15 *Pseudomonas fluorescens*: A Promising Biocontrol Agent and PGPR for Sustainable Agriculture

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Abstract

Indiscriminate use of chemicals as fertilizers and pesticide caused incredible harm to the environment and ecosystem including animals and humans. To replace such type of hazardous agrochemicals, biological solution is provided by nature in the form of microorganisms having capacity to promote the plant growth without substantially harming the environment. One of the biological approaches for the control of different phytopathogenic agents is the use of biocontrol plant growth-promoting rhizobacteria (PGPR), which is capable of suppressing or preventing the phytopathogen damage. The best characterized biocontrol PGPR belong to the bacteria genus *Pseudomonas*. Fluorescent pseudomonads are suitable for application as biological control agents due to their abundant population in natural soils and plant root system and their capability to utilize many plant exudates as nutrient. Fluorescent pseudomonads are known to have important traits in bacterial fitness such as the ability to adhere to soil particles and to the rhizoplane, motility and prototrophy, synthesis of antibiotics, and production of hydrolytic enzymes. Moreover, *Pseudomonas* also possesses plant growth-promoting traits such as nitrogen fixation, phosphate solubilization, iron chelation, and phytohormone production. Such multidimensional utility of fluorescent *Pseudomonas* makes them a bioagent of choice to be exploited in the field of agriculture.

Keywords

Biocontrol • Rhizobacteria • Phytopathogens • Fluorescent • Pseudomonas

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D.P. Singh et al. (eds.), *Microbial Inoculants in Sustainable Agricultural Productivity*, DOI 10.1007/978-81-322-2647-5_15

15.1 Need of Biocontrol Agents

Across the world, plant diseases are major cause of yield loss. The global market for phytosanitary products is dominated by synthetic pesticides (Thakore 2006). There are many disadvantages of using such chemical pesticides which include accumulation of toxic residues in environment and adaptation of pathogens to such chemicals which in turn reduce its efficiency and led to undesirable effect on nontarget organisms prevailing in the same niche. Moreover, nowadays, consumers are becoming more and more concerned about pesticide-free safer foods which results in emergence of eco-friendly strategies for plant disease management, i.e., biocontrol agents.

15.2 What Are Biocontrol Agents ?

Biocontrol agents can be defined as living organisms or natural products derived from living organisms (genetically modified crops, insects, nematodes, and microorganisms; Fig. 15.1) that are used to suppress plant pathogen pest populations.

Among these biocontrol agents, microorganism-based products (bacteria, fungi, virus, and yeasts) represent 30 % of total sales (Thakore 2006). Microbial biocontrol agents are having different modes of action for dealing with pathogens. The application of biocontrol agents and disease suppressing chemicals can reduce the possibility of resistance development among pathogen representing an integrated pest management strategy with the goal of minimizing the use of chemicals. Most of the bacterial strains exploited as biocontrol agents belong to the genera *Agrobacterium, Bacillus*, and *Pseudomonas* (Fravel 2005).

15.3 *Pseudomonas* as Biocontrol Agent

Research carried out at the University of California, Berkeley, during the late 1970s (Weller 1988) has awakened the global interest in the *Pseudomonas* sp. as biocontrol agents. Species of fluorescent *Pseudomonas* are capable of utilizing wide range of organic and inorganic compounds which imparts them capacity to live in varied environmental conditions. Members of this genus are found in large numbers in all the major natural environments, viz., terrestrial, freshwater, and marine, and they also form intimate associations with plants and animals. This widespread dispersal suggests a significant amount of physiological and genetic flexibility (Nowak-Thompson et al. 1997). The bacteria belonging to genus *Pseudomonas* are functionally diverse and ecologically noteworthy microorganisms because of their multiple utility as plant growth-promoting agents and bioremediators. Pseudomonads are gram-negative, chemoheterotrophic, and motile rods with polar flagella as defined by Palleroni (1984). *Pseudomonas* has been recognized as a complex collection of a large number of described species (Gardener et al. 2005). The functional and metabolic heterogeneity of *Pseudomonas* has been well documented from comprehensive studies dating to more than 45 years ago. Species of the genus *Pseudomonas* embodies an attractive biocontrol agent because of their catabolic adaptability, their outstanding root-colonizing abilities, and their capacity to produce a wide range of antifungal metabolites. Among various *Pseudomonas* spp., fluorescent pseudomonads have received particular attention as biocontrol agent of choice. *Pseudomonas* exerts its biocontrol activity through direct antagonism of phytopathogens and induction of disease resistance in the host plant (Cartieaux et al. 2003). Fluorescent *Pseudomonas* is a widely studied group among common inhabitants of the rhizosphere. They can be visually distinguished from the other *Pseudomonas* species of soil by their ability to produce water-soluble yellow-green pigments. They comprise of *P. aeruginosa*, the type species of the genus, *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, and the plant pathogenic species *P. cichorii* and *P. syringae* (Landa et al. 2003; De La-Funte et al. 2006). *Pseudomonas* spp. are well adapted for inhabiting in the rhizosphere. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller 1988). These include their ability to (1) grow faster which makes them

