Pest Technology ©2009 Global Science Books

Biological Control of Soilborne Plant Pathogens with Rhizosphere Bacteria

Satyavir S. Sindhu* • **Yuvraj S. Rakshiya** • **Govind Sahu**

Department of Microbiology, CCS Haryana Agricultural University, Hisar-125 004, India

Corresponding author: * **sindhuss@hau.ernet.in**

ABSTRACT

Many of the agrochemicals used in controlling pests and diseases are also implicated in ecological, environmental and human health hazards. To find an effective alternative approach with minimum deleterious effects, biological control of soilborne pathogens by application of specific antagonistic microorganisms to seeds, soil or planting material has been studied intensively in the last two decades. Certain bacteria were characterized from rhizosphere of different crop plants that inhibited deleterious and pathogenic bacteria and fungi by producing antibiotics, bacteriocins, siderophores, hydrolytic enzymes and other secondary metabolites. However, the use of these bacteria to protect crops sometimes fails because antagonistic rhizobacteria are unable to compete or colonize the rhizosphere of inoculated plants. Tremendous progress made in characterizing the process of rhizosphere colonization and competence, identification and cloning of bacterial genes contributing to pathogen suppression will contribute to our current understanding of the mechanisms involved in biocontrol. The limitations of these biocontrol products can be addressed by enhancing biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations and integration of biocontrol with other alternative methods that provide additive effects. These biocontrol agents will subsequently be utilized in sustainable agriculture for improving growth of crop plants.

Keywords: biocontrol, plant diseases, plant pathogens, *Pseudomonas*
Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; **AFMs**, antifungal metabolites; **AHLs**, *N*-acyl-homoserine lactones; **DAPG**, 2,4-diacetyl phloroglucinol; **HCN**, hydrogen cyanide; **ISR**, induced systemic resistance; **PCN**, phenazine-1-carboxamide; **PGPR**, plant growth-promoting rhizobacteria; **QS**, quorum sensing; **SA**, salicylic acid; **SAR**, systemic acquired resistance

CONTENTS

INTRODUCTION

The rhizosphere around the growing plant roots is a very dynamic environment and harbors a much higher number of total microorganisms than root-free soil. The different microbial populations interact with each other and with the plant through symbiotic, associative, neutralist or antagonistic effects and influence the plant growth accordingly. The outcome of colonization or penetration of the plant tissue with a microorganism varies from asymptomatic to disease and from associative to symbiosis, depending upon the mutual perception or recognition between the interacting cells and this interaction is also influenced greatly by the environment (Benizri *et al.* 2001). The microbes that do penetrate and colonize plants have evolved an elaborate system for subverting plant defense system. In the absence of appropriate microbial populations in the rhizosphere, plant growth may be impaired (Sturz *et al.* 2000).

In recent years, there has been a renewed interest in the use of rhizobacteria for inoculation of agricultural crops (Sindhu *et al.* 1997; Ahmad *et al.* 2008). The group of beneficial, root associative bacteria that stimulates the

Received: 6 October, 2008. Accepted: 22 February, 2009.

Review

growth of plant is termed as plant growth-promoting rhizobacteria (PGPR). Fluorescent pseudomonads and bacilli comprise major group among PGPR along with other bacteria like *Acetobacter*, *Actinoplanes*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Cellulomonas*, *Clostridium Enterobacter*, *Erwinia*, *Flavobacterium*, *Pasteuria*, *Rhizobium*, *Serratia* and *Xanthomonas*. Microbial populations in the rhizosphere may benefit the plant in a variety of ways, including: (i) increased recycling, solubilization and uptake of mineral nutrients, (ii) synthesis of vitamins, amino acids, auxins and gibberlins which stimulate plant growth and (iii) antagonism with potential plant pathogens to suppress the diseases (Goel *et al.* 2001a; Weller 2007; Ahmad *et al.* 2008). These rhizobacteria are ideal for use as biocontrol agents as they can provide the front line defense for plant roots against the attack by various plant pathogens (Dowling and O'Gara 1994; Compant *et al.* 2005).

The attempted infection of a plant by a pathogen, such as fungus, may be regarded as a battle whose major weapons are proteins and small chemical compounds produced by both organisms (Ferreira *et al.* 2006). Phytopathogens damage can reduce crop yields varying from 25 to 100%. The population of pytopathogens and severity of the disease is usually controlled by application of chemical agents and pesticides. Many of the pesticides that are used to control phytopathogens are hazardous to animals and humans, and persist and accumulate in natural ecosystems. It is, therefore, desirable whenever possible, to replace these chemicals with biological control agents that are more friendly to the environment. The biological approach for the control of phytopathogenic agents is to use plant growth-promoting bacteria as biocontrol agents to suppress or prevent phytopathogen damage. Thus, biocontrol involves harnessing of disease-suppressive microorganisms to improve plant health.

