Chapter 7

PLANT GROWTH-PROMOTING RHIZOBACTERIA: POTENTIAL GREEN ALTERNATIVE FOR PLANT PRODUCTIVITY

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Abstract: Use of plant growth promoting rhizobacteria (PGPR) for the benefits of agriculture is gaining worldwide importance and acceptance and appears to be the trend for the future. PGPR are bioresources which may be viewed as a novel and potential tool for providing substantial benefits to the agriculture. These beneficial, free-living bacteria enhance emergence, colonize roots, stimulate growth and enhance yield. PGPR are known to induce resistance against various plant pathogens in different crops ranging from cereals, pulses, ornamentals, vegetables, plantation crops, spices and some trees. Most studies have emphasized exploration and potential benefits of PGPR in agriculture, horticulture and forestry. The plausible mechanisms adopted by these rhizobacteria in growth promotion and resistance, though abundantly documented but still remains to be fully explored. Integrated use of PGPR allows the combination of various mechanisms thereby enhancing their beneficial abilities. However, their use has not been to the full potential due to inconsistency in their performance and their commercialization limited to few developed countries. Use of PGPR as bioinoculants, biofertilizers and biocontrol agents, advantages and disadvantages, practical potential in improved agriculture and future prospects are also discussed.

Key words: biocontrol agents; biofertilizers; bioinoculants; growth promotion; induced resistance; integrated pest management; rhizobacteria;

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1 INTRODUCTION

Many microorganisms are attracted by nutrients exuded from plant roots and this “rhizosphere effect” was first described by Hiltner (Hiltner, 1904)). He observed higher numbers and activity of microorganisms in the vicinity of plant roots. The rhizosphere and rhizoplane are colonized more intensively by microorganisms than the other regions of the soil. Some of these microorganisms not only benefited from the nutrients secreted by the plant roots but also beneficially influence the plants, resulting in a stimulation of their growth. For instance, rhizobacteria can fix atmospheric nitrogen, which is subsequently used by the plants, thereby improving plant growth in the soil deficient of nitrogen. Other rhizobacteria can directly promote the plant growth by the production of hormones. These rhizobacteria positively influence plant growth and health and often referred as plant growth promoting rhizobacteria (PGPR). However, their effects are complex and cumulative because of interactions of plants, pathogens, antagonists, and environmental factors (Schippers, 1992).

Genera of PGPR include *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Bacillus*, *Paenibacillus*, and some are members of the Enterobacteriaceae. Direct use of microorganisms to promote plant growth and to control plant pests continues to be an area of rapidly expanding research. Rhizosphere colonization is one of the first steps in the pathogenesis of soil borne microorganisms. It is also crucial for the microbial inoculants used as biofertilizers, biocontrol agents, phytostimulators, and bioremediators. *Pseudomonas* spp. are often used as model root-colonizing bacteria (Lugtenberg *et al*., 2001).

The beneficial effects of these rhizobacteria have been variously attributed to their ability to produce various compounds including phytohormones, organic acids, siderophores, fixation of atmospheric nitrogen, phosphate solubilization, antibiotics and some other unidentified mechanisms (Glick, 1995). Motile rhizobacteria may colonize the rhizosphere more profusely than the non-motile organisms resulting in better rhizosphere activity and nutrient transformation. They also eliminate deleterious rhizobacteria from the rhizosphere by niche exclusion thereby better plant growth (Weller, 1988). Induced systemic resistance has been reported to be one of the mechanisms by which PGPR control plant diseases through the manipulation of the host plant’s physical and biochemical properties.