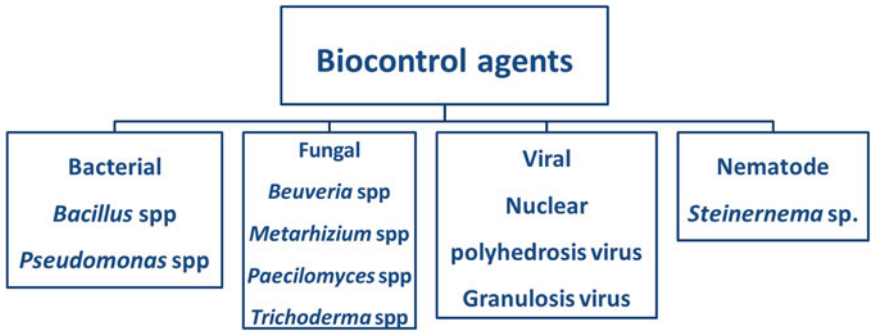

Fig. 15.1 Classification of biocontrol agents

easy to be mass produced in the laboratory, (2) readily consume seed and root exudates, (3) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant, (4) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances), (5) compete aggressively with other microorganisms, (6) adapt to environmental stresses, and (7) easily colonize plants upon subsequent reinoculation in soil by seed bacterization. The presence of pseudomonads in soil provides natural suppressiveness to the soil against some soil-borne pathogens (Weller et al. 2002).

Several strains live in commensal relationship with plants, protecting them from infection by pathogens that would otherwise cause disease. Control of root diseases by beneficial bacteria involves a blend of possible mechanisms that may complement each other. The primary mechanism of biocontrol includes production of antibiotics or inactivation of virulence trait of pathogens (Diby et al. 2005). Another important mechanism is the indirect inhibition of the pathogen by bacterial stimulation of defense responses in the plant host. Many of the plant-associated strains belong to fluorescent *Pseudomonas* group, which currently includes more than 50 named species (Yamamoto et al. 2000; Mulet et al. 2010).

Pseudomonas plays key role in better growth and development of plant through its capacity to protect plants against pathogens during various developmental stages. The above said benefit of pseudomonads depends on their ability to efficiently consume root exudates and resist predation by soil predators such as nematodes and protozoa (De Mesel et al. 2004; Abuzar and Haseeb 2010). Bacteria have evolved an array of antipredatory mechanisms, such as toxicity. Extracellular metabolites of *Pseudomonas* sp. drive complex interactions with predators, affecting their physiology and behavior. Secondary metabolite works specifically on predators, acting as repellents, stressors, or toxics. Production of such secondary metabolites by biocontrol bacteria serves multiple functions, and metabolites protecting plants against pathogens improve bacterial resistance (Gadoury et al. 1989).

Pseudomonas sp. can utilize variety of organic compounds as energy sources and produce an array of secondary metabolites foremost as 2, 4-diacetylphloroglucinol (DAPG, Phl), lipopeptides, phenazines, pyrrolnitrine, pyochelin, and hydrogen cyanide (Keel et al. 1992; Haas and Defago 2005). Biocontrol strains of *Pseudomonas* sp. with a proven effect in plant bioassays produce one or several antibiotic compounds. In vitro, these antibiotics have been proven as inhibitory compounds, and they are also showing active response for the plant health management in field conditions. Strains that produce the antifungal compound DAPG play an important role in the suppression of some root diseases when introduced into the rhizosphere via seed or soil treatments (Reddy et al. 2009). *Pseudomonas* sp. plays a key role in suppression of plant diseases and commercially exploited for plant disease management in agriculture sector. Biological control of plant diseases through antagonistic bacteria is less popular among the farming com-

munity in comparison to other disease control measures, but it has potential to transform plant disease management strategies.

