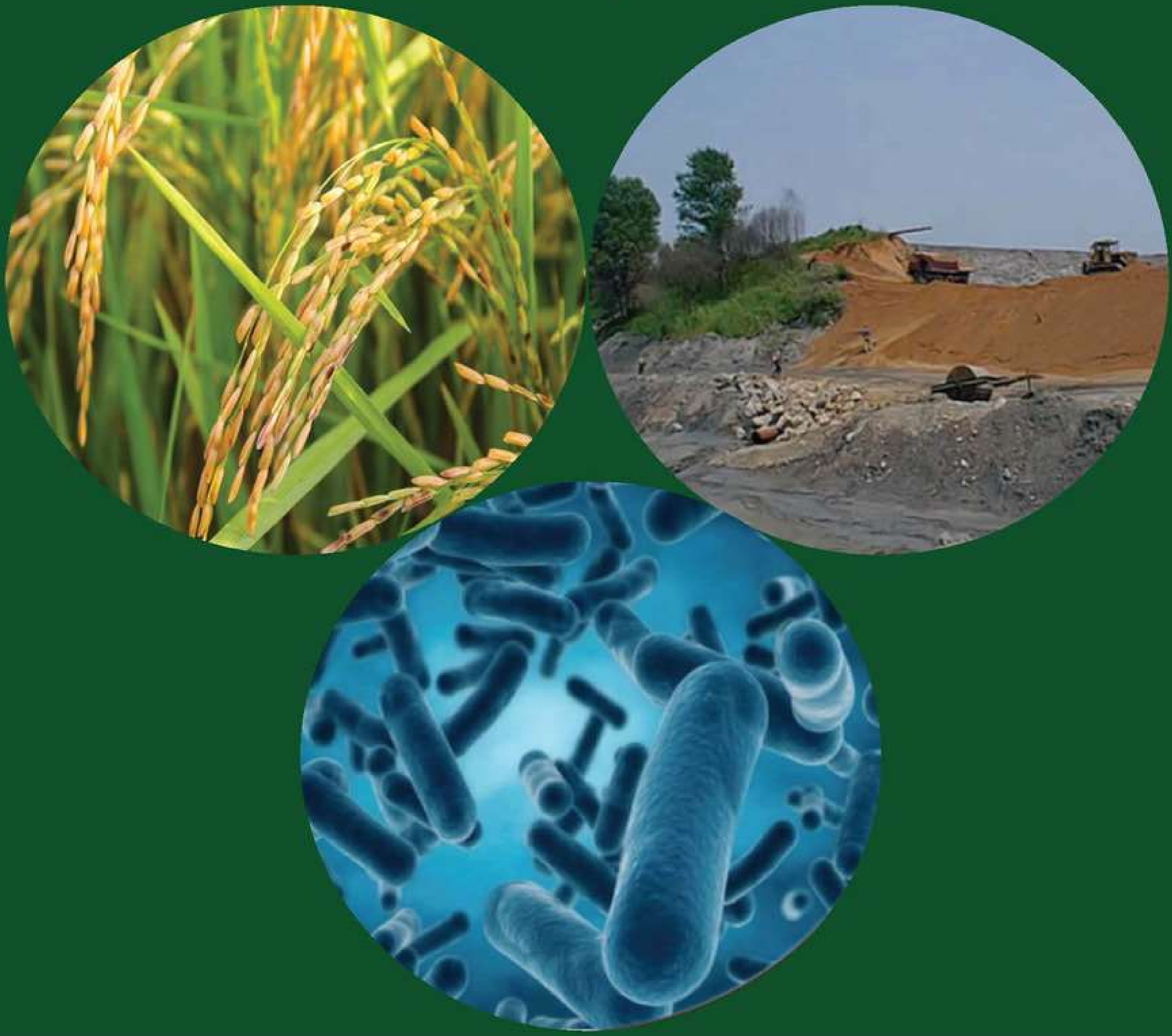
Microbes in Agriculture and Environmental Development



Edited by

Chhatarpal Singh • Shashank Tiwari
Jay Shankar Singh • Ajar Nath Yadav

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Mukesh Kumar
Department of Soil Science
Dr. Rajendra Prasad Central Agriculture University
Samastipur, India

V. K. Mishra
College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi, India

Subhrajyoti Mishra
Department of Fruit Science
College of Horticulture
JAU
Junagadh, India

Ajay Neeraj
School of Environment and Sustainable Development
Central University of Gujarat
Gandhinagar, India

Umesh Pankaj
College of Horticulture and Forestry
Rani Lakshmi Bai Central Agricultural University
Jhansi, India

Abhay Raj
Environmental Microbiology Laboratory, Environmental Toxicology Group
CSIR-Indian Institute of Toxicology Research (CSIR-IITR)
Lucknow, India

P. Rajarao
Department of Botany and Environmental Science
Osmania University
Hyderabad, India

P. M. Sameera
University College of Technology Osmania University
Hyderabad, India

Ganesh Dattatraya Saratale
Department of Food Science and Biotechnology
Dongguk University
Seoul, Republic of Korea

Mohd Arshad Siddiqqui
School of Environment and Sustainable Development
Central University of Gujarat
Gandhinagar, India

Shalini Singh
Department of Microbiology
School of Bioengineering and Biosciences
Lovely Professional University
Punjab, India