Several rhizosphere bacteria have the potential to control various root, foliage and post harvest diseases of agricultural crops and these rhizobacteria are ideal for use as biocontrol agents (Glick and Bashan 1997; Spadro and Cullino 2005). Weller (2007) reviewed the use of *Pseudomonas* as biocontrol agents of soilborne pathogens and emphasized the need for development of new formulations and on the testing and efficacy of biocontrol products. Sindhu *et al.* (2002) reported plant growth promoting effects of fluorescent *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions in chick pea. The coinoculation resulted in enhanced nodulation by *Mesorhizobium* sp. and shoot dry weight was increased by 3.92 to 4.20 times in comparison to uninoculated controls. The analysis of sugar-beet associated bacterial and fungal communities for antagonism towards fungal plant pathogens indicated that the majority of antagonistic microorganisms suppressed only one pathogen, whereas only 4-7% showed a broad antagonistic potential (Zachow *et al.* 2008).

Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment. Besides, some non-pathogenic rhizobacteria can induce physiological changes throughout the entire plants, making them more resistant to pathogens. The biological disease control organisms have various advantages, namely: (1) these organisms are considered safer than many of the chemicals now in use, (2) they do not accumulate in the food chain, (3) self replication circumvents repeated applications, (4) target organism seldom develop resistance as happens when chemical control agents are used, (5) where less effective than a chemical control agent, the two sometimes can be combined and (6) properly developed biocontrol agents are not considered harmful to the ecology.

SUPPRESSION OF GROWTH OF PATHOGENIC MICROORGANISMS

Rhizobacteria have been found to suppress diseases caused by various pathogenic bacteria and fungi, and these antagonistic rhizobacteria have the potential for use as biocontrol agents (Weller 1988; Thomashow and Weller 1996; Scherwinski *et al.* 2008). Biological control can be defined as "the control or suppression of a plant disease due to reduction in the number and activity of a plant pathogen by use of one or more organisms or with the product of a natural biological process." Disease suppression by biocontrol agents occurs due to interactions among the biocontrol agents with members of the rhizosphere or phyllosphere community and many microorganisms have been identified which are involved in specific pathogen suppression in soil (Borneman and Becker 2007).

MECHANISMS INVOLVED IN BIOCONTROL

The mechanisms by which rhizobacteria inhibit the growth of phytopathogenic microorganisms includes: (i) antibiotic production; (ii) production of bacteriocins; (iii) production of siderophores; (iv) production of hydrolytic enzymes such as β-1,3-glucanase and chitinases; (v) production of other metabolites; (vi) phytoalexins production; (vii) interference in quorum sensing; (viii) reduction in ethylene production; and (ix) induction of systemic resistance.

(i) Production of antibiotics

Antibiotic production by rhizobacteria is one of the major mechanisms postulated for antifungal activity and plant growth promotion. These antibiotics have been shown to play a role in disease suppression in many biocontrol systems by mutant analyses and biochemical studies using purified antibiotics. These antimicrobial compounds may act on plant pathogenic fungi by inducing fungistasis, inhibition of spore germination, lysis of fungal mycelia, or by exerting fungicidal effects. A large number of antibiotics including diacetyl phloroglucinol, oomycin A, phenazines, pyocyanine, pyrroles, pyoluteorin and pyrrolnitrin, etc. are produced by rhizobacteria (Bender *et al.* 1999), which help in suppression of pathogen growth (**Table 1**). Thus, antibiosis is one of the highly effective mechanisms for suppressing pathogens in the rhizosphere.

The first antibiotics clearly implicated in biocontrol by fluorescent pseudomonads were the phenazine derivatives

Table 1 Antifungal activity and antibiotics produced by fluorescent pseudomonads.

Antibiotics	Producing bacteria	Target pathogen	Target disease
Phenazine-1-carboxylic acid (PCA)	*P. fluorescens* 2-79	*G. graminis* var. *tritici*	Take-all disease of wheat
	P. aureofaciens 30-84	*G. graminis* var. *tritici*	Take-all disease of wheat
2,4-Diacetyl phloroglucinol (DAPG)	*P. fluorescens* CHA0	*G. graminins* var. *tritici*	Take-all disease of wheat
	P. fluorescens Q2-87	*Thielaviopsis basicola*	Black root- rot of tobacco
	P. fluorescens F113	*Pythium ultimum*	Damping-off of sugar beet
	P. fluorescens Pf5	*Rhizoctonia solani*	Sheath blight
Pyrrolnitrin (Prn)	*P. cepacia*	*Bipolaris maydis*	Southern maize leaf blight
	P. fluorescens Pf5	*Sclerotina homoecarpa*	Dollar spot of turf grass
Pyoluteorin (Plt)	*P. fluorescens* Pf5	*Pythium ultimum*	Damping-off of cotton
Iturin A and surfactin	*Bacillus subtilis* RB14	*Rhizoctonia solani*	Damping-off of tomato