2 GROWTH PROMOTION OF CROP PLANTS BY RHIZOBACTERIA AND THE MECHANISMS

PGPR are beneficial for plant growth and also referred as yield increasing bacteria (YIB). They can affect plant growth and yield in a number of ways and enhancement of vegetative and reproductive growth is documented in a range of crops like cereals, pulses, ornamentals, vegetables, plantation crops and some trees. Treatments with PGPR increase germination percentage, seedling vigor, emergence, plant stand, root and shoot growth, total biomass of the plants, seed weight, early flowering, grains, fodder and fruit yields etc., (van Loon *et al*., 1998; Ramamoorthy *et al*., 2001). Though the exact mechanisms involved in growth promotion are still unclear, various mechanisms have been suggested to explain the phenomenon of plant growth promotion. These include increase in the nitrogen fixation, production of auxins, gibberellins, cytokinins, ethylene, solubilization of phosphorous, oxidation of sulfur, increase in availability of nitrate, extra cellular production of antibiotics, lytic enzymes, hydrocyanic acid, increases in root permeability, strict competition for the available nutrients and root sites, suppression of deleterious rhizobacteria, and enhancement in the uptake of essential plant nutrients etc.(Subba Rao,1982; Pal *et al*., 1999; Enebak and Carey, 2000). However, experimental evidence suggests that bacterially-mediated phytohormone production is the most likely explanation for PGPR activity in the absence of pathogens (Brown, 1974; Tien *et al*., 1979; Holl *et al*., 1988) while siderophore production by PGPR may be important for plants growth stimulation when other potentially deleterious rhizosphere microorganisms are present in the rhizosphere (Kloepper *et al*., 1980; Bossier *et al*., 1988).

3 DISEASE CONTROL MECHANISMS

3.1 Biocontrol

Plant pathogens such as fungi, bacteria, viruses, nematodes etc., which cause various diseases in crop plants are controlled by PGPR (Raupach *et.al*., 1996; Hasky-Gunther *et.al*., 1998; Vidhyasekaran *et.al*.,2001;Viswanathan and Samiyappan,2002). Mechanisms of biocontrol may be competition or antagonisms; however, the most studied phenomenon is the induction of systemic resistance by these rhizobacteria in the host plant (van Loon *et al*., 1998; Ramamoorthy *et al*., 2001). PGPR control the damage to plants from pathogens by a number of mechanisms including: out-competing the pathogen by physical displacement, secretion of

siderophores to prevent pathogens in the immediate vicinity from proliferating, synthesis of antibiotics and variety of small molecules that inhibit pathogen growth, production of enzymes that inhibit the pathogen and stimulation of the systemic resistance in the plants. PGPR may also stimulate the production of biochemical compounds associated with host defense. Enhanced resistance may be due to massive accumulation of phytoalexins, phenolic compounds, increases in the activities of PR-proteins, defense enzymes and transcripts, and enhanced lignification. Biocontrol may also be improved by genetically engineered PGPR to over express one or more of these traits so that strains with several different anti-pathogen traits can act synergistically (Glick and Bashan, 1997). Rhizobacteria-mediated ISR has been reported to be effective against fungi, bacteria and viruses, but appears to involve different signaling pathways and mechanisms.

3.2 Structural mechanisms

PGPR can induce structural changes in the host and these changes were characterized by a considerable enlargement of the callose-enriched wall appositions deposited onto the inner surface of cell wall in the epidermis and outer cortex (Benhamou *et al*., 1998), callose deposition (M'Piga *et al*., 1997) and lignification (Kloepper, 1993). A strain of *Pseudomonas fluorescens* functions as an activator of plant disease resistance by inducing callose synthesis in tomato (M'Piga *et al*., 1997). Bean roots bacterized with a saprophytic fluorescent pseudomonad, had higher lignin content than control (Anderson and Guerra, 1985).

Treatment of PGPR significantly reduced germination of sporangia and zoospores of *Phytophthora infestans* on the leaf surface of tomato than the leaves of the non-induced control. *Serratia plymuthica* strain R1GC4 sensitizes susceptible cucumber plants to react more rapidly and efficiently against *Pythium ultimum* attack through the formation of physical and chemical barriers at sites of fungal entry (Benhamou *et al*., 2000). *Pseudomonas fluorescens* induced accumulation of lignin in pea roots (Benhamou *et al*., 1996a,b). *Bacillus pumilus* SE34 showed a rapid colonization of all tissues including the vascular stele in tomato and induced resistance against *Fusarium oxysporum* (Benhamou *et al.,* 1998). The main facets of the altered host metabolism concerned the induction of a structural response at sites of fungal entry and the abnormal accumulation of electron-dense substances in the colonized areas.