Snigdha Singh
School of Environment and Sustainable Development
Central University of Gujarat
Gandhinagar, India

Sunil Soni
School of Environment and Sustainable Development
Central University of Gujarat
Gandhinagar, India

M. Soniya
College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi, India

A. Suresh
University College of Technology Osmania University
Hyderabad, India

9 Plant Growth-Promoting Rhizobacteria (PGPR) Activity in Soil

A. Suresh, P.M. Sameera, J. Chapla, and P. Rajarao

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9.1 INTRODUCTION

Soil is considered a good host of microbial activities; the microbes occupy less than 5 percent of total space in soil. Soil is the natural growth medium for plants and microorganisms. The dynamic environment, which harbors the diverse groups of microbes, is confined to the aggregates with accumulated organic matter. The major microbial habitat around the plant root is called the rhizosphere and it was defined by Hiltner (1904) as the volume of soil, influenced by the presence of living plant roots, whose extension may vary with soil type, plant species, age and other factors (Foster 1988). The rhizosphere is a hot spot of microbial interactions as exudates released by plant roots are the main food sources for microorganisms and a driving force of their population density and activity. The ratio of the microbial load of the rhizosphere soil to the non-rhizosphere soil is generally known as the rhizosphere effect. Several factors such as soil type, soil moisture, pH, temperature, age and conditions of the plant are known to influence the effect of rhizosphere. The actual root surface colonized by microorganisms is often referred to as rhizoplane. A specific group of microorganisms inhabits this microhabitat. Most of the fungi inhibit the root surface in a mycelial state. Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates, and also the corresponding ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere Curl and Truelove 1986). There is a continuum of bacterial presence in the soil rhizosphere, rhizoplane and internal plant tissues Hallmann et al. 1997).

Microorganisms are the main foundation for the maintenance of soil ecosystems and microbial diversity. Microorganisms are bacteria, actinomycetes and fungi, occupying an important niche in every ecosystem. They are essential in N, P, K and S cycles in nature, but also play an important part in the decomposition of organic matter. Microorganisms such as bacteria are the most common and grow rapidly, having the ability to utilize a wide range of substances, such as carbon or nitrogen sources. The most prominent group of bacteria in the rhizosphere are the non-sporulating gram-negative rod-shaped bacteria. Though fungi and actinomycetes are present, their populations are negligible compared to bacteria.

9.2 PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR)

Numerous species of soil bacteria which flourish in the rhizosphere of plants may be grown in or around plant tissues, and stimulate growth with a plethora of mechanisms. These bacteria are collectively known as plant growth-promoting rhizobacteria (PGPR). The abbreviation PGPR has become common usage ever since it was first used by Kloepper and Schroth (1978).

Rhizobacteria that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as plant growth-promoting rhizobacteria. Rhizosphere bacteria that favorably affect plant growth and yield of crops are referred to as plant growth-promoting rhizobacteria (PGPR).

Soil is the natural growth medium for living plants and microorganisms. A soil-plant ecosystem depends on microbial activities which in turn contribute towards improving soil health, environmental quality and crop production. Bacteria are the most dominant group of microorganisms in the soil and probably equal to one half of the microbial biomass present. The most abundant genera of bacteria present in the soil are *Acetobacter*, *Actinoplanes*, *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Cellulomonas*, *Clostridium*, *Flavobacterium*, *Pasteuria*, *Rhizobium* and *Bradyrhizobium,* fluorescent pseudomonads, *Micrococcus*, *etc*., which are large groups among PGPRs.

Thus, the plant growth-promoting microorganisms (PGPMs) are defined by three intrinsic characters: (i) ability to colonize the root, (ii) survive and multiply in microhabitats associated with the root, in competition with other microbiota, to express their plant growth and protection activities and (iii) promote plant growth Gamalero et al. 2004).

In recent years it has been proven that root colonization indeed is required for some biocontrol mechanisms, such as antibiosis and competition for nutrients and niches (CNN) (Uren NC 2007). Beneficial effects of rhizospheric bacteria have often been based upon increased plant growth, faster seed germination, better seedling emergences, enhanced nodulation and nitrogen fixation in leguminous crops and suppression of disease. Then, PGPR have been further divided into subsets like emergence-promoting rhizobacteria (EPR); nodulation-promoting rhizobacteria (NPR) and disease-suppressing rhizobacteria (DSR). Beneficial plant-microbe interactions in the rhizosphere are the determinants of plant health and soil fertility (Jaffries et al. 2003). They include different PGPR bacteria like *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Acetobacter*, *Burkholderia* and Bacilli (Glick 1995).

9.3 MECHANISM OF GROWTH PROMOTION OF PGPR

Plant growth-promoting bacteria are rhizospheric bacteria with the ability to stimulate and enhance plant growth through different mechanisms (Glick et al. 1999). Plant growth-promoting bacteria may be important for plant nutrition by increasing N and P uptake by the plants and playing a significant role as PGPR in the biofertilization of crops (Cakmakci et al. 2005). These are thought to improve plant growth by colonizing the root system and pre-empting the establishment of suppressing deleterious rhizosphere microorganisms on the roots (Figure 9.1).