that contributed to disease suppression by *Pseudomonas fluorescens* strain 2-79 and *P. aureofaciens* strain 30-84, which control take-all of wheat (Weller and Cook 1983). Gurusiddaiah *et al.* (1986) isolated a strain of *Pseudomonas fluorescens* 2-79 (NRRL B-15132) from wheat rhizosphere, which was suppressive to the take-all disease of wheat root caused by *Gaeumannomyces graminis* var. *tritici*. The antibiotic was isolated from potato glucose broth culture of this strain. This antibiotic was found active against several fungi including *G. graminis* var. *tritici*, *Rhizoctonia solani* and *P. aristesporum.* Evidence for the role of phenazines includes an analysis of transposon insertion mutants that lack the ability to produce phenazine-1-carboxylate are reduced in disease suppressiveness (Thomashow and Weller 1988; Pierson and Thomashow 1992). Furthermore, the antibiotic is produced on roots and rhizosphere of wheat grown in raw soil and treated with *P. fluorescens* strains 2-79 and 30-84 (Mazzola *et al.* 1992). The production of antibiotics and their role in disease control is presented in **Table 1**. Bull *et al.* (1991) reported the production of phenazine-1-carboxylic acid by *P. fluorescens* 2-79 RN_{10}, which acted as biocontrol agent of take-all of wheat. They found an inverse relationship between the population size of phenazine-producing 2-79 RN_{10} and the number of lesions formed by *G. graminis* var. *tritici*. They also reported that phenazine-1-carboxylic acid is a major factor in suppression of *G. graminis* var. *tritici* during primary infection of roots.

Shanahan *et al.* (1992) isolated a *Pseudomonas* sp. strain F113 from the rhizosphere of sugar beets which was found to inhibit a range of plant pathogenic fungi. The antibiotic-like compound was isolated and identified as 2,4-diacetyl phloroglucinol (DAPG). An antibiotic-negative mutant strain F113G22 derived by transposon mutagenesis lost the ability to inhibit bacterial and fungal microorganisms. *P. fluorescens* strain CHA0 was isolated from rhizosphere of tobacco grown near Payerne, Switzerland, in a soil naturally suppressive to black root rot of tobacco caused by *Thielaviopsis basicola* (Stutz *et al.* 1986). It was found to produce a variety of secondary metabolites i.e. pyoluteorin, DAPG, hydrogen cyanide (HCN), salycilic acid, pyochelin and pyoverdine, and protected various plants from diseases caused by soil-borne pathogenic fungi (Keel *et al.* 1992; Maurhofer *et al.* 1992). Mutant strain CHA625 lacking the production of DAPG metabolite showed reduced suppressive effects and the antibiotic-overproducing strains showed improved biocontrol abilities in several host-pathogen systems. Complementation of mutant CHA625 with an 11-kb fragment from a CHA0 genomic library coordinately restored DAPG production, fungal inhibition and disease suppression. The production of DAPG was found to be primary mechanism of take-all suppression by *Pseudomonas* strain CHA0. Later on, enhanced antibiotic production and improved protection against damping-off of cucumber by this strain were found due to amplification of a single gene encoding the house keeping sigma factor σ^{70}. Furthermore, a quantitative relationship between antibiotic production and disease suppressiveness is suggested by the enhancement of production of DAPG and pyoluteorin accomplished by adding extra copies of a 22-kb fragment of DNA that improves suppression of *Pythium* on cucumber (Maurhofer *et al.* 1992). The genes for the biosynthesis of many of the metabolites involved in disease suppression by fluorescent pseudomonads have been isolated, and their regulation has been studied (Pierson and Thomashow 1992; Pierson *et al.* 1995; Bangera and Thomashow 1996; Haas and Keel 2003).

Schmidli-Sacherer *et al.* (1997) obtained a derivative of *P. fluorescens* CHA0 with a mutation in the global regulator gene *gac*A ($GacA^-$) that did not produce phloroglucinol, pyoluteorin and HCN but $GacA^-$ mutant was found to overproduce pyochelin and pyoverdine. $GacA^-$ mutant failed to protect the dicotyledonous plant cress and cucumber against damping-off caused by *Pythium ultimum*. In contrast, the $GacA^-$ mutant could still protect the gramineae wheat and maize against damping-off mediated by *P. ultimum* and wheat against *G. graminis* var. *tritici*. $GacA^-$ Pvd^- double mutant CHA496 overproduced pyochelin and salicylic acid compared with CHA0 and protected wheat against *P. ultimum* and *G. graminis* var. *tritici*, whereas, cress and cucumber were not protected. So, a functional *gac*A gene was necessary for the protection of dicotyledons against root diseases, but not for that of gramineae. Hammer *et al.* (1997) described the cloning and characterization of a 6.2-kb DNA region, which encoded the pyrrolnitrin biosynthetic pathway. This DNA region contained 4 genes, *prn*ABCD, each of which was required for pyrrolnitrin production and conferred the ability to produce pyrrolnitrin when expressed heterologously in *E. coli*.