3.3 Biochemical mechanisms

PGPR are known to produce antibiotics, antifungal metabolites, enzymes, phenolics, signal compounds and other determinants of defense in response to pathogen attack. Various antibiotics like bacilysin, iturin-like lipopeptides, diacetylphloroglucinol and pyrrolnitrin, HCN, phenazine-1-carboxylate are produced by rhizobacteria (Thomashow *et al*., 1990). Rhizosphere colonization by *Pseudomonas aeruginosa* 7NSK2 activated phenlyalanine ammonia lyase (PAL) in bean roots and increased the salicylic acid levels in leaves (De Meyer *et al*., 1999). Increased activity of PAL was observed in *P. fluorescens* treated tomato and pepper plants in response to infection by *F. oxysporum* f. sp. *lycopersici* and *Colletotrichum capsici* (Ramamoorthy and Samiyappan, 2001). In bean, rhizosphere colonization of various bacteria induced peroxidase (PO) activity (Zdor and Anderson, 1992), The higher PO activity was noticed in cucumber roots treated with *Pseudomonas corruga*ta and inoculated with *Pythium aphanidermatum* (Chen *et al*., 2000). Foliar application of *P. fluorescens* increased chitinase and glucanase activities in rice (Meena *et al*., 1999). Groundnut plants, sprayed with *P. fluorescens* strain Pf1, showed significant increase in activities of PAL, phenolic contents, chitinase and glucanase 23-kDa thaumatin-like protein (TLP) and a 30-kDa glucanase (Meena *et al*., 2000). Earlier and increased activities of phenylalanine ammonia lyase (PAL), peroxidase (PO) and polyphenol oxidase (PPO) were observed in *P. fluorescens* Pf1 pretreated tomato and hot pepper plants challenged with *P. aphanidermatum*. Phenolic compounds are toxic to pathogens in nature and may increase the mechanical strength of the host cell wall. Accumulation of phenolics by prior application of *P. fluorescens* in pea has been reported against *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou *et al*., 1996a). Similarly, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum* (Benhamou *et al*. 2000). Moreover, *P. fluorescens* Pf1 isolate also induced the accumulation of phenolic substances and PR-proteins in response to infection by *F. oxysporum* f. sp. *lycopersici* in tomato (Ramamoorthy *et al*., 2001) and *C. capsici* in pepper (Ramamoorthy and Samiyappan, 2001). The levels of a PR-protein increased in bean leaves following seed treatment with PGPR strains (Hynes and Lazarovits, 1989) while PR-proteins viz., PR-1a, 1b, 1c, endochitinase and b-1,3-glucanases were induced in intercellular fluid in the leaves of tobacco plants grown in the presence of *P. fluorescens* strain CHA0(Maurhofer *et al*., 1994). Increase in lignin content, peroxidase activity and 4-coumarate CoA ligase activity were observed after inoculation with *Xanthomonas oryzae* pv. *oryzae* in rice leaves pre-treated with *P. fluorescens* (Vidhyasekaran *et al*., 2001). Inoculation of PGPR can induce phytoalexin synthesis (Van Peer *et al*., 1991) and phenol accumulation

(M'Piga *et al*., 1997). Moreover, PGPR-mediated ISR triggered the hypersensitive reaction (HR), causing death of individual cell of leaves following inoculation with the pathogen. Analysis of H_2O_2 content, showed that H_2O_2 increased significantly in all treatments 12 h after pathogen inoculation, compared to non-induced control (Yan *et al*., 2002).

3.4 Molecular mechanisms

Mechanisms of rhizobacteria-mediated induced systemic resistance (ISR) to the large extent are unknown. ISR in *Arabidopsis* mediated by rhizobacteria is not associated with a direct effect on expression of known defense-related genes but stimulated the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Gene expression studies were performed with *Arabidopsis* gene-specific probes for the defense-related genes *PR-1*., *PR-2*., *PR-5*., *Hel*., *ChiB*, *Pdf*1.2, *Atvsp*, *Lox1*, *Lox2*, *Pal1*, and *Pin2*. Responsiveness of genes to the defense signaling molecules SA, ethylene, and jasmonate was verified by analyzing their expression in leaves treated with SA, the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC), or methyl jasmonate (MeJA). Although variation in the expression of most genes was apparent, roots and leaves of *P. fluorescens* WCS417r-treated plants never showed an enhanced expression of any of the genes, at any time tested (van Wees *et al*., 1997).

