Biological Control of Pest and Diseases Using Fluorescent Pseudomonads

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Abstract

Bacteria which are shown to have potential for biological control of destructive diseases are distributed in many genera. Among them, fluorescent pseudomonads are currently considered as the most effective bacteria for biological control of soil and foliar diseases. Fluorescent pseudomonads enhance the plant growth parameters, and hence, they are called plant growth-promoting rhizobacteria (PGPR). PGPR are known to control a wide range of phytopathogens like fungi, bacteria, viruses, insect pests and nematodes, and they are known to control these pathogens by biocontrol mechanism which may be by competition, or antagonism, induction of systemic resistance by these bacteria in the host plant, thereby containing the invading pathogens. For the management of pest and diseases of crop plants, applications of strain mixtures of PGPR formulations perform better than individual strains. Fluorescent pseudomonads showing various modes of action especially rhizosphere colonization, antibiotic production and induction of systemic resistance would certainly be potential biocontrol agents for the management of pest and diseases of crop plants.

Keywords

Biocontrol • PGPR • Formulations • Delivery systems • Mechanisms

2.1 Introduction

Biological control of plant diseases opened an era of new technology to manage crop diseases and received the attention of researchers throughout the world, which will enhance the sustainability of agricultural production systems, and to reduce the use of chemical pesticides. The most promising group of plant growth-promoting

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rhizobacteria (PGPR) for biocontrol of plant diseases is fluorescent pseudomonads. Fluorescent pseudomonads associated with plants include *Pseudomonas fluorescens, P. putida, P. aeruginosa* and *P. aureofaciens*. Many of the fluorescent pseudomonads, predominantly *P. fluorescens*, have been isolated from suppressive soil for the management of soilborne and foliar diseases. *P. fluorescens* and *P. putida* are easy to isolate and grow in the laboratory, and they are not fastidious in their requirement of nutrition. They are normal inhabitants of the soil and especially of root surface of plants, so they grow well on the root surfaces when introduced artificially. Their generation time is as low as 5.2 h. Among the various biocontrol agents, fluorescent pseudomonads are known to survive both in rhizosphere and in phyllosphere (Wilson et al. 1992).

Rabindran (1994) highlighted that the culture filtrate of *P. fluorescens* isolate PfALR2 caused 100 % reduction in the germination of sclerotia and reduction in the virulence of sclerotia of *R. solani*. Development of resistance in cucumber against *P. syringae* pv. *lachrymans* as well as to the fungal pathogens *Fusarium oxysporum* f. sp. *cucumerinum* and *Colletotrichum orbiculare* by application of *P. fluorescens* 89B-27 was reported (Liu et al. 1995). Meena et al. (2000) reported that foliar application of *P. fluorescens* strain Pf1 induced accumulation of phenolics and PR proteins in groundnut. Significant increase in yield of several crops due to *P. fluorescens* had been reported (Saravanakumar et al. 2007). Uppal et al. (2008) reported that *P. fluorescens* Biotype F isolate DF37 significantly reduced the disease incidence, severity and vascular discoloration of Verticillium wilt in both cultivars Kennebec and Russet Burbank.

Several studies have indicated that PGPR may stimulate the production of biochemical compounds associated with host defence; massive accumulation of phytoalexins and phenolic compounds; increase in the activities of PR proteins, defence enzymes and transcripts; and enhanced lignification. The induction of SAR using various ISR inducers has been of recent interest with quite reasonable success. Induced resistance against Fusarium wilt of carnation caused by *Fusarium oxysporum* f. sp. *dianthi* was found by prior application of *Pseudomonas* spp. strain WCS417r (Van Peer et al. 1991). Verhagen et al. (2009) studied the ability of *P. fluorescens* CHAO to induce resistance in grapevine against *Botrytis cinerea* and highlighted the importance of salicylic acid, pyochelin and pyoverdin in priming phytoalexin responses and induced resistance. Vleesschauwer et al. (2008) demonstrated the ability of *P. fluorescens* WCS374r to trigger ISR in rice (*Oryza sativa*) against the leaf blast pathogen, *Magnaporthe oryzae*, and found that the induced resistance is regulated by an SA-independent but jasmonic acid/ethylene-modulated signal transduction pathway.