15.4 Concept of Disease Suppressive Soil

Suppressive soils are soils in which phytopathogens are unable to persist or are present but fail to induce severe disease symptoms on susceptible crops. Plants are protected from diseases generally caused by soil-borne phytopathogens such as bacteria, fungi, and even nematodes in suppressive soils. Suppressiveness in soil is mainly attributed to the presence of high number of antagonistic bacteria having disease suppressive properties. Here the plant roots harbor plant-beneficial microbial communities which are having general beneficial effect on plant health and thereby also known as plant probiotics. Pasteurization of soil results into loss of disease suppressiveness which proves that microorganisms play an important role in disease suppressiveness of soil. Most of the soil pathogens such as fungi, bacteria, and plant-deleterious nematodes get suppressed in such soils. Dominant microfloras of suppressiveness in soil are *Trichoderma*, *Pseudomonas*, and *Bacillus* species. How these bacteria achieve this and what they have, to protect plant from pathogenic fungi, have been analyzed in biocontrol strains of fluorescent *Pseudomonas. Pseudomonas* competitively colonizes plant roots and stimulates plant growth and/or reduces the incidence of plant disease. *Pseudomonas* acts by production of antibiotics or by induction of systemic resistance within the plants during its colonization. It also has reported that growth regulatory compounds and beneficial enzymes are present in them (Haas and Defago 2005). *Pseudomonas* owes their fluorescence due to extracellular diffusible pigments such as pyoverdin (Pvd), pyochelin, and ferripyoverdin (Pvd Fe^{3+} complex) (Paez et al. 2005). The phenomenon of natural suppressive soils has been described for *Gaeumannomyces graminis* var. *tritici* (take-all of wheat), *Fusarium oxysporum* (wilt), *Phytophthora cinnamon* (root rot), *Pythium* spp. and *Rhizoctonia solani* (damping-off of seedling), *Thielaviopsis basicola* (black root rot), *Streptomyces scabies* (bacterial scab), *Ralstonia solanacearum* (bacterial wilt), and *Meloidogyne incognita* (root swelling and root-knot galls) (Haas and Defago 2005).

15.5 Mechanism of Biocontrol by *Pseudomonas*

Over the last few years, a great diversity of rhizosphere microorganisms has been described, characterized, and, in many cases, tested for activity as biocontrol agents against soil-borne plant pathogens. Such microorganisms can produce substances that may limit the damage caused by phytopathogens, e.g., by producing antibiotics, siderophores, and a variety of enzymes or by induction of systemic resistance in host plants. These microorganisms can also function as competitors of pathogens for colonization sites and nutrients. The major mechanisms by which *Pseudomonas* exerts its biocontrol effect are:

1. Competition for niche and nutrient acquisition
2. Antibiotic production
3. Induced systemic resistance

15.5.1 Competition for Niche and Nutrient Acquisition

The high microbial diversity, density, metabolic activity, and competition occurring in the rhizosphere environment represent a challenging "biological buffering" (Keel et al. 1996) that generally limits the establishment of exogenous, foreign microorganisms into the rhizosphere. Thereby, it is essential to evaluate the ability of introduced pseudomonads to colonize roots and provide protection against major and minor soil-borne pathogens. Several definitions of root colonization by rhizobacteria were proposed (Lemanceau et al. 1995; Van Loon et al. 1998), and that defines microbial colonization of plant as movement of the rhizobacteria from an inoculum source to the roots, multiplication, and persistence in the presence of native soil microflora. Weller et al. (2002) defined root colonization as the process