PPO transcript levels increased in young leaves of tomato when mature leaflets were injured (Thipyapong and Steffens, 1997). Increase in mRNAs encoding PAL and chalcone synthase were recorded in the early stages of the interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992). ISR in A. *thaliana* by *P. fluorescens* WCS417r and subsequent inoculation of *Pseudomonas syringae* pv. *tomato* Dc3000(ISR) functions independently of salicylic acid but requires an intact response to the plant hormones jasmonic acid and ethylene. Rhizobacteria-mediated ISR is not based on the induction of changes in the biosynthesis of either JA or ethylene. ISR-expressing plants have the capacity to convert 1-aminocyclopropane-1-carboxylate (ACC) to ethylene providing a greater potential to produce ethylene upon pathogen attack (Pieterse *et al*., 2000). Fluorescent pseudomonads are also known to produce salicylic acid, which acts as local and systemic signal molecules in inducing resistance in plants (De Meyer and Hofte, 1997).

4 SIGNALING COMPOUNDS AND PATHWAYS

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are involved in the regulation of basal resistance against different pathogens. These three signals play important roles in induced resistance as well. SA is a key regulator of pathogen-induced systemic acquired resistance (SAR) whereas JA and ET are required for rhizobacteria-mediated induced systemic resistance (ISR). Both types of induced resistance are effective against a broad spectrum of pathogens. Comparison of the effectiveness of SAR and ISR using a fungal, a bacterial, and a viral pathogen in non-induced *Arabidopsis* plants, these pathogens are primarily resisted through either SA-dependent basal resistance (*Perenospora parasitica* and Turnip crinkle virus (TCV)), JA/ET-dependent basal resistant responses (*Alternaria barssicicola*), or a combination of SA-, JA-, and ET-dependent defenses (*Xanthomonas campestris* pv. *armoraciae*). Activation of ISR resulted in a significant level of protection against *Alternaria brassicicola*, whereas SAR was ineffective against this pathogen. Conversely, activation of SAR resulted in a high level of protection against *Phytophthora parasitica* and TCV, whereas ISR conferred only weak and no protection against *P. parasitica* and TCV, respectively. Induction of SAR and ISR was equally effective against *X. campestris* pv. *armoraciae*. These results indicate that SAR is effective against pathogens that non-induced plants are resisted through SA-dependent defenses, whereas ISR is effective against pathogens in non-induced plants and resisted through JA/ET-dependent defenses. This suggests that SAR and ISR constitute a reinforcement of extant SA- or JA/ET-dependent basal defense responses, respectively (Ton *et al*., 2002).

Serratia marcescens 90-166 mediates induced systemic resistance to fungal, bacterial, and viral pathogens by producing salicylic acid (SA), using the salicylate responsive reporter plasmid pUTK21. High-pressure liquid chromatography analysis of culture extracts confirmed the production of SA in broth culture. Mini-Tn5phoA mutants, which did not produce detectable amounts of SA, retained ISR activity in cucumber against the fungus *Colletotrichum orbiculare*. Strain 90-166 induced disease resistance to *P. syringae* pv. *tabaci* in wild-type *Xanthi*-nc and transgenic NahG-10 tobacco expressing salicylate hydroxylase. Results of the study indicate that SA produced by 90-166 is not the primary bacterial determinant of ISR and the bacterial-mediated ISR system is affected by iron concentrations (Press *et al*., 1997).

Several genera of bacteria including pseudomonads are known to synthesize SA and SA is an intermediate in the biosynthesis of pyochelin siderophores (Ankenbauer and Cox, 1988). There are some indications that SA may be involved in bacterially mediated ISR since *Pseudomonas fluorescens* strain CHAO, which provides ISR in tobacco to tobacco necrosis

virus (Maurhofer *et al*., 1994), produces SA (Meyer *et al*., 1992; Visca *et al*. 1993). However, the role of SA production in CHAO-mediated ISR has not been reported. Leeman *et al*., (1996) reported that *P. fluorescens* strain WCS374, which provides ISR in radish against *F. oxysporum* f. sp. *raphani*, produced SA in quantities that were iron dose-dependent, and they suggested that ISR was due to bacterial SA production. Recently, the involvement of SA produced by *P. aeruginosa* 7NSK2 in the induction of resistance against *Botrytis cinerea* on *Phaseolous vulgaris* has been reported (De Meyer and Hofte,1997).