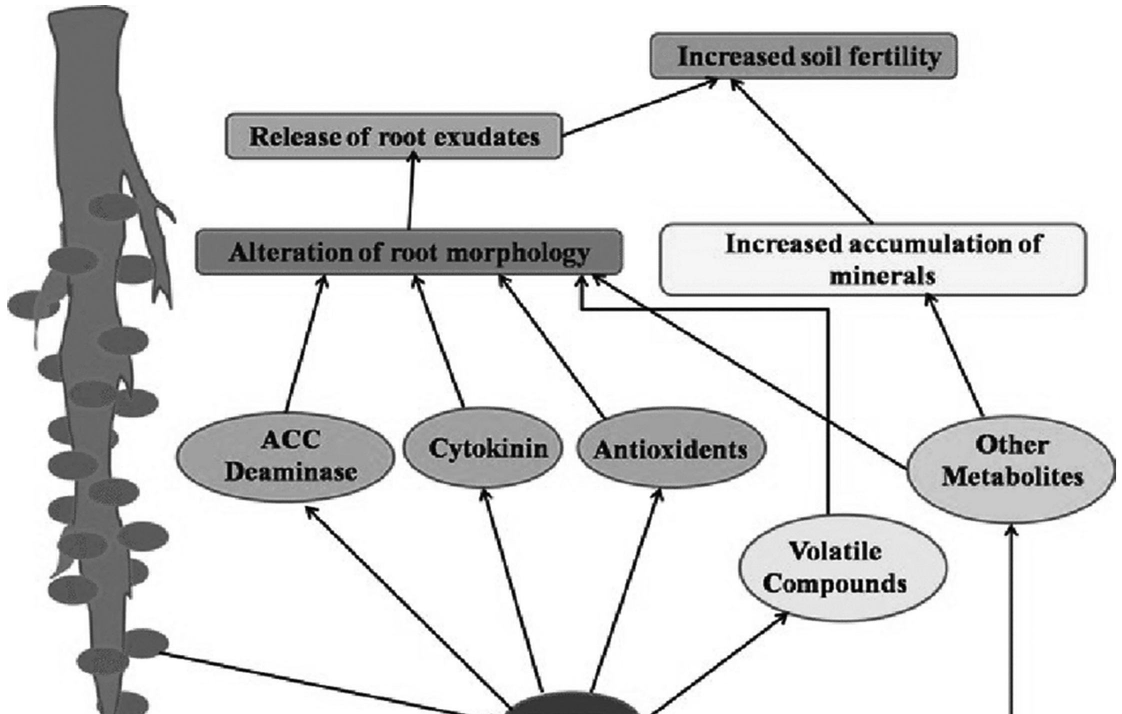


FIGURE 9.1 Soil fertility enhancement mechanism through PGPRs.

These mechanisms include the production of plant growth hormones, auxin (Khakipour et al. 2008) and 1-aminoacyclopropane-1-carboxylic acid deaminase (ACC) increased solubility of immobile nutrients such as P and Fe (by producing siderophores), fixation of atmospheric N and neutralizing the unfavorable effects of pathogens on plant growth (Jalili et al. 2009).

Plant hormones are very effective on plant growth and development, and among them is IAA (auxin), one of the most important growth regulators (Stepanova et al. 2008), often produced by rhizospheric bacteria. Production of auxin at the rates higher than the plant's requirement produces extra amounts of ACC, which is a prerequisite for ethylene production and is catalyzed by ACC-oxidase (Table 9.1). However, in some plants, ethylene can increase seed germination and interrupt seed dormancy (Glick et al. 1994). Under soil stress conditions, there is an increase in the ethylene level and a decrease in the plant growth. PGPR are able to alleviate such stresses on plant growth by producing ACC-deaminase enzyme, which turns ACC into ammonium and α-ketobutyric acid (Penrose and Glick 2003).

PGPR can affect plant growth in two different ways: direct and indirect. The direct promotion of plant growth by PGPR for the most part entails either providing the plant with a compound that is synthesized by the bacterium, or facilitating the uptake of certain nutrients from the environment. The indirect promotion of plant growth is when PGPR prevent the deleterious effect on one or more phytopathogenic organisms. These two mechanisms by which PGPR promote plant growth have been reviewed (Lata et al. 2002). It has been suggested that the production of plant hormone-enhanced nutrient uptake and suppression of phytopathogenic microorganisms such as auxins (Asghar et al. 2002), cytokinins (Arkhipova et al. 2005) and gibberellins (Joo et al. 2004) as well as through the solubilization of phosphate minerals. Indirect growth promotion occurs through the elimination of pathogens by the production of ß 1,3-glucanase (Fridlender et al. 1993), antibiotics (Raaijmakers et al. 1997), cyanide (Owen and Zdor 2001) and siderophores (Pidello 2003). Many PGPR stimulate the growth of plants by helping to control pathogenic organisms (Zehnder et al. 2001) (Table 9.1).