Fluorescent pseudomonads are shown to be effective against certain insect and nematode pests (Ramamoorthy et al. 2001). Tian et al. (2007) reported that fluorescent pseudomonads promote plant growth, increase rhizosphere colonization and suppressed nematodes. Pechy-Tarr et al. (2008) found that when *P. fluorescens* CHAO or Pf5 injected into the haemocoel of the tobacco hornworm, *Manduca sexta*, even low doses killed the larvae of *Manduca sexta*. Karthiba et al. (2010) reported that rice plants when treated with bioformulations containing *P. fluorescens* strains Pf1 and AH1 and *Beauveria bassiana* isolate B2 showed a greater accumulation of defence enzymes, lipoxygenase and chitinase activity against leaf folder.

2.2 Characteristics of an Ideal Microbial Pathogen

The ultimate aim of biological control is to achieve control over the disease by biological means. A biocontrol agent should grow and persist, or "colonize", the surface of the plant it protects. Usually colonization or even the initial population size of the biocontrol agent suppresses/modulates the pathogen from farther distances by production of allelochemicals. The ideal characteristics of a biocontrol agent include high rhizosphere competence, high competitive saprophytic ability, enhanced plant

growth, ease for mass multiplication, broad spectrum of action, reliable control, safe to environment and compatibility with other rhizobacteria and should tolerate desiccation, heat, oxidizing agents and UV radiation.

2.3 Plant Growth-Promoting Activity

The bacteria that provide some benefit to plants are of two general types: those that form a symbiotic relationship with them and those that are free-living in the soil but are often found near, on or even within the roots of plants. Plant growth-promoting rhizobacteria (PGPR) have beneficial effects which have been variously attributed to their ability to produce various compounds including phytohormones, organic acids and siderophores, to fix atmospheric nitrogen, to solubilize soil phosphate, to produce antibiotics that suppress deleterious rhizobacteria or to show some other unidentified mechanisms (Glick 1995).

There are several reports that PGPR have promoted the growth and reproductive parameters of plants ranging from cereals, pulses, ornamentals, vegetable crops, plantation crops and even tree species. Hofte et al. (1991) highlighted that plant growth-promoting strains of *P. aeruginosa* 7NSK2 and *P. fluorescens* ANP15 significantly increased the germination of maize seeds. The growth promotion of winter wheat by treating seeds with several strains of *Pseudomonas* spp. under greenhouse and field condition was reported by De Freitas and Germida (1992). The grain yield of wheat was increased by 46–75 % under greenhouse condition and 11 % under field condition. Dubeikovsky et al. (1993) suggested that indoleacetic acid (IAA) production by *P. fluorescens* might influence the development of blackcurrant cuttings. The strongest effect was observed as changes of root system weight and morphology. The stimulating IAA-mediated effect of bacterial inoculation on the development of the roots of the cuttings was observed. Increase in growth of rice plants by seed treatment with *P. fluorescens* was also reported (Muthamilan 1994).

Tosi and Zazzerini (1994) recorded an increase in the length of sunflower seedlings by seed treatment with *P. fluorescens* strain 14. Significant plant growth promotion with increased runner length and increased leaf number per plant in cucumber by seed and soil application of PGPR was reported (Wei et al. 1996). Williams and Asher (1996) achieved improvement in seedling emergence in proportion of healthy seedling in sugar beet by *Pseudomonas* sp. when compared to seedlings from untreated seeds. Seed coating with pseudomonad isolates like BHU1, A19 and C185 resulted in significantly greater root length, root and shoot biomass, pod yield and nodule number of groundnut compared with the control (Pal et al. 1999). This may be attributed to various factors such as ACC (1-aminocyclopropane-1-carboxylate, sigma) deaminase activity, siderophore production and increase in root length. These bacteria might have enhanced the uptake of nutrients resulting in healthier and better root system and resulting in improved plant growth.

Seed and soil application of PGPR strains showed significant plant growth promotion with increased runner length and increased leaf number per plant in cucumber (Wei et al. 1996). Meena and Marimuthu (2012) observed the boosting effect of *P. fluorescens* Pf formulation on plant growth promotion like plant height, leaf area index, root length, nodules per plant and dry matter production. The growth substrates used in micropropagation are usually devoid of beneficial microorganisms. By introducing such microorganisms to the substrates, it would be possible to lower fertilizer and pesticide inputs and grow the plants in a more sustainable way.