Root colonization of *A. thaliana* by the nonpathogenic, rhizosphere-colonizing bacterium *P. fluorescens* WCS417r has been shown to elicit induced systemic resistance (ISR) against *P. syringae* pv. *tomato* (Pst)(Knoester *et al*., 1999). Several ethylene-response mutants were tested and showed essentially normal symptoms of Pst infection. ISR was abolished in the ethylene-insensitive mutant *etr1-1*, whereas SAR was unaffected. Similar results were obtained with the ethylene mutants *ein2* through *ein7*, indicating that the expression of ISR requires the complete signal-transduction pathway of ethylene known so far. The induction of ISR by WCS417r was not accompanied by increased of ethylene production in roots or leaves, and neither by increases in the expression of the genes encoding the ethylene biosynthetic enzymes 1-aminocyclopropane-1-carboxylic (ACC) synthase and ACC oxidase. The *etr1* mutant, displaying ethylene insensitivity in the roots only, did not express ISR upon application of WCS417r to the roots, but did exhibit ISR when the inducing bacteria were infiltrated into the leaves. These results demonstrate that, for the induction of ISR, ethylene responsiveness is required at the site of application of inducing rhizobacteria (Knoester *et al*., 1999).

The *Bacillus amyloliquefaciens* EXTN-1 treated tobacco plants showed augmented, rapid transcript accumulation of defense related genes including PR-1a, phenylalanine ammonia-lyase, and 3-hydroxy-3methylglutaryl CoA reductase (HMGR) following inoculation with Pepper Mild Mottle Virus (PMMoV). Thus, their expression is associated with the development of both local and systemic resistance. All these results may indicate that EXTN-1 induces systemic resistance via salicylic acid and jasmonic acid-dependent pathways and timely recognition followed by rapid counter attack against the viral invasion is the key differences between incompatible interaction and compatible one (Ahn *et al*., 2002).

PGPR strains *B. pumilus* SE34 and *P. fluorescens* 89B61, elicited systemic protection against the blight on tomato and reduced disease (Yan *et al*., 2002). Induced protection elicited by both PGPR strains was SA-independent but ethylene- and jasmonic acid-dependent. In *Arabidopsis*, selected bacterial strains trigger a SA-independent but JA and ethylene dependent pathway that nevertheless, is dependent on the regulatory factor

NPR1, which is also part of the SA-dependent pathway. Two non-inducible ecotypes of *Arabidopsis* are impaired in the same gene (ISR1) and have reduced sensitivity to ethylene, confirming the importance of ethylene sensitivity in ISR signaling (Hammerschmidt *et al*., 2001).

5 USE OF PGPR ON COMMERCIAL SCALE

The development of biological products based on beneficial micro-organisms can extend the range of options for maintaining the health and yield of crops. As early as 1897 a "bacteriological fertilizer for the inoculation of cereals" was marketed under the proprietary name Alinit by Farbenfabriken vorm. Friedrich Bayer & Co." of Elberfeld, Germany, Today's Bayer AG. The product was based on a *Bacillus* species now known by the taxonomic name *Bacillus subtilis* (Kilian *et al*., 2000). In the mid-1990s in the USA, *B. subtilis* started to be used as seed dressing, with registrations in more than seven crops and application to more than 2 million ha (Backmann *et al*., 1994). This was the first major commercial success in the use of an antagonist. In Germany, FZB 24 *B. subtilis* has been on the market since 1999 and is used mainly as a seed dressing for potatoes (Kilian *et al*., 2000).

In response to environmental and health concerns about extended use of pesticides, there is considerable interest in finding alternative control approaches for use in integrated pest management strategies for crop diseases (Reuveni, 1995). It seems inevitable that fewer pesticides will be used in the future and that greater reliance will be placed on biological technologies including the use of microorganisms as biocontrol agents (Backman *et al*., 1997; Budge *et al*., 1995). However, microorganisms as biocontrol agents typically have a relatively narrow spectrum of activity compared with synthetic pesticides (Baker, 1991; Janisiewicz, 1988) and often exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for suppression of plant pathogens (Backman *et al*., 1997).

Commercial development has already been accomplished with two products marketed as Kodiak and Epic (Gustafson inc.), in which two different *Bacillus subtilis* biocontrol strains were combined with a fungicide (Carboxin-PCNB-metalaxyl) for use against soil borne diseases. During the 1996 season, approximately 5 million ha of crops were treated with these products, targeting diseases of roots caused by *Rhizoctonia solani* and *Fusarium* spp. plus promoting root mass and plant vigor through hormone-like responses and disease control.