TABLE 9.1

Influence of Inoculations of PGPRS on Growth of Chili Cultivar under Greenhouse Conditions (45 DAS)

Isolate	Plant Height (cm)	Shoot Dry Weight (grams)	Root Length (cm)	Root Dry Weight (cm)
PGPR1	15.5	10.02	3.7	2.10
PGPR2	6.4	7.05	3.3	1.0
PGPR3	11.5	7.6	6.6	2.50
PGPR1004	8.5	6.89	2.9	1.57
Control	6.3	5.86	4.8	1.02
LSD(0.05)	0.003	0.020	0.016	0.010

Values are mean of three replicates and significant at $P<0.05$

PGPR enhance the nutrient status of host plants, and these improvements may be categorized into:

1. Biological nitrogen fixation
2. Increase of the availability of nutrients in the rhizosphere
3. Enhancement of the root surface areas
4. Beneficial symbiosis of the host
5. Combination of modes of action

Many PGPR increase the availability of nutrients for the plant in rhizosphere. It involves the solubilization of unavailable nutrients or siderophores production, which facilitates the transport of certain nutrients. Bacterial-associated root increases in height are reported in response to PGPR. Most incidents noted an increase in root length and root surface area (Singh and Purohit 2008).

The PGPR and the mechanisms involved by which it promotes plant growth are ambiguous and not fully understood, but are thought to include the following characters, termed "PGPR traits" (Cattelan et al. 1999):

- i) The ability to produce or change the concentration of plant hormones like indoleacetic acid (Mardukhova et al. 1984; Abbas et al. 2009; Karnwal 2009), gibberellic acid (Mahmoud et al. 1984; Gutierrez et al. 2001), cytokinins (Tein et al. 1979; Saleena et al. 2001) and ethylene (Arshad and Frankenberger 1991; Glick et al. 1995).
- ii) Asymbiotic nitrogen fixation (Boddey and Dobereiner 1995; Kennewdy et al. 1997) and symbiotic nitrogen fixation.
- iii) Antagonism against phytopathogenic microorganisms by the production of siderophores (Scher and Baker 1982; Alexander and Zuberer 1991), β-1,3-glucanase (Fridlender et al. 1993) chitinases (Renwick et al. 1991), antibiotics (Shanahan et al. 1992) and cyanide (Flaishman et al. 1996; Souza and Raaijmakers 2003; Sunish Kumar et al. 2005).
- iv) Solubilization of mineral phosphates and other nutrients (Sperber 1958a, b; Chen et al. 2006; Pandey et al. 2006).

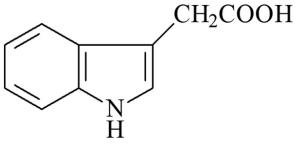
The microorganisms that fall under the broad category of PGPR are: *Rhizobia*, *Azotobacters*, *Azospirillum*, *Acetobacter*, phosphate solubilizers, *Mycorrhizae*, *Pseudomonas*, especially fluorescent pseudomonads.

9.4 MECHANISMS OF DIRECT GROWTH PROMOTIONS

9.4.1 PRODUCTION OF PLANT GROWTH REGULATORS

The mechanism most commonly invoked to explain the various effects of PGPR on plants is the production of phytohormones, the most common and well-characterized is IAA, which is known to stimulate both rapid and long-term response in plants. Many rhizosphere bacteria produce IAA in culture media, especially in the presence of tryptophan.

Naturally occurring substances with indole nucleus possessing growth-promoting activity are referred to as auxins. In many host-parasite interactions, definitive alterations in auxins (indole-3-yl-acetic acid, IAA) have been reported (Mahadevan 1984).



Chemical structure of Indole-3-acetic acid

Plant extracts, plant growth hormones or other natural products recently have recently been substituted for the chemical control of disease (Bekheit 2002).Indole-3-acetic acid (IAA) is well known for being a bioactive growth regulator, controlling stem elongation, geotropism, apical dominance, root initiation, etc.It has also been established that it is able to induce a concomitant resistance against phytopathogen attack through the regulation of defense mechanisms in plants (Mayda et al. 2000). IAA, in controlling diseases, not only induces resistance in a host but may also extend to the pathogen itself (Sharaf and Farrag 2004). The inhibitory activity was detected for several pathogenic fungi like *Gaeumannomyces graminis* var. *triticii*, *Rhizoctonia cerealis*, *Helminthosporium sativum* and *Phytophthora capsici*.

There are numerous soil microorganisms involved in the synthesis of auxins in pure culture and soil (Barazani and Friedman 1999). Some microorganisms produce auxins in the presence of a suitable precursor such as L-tryptophan. The concentration effects of auxins on plant seedlings are that low concentration may stimulate growth, while high concentrations may be inhibitory (Arshad and Frankenberger 1991). Different plant seedlings respond differently to variable auxin concentrations (Sarwar and Frankenberger 1994) and also different types of microorganisms.

Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxin as secondary metabolites because of rich supplies of substrates exuded from the roots compared with non-rhizospheric soils. Plant morphogenetic effects may also be a result of different ratios of plant hormones produced by roots as well as by rhizosphere bacteria. Diverse soil microorganisms including bacteria, fungi and algae are capable of producing physiologically active quantities of auxins which may exert pronounced effects on plant growth. Plant hormones are very effective for plant growth and development, and among them auxin (IAA) is one of the most important growth regulators often produced by rhizospheric bacteria (Mayak et al. 2004). Production of auxin at a rate higher than the plant requirement results in extra amounts of 1-aminoacyclopropane-1-carboxylic acid deaminase (ACC), which is a prerequisite for ethylene production and is catalyzed by ACC-oxidase.

