inoculation with exopolysaccharides containing PGPR results in significantly decreased Na^+ and increased K^+ concentration and alleviates salt stress by potentially binding cations such as Na^+ and decreasing the level of Na^+ available for uptake (Nadeem et al. 2010; Kang et al. 2014). Gururani et al. (2013) reported that some free-living PGPR strains produce osmolytes which help plants to increase their osmotic potential within the cell thereby relieving the stress.

Abiotic stress in plants resulting from water deficiency and drought could be caused by the formation of reactive oxygen species (ROS) as a result of misdirection of electrons during photosystems. In one experiment, inoculating plants suffering from oxidative stress with *Azotobacter chroococcum* strain, that produce cytokinin and antioxidants, resulted in the accumulation of abscisic acid (ABA) that resulted in the degradation of ROS (Grover et al. 2011). In a related report, a significant increase in the activities of the antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) was observed after treatment of stressed plant with the diazotrophic bacteria *Azospirillum* and *Azotobacter* (Karthikeyan et al. 2012).

Inoculating plants with PGPR increases proline biosynthesis that acts as reactive oxygen scavenger which can improve plant growth under stress. Proline accumulates in different legumes such as *Glycine max* and *Phaseolus vulgaris* as a characteristic response to prolonged severe water

stress, and it was shown that there is a direct correlation between proline accumulation and drought tolerance (Zahran 1999; Sharma et al. 2013). Proline production in *Zea mays* due to co-inoculation of *Rhizobium* and *Pseudomonas* resulted in salt tolerance as a result of maintenance of relative water content and selective uptake of K^+ ions (Bano and Fatima 2009). Enhanced uptake of nutrients and improving plant health under stress condition can be achieved by inoculating PGPR capable of producing IAA and gibberellins that result in increased root length, root surface area, and number of root tips (Egamverdieva and Kuchrova 2009)

2.4.3 Abiotic Stress Tolerance in Legume-Rhizobium Interaction

Abiotic stress is a common phenomenon in the legume-rhizobium symbiosis which greatly affects the nodulation process and thus that of nitrogen fixation. Legume nodules face abiotic stress including water stress, salinity, soil nitrate, temperature, acidity, and heavy metals (Walsh 1995). Inoculating legumes with mixed cultures of rhizobium and ACC deaminase-positive PGPR promotes nodulation through inhibition of ethylene biosynthesis thereby enhancing nodulation and nitrogen fixation. A few examples include early growth and promotion of nodulation in *Glycine max* by ACC deaminase rhizobacteria and enhanced nodulation in *Pisum sativum* by ACC deaminase *Rhizobium leguminosarum* bv. Viciae 128C53K (Cattelan et al. 1999).

Water stress caused by soil moisture deficiency has a serious negative effect on nodule initiation and thus on N_2 fixation. Since the sensitivity to moisture stress varies for a variety of rhizobial strains such as *R. leguminosarum* bv. trifoli, *Sinorhizobium meliloti*, cowpea *Bradyrhizobium*, and *B. japonicum* strains, it is possible to select the most stress-tolerant rhizobial strains within the range of their legume host (Zahran 1999). The above abiotic stresses added up together with aluminum (Al^+) toxicity and P

deficiency hugely affect nodulation and nitrogen fixation. A number of *Rhizobium* strains have evolved some sort of adaptation to saline conditions by the accumulation of low molecular weight organic solutes called osmolytes which counteract the dehydration effect of low water activity (Zahran 1999). To summarize, with the increasing research on the beneficial aspects of plant-microbe interaction including the legume-rhizobium symbiosis, there exist tremendous perspectives of the development and application of rhizobium inoculants that can sustain high levels of N_2 fixation even in the presence of these adverse environmental factors.

2.5 Conclusion

Two major problems trigger the adoption of microbial inoculants for use in sustainable agriculture: (i) the prolonged and indiscriminate use of agrochemicals to improve plant growth and crop yield which leads to ecological imbalance and affects the environment negatively and (ii) environmental stresses that affect plant growth and productivity. The rhizosphere, with its high microbial diversity, is a vital source of beneficial plant growth-promoting rhizobacteria that could be screened and developed into potential microbial inoculants for sustainable agriculture. One of the most important problems however is the inconsistency in the field performance of PGPR inoculants that still warrants intensive research in the field. It is hence very essential to explore the soil microbial diversity and the various modes of actions involved in direct and indirect plant growth promotion and develop consortium of two or more PGPR to attain maximum benefits from microbial inoculation. With regard to developing microbial inoculants for biocontrol, isolation of bacteria from soils suppressive to a number of soilborne plant diseases where the disease development is minimal even in the presence of a virulent pathogen and susceptible host could be a strategy. In developing a PGPR strain into potential microbial inoculants, it is vital to elucidate new associations between different strains and/or species in the population and study

the various plant bacterial signal exchange. The choice of strains beneficial to both biocontrol and plant growth-promoting potentials is very essential which focuses on isolation and screening PGPR strains that exhibit various types of beneficial traits such as production of antibiotics, siderophores, indole-3-acetic acid, acc-deaminase activity, nodulation and nitrogen fixation and by detection of the genes involved in the regulation, and synthesis of these beneficial traits and compounds.

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