Many root-colonizing bacteria are known to promote plant growth by producing gibberellins, cytokinins and indole acetic acid (Dubeikovsky *et*

al., 1993) and hence are called as PGPR. The application of five commercial chitosan-based formulations of carefully chosen PGPR developed at Auburn University, USA has previously shown demonstrable increase in the growth of nursery-raised plants such as cucumber, pepper and tomato among others. Later, seedlings of three indica rice cultivars, IR24, IP50 and Jyothi raised in rice field soil amended with each of the formulations in a 1:40 (formulation: soil) ratio have shown significant two-fold increase in root and shoot length, and grain yield. The observations do suggest that application of such commercial bacterial formulations can serve as microbial inoculants for the improvement of rice growth (Vasudevan *et al*., 2002).

6 INTEGRATION AND MIXTURES OF PGPR

In nature biocontrol results from mixtures of antagonists, rather from high populations of a single antagonist. Moreover, mixtures of antagonists are considered to account for protection of disease-suppressive soils (Lemanceau and Alabouvette, 1991; Schippers, 1992). Consequently, application of a mixture of introduced biocontrol agents would more closely mimic the natural situation and may broaden the spectrum, enhance the efficacy and reliability of biocontrol (Duffy and Weller, 1995). Strategies for forming mixtures of biocontrol agents could be envisioned including mixtures of organisms with differential plant colonization patterns; biocontrol agents that control different pathogens; antagonists with different mechanisms of disease suppression; taxonomically different organisms and antagonists with different optimum temperature, pH and moisture conditions for plant colonization (Backman *et al*., 1997). Combination of various mechanisms of biocontrol is useful in achieving the goal without genetic engineering (Janisiewicz, 1996). PGPR strains INR 7 (*Bacillus pumilus*). GBO3 (*Bacillus subtilis*), and ME1 (*Curtobacterium flccumfaciens*) were tested alone and in combinations for biocontrol against *Colletotrichum orbiculare* (causing anthracnose), *Pseudomonas* syringae pv. *lachrymans* (causing angular leaf spot), and *Erwinia tracheiphila* (causing cucurbit wilt disease). Greater suppression and enhanced consistency was observed against multiple cucumber pathogens using strains mixture (Raupach and Kloepper, 1998). Studies on combinations of biocontrol agents for plant disease control have included mixtures of fungi (Budge *et al*., 1995; Datnoff *et al*., 1993, 1995; De Boer *et al*., 1997; Paulitz *et al*., 1990), mixtures of fungi and bacteria (Duffy *et al*., 1996; Duffy and Weller, 1995; Hassan *et al*., 1997; Janisiewicz, 1988; 1996; Leeman *et al*., 1996; Leibinger *et al*., 1997; Lemanceau and Alabouvette, 1991; Park *et al*., 1988) and mixtures of bacteria (De Boer *et al*., 1997; Janisiewiez and Bors., 1995; Johnson *et al*.,

1993; Mazzola al., 1995; Pierson and Weller, 1994; Raaijmakers *et al*., 1995; Roberts *et al*., 1997; Schisler *et al*., 1997; Stockwell *et al*., 1996; Sung and Chung, 1997; Waechter-Kristensen *et al*., 1994; Wei *et al*., 1996). Combinations of a strain of *Trichoderma koningii* with different *Pseudomonas* spp. isolates provided greater suppression of take-all disease than either the fungus or the bacterium alone (Duffy *et al*., 1996). Increased suppression of Fusarium wilt of carnation was observed by combining *P. putida* WCS358 with non-pathogenic *Fusarium oxysporum* Fo47 (Lemanceau *et al*., 1992, 1993). The enhanced disease suppression may be due to siderophore-mediated competition for iron by WCS358, which makes the pathogenic *F. oxysporum* strain more sensitive to competition for glucose by the non-pathogenic strain Fo47. Furthermore, strains of nonpathogenic *Verticillium lecanii*, *Acremonium rutilum* or *Fusarium oxysporum* with the fluorescent *Pseudomonas* spp. strains WCS358, WCS374 or WCS417 resulted in significantly better suppression of Fusarium wilt of radish compared to the single organism (Leeman *et al*. 1996). Mixtures of fluorescent pseudomonads were significantly more suppressive of take-all than either used alone (Pierson and Weller, 1994; and Duffy and Weller, 1995). Similarly, chitinase-producing *Streptomyces* spp. and *Bacillus cereus* isolates used in conjunction with antibiotic-producing *P. fluorescens* and *Burkholderia cepacia* isolates had a synergistic effect on the suppression of rice sheath blight (Sung and Chung, 1997). Limited numbers of compatible and effective mixtures of biocontrol agents are available. The majorities of mixtures have no benefit or detrimental effects on biocontrol activity. Further, a mixture that improves activity under one set of conditions may be antagonistic under another set of conditions. A biocontrol product composed of a mixture of strains has a potential economical constraint. Production and registration of such a product will be more costly than a product composed of single strain. Development of mixtures of biocontrol agents should be emphasized, because these may result in better plant colonization, better adapt to the environmental changes that occur throughout the growing season, have a larger number of pathogen-suppressive mechanisms and protect against a broader range of pathogens.