2.4 Formulation Development

Major research on biocontrol is centred with the use of cell suspensions of PGPR directly to seed. Technologies become viable only when the research findings are transferred from lab to field. Bacterial cell suspension cannot be used for large-scale field use due to difficulty in storage, transport and handling. Commercial

application of PGPR either to increase crop health or to manage plant diseases depends on the development of commercial formulations with suitable carriers that support the survival of bacteria for a considerable length of time. Carriers should be economical and easily available. The organic carriers used for formulation development are peat, lignite, talc, kaolinite, zeolite, alginate, press mud, sawdust, vermiculite, etc. The carriers with smaller particle size increased the surface area and thereby increased the resistance to desiccation of the bacteria by the increased coverage of the bacterial cells (Dandurand et al. 1994).

The major concern in commercial production systems is the achievement of adequate growth of the biocontrol agent. Mass production is achieved through liquid, semisolid and solid fermentation systems. A powder formulation with a longer shelf life would be beneficial. Talc is chemically referred to as magnesium silicate [$Mg_3Si_4O_{10}(OH_2)$] and available as powder form from industries suited for wide range of applications. It has very low moisture equilibrium, relative hydrophobicity and chemical inertness, reduced moisture absorption and prevents the formation of hydrate bridges that enable longer storage periods. Owing to the inert nature of talc and easy availability as raw material from soapstone industries, it is used as a carrier for formulation development in a large scale.

Addition of certain gums and polysaccharides as a sticker to the bacterial formulations did not reduce the viability of bacterial population (Suslow et al. 1979). Kloepper et al. (1980) described dry gum-talc formulations that permitted the successful introduction of pseudomonads onto potato seeds. *P. fluorescens* strains grown on King's B broth for 48 h were mixed with 100 g of sterilized peat soil at the rate of 50 ml broth per 100 g of soil gave good control of root rot of black gram when it was applied to soil (Samiyappan 1988).

Hofte et al. (1991) reported that *P. fluorescens* was multiplied on modified King's B medium and the cell pellet after centrifugation was resuspended in 10 ml of $MgSO_4$ (0.1 M) and 2 ml of carboxymethyl cellulose (CMC) (2 %). This suspension when treated to maize seeds improved the seedling emergence. Seed treatment of winter wheat by fluorescent pseudomonads multiplied in King's B broth for 72 h mixed with 1 % carboxymethyl cellulose and talc improved the plant growth (De Freitas and Germida 1992). Fluorescent pseudomonads multiplied on 10 % trypticase soy broth was mixed with preneutralized, sterile class 3 peat, and pH was adjusted to 6.8 by using fine-grade calcium carbonate. This peat-based inoculum when applied to cotton seeds at the rate of 110 g kg^{-1} seed along with CMC (1 %) as sticker (55 ml kg^{-1}) controlled the seedling diseases in cotton under field conditions (Hagedorn et al. 1993).

Talc- and peat-based formulations of *P. chlororaphis* and *Bacillus subtilis* were prepared and used for the management of rhizome rot of turmeric (Nakkeeran et al. 2005). *P. putida* strain 30 and 180 survived up to 6 months in talc-based formulations. The population load at the end of 6th month was 10^8 cfu $g^{-1}$of the product (Bora et al. 2004). The feasibility of the technique and shelf life of the product have to be evaluated to make the technology as a viable component in disease management. The commercially available formulations of *Pseudomonas* spp. are listed in Table 2.1.

2.5 Delivery Systems

PGPR are delivered through several means based on the nature of survival and mode of infection of pathogens. It is delivered through seed, soil, foliage, rhizomes or setts or through combination of several methods of delivery.