whereby rhizobacteria introduced into the seeds, vegetative propagated plant parts, or soil become distributed along roots growing in raw soil, multiply, and then survive for several weeks in the presence of indigenous soil microflora. Root colonization included colonization of the rhizosphere, rhizoplane, and/or inside the root. Rhizosphere competence describes the relative root-colonizing ability of a rhizobacterium. Bacterial inoculants become more powerful when they multiply on the root and colonize it. So the establishment of inoculant is an important factor for the disease suppression by bio-inoculant. Root colonization not only results in high population densities on the root system, it also functions as the delivery system of antifungal metabolites along the whole root. The extent of colonization ability of applied strain may also be dependent on the mechanism by which a biocontrol agent performs its action. The biocontrol of plant disease can be achieved by antibiosis wherein optimum colonization is needed for delivery of antifungal compounds to entire root system, whereas for ISR colonization of plants by limited number of bacteria is sufficient to induce ISR response in plant. The speed and degree of colonization by biocontrol is supposed to be an important trait. Most of the *Pseudomonas* strains are having short generation time. Microcolonies of *P. fluorescens* WCS365 appeared on the tomato root (Chin-A-Woeng et al. 1997; Bloemberg et al. 2000) 1 day after seed inoculation. Bacterial antagonist generally colonizes intracellular junction between root epidermal cells as they are nutritionally rich which represent small surface area of total root surface area (Chin-A-Woeng et al. 1997). Dhingani et al. (2013) studied colonization of fluorescent *Pseudomonas* isolates as a plant growth-promoting attribute. They isolated 30 isolates of fluorescent *Pseudomonas* from six different locations of Junagadh district, Gujarat, India, and confirmed various PGPR traits present in the fluorescent *Pseudomonas* which may help in the improved plant growth promotion during colonization with suppressive rhizospheric soils. Many of the biocontrol systems are dependent on positive relationship between colonization and pathogen suppression. During the last 40 years, the process of root colonization, the biotic and abiotic factors affecting colonization, and the bacterial genes and traits that contribute to rhizosphere competence has been clearly elucidated from the experimental systems using *Pseudomonas* sp.

Soil area around the root and influenced by root is known as rhizosphere (Hiltner 1904) which is richer in microbes than bulk soil. The rhizospheric microflora is mainly affected by root exudates that contain organic acids, sugars, and amino acids. Biocontrol agents applied to the soil have to race with injurious microorganisms and pathogens for limited available nutrients in root exudates and suitable colonization niches and finally outnumber them. After inoculation, the biocontrol agent can cause inhibition of soil pathogen only for a short period of time. Soil microorganisms have to become highly dependent upon nutrients present in the rhizosphere or root exudates. So, we can assume that there must be strong competition for nutrients between the biocontrol agent and the indigenous microflora in the rhizosphere of the host plant. Native microbial strains or aggressively colonizing biocontrol bacteria can therefore prevent the establishment and consequent deleterious effects of a pathogen. The ability of pseudomonads to establish in niche and rapidly compete for nutrient acquisition is thought to be a general mechanism for antagonistic activity dispersed by biocontrol strains of pseudomonads and thereby acting as plant probiotic. Fungal pathogens can be eliminated from the soil by increasing competition for nutrients such as carbon, nitrogen, or iron which in turn reduce the ability of fungal pathogens to proliferate in the soil (Leong 1986; Loper and Buyer 1991). The generation time of pseudomonads is 3–6 h in rhizosphere which is slower than that in nutrient-rich laboratory media as microorganism in the rhizosphere live under nutrient limiting (Lugtenberg and Kamilova 2009; Haas and Defago 2005). Populations of *Pseudomonas* established on the plant roots could act as a sink for the accessible nutrients and limit the nutrient availability for pathogen and its successive root colonization. This mechanism is generally used by fluorescent pseudomonads because of their nutritional versatility and high growth rates in the rhizosphere

(Walsh et al. 2001). Moreover, the pseudomonads compete with indigenous microbial populations for nutrition in the rhizosphere for successful removal of the pathogens. Siderophores are organic compounds produced by pseudomonads which sequester most of the available Fe^{3+} in the rhizosphere and starve the pathogens for their iron requirement and thereby play a main role in defeating pathogens in the same ecological niche (O'Sullivan and O'Gara 1992). Fluorescent siderophores have high affinity for ferric iron, which forms ferric-siderophore complex that becomes unavailable to other organisms, but the producing strain can utilize this complex via a very specific receptor in its outer cell membrane (Koster et al. 1993, 1995; Buyer and Leong 1986). In this way, fluorescent *Pseudomonas* strains may restrict the growth of deleterious bacteria and fungi on the plant root (Loper and Buyer 1991).