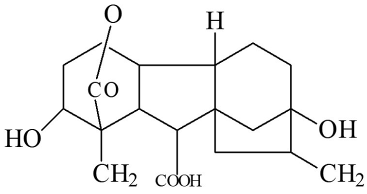
Plant growth-promoting rhizobacteria (PGPR) producing plant growth regulators play an important role in plant growth promotion. The effect of these plant growth regulators on the plant are concentration-dependent. Moreover, IAA of *pseudomonas* origin induces some resistance in plants, for instance it stimulates resistance in *Phaseolus vulgaris* against *Colletotrichum vulgari s*(Huges and Dickerson 1990; Singh et al. 2018, 2017a, b, c, 2019; Tiwari et al. 2018, 2019a, b; Kour et al. 2019a).

The inhibitory activity of IAA was also detected for several pathogenic fungi like *Gaeumannomyca graminis var tritici*, *Rhizoctonia cerealis*, *Helminthosporium sativum* and *Phytophthiriacapsici* (Lu et al. 2000). The PGPR-producing IAA hormones are known to have a dual role in influencing plant growth by involvement in the biocontrol, together with glutathione-s-transferases in defense-released plant reactions and inhibit the germination of spore and growth of mycelium of different pathogenic fungi. IAA hormone, when supplied to excised potato leaves, eventually reduced the severity of the disease provoked by *Phytophthora infestans* (Martinez et al. 2001). Hence plant growth hormones are seen as potential biocontrol agents.

The ability of pseudomonad-produced auxin can very much affect plant growth (Khakipour et al. 2008), as it has some very important functions in plants such as hormonal adjustment, plant cell division, development and nodule formation. Since tryptophan is necessary for auxin production, its production at the control medium can be related to bacterial cell degradation (Frankenberger and Brunner 1983). According to Benizri et al. (1998), although there was not any tryptophan in corn root exudates, the *Pseudomonas fluorescens* strain M31 was able to produce auxin. Table 9.1 shows PGPR strains involved in phytohormone production in different plants (Jha and Bhattacharyya 2012).

9.4.2 PRODUCTION OF GIBBERELLIC ACID

Gibberellins are cyclic diterpens containing 20-carbon in their structure. They contain the skeleton enantiomers of gibberallane, which proves to be advantageous, as it enables the use of a uniform numbering system. Similar to other cyclic diterpens, so far more than 110 gibberellins have been identified.



Chemical structure of Gibberellic acid (GA_1)

Phytohormone production including gibberellins (Fulchieri et al. 1993; Lucangeli and Bottini 1997) is one mechanism that has been proposed. Gibberellic acids are a class of phytohormones with many demonstrated effects on a number of physiological processes (Davies 1995). Among 130 gibberellic acids identified thus far from plants, fungi and bacteria, GA_1, GA_3 and GA_4 are the three most common directly effective gibberellic acid shoot elongation promotions.

Gibberellic acid, which comes from a naturally produced growth hormone, is a member of a type of plant hormone called gibberellins, which regulate the growth rate of plants. Gibberellins are involved in several plant development processes and promote a number of desirable effects including stem elongation, uniform flowering, reduction in the time of flowering and increase in the flower number and size (Jaleel and Gopi 2009). Gibberellic acid is known to increase the antioxidant metabolism and alkaloid production in *Catharanthus roseus* (Jaleel et al. 2009).

Leaf shape and plant structure also be affected by altered levels of gibberellic acids and the application of exogenous GA_3 (Aguirre and Blanco 1992). Since GA_1 is thought to stimulate cell elongation, thus higher GA_1 content in tissues may also be associated with stem elongation and subsequent bloating (Jaleel et al. 2009). Stem swelling and alterations in leaf anatomy with gibberellic acid applications were reported in mustard (Xu et al. 2008; Singh et al. 2016).

Gibberellins are economically and industrially important products. They are commonly used in agriculture, viticulture, gardens and horticulture (Bandelier and Renaud 1997). Gibberillins are naturally present in plants in which they act as growth regulators. At an industrial scale, they are produced primarily by submerged fermentations using *Gibberella fujikorii*. They can also be obtained from several bacterial sources such as *Azotobacter*, *Pseudomonas* and *Azospirillum* (Basiacik and Nilufer 2004). Gibberellins are produced by microorganisms as typical secondary metabolites; upon exhaustion of nitrogen sources, exponential growth ceases and secondary metabolism is triggered (Gelmi and Perez 2000). More than ninety different types of gibberellins are known to occur in higher plants and microorganisms (Mander and Owen 1996).

9.4.3 NITROGEN FIXATION

Some diazotrophic PGPR supply a portion of the fixed nitrogen required to their plant hosts. Nitrogen-fixing organisms can contribute to nutrition for nitrogen and increased efficiency in the use of the nitrogen plant *Rhizobium*.

9.4.4 UPTAKE OF MINERALS

Several reports suggested that PGPR stimulates plant growth by facilitating the absorption of minerals into the plant, particularly phosphate. PGPR was found to be involved in inorganic phosphate solubilization, most of which were *Pseudomonas* and *Bacillus* species. Toro et al. (1997) studied the effects of mycorrhizal and nodulated *Pueraria phaseoloids* on the yield and nutrition exerted by rhizobacterium P-solubilizing. Significant nutrient stimulation (N, P, K and Ca) uptake was observed with *Azospirillum* sp. fungus-bacteria combinations. A majority of agricultural soils contain large reserves of phosphorus, of which a considerable part is accumulated as a result of regular application. The phenomenon of fixation and precipitation of P is soil dependent on pH. In acidic soils, P is precipitated as Al and Fe phosphates, whereas in calcareous soil, high Ca concentration results in P precipitation. The soil is actually a habitat for a range of organisms that use a variety of solubilization reactions to release soluble phosphorus from insoluble phosphates. The potential of these phosphate-solubilizing microorganisms has been used as bioinoculants for crops grown in less nutrient-present soils and modified to tricalcium phosphate with rock phosphate.