2.5.1 Seed Treatment

Seed bacterization means treating seeds with bacterial cultures that will improve plant growth; such bacterial cultures that improve plant growth are called as bacterial fertilizers. Protection against damping off of sweet corn incited by *Pythium ultimum* (Pythiaceae) was observed due to biopriming (Callan et al. 1990). They

Table 2.1 Commercially available formulations of fluorescent pseudomonads

Commercial product	Antagonistic bacteria	Target pathogens	Manufacturer
Bio-Save 10 Bio-Save 100 Bio-Save 1000	*P. syringae* ESC-100	*Botrytis cinerea* *Geotrichum candidum*	Eco Science Corp, Produce Systems Div., Orlando
BlightBan A506	*P. fluorescens* A506	*Erwinia amylovora*	Plant Health Technologies, USA
Cedomon	*P. chlororaphis*	*Fusarium sp.*	BioAgri AB, Sweden
Conquer	*P. fluorescens*	*P. tolaasii*	Mauri Foods, Australia
Victus	*P. fluorescens*	*P. tolaasii*	Mauri Foods, Australia
BioJect Spotless	*P. aureofaciens*	*Pythium aphanidermatum*	Eco Soil Systems, San Diego, CA
Deny	*P. cepacia*	*Rhizoctonia* sp. *Fusarium* sp. *Pythium* sp.	Stine Microbial Products, Shawnee, KS
Intercept	*P. cepacia*	*Rhizoctonia* sp. *Fusarium* sp. *Pythium* sp.	Soil Technologies Corp, USA
Biocoat	*P. fluorescens* WCS374r	*Fusarium oxysporum* f. sp. *raphani* *Fusarium oxysporum* f. sp. *dianthi*	S&G seeds, BV, Netherlands

also observed that *Pseudomonas aeruginosa* strain 7NSK2 and *P. fluorescens* strain ANP15 inoculated onto maize seeds protected seeds from cold-shock damage and increased the germination. Weststeijn (1990) reported that tulip root rot caused by *P. ultimum* was suppressed by dipping tulip bulbs in *Pseudomonas* suspension of 2×10^9 cells ml^{-1}. Transfer of technology for commercial use could be possible if PGPR strains are available as a product. Seed bacterization with peat-based formulation of *P. fluorescens* strain 1 at the rate of 10 g kg^{-1} seed reduced rice blast and sheath blight disease (Muthamilan 1994; Rabindran 1994; Rabindran and Vidhyasekaran 1996). Meena et al. (2001) reported that seed treatment with powder formulation of *P. fluorescens* resulted in significant reduction in root rot incidence of groundnut under field conditions. Gamliel and Katan (1993) reported that tomato root when inoculated with *P. alcaligenes* reduced the wilt caused by *F. oxysporum* f. sp. *vasinfectum*. In cotton, seedling disease caused by *Rhizoctonia solani* and *P. ultimum* was suppressed by *Pseudomonas* spp. under field conditions (Hagedorn et al. 1993). Treatment of tomato seeds with powder formulation of PGPR (*Bacillus subtilis, B. pumilus*) reduced symptom severity of tomato mosaic virus and increased the fruit yield (Murphy et al. 2000). Nagaraj et al. (2004) tested *P. fluorescens* strains isolated from the rice rhizosphere for their antagonistic effect towards rice sheath blight fungal pathogen, *R. solani*. Meena et al. (2006) found that when the groundnut seeds were treated with *P. fluorescens* and sown in soil, the antagonist colonized well in the groundnut rhizosphere.

2.5.2 Soil Application

Soil being as the repertoire of both beneficial and pathogenic microbes, delivering of PGPR strains to soil will increase the population dynamics of augmented bacterial antagonists and thereby would suppress the establishment of pathogenic microbes onto the infection court. Weststeijn (1990) found that root rot in tulip caused by *P. ultimum* was reduced by mixing *Pseudomonas* suspensions thoroughly through the soil to a concentration of 10^8 cells g^{-1} dry soil before planting the bulbs. Wilt disease of sunflower was found to be suppressed when *P. cepacia* strain N24 was applied to the seedbeds at the rate of 500 ml m^{-2} under greenhouse conditions (Hebber et al. 1991). Take-all disease of wheat

was found to be suppressed by applying 120 ml of *P. aureofaciens* suspension to 13 kg of soil as atomized mist produced by use of chromatography sprayer and compressed air (Mazzola et al. 1992). Hagedorn et al. (1993) highlighted that furrow application of *Pseudomonas* spp. at the rate of 14 ml m^{-1} increased the seedling stand in cotton. The improved seedling stand was due to the suppression of seedling disease in cotton by the antagonistic bacteria.