In few cases combinations of biocontrol agents do not result in improved suppression of disease (Hubbard *et al*, 1983; Sneh *et al*, 1984; Miller and May, 1991; Dandurand and Knudsen, 1993). Tomato seedlings were treated with the potential biocontrol agents such as nonpathogenic strains of *Fusarium* spp., *Trichoderma* spp., *Gliocladium virens*, *Pseudomonas fluorescens*, *Burkholderia cepacia*, and others in the greenhouse and transplanted into pathogen-infested field soil. Combinations of antagonists like multiple *Fusarium* isolates, *Fusarium* with bacteria, and *Fusarium* with other fungi, also reduced disease, but did not provide better

control than the nonpathogenic *Fusarium* (Larkin and Fravel, 1998). Use of a *T. harzianum* strain with a strain of *P. fluorescens* were able to suppress root rot of pea caused by *Aphanomyces euteiches* f. sp. *pisi* but did not result in better disease suppression (Dandurand and Knudsen, 1993). Positive and negative interactions of introduced microorganisms and indigenous microflora can influence their performance in the rhizosphere. For example, two groups of microorganisms that occupy the same ecological niche and have the same nutritional requirements are bound to compete for nutrients (Bakker *et al*., 1988; Fukui *et al*., 1994; Janisiewicz and Bors, 1995; Raaijmakers *et al*., 1995). Siderophore-mediated competition for iron between the two biocontrol agents *P. putida* WCS358 and *P. fluorescens* WCS374 decreased colonization of radish roots by the latter strain (Raaijmakers *et al*., 1995). Hubbard *et al*., (1983) described negative effects of endemic *Pseudomonas* spp. on *T. harzianum*. They suggested that negative effects were caused by effective competition for iron by the *Pseudomonas* spp. because addition of iron to naturally infested soil suppressed growth inhibition of *T. harzianum* and also suppressed *Pythium* seed rot of pea. Negative interaction between two biocontrol agents may also be due to detrimental effects of secondary metabolites produced by one organism on the other (Mew *et al*., 1994). Thus, an important pre-requisite for the desired effectiveness of strains appears to be compatibility of the co-inoculated microorganisms (Li and Alexander, 1988; Baker, 1990; Raaijmakers *et al*., 1995). Numerous biotic and abiotic factors contribute to this inconsistent performance of biocontrol agents (Weller, 1988). Inadequate colonization of the rhizosphere, limited tolerance to changes in environmental conditions and fluctuation in the production of antifungal metabolites are among the most important factors (Duffy *et al*., 1996; Pierson and Weller, 1994). Antagonism between the indigenous microbial population and biocontrol agent or mixture of biocontrol agents applied can also influence the performance of a biocontrol agent in the rhizosphere.