9.4.5 ROOT COLONIZATION

The distribution of bacteria in the rhizosphere can be considered from two angles: from the outside to the inside of the root, or from the root base (seed) to the root tip longitudinally. Distribution results from several dynamic phenomena that occur during the establishment of rhizosphere bacterial populations, such as post-inoculation bacterial migration, attachment to the roots and the ability of the roots to survive and proliferate. It should be remembered before interpreting distribution patterns that the types of measurement procedures and statistical analyzes used partly affect the results (Kloepper and Beauchamp 1992).

It is assumed that PGPR stimulation of plant growth generally requires binding of the bacterium to the plant root. The successful use of either rhizobial or PGPR inoculants in agriculture depends on the inoculation of viable bacteria to the root zones, most commonly achieved by inoculating seeds with the preparation of dormant bacterial cells through coated seeds or bulk inoculants.

9.5 INDIRECT GROWTH PROMOTION MECHANISMS

9.5.1 Production of Siderophores

The availability of iron is extremely limited in the rhizosphere, which is one of the most important nutrients required for the growth of nearly all living organisms. It has been found that Fe-binding ligands called siderophores with high affinity to sequester Fe from the microenvironment have been secreted to survive environmental organisms. One way the plant growth-promoting rhizobacteria can prevent phytopathogens from prolifying and facilitate plant growth by producing and secreting siderophore with a very high affinity to Fe. The secret siderophore molecules bind most of the Fe^{3+} available in the rhizosphere, effectively preventing any pathogens in their immediate vicinity from proliferating due to lack of Fe. As a mechanism for promoting plant growth and the biological control of pathogens, Kloepper et al. (1980) first demonstrated the importance of siderophore production.

The scarcity of bioavailable iron in soil habitats and on plant surfaces foments a furious competition (Loper and Henkels 1997). Under iron-limiting conditions, plant growth-promoting bacteria produce low molecular weight compounds called siderophores to competitively acquire ferric ion (Whipps 2001). Although various bacterial siderophores differ in their abilities to sequester iron, in general they deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity (Loper and Henkels 1999). Some PGPB strains go one step further and draw iron from heterologous siderophores produced by cohabiting microorganisms (Lodewyekx et al. 2002).

9.5.2 Antibiotics

The synthesis of antibiotics is one of the most effective mechanisms a PGPR can use to prevent the proliferation of phytopathogens. Most antibiotics belong to the nitrogen class that contains heterocycles such as phenazines and antibiotics of the type pyrrolintrin. Howell and Stipanovic (1980) reported *P.fluorescences* Pf-5 as the purified antibiotics pyoluteorin and pyrrolnitrin. The cotton damping could be suppressed by *Pythium ultimum* or *Rhizoctonia solani* such as strain production.

The usefulness of *Bacillus* as a source of antagonism to many plant pathogens is well known. Several potent strains from different *Bacillus* species have been tested for their ability to control multiple diseases on a wide variety of plant species. *Bacillus* has ecological benefits as it produces endospores that are tolerant to extreme conditions such as heat and desiccation. *Bacillus* species and actinomycetes share several characteristics that make them attractive to biological control agents including their abundance in the soil by producing various biologically, potentially active metabolites against a range of fungi.

The field of biological control of soil-borne plant pathogenic fungi was revolutionized by fluorescent pseudomonads. They have emerged over the past three decades as the largest and potentially most promising plant growth group promoting rhizobacteria involved in plant disease biocontrol. For several compelling reasons, fluorescent pseudomonads have received the greatest attention as they colonize roots readily in nature. Simple nutritional requirements and the ability to use many carbon sources that exude from roots and compete with indigenous microflora may explain their ability to colonize the rhizosphere. Pseudomonads are also suitable for genetic manipulation. These features make them useful vehicles for supplying the rhizosphere with antimicrobial and insecticidal compounds and plant hormones. Suresh et al. (2016) examined the characteristics of fluorescent pseudomonads such as antibiotic production, hydrogen cyanide, siderophore involved in plant pathogen suppression.

9.5.3 AMMONIA AND CYANIDE PRODUCTION

In 1988 Howell et al. reported volatile compounds such as ammonia produced by *Enterobacter cloacae* were involved in the suppression of *Pythium ultimum*, induced by the damping off of cotton. Hydrocyanic acid (HCN) is produced by many rhizobacteria and postulated to play a role in the biological control of pathogens. Cyanide production (Fig 9.2) in one study was thought to be detrimental to plant growth

FIGURE 9.2 Production of siderophore on CAS agar plate.