2.5.3 Foliar Application

The efficacies of biocontrol agents for foliar diseases are greatly influenced by microclimate. The concentration of nutrients like amino acids, organic acids and sugars exuded through stomata, lenticels, hydathodes and wounds varies highly. It affects the efficacy and survival of antagonist in phylloplane. Kelly Cartwright (1995) reported that three spray applications of *P. cepacia* to cuttings during a two-week period were more effective than either one or two bacterial sprays in the control of *Rhizoctonia* stem rot of Poinsettia. Rice blast (*P. oryzae*) can be effectively controlled by foliar spray of talc-based powder formulation of *P. fluorescens* strain Pf1 (1 kg ha^{-1}). The effectiveness of spraying persisted up to 15 days. When the bacterial product was sprayed on plants grown from treated seed, the effectiveness was higher than when spraying was carried out without any prior seed treatment (Vidhyasekaran et al. 1997). The dosage and frequency of application has to be standardized based on the crop value, which could be a reliable and practical approach. Selected strains from many genera of bacteria isolated from these suppressive soils have the potential to reduce plant diseases when applied to the plant root environment (Weller et al. 2002). The biological control of plant pests and diseases using a single organism has been reported to give inconsistent and poor performance. Bioformulations combining *P. fluorescens* Migula strains Pf1 and AH1 and *Beauveria bassiana* (Balsamo) Vuill. isolate B2 effectively reduced the incidence of leaf folder insect and sheath blight disease on rice plants and showed the possibility of controlling both pest and disease using a single bioformulation (Karthiba 2010).

2.5.4 Multiple Delivery Systems

Plant pathogens establish host-parasite relationship by entering through infection court such as rhizosphere, spermosphere and phyllosphere. Hence, protection of sites vulnerable for the entry and infection of pathogens would offer a better means for disease management. Meena et al. (2002) reported that combined application of *P. fluorescens* formulation to seed and foliage effectively controlled foliar diseases of groundnut and increased the pod yield. In rice, seed treatment followed by root dipping and foliar spray with *P. fluorescens* showed higher induction of ISR against sheath blight pathogen, *R. solani* (Radjacommare et al. 2004). Similarly, Viswanathan and Samiyappan (2001) reported PGPR-mediated ISR against red rot disease in sugarcane. Application of PGPR strains showed enhanced resistance to bacterial speck and spot of tomato (Kavitha and Umesha 2007).

The influence of plant growth promotion and induced systemic resistance (ISR) resulted in enhancing the disease resistance in tea plants against blister disease by PGPR bioformulations (Saravanakumar et al. 2007). Delivering of rhizobacteria through combined application of different delivery systems will increase the population load of rhizobacteria and thereby suppress the pathogenic propagules. PGPR formulations comprising of bacterial strain mixtures having the capability to induce chitinase in plant play an important role in hydrolyzing chitin, the structural component in gut linings of insects, and would lead to better control of insect pest (Broadway et al. 1998). In addition, certain PGPR strains also activate octadecanoid, shikimate and terpenoid pathways. This in turn alters the volatile production in the host plant leading to the attraction of natural enemies. Identification of entomopathogenic PGPR strains that have the capability to colonize

phylloplane in a stable manner will be a breakthrough in the management of foliar pests (Otsu et al. 2004). Combined application of entomopathogenic strains with compatible PGPR strains which have the ability to suppress plant diseases has to be developed for broad-spectrum action.

2.6 Mechanisms of Biocontrol

Besides the capacity to colonize roots intensively for an extended period of time, other mechanisms are involved that make the fluorescent pseudomonads an effective biocontrol agent. PGPR that indirectly enhance plant growth via suppression of phytopathogens do so by a variety of mechanisms. These include the ability to produce siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize antifungal metabolites such as antibiotics, fungal cell wall-lysing enzymes or hydrogen cyanide, which suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root; and the ability to induce systemic resistance. Multiple interactions occur between the bacteria and between bacteria and other microorganisms involving competition, antibiosis, parasitism and predation. Various interactions also occur between bacteria and plant roots that can be beneficial, neutral or harmful to the plant. Biochemical and molecular approaches are providing new insight into the genetic basis of these traits, the biosynthetic pathways involved, their regulation and importance for biological control in laboratory and field studies (Nelson 2004). The beneficial effects of these bacteria, in most cases, have been related to their ability to produce plant growth hormones and/or antimicrobial substances and to protect growing roots from deleterious root microbes present in the rhizosphere (Harish et al. 2008).