Although bacilli have received less attention as potential biocontrol agents than the pseudomonads, evidence indicating that they may promote effective disease suppression is accumulating. The bacilli are particularly attractive for practical use because they produce stable endospores, which can survive the heat and desiccation conditions that may be faced by biocontrol agents (Turner and Backman 1991; Osburn *et al.* 1995). One well-studied example is *Bacillus cereus* strain UW85, which suppresses diseases caused by the oomycetes, a group of protists that cause severe plant diseases. Analysis of mutants of *B. cereus* shows a significant quantitative relationship between disease suppressiveness and the production of two antibiotics, zwittermicin A and kanosamine (Silo-Suh *et al.* 1994; Milner *et al.* 1996). Zwittermicin A is an aminopolyol representing a new class of antibiotic and kanosamine is an aminoglycoside. The purified antibiotics suppress disease and inhibit development of oomycetes by stunting and deforming germ tubes of germinating cysts. *Bacillus subtilis* RB14, which produced antibiotics iturin A and surfactin was found to suppress damping-off disease of tomato seed-lings caused by *Rhizoctonia solani* (Asaka and Shoda 1996). Thorough analysis of antibiotic biosynthesis and regulation in the bacilli will depend on the development of genetic techniques, such as high frequency transformation, transposon mutagenesis and reporter gene fusions similar to those available for the pseudomonads.

Trichoderma and *Gliocladium* are closely related fungal biocontrol agents. Each produces antimicrobial compounds and suppresses disease by diverse mechanisms, including the production of the structurally complex antibiotics gliovirin and gliotoxin (Howell *et al.* 1993; Zachow *et al.* 2008). Mutants of *Gliocladium virens* that do not produce gliotoxin are reduced in their ability to control Pythium damping-off (Wilhite *et al.* 1994). Mutants with increased or decreased antibiotic production show a corresponding effect on biocontrol (Howell and Stipanovic 1983).

(ii) Production of bacteriocins

Another important class of antibiotics produced by bacteria is the bacteriocins. The bacteriocins are usually proteins produced by many Gram-negative and Gram-positive bacteria, and they are inhibitory to other related strains of the same species because of their high degree of specificity. One of the first commercial applications of biological control for root diseases has been the use of *Agrobacterium radiobacter* K84 to control the crown gall disease of dicotyledonous plants caused by *Agrobacterium tumefaciens* (New and Kerr 1972). *P. fluorescens* strain BC8 produced a bacteriocin fluorescein-BC8 and inhibited the growth of virulent *P. solanacearum* strains under *in vitro* conditions (Gallardo *et al.* 1989). The avirulent *P. solanacearum* strain having the ability to produce bacteriocin suppressed root colonization by the virulent strain of same species, resulting in reduced incidence of bacterial wilt in tomato and in better plant growth (Arwiyanto *et al.* 1994). Recently, a novel lectin-like bacteriocin related to LipA has been reported from biocontrol *P. fluorescens* Pf5 (Parret *et al.* 2005).

Schwinghamer and Brockwell (1978) reported suppression of growth of sensitive *R. trifolii* strains on coinoculation with bacteriocin-producing *R. trifolii* strains in sterile broth culture and peat culture conditions. Triplett and Barta

(1987) reported that trifolitoxin production by *R. leguminosarum* bv. *trifolii* strain T24 inhibits growth of strains of *R. leguminosarum* bvs. *trifolii*, *phaseoli* and *viciae* as well as *R. fredii*. The genes encoding trifolitoxin production have been cloned from genomic library of strain T24 (Triplett 1988) and the transfer of these genes into an effective *R. leguminosarum* bv. *trifolii* strain TA1 conferred trifolitoxin production. The exconjugants occupied significantly more nodules on clover roots on coinoculation with trifolitoxin sensitive reference strain (Triplett 1990).

(iii) Production of siderophores

Iron is essential element for all living organisms, with the possible exceptions of certain lactobacilli. Iron is abundant in Earths crust, but most of it is found in the insoluble form of ferric hydroxide; thus, iron is only available to organisms at concentrations at or below 10^{-18} M in soil solutions at neutral pH. This presents a challenge for bacteria, which require iron at micromolar concentrations for growth. To cope with its solubility, many microorganisms synthesize extracellular siderophores, in response to low iron stress (Neilands 1981; Neilands and Nakamura 1991). Siderophores are low molecular weight, high affinity Fe^{3+} chelators that transport iron into bacterial cells. Nearly all-aerobic and facultative anaerobic bacteria have been found to produce siderophores. PGPR produce different types of siderophores, which are involved in disease suppression and plant growth promotion (Leong 1986). Two important siderophores-mediated iron uptake systems have been found in the rhizobacteria: one involving the fluorescent pseudobactin (pyoverdine) and other pyochelin. Plant growth promoting rhizobacteria appear to exert their beneficial effects in part by producing extracellular siderophores under iron limiting conditions that efficiently chelate environmental iron, making it less available to endemic microorganisms, thus inhibiting their growth. The various categories of siderophores produced by PGPR include catechol, hydroxamate, pyoverdine and some other types like azotochelin, anthranilic acid and azotobactin.