Failure of a pathogen to compete effectively with the biocontrol strain and use the available nutrient sources in same ecological niche will restrict the pathogen's spread. A classical example of niche exclusion is the control of leaf frost injury caused by *P. syringae*, which has an ice nucleation protein on its cell surface (Lindow 1983a, b; Lindow et al. 1983). Well-known example of competition for nutrients is limitation of iron as iron – an essential cofactor for growth in all organisms. The availability of Fe^{3+} in soils is lower at neutral and alkaline pH, which in turn leads to Fe^{3+} limitation. Fluorescent *Pseudomonas* species utilize Fe^{3+} by production of siderophores which are high-affinity iron chelating compounds. The capacity of iron scavenging under iron limitation gives the biocontrol organism a selective advantage over phytopathogens that possess less efficient iron binding and uptake systems. As compared to wild-type parental strains, siderophore-deficient mutants were found to be less effective against pathogens (Bakker et al. 1986).

15.5.2 Antibiotic Production

Antibiotic-producing bacterial biocontrol agents occur frequently and are efficient agents for plant disease management as they can be easily isolated from soil. Many factors affect the production of antibiotics such as temperature, pH, and the levels of various metal ions, particularly of Zn^{2+} (Duffy and Defago 1997). Among the variety of *Pseudomonas* species inhabiting the rhizosphere, certain strains of fluorescent pseudomonads have received particular attention because of their potential to control seed- and soil-borne pathogenic fungi and oomycetes (Keel et al. 1992, 1996). Plant-beneficial microorganisms help in exclusion of plant pathogens from rhizosphere through secretion of antimicrobial metabolites which in turn improves plant health (Haas and Keel 2003; Handelsman and Stabb 1996; Raaijmakers et al. 2002; Thomashow and Weller 1996). A triangular interaction occurs among plants, pathogens, and bacteria for regulation of antifungal traits of *Pseudomonas* (Jain et al. 2011). Due to this reason, efficient colonization is required for antibiosis (Chin-A-Woeng et al. 2003), and that's why it is not unexpected that some strains, which show antifungal activity under laboratory conditions, do not act as biocontrol agents in vivo. The identification and quantification of the antibiotics which are produced during biocontrol in situ are a challenge and have been shown only for a few cases (Thomashow and Weller 1996). The slow growth rate of bacteria in the rhizosphere favors the production of secondary metabolites (Haas and Defago 2005). Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites, of which DAPG, phenazines, pyrrolnitrin, pyoluteorin, and volatile hydrogen cyanide are the most frequently detected classes. However, novel antifungal metabolites viscosinamide (Nielsen et al. 1999) and tensin (Nielsen et al. 2001) have been discovered and play a role in protection of plants against phytopathogens. Fluorescent pseudomonads producing antibiotic DAPG are an important group of biocontrol agents for suppressing diseases of roots and young seedlings of various crops, e.g., suppression of black root rot of tobacco by *P. fluorescens* CHA0 (Stutz et al. 1986), take-all of wheat (Keel et al. 1992), and *Fusarium* wilt, crown, and root rot of tomato (Duffy and Defago 1997; Tamietti et al. 1993). Moreover, *Pseudomonas* sp. F113 is found to suppress damping-off of sugar beet (Fenton et al. 1992; Shanahan et al. 1992), and *P. fluorescens* Q2-87 (Harrison et al. 1993;

Pierson and Weller 1994) and Q8r1-96 (Raaijmakers and Weller 1998) suppress take-all of wheat. DAPG-producing strains of *P. fluorescens* are also having a key role in the natural biocontrol of take-all disease (Raaijmakers and Weller 1998; Raaijmakers et al. 1997). The exact mechanism of action of DAPG on pathogens is yet to be discovered. The importance of DAPG as biocontrol molecule has been demonstrated by genetic approaches (Thomashow 1996) as well as direct isolation of disease suppressive strains producing DAPG from rhizosphere of crop plants (Bonsall et al. 1997; Duffy and Defago 1997; Raaijmakers and Weller 1998).