2.6.1 Antibiosis

Antibiosis is now often implicated as an important mechanism of biological control, resulting from the fact that it is an attractive mechanism to study and can provide a highly effective mode of action. Single strains of *Pseudomonas* produce several different antibiotics. Since PGPR being a potential candidate in disease management through multiple modes of action, it becomes highly imperative to know about the role of antibiotics in the management of plant pathogens. Production of antibiotics by rhizosphere bacteria is controlled by complex regulatory networks, in which plant, bacterial and environmental signals are involved. Environmental factors have a significant influence on the production of specific metabolites by fluorescent pseudomonads. Inhibition of pathogens of several crops by the release of diffusible or volatile metabolites such as pyrrolnitrin, pyoluteorin, phenazine or cyanide was confirmed by application of DNA technology and biochemical reaction techniques (Gutterson 1990).

The compound 2,4-diacetylphloroglucinol (DAPG) is a phenolic molecule produced by certain plant-associated fluorescent pseudomonads of worldwide origin (Thomashow et al. 1997). It has antifungal, antibacterial, antihelminthic and phytotoxic properties. DAPG is synthesized by condensation of three molecules of acetyl coenzyme A with one molecule of malonyl coenzyme A to produce the precursor monoacetylphloroglucinol, which is subsequently transacetylated to generate DAPG by a biosynthetic route utilizing chalcone synthase (CHS)-type enzyme (Shanahan et al. 1992). *P. fluorescens* strain CHAO suppressed black root rot of tobacco caused by *Thielaviopsis basicola* and take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. The suppression was due to the production of 2,4-diacetylphloroglucinol (Keel et al. 1992). Phenazine comprises a large family of heterocyclic nitrogen containing brightly coloured pigments with broad-spectrum antibiotic activity. Several strains of fluorescent pseudomonad produce antifungal metabolites, namely, phenazines (Thomashow et al. 1997). Suppression of take-all of wheat by *P. fluorescens* strain 2-79 was mainly due to the production of antibiotic phenazine-carboxylic acid (Thomashow and Weller 1990). Though phenazine plays a vital role in the management of soilborne pathogens, the chemotaxis

and motility of the bacteria decides the antifungal action of the antibiotic producers.

Pyrrolnitrin (3-chloro-4-(2′-nitro-3′-chlorophenyl)pyrrole) is a broad-spectrum antifungal metabolite produced by many fluorescent strains of the genus *Pseudomonas*. The biological control agent *P. fluorescens* BL915 contains four gene clusters involved in the biosynthesis of antifungal molecule. Pyoluteorin is an aromatic polyketide antibiotic consisting of a resorcinol ring, which is derived through polyketide biosynthesis. It is produced by several *Pseudomonas* spp. that suppress plant diseases incited by phytopathogenic fungi (Maurhofer et al. 1994). It mainly inhibits the oomycetous fungi, including *P. ultimum* against which it is strongly active. Paul and Sharma (2006) reported the production of two antibiotics—pyoluteorin and pyrrolnitrin by *P. fluorescens*—which inhibits the growth of *Phytophthora capsici*, the pathogen on black pepper. Sreenivasulu et al. (2006) reported that the volatile metabolite of *P. fluorescens* completely inhibited the pathogen of basal stem rot of coconut, *Ganoderma lucidum*.

2.6.2 Hydrogen Cyanide Production

Hydrogen cyanide production by certain fluorescent pseudomonads was found to influence the plant root pathogens. Suppression of black root rot of tobacco (*Thielaviopsis basicola*) by *P. fluorescens* CHAO was mainly due to the production of hydrogen cyanide (Stutz et al. 1986). Voisard et al. (1989) reported that mutants of CHAO deficient in HCN production were less suppressive than the parental strain to *T. basicola* in tobacco. Defago et al. (1990) highlighted that cyanide secreted by *P. fluorescens* strain CHAO played a role in the suppression of take-all (*G. graminis* var. *tritici*) and root rot (*R. solani*) of wheat. Root rot suppression by *P. fluorescens* strain E-11-3 was due to the production of HCN which influences the pathogen or the host or both. Wei et al. (1991) reported that four PGPR strains of *P. fluorescens*, namely, G8-4, *P. aureofaciens* 28-9 and 36-5 and *P. putida* 34-13, produced HCN in vitro, whereas two strains *P. aureofaciens* 25-33 and *Serratia plymuthica* 2-67 that induced resistance in the host showed no HCN production. There are suggestions that the biocontrol of pathogens through HCN production by certain fluorescent *Pseudomonas* may be due to the induction of plant resistance against certain pathogens.