Kloepper *et al.* (1980) were the first to demonstrate the importance of siderophores production in biocontrol of plant pathogens with pseudobactin, a siderophore produced by plant growth promoting *Pseudomonas* strain B10. Antagonism *in vitro* and fluorescence by the PGPR were not observed when one 10 μM ferric chloride ($FeCl_3$) was added to iron-deficient medium. Also, PGPR did not enhance plant growth when ferric iron was added to field soil in the form of Fe-EDTA. Moores *et al.* (1984) constructed a gene bank from plant growth promoting *Pseudomonas* sp. strain B10. Non-fluorescent mutants of *Pseudomonas* sp. strain B10 were obtained by mutagenesis with nitrosoguanidine (NTG), ethylemethano-sulphonate (EMS), or UV light that were defective in the biosynthesis of yellow-green fluorescent siderophore pseudobactin. No yellow-green, fluorescent mutant defective in the production of pseudobactin was identified. Complementation analysis showed that a minimum of 12 genes, arranged in four gene clusters were required for the biosynthesis of pseudobactin.

Buyer and Leong (1986) reported that growth inhibition of certain deleterious fluorescent pseudomonads by specific beneficial fluorescent pseudomonads was due to in part to the inability of susceptible strains to utilize siderophores from beneficial strains to transport iron (III). Some deleterious strains, which were able to utilize siderophores from beneficial strains, were not inhibited. They suggested that the ability of a given pseudomonad to utilize an exogenous siderophores from another pseudomonad may depend upon its possessing a specific outer membrane receptor protein for that pseudomonad's ferric siderophores. Siderophore-negative mutants were derived from *P. fluorescens* strain 3551 and B224 by chemical, Tn5 insertion and UV mutagenesis. Sid^- mutants of strain 3551 provided less biocontrol than parent strain against *Pythium ultimum* causing damping-off disease of cotton whereas Sid^- mutants of strain B224 showed less increase in growth of wheat than Sid^+ parent strain in presence of wheat pathogen *P. ultimum* var. *sporangiferum* (Schippers *et al.* 1987).

An interesting aspect of siderophore biology is that diverse organisms can use the same type of siderophore. Microorganisms may use each other's siderophores if they contain the appropriate uptake protein (Koster *et al.* 1993; Raaijmakers *et al.* 1995), and plants can even acquire iron from certain pseudobactins (Duijff *et al.* 1995). Studies with various siderophore-negative Tn5 mutants showed that pseudobactin of either pyoverdine and pyochelin was necessary to achieve wild-type levels of protection against *Pythium*-induced damping-off (Buysens *et al.* 1996). Further work is needed to characterize the ability of soilborne organisms to utilize siderophores produced by biocontrol agents. Rapid breakdown of biocontrol would be expected if the target pathogens could circumvent disease suppression predicated on iron deprivation by acquiring the ability to utilize the siderophores from their neighbors in the soil.

A spontaneous mutant of *P. fluorescens* RS111 was isolated that was less sensitive to antagonism by other strains of fluorescent *Pseudomonas* (Bakker *et al*. 2002). This mutant, designated as RS111a, appeared to be more effective in suppression of Fusarium wilt than the parental strain. To evaluate the modes of action of these strains, Tn5 transposon mutant genome banks of RS111 and RS111a were generated. Both strains produced an antifungal compound and non-producing mutants were identified. The antifungal activity of RS111a was less than that of RS111, indicating that the antifungal factor is not very important in disease suppression. Differences in siderophore production were observed between RS111 and RS111a. On CAS medium, non-fluorescent pseudobactin mutants of RS111 produced halo zone, indicating that RS111 produces more than one siderophore. On the other hand, RS111a only produced pseudobactin, as the non-fluorescent mutants did not produce halos on chromo-azurol S (CAS)-supplemented medium.

The phorate-degrading *Pseudomonas* species were isolated from agricultural soil (Bano and Musarrat 2003). It was found that *Pseudomonas* isolates (PS-1, PS-2 and PS-3) exhibited substantial phosphate solubilisation, produced indole acetic acid and siderophore. The isolate PS-3 also showed antifungal activity against *Fusarium oxysporum.* Bano and Musarrat (2004) found that *Pseudomonas* sp. NJ-101 obtained from agricultural soil exhibited efficient degradation of the insecticide carbofuran. The ability to produce hydrogen cyanide and siderophore stipulated its role in biological control. The growth inhibition of *Fusarium* sp. validated the antagonistic activity of NJ-101 against the common phytopathogens. Concurrent production of indole acetic acid and solubilisation of inorganic phosphate revealed its plant growth promotion potential and its significance in management of the agro-environmental and phytopathological problems.