These results indicate that specific interactions of biocontrol agents influence disease suppression in combination. It is necessary, therefore to further investigate microbial interactions that enhance or detract biocontrol efficacy (Handelsman and Stabb, 1996) to understand and predict the performance of mixtures of biocontrol agents. Increasing the genetic diversity of biocontrol systems by the mixture of microorganisms may persist longer in the rhizosphere and utilize a wider array of biocontrol mechanisms (e.g. induction of resistance, production of antibiotics and competition for nutrients) under a broader range of environmental conditions (Pierson and Weller, 1994). Multiple organisms may enhance the level and consistency of control by providing multiple mechanisms of action, a more stable rhizosphere community, and effectiveness over a wide range of environmental conditions. In particular combinations of fungi and bacteria

may provide protection at different times or under different conditions, and occupy different or complementary niches. Such combinations may overcome inconsistencies in the performance of individual isolates. Several researchers have observed improved disease control using combinations of multiple compatible biocontrol organisms (Duffy *et al*., 1996; Pierson and Weller, 1994; Lemanceau, 1991; Lemanceau and Alabouvette 1991; Leeman *et al*., 1996; Park *et al*., 1988) and have demonstrated enhanced biocontrol of Fusarium wilt by combining certain nonpathogenic strains of *F. oxysporum* with fluorescent strains of *Pseudomonas.*

7 DELIVERING PGPR: PROS AND CONS

Advantages of a seed treatment with PGPR in a biocontrol system are: 1) their saprophytic nutritional status makes large scale production feasible, 2) only small amounts of inoculum are required, 3) application is simple, 4) independence from energy sources for survival, 5) systemic spread along the surface of the developing root system, and 6) antagonistic activity on the root surface during the economically important phase of early root infection by the pathogens. Their versatile metabolism, fast growth, active movement, and ability to readily colonize the root surface make these rhizobacteria suitable for seed bacterization. Further, seed treatments provide targeted application of PGPR, allowing earlier protection than with foliar sprays. The additional plant growth-promotion by PGPR treatments in comparison to chemical pesticides adds another advantage. However, microorganisms as biocontrol agents have a relatively narrow spectrum of activity compared with synthetic pesticides (Baker, 1991; Janisiewicz, 1988) and often exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for suppression of plant pathogens (Backman *et al*., 1997). However, growing popularity of biocontrol is its record of safety during the past 100 years. No microorganism or beneficial insect deliberately introduced or manipulated for biocontrol purposes has, itself, become a pest and there is no evidence for negative effects of biocontrol agents on the environment. Effective biocontrol demands thorough knowledge of biological interactions at the ecosystem, organismal, cellular, and molecular levers. Biocontrol is also likely to be less spectacular than most physical or chemical controls but usually more stable and long lasting (Baker and Cook, 1974). Although biocontrol is having been used in agriculture for centuries, as an industry biocontrol is still in its infancy.

8 FUTURE PROSPECTS

Diseases are very common in plants and are responsible for the loss of approximately one third of the crop yield (Lugtenberg *et al.*, 1994). Chemical pesticides that control plant diseases have become a threat to health and the environment and hence being banned worldwide. This has increased the interest in biocontrol of plant diseases. PGPR mediated agriculture is now gaining worldwide importance and acceptance for an increasing number of crops and managed ecosystems as the safe method of pest control. Biocontrol has untapped potential and is underused, under exploited, underestimated, often untried and therefore unproven. The new tools of recombinant DNA technology, mathematical modeling, and computer technology combination with a continuation of the more classical approaches such as importation and release of natural enemies and improved germplasm, breeding, and field testing should quickly move biocontrol research and technology into a new era. Although activity and effects of biocontrol have been reported for a number of antagonists, the underlying mechanisms are not fully understood. This deficiency in our knowledge often hinders attempts to optimize the biological activity by employing tailored application strategies. One can envision a number of different ways in which biocontrol efficacy of PGPR might be improved. Biocontrol efficacy of PGPR may be improved by genetically engineering them to over express one or more of these traits so that strains with several different anti-phytopathogen traits can act synergistically. More detailed studies are needed on the composition of the rhizosphere population, the effect of cultivar on bacterial population dynamics, the influence of inoculum density on antagonistic activity, the survival of inoculum under adverse conditions, and the role of environmental conditions in altering the activity of rhizobacteria. An attempt to overcome problems of varying efficacy may be attained by strain mixing, improved inoculation techniques, or gene transfer of active genetic source of antagonists to the host plant (Oostendorp and Sikora, 1986). The soil microbes are active elements for soil development and the basis of sustainable agriculture. Form the point of sustainable agricultural development and good eco-environment establishment, we propose a scientific fertilizer that is to apply organic, inorganic and microbial fertilizers in a balance and rational way to keep high and stable yield.

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