(Baker and Schippers 1987). However, in a standardized gnotobiotic system, cyanide has been shown to be involved in the suppression of the black root rot (Voisard et al. 1989) and several other pathogens like *Gaeumanomyces graminis*, causing disease in cereals (Paszkowski 1998). HCN is produced in certain conditions as well as specific growth stages of bacteria. The contribution of this compound in the disease-controlling ability of the producer strain varies among different species and strains (Anith et al. 1999). This volatile substance is studied specifically in pseudomonads for their biocontrol ability. Indeed, many rhizosphere-inhabiting fluorescent pseudomonads exhibit antagonistic effects towards fungal pathogens of plant roots, thereby protecting the plant from disease (Schisler et al. 1997). Among the different mechanisms involved in disease suppression, the production by fluorescent pseudomonads of antimicrobial secondary metabolites such as hydrogen cyanide (HCN) or 2,4-diacetylphloroglucinol (Phl) (Keel et al. 1992) is recognized to be significant for effective biocontrol.

9.5.3.1 Competition

Fluorescent pseudomonads are reported to be nutritionally versatile and grow rapidly in the rhizosphere, thereby excluding the other organisms from reaching the niche.

9.5.4 Lytic Enzymes

Some PGPR strains have been found to produce enzymes that can lyse fungal cells. These enzymes are able to digest and lyse *Fusarium solani* mycelia, thereby preventing the fungus from causing crop loss owing to root rot. Chitinase produced by *S. plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea* (Frankowski et al. 2001). The ability to produce extracellular chitinases is considered crucial for *Serratia marcescens* to act as an antagonist against *Sclerotium rolfsii* (Ordentlich et al. 1988), and for *Paenibacillus* sp. strain 300 and *Streptomyces* sp. strain 385 to suppress *Fusarium oxysporum* f. sp. *cucumerinum*. It has been also demonstrated that extracellular chitinase and laminarinase synthesized by *Pseudomonas stutzeri* digest and lyse mycelia of *F. solani* (Lim et al. 1991). Although chitinolytic activity appears less essential for PGPB such as *S. plymutica* IC14 when used to suppress *S. sclerotiorum* and *B. cinerea*, the synthesis of proteases and other biocontrol traits are involved (Kamensky et al. 2003). The β-1,3-glucanase synthesized by *Paenibacillus* sp. strain 300 and *Streptomyces* sp. Strain 385 lyse fungal cell walls of *F. oxysporum* f. sp. *Cucumerinum* (Singh et al. 1999). *B. cepacia* synthesizes β-1,3-glucanase that destroys the integrity of *R. solani*, *S. rolfsii* and *Pythium ultimum* cell walls (Fridlender et al. 1993). Similar to siderophores and antibiotics, the regulation of lytic enzyme production (proteases and chitinases in particular) involves the GacA/GacSor GrrA/GrrS regulatory systems and colony phase variation (Lugtenberg et al. 2001).

9.5.5 Induction of Systemic Resistance

In many plants, long-lasting and broad spectrum systemic resistance to disease-causing agents including fungal pathogens can be induced by treating the plant or seed with a PGPR. In this case the PGPR appear to turn on the synthesis of some antipathogenic metabolites within the plants in a mechanism that does not involve any direct interaction between the PGPR and the pathogen. Ramamoorthy et al. (2001) identified how many strains of pseudomonads can indirectly protect the plants by inducing systemic resistance against various diseases.

9.5.6 Promotion of Symbiosis/Enhancement of Legume Nodulation

The symbiosis between microorganisms and plants is also influenced by free-living bacteria and thus indirectly stimulates plant growth. Some plant growth-promoting bacterial inocula may interact positively with various symbiotic plant microorganisms such as *Rhizobium*, *Bradyrhizobium*, *Frankia* and mycorrhizal fungi.

9.5.7 Phosphate Solubilization

Phosphorous is considered an essential micronutrient and a great portion of phosphorous from chemical fertilizers becomes insoluble by its conversion to calcium or magnesium salts in soils, and so becomes unavailable to plants. Soil microorganisms transform the insoluble forms of phosphorous into soluble forms and thus influence the subsequent availability of phosphate to plant roots. (Richardson et al. 2001). Phosphate-solubilizing microorganisms have been employed in agriculture and horticulture and have been considered highly significant due to their potential in ecological amelioration. It is believed that microbial-mediated solubilization of insoluble phosphates in soil is through the release of organic acid microbial metabolites (Rodriguez et al. 2004). However, in addition to acid production, other mechanisms can cause phosphate solubilization (Nautiyal et al. 2000). Phosphate solubilization has been reported to depend on the structural complexity and particle size of phosphates and the quantity of organic acid secreted by microbes (Gaur 1990).

Different soil microorganisms including fluorescent pseudomonads play an important role in solubilizing inorganic phosphates that are chemically fixed. This is accomplished mainly by the secretion of organic and inorganic acids by microorganisms. These acids form a chelate with calcium ion in addition to the lowering of pH. Carbon and nitrogen sources greatly influence this process. The secretion of organic acids is intimately related to substrate metabolism by the organisms. The carbon and nitrogen sources are an important parameter for the active proliferation of organisms and the production of organic and inorganic acids (Bagyaraj et al. 2000). Recently, the solubilization of tricalcium and rock phosphates by *Pseudomonas fluorescens* and the influence of different carbon and nitrogen sources have been studied (Patel and Dare 2003).Scientific reports on phosphate solubilization by fluorescent pseudomonads in general are scanty. But we can observe an increase in the surface area covered by the root system and mineral solubilization ability of fluorescent pseudomonads, thus facilitating an increased nutrient uptake, which may increase seedling biomass (Nautiyal 1999).