Pseudomonas fluorescens strains isolated from the rice rhizosphere were tested for their antagonistic effect towards rice sheath blight fungal pathogen, *Rhizoctonia solani* (Nagraj *et al.* 2004). Production of chitinase, β-1,3-glucanase, siderophores, salicyclic acid and hydrogen cyanide by *P. fluorescens* were recorded with strain MDU2. A significant relationship between the antagonistic potential of *P. fluorescens* against *R. solani* and its level of β-1,3-glucanase, salicyclic acid and hydrogen cyanide was observed.

(iv) Production of hydrolytic enzymes

Lysis by hydrolytic enzymes excreted by microorganisms is a well-known feature of mycoparasitism. The process of destruction of pathogens by the action of cell wall lysing enzymes is known as parasitism. Extracellular chitinase and laminarinase produced by *P. stutzeri* have shown marked effect on mycelial growth inhibition rather than spore germination and also caused lysis of *F. solani* mycelia and germ tube. *P. cepacia* decreased the incidence of diseases

caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium ultimum* due to production of β-1,3-glucanase (laminarinase).

Chet *et al*. (1990) cloned the gene encoding chitinase enzyme from *Serratia marcescens* and transferred it into *E. coli*. The partially purified chitinase caused extensive bursting of the hyphal tips. This chitinase preparation was effective in reducing disease incidence caused by *R. solani* in cotton under green house condition. Lim *et al.* (1991) reported that *P. stutzeri* strain YPL-1 produced extracellular chitinase and laminarinase which markedly inhibited mycelial growth of *F. solani* rather than spore germination and also caused lysis of mycelia and germ tube. Chet *et al*. (1993) isolated three different chitinase genes from *Serratia*, *Aeromonas* and *Trichoderma.* The cloned genes were expressed in *E. coli* and subsequently introduced into *R. meliloti, P. putida* and *Trichoderma* strains resulting in increased chitinolytic activity of transformants against *Sclerotium rolfsii* and *Rhizoctonia solani*.

Similarly, Khot *et al.* (1996) reported that certain *Pseudomonas* and *Bacillus* isolates produced chitinase, β-1,3 glucanase and siderophores, and reduced the wilt incidence by 31.6% respectively in chickpea under field conditions. Singh *et al*. (1999) reported production of chitinase and β-1,3-glucanase from two chitinolytic bacterial strains, *Paenibacillus* sp. 300 and *Streptomyces* sp. 385, when grown in the presence of colloidal chitin as the sole carbon source. Suppression of *Fusarium* wilt of cucumber by a combination of these two bacteria might be due to the hydrolytic enzyme. Zhang and Yuen (2000) studied the role of chitinase produced by *Stenotrophomonas maltophila* strain C3 in biological control of leaf spot on tall fescue (*Cestuca arudinacea*), caused by *Bipolaris sorokiniano*. A high molecular weight fraction (78 kDa) was chitinolytic and more inhibitory than a low molecular weight, non chitinolytic fraction to conidial germination and hyphal growth of *B. sorokiniana* and to leaf spot development. When boiled, the chitinolytic fraction lost chitinase activity along with most of the antifungal properties.

Serratia plymuthica IC14 was isolated from soil around melon roots (Kamensky *et al*. 2003) and it possessed chitinolytic and proteolytic activities, produced antibiotic pyrrolnitrin as well as siderophores and secreted plant growth hormone, indole-3-acetic acid. Foliar application of strain IC14 protected cucumber against *Botrytis cinarea* grey mold and *S. sclerotiorum* white mold disease of leaves under greenhouse conditions reducing disease incidence by 76 and 84%, respectively. Two mutants were obtained from Tn-5 insertion mutagenesis having increased and second one with low chitinolytic activity. Neither of them differed appreciably from the parent strain in the production of other antifungal compounds or in suppression of *B. cinerea* and *S. sclerotiorum* on plates or in greenhouse conditions. Gohel *et al*. (2004) using above procedure screened *Pseudomonas* sp., *Pantoeadispersa* and *Enterobacter ammrenus* for chitinase and protease activity. These strains inhibited the growth of *Fusarium* sp. and *M. phaseolina*. The culture filtrate inhibiting hyphal elongation was observed microscopically.