Development of resistance among the human and animal pathogens against the antibiotics used for treatment is believed to be the main risk of using an antibiotic-producing biocontrol agent. Moreover, there is also possibility of transfer of genes encoding the antibiotic production to related strains (Zhang et al. 2003), which seems to be realistic as some conjugative transfers require quorum sensing that are dependent on a high density of microbes. This type of cross transfer of genes is possible in root where pseudomonads form microcolonies under a mucoid layer (Chin-A-Woeng et al. 1997). The genetic material is exchanged at a high frequency in the rhizosphere. These are the reasons for slow process of registration of biocontrol products based on antibiotic-producing microbes.

15.5.3 Induced Systemic Resistance (ISR)

In simple words, ISR can be defined as a broad spectrum plant immune response activated by plant-beneficial bacteria that live in association with plant roots. Few strains of pseudomonads such as *P. fluorescens* (van Loon and Bakker 2006; van Wees et al. 1997; Kamilova et al. 2005) trigger ISR response to combat against a broad spectrum of plant pathogens. Such immunized plants express defense responses faster and stronger after pathogen attack, which results in enhanced level of protection (Van Peer et al. 1991). Such beneficial microbes induce resistance in distant parts of the plants such as leaves, and that's why it is known as ISR response. ISR response induced by beneficial microbes is effective against broad range of pathogens, viz., bacteria, fungi, and viruses (van Loon et al. 1998; van Loon 2007), but the response is believed to be random (Verhagen et al. 2003). There exists the host specificity among the ISR-inducing microbial strains as the ISR induction was found to be dependent on the plant species and cultivar (van Loon and Bakker 2006; van Wees et al. 1997). Generally the plant hormones, viz., jasmonate and ethylene, are believed to be key regulators of ISR response (van Wees et al. 2000). ISR response was observed in many plant-pathogen systems wherein the bacterium and the challenging pathogen remained spatially separated. Many effective biocontrol pseudomonads provoke ISR (Ongena et al. 2004; Ton et al. 2002; Zehnder et al. 2001). ISR does not require complete root colonization. In addition to live microbes, such as *Bacillus*, *Pseudomonas*, and *Trichoderma*, dead microbial cells and some of the products of bacterial metabolites, viz., siderophores, lipopolysachharides, salicylic acid, pyocyanin, and pyochelin as well as organelles such as flagella, are the main inducers of ISR response in plants (Audenaert et al. 2002). Moreover, the volatile 2,3-butanediol (Ryu et al. 2003), the signal molecule AHL (Schuhegger et al. 2006), the antibiotic phloroglucinol (Iavicoli et al. 2003), and some c-LPs (Ongena et al. 2002; Pérez-García et al. 2011) are also believed to be important triggering molecules of ISR response.

15.6 Role of *Pseudomonas* for Plant Growth Promotion

Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller 2007). There are several ways in which different plant growth-promoting *Pseudomonas* have been reported to directly facilitate the proliferation of their plant hosts. The direct promotion of plant growth by PGPR generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment. Direct mechanisms of plant growth

promotion are (1) phytohormone production, (2) nitrogen fixation, (3) siderophore production, and (4) phosphate solubilization.

15.6.1 Phytohormone Production

15.6.1.1 Indole 3 Acetic Acid

Many rhizospheric strains of *Pseudomonas* produce indole acetic acid (IAA) which helps in stimulating plant growth (Loper and Schroth 1986). The phytohormone indole-3-acetic acid (IAA) is known to be involved in root initiation, cell division, and cell enlargement. IAA production by microorganisms increases root length and surface area which in turn enables plants to increase absorption of water and nutrients from their ecosystem (Salisbury 1994). Increase in root length as well as the number of secondary roots in young seedlings through IAA production by microorganisms increases the chances of survival of seedlings due to enhanced capacity to anchor to the soil and absorb water and nutrients from the surroundings (Patten and Glick 2002). In IAA-producing bacteria, L-tryptophan-dependent auxin production was observed and reported to increase the grain yield and the number of branches (Asghar et al. 2002, 2004). Patten and Glick (2002) reported the role of IAA-producing *P. putida* in the development of the host plant root system.