2.6.3 Competition for Nutrients and Space

Competition between pathogenic and saprophytic microorganisms for organic materials released from the roots can reduce growth and/or pathogenic activity of the pathogens. Many successful antagonists that do not produce antibiotics are able to grow rapidly at the wound sites and are better to extreme nutrients and environmental conditions compared with postharvest pathogens. Unless an organism can compete favourably with other organisms and effectively scavenge and utilize favourable nutrients, it will not constitute a significant proportion of the rhizosphere population. The involvement of competition for nutrients in biological control by fluorescent *Pseudomonas* spp. was suggested in several studies. It was found that in vitro antagonistic activity is based on competition and correlated with disease suppression. Moreover, addition of specific substrates to the plant pathogen system reduced biological control (Elad and Chet 1987). All disease-suppressive mechanisms exhibited by fluorescent pseudomonads are essentially of no real value unless these bacteria can successfully establish themselves at the root environment. Nutrient competition varies in different rhizospheres, depending on the available sources of carbon, nitrogen, sulphur, phosphate and micronutrients. It is not yet very clear whether a superior ability utilizes a particular type of nutrient or nutrients provide advantage to fluorescent pseudomonads.

2.6.4 Siderophore Production

Siderophores are low-molecular-weight molecules that are secreted by microorganisms to take up iron

from the environment, and their modes of action in suppression of disease were thought to be solely based on competition for iron with the pathogen. These siderophores have been classified as either pyoverdins or pseudobactins. The production of these siderophores has been linked to their disease suppression ability (Loper and Buyer 1991). Production by certain fluorescent pseudomonads of extracellular, water soluble, yellow and green pigments in KB medium that fluoresce in UV light is known for a hundred years. All such pigment (or siderophore)-producing pseudomonads *P. aeruginosa, P. fluorescens* and *P. putida* belong to one intrageneric homology group. Pseudobactin, the first siderophore, was isolated, purified and characterized by X-ray crystallography from *P. fluorescens* strain B10. The involvement of pseudobactin production by fluorescent pseudomonads in biological control was reported by several workers (Weller 1988; Loper and Buyer 1991). Lemanceau et al. (1992) indicated that the production of pseudobactin 358 by *P. putida* WCS358 appeared to be responsible for the suppression of Fusarium wilt of carnation. Bhavani and Abraham (2005) found that two strains of *P. fluorescens* antagonists of *Phytophthora palmivora* causing pod rot of cocoa produced appreciable quantities of siderophores to suppress the pathogen. The production of siderophores by bacterial antagonist as one of the mechanism of antagonism against *Fomes lamaoensis* in tea was reported (Chakraborty et al. 2006).

2.6.5 Induced Systemic Resistance

Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated (Audenaert et al. 2002). Induced resistance results from perception of rhizobacteria by plant roots which give rise to an increased level of resistance which expressed upon subsequent infection by a pathogen. Localized induction of resistance at the site where eliciting bacteria are present on the roots is difficult to demonstrate, because a challenging pathogen will also be subject to bacterial antagonism at this same location. In contrast, no direct interaction between inducing bacteria and a challenging pathogen is possible when each organism is present at spatially separated sites and no contact between the two is established. Enhanced resistance due to ISR by PGPR is achieved by induction of defence compounds of phenylpropanoid pathway and PR proteins (pathogenesis-related proteins). Induced systemic resistance triggered in some rhizobacterial strains depends on salicylic acid (SA) signalling in the plants. Induced resistance by *P. aeruginosa* 7NSK2 was found to be iron regulated and involved three siderophores, pyoverdin, pyochelin and salicylic acid. Salicylic acid is also a precursor in the production of SA-containing siderophores, such as pseudomonine in *P. fluorescens* WCS374 (Audenaert et al. 2002). The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis* revealed that root colonization by *P. fluorescens* WCS417r did not lead to transcriptional changes in the leaves, whereas in the roots there is a large set of genes that are differentially transcribed (Verhagen et al. 2004).