Radjacommare *et al*. (2004) isolated 22 strains of *P. fluorescens* from rhizosphere of nine plants species. Two strains Pf 1 and Pf 7 of *Pseudomonas* induced systematic resistance in rice CVIR 50 which was susceptible to sheath blight caused by fungus *R. solani*. Inoculation of rice with sheath blight pathogen and *Pseudomonas* showed an increase in chitinase activity in treated plants than that of untreated control plants. Kohli *et al.* (2006) obtained forty bacterial isolates from rhizosphere of sunflower and screened for their antagonistic activity against two root-rot pathogens *Rhizoctonia solani and sclerotinia sclerotiorum*. *Pseudomonas maltophila* was identified as biocontrol agent against the two root-rot pathogens. It was found to produce chitinase, which was responsible for the lysis of mycelial biomass. *Pseudomonas maltophila* showed good growth on chitin and cell wall preparations of both the fungi when supplied as a sole source of carbon.

Downing *et al.* (2000) transferred cloned *chi*A genes of *Serratia marcescens* and *cry*1Ac7 of *Bacillus thuringiensis* in the sugarcane-associated endophytic bacterium *Herbaspirillum seropodicae*. Expression of the genes resulted in biocontrol of sugarcane borer *Eldana saccharina*. Recombinant strains of *Rhizobium meliloti* have been constructed which carry genes to produce chitinase and expressed it during symbiosis in alfalfa roots (Sitrit *et al.* 1993).

(v) Production of secondary metabolites

Besides antibiotics, siderophores and hydrolytic enzymes, a number of other metabolites are also produced by PGPR, which play important roles in plant growth promotion and resistance to diseases in plants. Hydrogen cyanide (HCN) is the other secondary metabolite, which is known to be produced by many rhizosphere bacteria and has been demonstrated to play a role in the biological control of the pathogens (Voisard *et al.* 1989). HCN overproducing bacterial strains resulted in small but statistically significant increase in the suppression of symptoms caused by *Mycophaerella graminicola* and *Puccinia recondita* f. sp. *tritici* on wheat seedling leaves. Tn5-derived mutant strain CHA5 lacking HCN production was used alongwith parent strain CHA0 to assess the role of HCN production in control of *Thielaviopsis basicola* on tobacco. CHA5 brought about significantly less control of tobacco root rot and did not reduce the percentage of infected root surface. Complementation of the strain CHA5 with HCN^{+} genes restored full biological control activity (Keel *et al.* 1989). Volatile compounds such as ethylene and ammonia gas present in the soil atmosphere have been reported to inhibit germination of fungal spores. But, the production of volatile inhibitors by disease-control bacteria is probably not a significant mechanism in the control of root pathogens.

(vi) Control of ethylene production and suppression of disease

The gaseous plant hormone ethylene is important for normal development in plants as well as for their response to stress. Ethylene mediates a wide range of plant responses and developmental steps (Glick 2004). One of the mechanisms that a number of plant growth-promoting bacteria use to facilitate plant growth and development is the lowering of plant's ethylene concentration through the action of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick 1995). The indole acetic acid (IAA) producing rhizobacteria can stimulate plant cell proliferation and/or can induce the synthesis of the plant enzyme ACC synthase that converts S-adenosylmethionine to ACC (Kende 1993). A portion of the ACC exuded from plant roots is then taken by the bacteria and subsequently converted by the enzyme, ACC deaminase, to ammonia and α-ketobutyrate, both of which are readily metabolized by most soil bacteria (Glick 2004). As a result of lowering the ACC level within a plant, the amount of ethylene that can be subsequently formed in the plant, is also reduced.

Bacterial and plant pathogens not only directly inhibit plant growth, but they also cause the plant to synthesize ethylene (van Loon *et al.* 2006). For example, the exogenous ethylene often increases the severity of a fungal infection, while some ethylene synthesis inhibitors significantly decrease the severity of a fungal infection (Elad 1990; Robinson *et al.* 2001). In one series of experiments, two biocontrol bacterial strains were transformed with the *Enterobacter cloacae* UW4 ACC deaminase gene and the effect of the transformed and nontransformed bacteria on the damage to cucumbers caused by *Pythium ultimum* was assessed (Wang *et al.* 2000). The ACC deaminase-containing biocontrol bacterial strains were more effective than biocontrol strains that did not possess this enzyme. In addition, one ACC deaminase-transformed biocontrol strain significantly reduced the extent of soft rot of potato slices caused

by the bacterial pathogen *Erwinia carotovora* in sealed plastic bags. The nontransformed bacterial strains did not prevent the damage to the potato slices.

(vii) Interference in quorum sensing and inhibition of plant pathogen growth

The best-studied signaling compounds are *N*-acyl-homoserine lactones (AHLs), which are involved in quorum sensing (QS) regulation and are produced by a diverse range of bacterial taxa in nature (Pierson III and Pierson 2007). Dong *et al.* (2004) showed that application of *B. thuringiensis* to potato tubers resulted in inhibition of QS-regulated soft-rot symptom development caused by *E. carotovora*. The net result was control of *E. carotovora* by the AHL-degrading *Bacillus* sp., presumably through interference with the pathogen's ability to express QS-dependent virulence traits.