15.6.1.2 Cytokinins

Cytokinins promote cell divisions, cell enlargement, and tissue expansion and are believed to be the signals for mediation of environmental stress from roots to shoots. *P. fluorescens* can produce cytokinins as reported by Garcia et al. (2001).

15.6.1.3 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

The stress hormone ethylene is the only gaseous phytohormone and produced upon physical or chemical to the plants which causes inhibition of plant root growth. Glick et al. (1998) reported that some of the PGRP strains can produce a stress-relieving enzyme named as ACC deaminase that breaks down ACC, which is the precursor for biosynthesis of ethylene in plants. Production of ACC deaminase enzyme by microorganisms can decrease the concentration of ethylene in the plant roots and thereby elongates plant roots (Glick et al. 1994). Shah et al. (1998) reported that insertion of ACC deaminase gene within *Pseudomonas* spp. aided bacteria with capacity to produce ACC deaminase enzyme and thereby release stress which in turn elongates seedling roots. Pseudomonas strains having capacity to produce ACC deaminase enzyme were reported to promote plant growth under stressful condition such as flood (Grichko and Glick 2001) or heavy metal contamination (Burd et al. 1998).

15.6.2 Nitrogen Fixation

The first evidence for nitrogen fixation by *Pseudomonas* like microorganisms has been reported by Anderson in 1955. Nitrogen-fixing ability of members of the genus *Pseudomonas* is poorly understood. The mechanism of nitrogen fixation and the protection of nitrogenase against oxygen deactivation were also not revealed (Young 1992). However, recently several workers demonstrated among the strains of pseudomonads (Desnoues et al. 2003; Krotzky and Werner 1987). The optimum conditions for the nitrogen fixation and structure of genes encoding nitrogenase enzyme in *Pseudomonas* sp. were studied in detail using *P. stutzeri* A15 (A1501), isolated from rice paddies in China (Desnoues et al. 2003). So, one can classify the *Pseudomonas* spp. as nitrogen fixers based on their physiological properties, nitrogenase assays, phylogenetic studies, and detection of *nifH* DNA by hybridization or PCR amplification (Chan et al. 1994; Vermeiren et al. 1999). After detection presence of nitrogen-fixing traits among the species of *Pseudomonas* genus, nitrogen-fixing strains of *Pseudomonas* spp. were reassigned genera in α- and β-proteobacteria (Chan et al. 1994). Krotzky and Werner in 1987 isolated two nitrogen-fixing *Pseudomonas* strains, viz., *P. stutzeri*. and *P. stutzeri* CMT.9.A, from the roots of sorghum, and You et al. (1991) isolated *P. stutzeri* strain A15 from rice paddies from China (You et al. 1991).

15.6.3 Solubilization of Phosphorus

The second important macronutrient required for plant growth is phosphorous. Phosphorous is present in insoluble forms such as iron and aluminum phosphates in acidic soils and calcium phosphates in alkaline soils. In phosphorous-rich soil, only a small proportion of phosphate (~0.1 %) is available to plants (Stevenson and Cole 1999). Phosphate-solubilizing bacteria (PSB) secrete organic acids and phosphatase enzymes to convert the insoluble phosphates into soluble forms. This process is known as phosphate solubilization which leads to an increase in the content of available phosphate for plants (Gyaneshwar et al. 2002). Almost all the soil types contain phosphate-solubilizing bacteria (Gyaneshwar et al. 2002), among which *Bacillus*, *Enterobacter, Erwinia*, and *Pseudomonas* spp. are most prevalent. Generally rhizospheric region of plant is colonized by phosphate-solubilizing bacteria where they bring about solubilization of insoluble inorganic phosphatic compounds. Most commonly the phosphate-solubilizing ability of PGPR strains is dependent on the availability of other macronutrients such as carbon and nitrogen as well as metal ions (Kim et al. 1998). Generally, phosphate-solubilizing bacteria produce various types of organic acids, among which the most abundant is β-ketogluconic acid, a secondary oxidation product of glucose metabolism. The oxidation of glucose is catalyzed by an enzyme glucose dehydrogenase (GDH) present in cytoplasmic membrane of bacteria, and as a result of the enzyme activity, gluconic acid and β-ketogluconic acid are produced which bring about phosphate solubilization.