Phosphate-solubilizing bacteria have been reported for promoting plant growth and enhancing yield (Kapoor et al. 1989). The secretion of organic acids and phosphatase enzymes are common mechanisms that facilitate the conversion of insoluble forms of phosphorous to plant available forms (Richardson 2001). The solubilization of insoluble phosphorous to accessible forms like orthophosphate is one of the important traits of plant growth-promoting rhizobacteria (PGPR).

9.6 PLANT GROWTH PROMOTION AND YIELD

There is an enormous body of work on the application of bacteria for the improvement of plant performance but few bacteria like *Azotobacter*, *Azospirillum* and *Pseudomonas* have been developed as commercial products. The organisms for potential use in agriculture are bacteria belonging to the genera *Pseudomonas* and *Bacillus* sp.

The effects of PGPR on crop growth have been reviewed (Goel et al. 2001). Inoculation of three PGPR isolates each belonging to *Proteus vulgaris*, *Klebsiella planticola* and *Bacillus subtillis* markedly increased the seed yield of sunflower and maize.

Microbial formulations are carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application. They are designed to improve soil fertility and help plant growth by increasing their numbers and thus their biological active root environment (Bashan 1989). The inoculation of seeds (Table 9.1) with PGPR is known to increase nodulation, nitrogen uptake and the growth and yield response of crop plants (Dorosinsky and Kadyrov 1975). Phosphate-solubilizing bacteria are also known to enhance phosphorus uptake, resulting in better growth and a higher yield of crop plants (Johri et al. 2003).

Dobbelare et al. (2002) assessed the inoculation effect of PGPR *Azospirillum brasilense*on the growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering and also an increase in the dry weight of the root system and upper plant parts. Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR were reported by Gravel et al. (2007). Inoculation of maize seeds with *Pseudomonas* strains under experiment conditions resulted in a more visible increase in shoot development, especially during the establishment of the plant. Khalid et al. (2004) showed that responses of wheat growth to inoculation with rhizobacteria depended on the plant genotype and PGPR strains, as well as the environmental conditions.

Burd et al. (2000) reported that plant growth-promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients and facilitating the uptake of nutrients by the plants by decreasing heavy metal toxicity in the plants' antagonizing plant pathogens. The enhancing effect of seed inoculation with rhizobacteria on shoot dry weight and yield of maize were reported by Pandy et al. (1998) and Shaharoona et al. (2006). Such an improvement might be attributed to the nitrogen-fixing and phosphate-solubilizing capacity of bacteria, as well as the ability of these microorganisms to produce growth-promoting substances (Salantur et al. 2006).

The inoculation of three PGPR isolates each belonging to *Bacillus, Pseudomonas* and *Rhizobium* increased yields of sunflower and maize over uninoculated plants grown in fields. Increases in plant height and root and shoot biomass were reported in different isolates of PGPR belonging to fluorescent pseudomonads (Suresh et al. 2016). Similarly, treatment of sunflower seeds with fluorescent pseudomonads resulted in yield increases of 30% in field trails. PGPR are potent inoculants but are not commercialized due to lack of consistency under field conditions. Researches indicate that a combination of PGPR strains (2 or more) which have diverse modes of plant growth promotion or antagonism against soil-borne pathogens are more effective than single strain inoculums. IAA-producing *Bacillus* isolates promoted root growth or nodulation when co-inoculated with Rhizobium species on a *Phaseolus vulgaris* contender in growth chambers. Gupta et al. (1998) reported that two strains of Enterobacter and one strain each of Pseudomonas fluorescens and Bacillus sp. have been found to promote the growth of green gram. Field studies also indicated that PGPR strains in conjugation with Rhizobium or Azospirillum increased the grain yield of chickpea and wheat.

The effects of PGPR on crop growth have been reviewed (Goel et al. 2001). Inoculation of three PGPR isolates each belonging to *Proteus vulgaris*, *Klebsiella planticola* and *Bacillus subtilis* markedly increased the seed yield of sunflower, maize and cotton over uninoculated plants grown in field (Malik et al. 1998). In an exploratory experiment, the inoculation of *Kurthia* spp. a gram-positive, ammonifying, urea hydrolyzing and thermo-tolerant organism isolated from the rhizosphere of wheat resulted in the improvement in growth and yield of rapeseed (*Brassica campestris* var *toria*) in pot culture studies under alluvial soil conditions (Malik et al. 1999).

9.7 CONCLUSIONS

Only those microorganisms which grow in the rhizosphere act as bio-control agents. The rhizosphere functions as a first line of defense for the roots of plants against the attacks on the soil-borne pathogens. Thus, there is a strong need to screen the rhizospheric bacteria having plant growth-promoting ability and to develop the PGPRs. Not only this, the bioinoculants with PGP and bio-control activity will be successful only when rhizosphere competence is able to exert the desired effect on the plants. It is necessary to introduce education to farmers in this regard. In doing so, the negative effects from the use of chemical fertilizers would be replaced by encouraging in the use of PGPRs in crop fields, resulting in high yields, the maintenance of soil fertility and sustainable development, all achieved in a natural manner.

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