Several studies have indicated that PGPR may stimulate the production of biochemical compounds associated with host defence; massive accumulation of phytoalexins and phenolic compounds; increase in the activities of PR proteins, defence enzymes and transcripts; and enhanced lignification. Peroxidase (PO) catalyzes the last step in the biosynthesis of lignin and other oxidative phenols, and it is associated with disease resistance in plants. In groundnut, increased activity of PO was observed due to application of *P. fluorescens*, and PO isoforms were expressed at higher levels (Meena et al. 2000). Phenylalanine ammonia lyase (PAL) is the first enzyme involved in phenylpropanoid pathway and plays a key role in the biosynthesis of phenolics and phytoalexins. When cucumber roots were treated with *P. corrugata* 13 or *P. aureofaciens* 63-28, PAL activity was stimulated in root tissues in 2 days, and this activated accumulation lasted for 16 days after bacterization (Chen et al. 2000).

Induction of higher PPO activity was noticed in tomato and hot pepper pretreated with fluorescent pseudomonads strain against *Pythium* diseases (Ramamoorthy et al. 2002). Phenolics are fungitoxic in nature and increase the physical and mechanical strength of the host cell wall. M'Piga et al. (1997) reported that application of *P. fluorescens* strain 63-28 brought about cell wall thickening, deposition of phenolic compounds and formation of callose resulting in restricted growth of *F. oxysporum* f. sp. *radicis-lycopersici*. Such rapid defence reactions at the site of fungal entry delay the infection process and allow sufficient time for the host to build up other defence reactions to restrict pathogen growth. Recently reports on mechanism of biological control revealed that several microbial strains protect plants from various pests, diseases and phytonematodes in several crops by activating defence genes; encoding chitinase, glucanase, peroxidase and synthesis of phytoalexins; and inducing physiological changes (Kavino et al. 2007; Rajendran et al. 2007; Saravanakumar et al. 2007; Harish et al. 2008).

Pathogenesis-related proteins are designated as PRs and are defined as proteins coded by the host plant but induced specifically in pathological or related situations. They are not only accumulated locally in the infected leaves but also induced systemically associated with the development of systemic induced resistance against further infection by pathogens (van Loon et al. 1994). Induced resistance by PGPR is associated with the accumulation of PR proteins (Radjacommare et al. 2004). Application of PGPR strains showed enhanced resistance to bacteria speck and spot of tomato (Kavitha and Umesha 2007) and anthracnose disease in mango (Vivekananthan et al. 2004). The influence of plant growth promotion and ISR resulted in enhancing the disease resistance in tea plants against blister disease by PGPR bioformulations (Saravanakumar et al. 2007).

2.7 Conclusion

PGPR have gained worldwide importance and acceptance for agricultural benefits. These microorganisms are the potential tools for sustainable agriculture and the trend for the future. Scientific researches involve multidisciplinary approaches to understand adaptation of PGPR to the rhizosphere, mechanisms of root colonization, effects on plant physiology and growth, biofertilization, induced systemic resistance, biocontrol of plant pathogens, production of determinants, etc. The technology of commercial use of biocontrol agents has tremendous potentials. The inconsistency in performance of these PGPR strains is a major constraint to their widespread use as biocontrol agent in commercial agriculture. However, genetic manipulation of PGPR has the potential to construct significantly better strains with improved biocontrol efficacy. The applications of mixture of biocontrol agents may be a more ecologically sound approach because it may result in better colonization and better adaptation to the environmental changes occurring throughout the growing season.

2.8 Future Recommendations

Future strategies are required to clone genes involved in the production of antibiotics, siderophores and other metabolites and to transfer these cloned genes into the strains having the good colonization potential along with other beneficial characteristics. PGPR offer an environmentally sustainable approach to increase crop production and health. The application of molecular tools is enhancing our ability to understand and manage the rhizosphere and will lead to new products with effectiveness. Trends in research include the increased use of biorational screening processes to identify microorganisms with potential for biocontrol, increased testing under semicommercial and commercial production conditions and increased emphasis on combining biocontrol strains with each other and with other control methods, integrating biocontrol into an overall system. Research should be initiated on the development of improved formulations, adjuvants and protectants for microbial fungicides. Critical studies on the field survival of the antagonists based on biochemical and molecular methods have to be strengthened.

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