15.6.4 Sequestering Iron by Siderophores

Iron is essential for life for all living organisms and is required as a component of proteins involved in important processes such as respiration, photosynthesis, and nitrogen fixation.

Despite the abundance of this element on the earth's surface, soil organisms such as plants and microbes have difficulty in obtaining enough iron to support their growth because iron in soil is largely present as insoluble, ferric hydroxides, which cannot be readily transported into cells. Microorganisms and some plants can secrete low molecular weight, organic, iron binding molecules known as siderophores which help in iron scavenging from soil. Each functional group presents two atoms of oxygen or less commonly nitrogen that bind to iron. In general, catecholate-type siderophores are typical to bacteria. It is known that many bacteria, including *Pseudomonas* spp., react to limiting Fe^{3+} concentrations by inducing a high-affinity iron uptake system (Braun 1985; Neilands 1982) consisting of siderophores, Fe^{3+} chelating molecules, and outer membrane receptor proteins with a high affinity for the matching Fe^{3+} siderophore complex (De Weger et al. 1986). Production of siderophores by plant growth-promoting *Pseudomonas* spp. during iron starvation is considered as the one of the mechanism in inhibition of phytopathogens. But whenever the concentration of iron in the medium is sufficient, such antagonism will not be observed (Geels and Schippers 1983). The following scenario was proposed to account for the enhancement of plant growth by the *Pseudomonas* spp. (Kloepper et al. 1980). After the inoculation of seeds, the *Pseudomonas* bacteria rapidly colonize the roots of the developing plant. The limiting Fe^{3+} concentration in the soil induces the high-affinity iron uptake system. The siderophores bind Fe^{3+}, and as an uptake of this Fe^{3+}, siderophore complex requires a very specific uptake mechanism; this binding makes this essential element unavailable for many other rhizomicroorganisms. These microorganisms, including deleterious species, then are unable to obtain sufficient iron for optimal growth since they produce either no siderophores at all or less efficient ones (Raaijmakers et al. 1995). Thus, the population of deleterious microorganisms is reduced, creating a favorable environment for the development of the plants (De Weger et al. 1986).

Several species of fluorescent pseudomonads produce siderophores, and there is evidence that a number of plant species can absorb bacterial siderophore complexes (Bitter et al. 1991).

Pyoverdines (PVDs) or pseudobactins are fluorescent yellow-green siderophores (Budzikiewicz 1997). *P. aeruginosa* produces siderophore pyochelin having lower affinity for iron. Fluorescent pseudomonad species, viz., *P. fluorescens*, *P. stutzeri*, and *P. putida*, produce siderophore named as pseudonlonine (Lewis et al. 2000; Mossialos et al. 2000; Mercado-Blanco et al. 2001).

15.7 Scope of *Pseudomonas* as Biocontrol Agent

The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria, i.e., *P. fluorescens*, to increase plant growth has shown considerable promise in laboratory and greenhouse studies. The potential environmental benefits of this approach, leading to a reduction in the use of agricultural chemicals, fit with sustainable management practices. We can expect to see new *P. fluorescens* products becoming available to farmers as a biofungicides. The success of these products will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Sequencing the genome provided further information of its environmental interactions and its metabolic capabilities, which can be used to control plant diseases. Though *P. fluorescens* is the most widely used biocontrol agent, the major limitation is not only its shelf life but also inconsistent field performance.

15.8 Conclusion

Unlike chemical pesticides, biocontrol agents need support even after their application to get established in targeted niche. Therefore, for the success of biological control, one has to ensure not only the quality of biocontrol agent applied but also its establishment in natural ecosystem to thrive and compete well with the pathogens. Development of better formulations to ensure survival of activity in the field and compatibility with chemical and biological seed treatments is another area of focus. *P. fluorescens* as bioagent has good prospectus in the future as it gives very high cost-benefit ratio. In view of this, the first assumption is to isolate the *P. fluorescens* bacteria from the rhizosphere of various field crops with enhanced antagonistic activity against soil-borne fungal pathogens under native environmental conditions and determine the ability of selected bacterial isolates to suppress the soil-borne fungal pathogens under in vitro conditions.

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