A *phz*I mutant of biological control *P. chloroaphis* strain 30-84, defective in AHL production, produces no phenazines and is no longer inhibitory to the fungal pathogen (Wood and Pierson III 1996). Addition of the extracts of the *yen*I-engineered tobacco plants restored phenazine production and fungal inhibition, whereas extracts of control plants that did not express *yen*I did not restore phenazine production or pathogen inhibition (Fray *et al.* 1999). Thus, the production of bacterial AHL signals by the plant host complemented an AHL-biological control bacterium and restored its ability to inhibit a plant pathogen. Similarly, tobacco and potato plants were engineered to express the *Bacillus aii*A AHL lactonase enzyme and then challenged with *E. carotovora.* The engineered leaves and tubers showed little pathogen damage, whereas nontransgenic control plants had severe soft rot symptoms (Dong *et al.* 2000), demonstrating that engineered plants could degrade AHL signals effectively to block bacterial communication required for pathogenicity.

(viii) Phytoalexins production

Some non-pathogenic rhizobacteria can induce physiological changes in the plants due to production of phytoalexins, making them more resistant to pathogens (Kuc 1995). Phytoalexins are low molecular weight antimicrobial compounds that are synthesized by and accumulated in plants after exposure to microorganisms. Most phytoalexins are flavonoids or isoflavonoid-like compounds and their occurrence is wide spread in the plant kingdom. Phytoalexins synthesis can be used as indicator of enhanced defense mechanism in bacteria-treated plants. Some polyphenolic compounds have been identified in root exudates of legumes grown under sterile conditions. In lentil, three desoxy-5-flavones and in soybean two isoflavonoids, coumastrol and daidzein were found. Higher phytoalexins concentration was observed in nodules of field grown plants than that from sterile pot cultured plants inoculated with different *R. leguminosarum* strains, suggesting that a plant defense mechanism is induced under field conditions. Flavonoid molecules secreted by legume roots act as inducers for the transcription of rhizobial nodulation genes and thus help in enhancement of nodulation. In beans, coumestrol is released in response to both pathogens and symbiotic rhizobia, in the latter case; it induces the nodulation genes at roughly the same concentration, as do other *nod*-gene inducers.

van Peer *et al.* (1991) showed involvement of induced resistance and phytoalexin accumulation in biological control of *Fusarium oxysporum* f. sp. *dianthi* carnation by *Pseudomonas* sp. strain WCS417r. Bacterization of roots with WCS417r decreased the number of diseased plants with cultivars 'Pallas' and 'Lena' by about 50-20 and 69-38% respectively and there was an increased accumulation of phytoalexins in stems of bacterized and inoculated plants compared with non-bacterized, fungus-infected plants. Sundaresan *et al.* (1993) showed induction and accumulation of phytoalexins in cowpea roots infested with mycorrhizal fungus *Glomus fasciculatum* and their resistance to *Fusarium* wilt disease. In mycorrhizal plants, the production of phytoalexins compounds was always higher than in non-mycorrhizal plants. Inoculation of the vesicular-arbuscular mycorrhiza (VAM) fungus also improved plant growth of cowpea along with imparting tolerance of the plant to wilt disease. Goel *et al.* (2001b) reported that inoculation of fluorescent *Pseudomonas* strain MRS13 in chickpea caused a 61.1 and 35.1% increase in the accumulation of flavonoid-like compounds in cvs. 'C235' and 'H8618', respectively compared to the uninoculated control.

(ix) Induction of systemic resistance

Some biocontrol agents induce a sustained change in the plant, increasing its tolerance to infection by a pathogen, a phenomenon known as induced systemic resistance (ISR). In some cases, it is clear that induced resistance by biocontrol agents involves the same suite of genes and gene products involved in the well-documented plant response known as systemic acquired resistance (SAR), but this is not always the case. SAR is typically a response to a localized infection or an attenuated pathogen, which is manifested in subsequent resistance to a broad range of other pathogens (Uknes *et al.* 1992).

Various non-pathogenic *Pseudomonas* rhizobacteria have the ability to induce a state of systemic resistance in plants, which provides protection against a broad spectrum of phytopathogenic organisms including fungi, bacteria, and viruses. ISR brought about by prior inoculation of the host by a pathogen, avirulent or incompatible forms of a pathogen, or heat killed pathogens has been attributed to induce physiological response of the host plant against subsequent inoculation by the virulent pathogens (Hoffland *et al.* 1996). Induced systemic resistance in plant has been demonstrated in over 25 crops, including cereals, cucurbits, legumes, solanaceous plants and trees against a wide spectrum of pathogens. Inoculation of PGPR strains 98B-27 (*P. putida*) and 90-166 (*Serratia marcescens*) and the pathogen (*F. oxysporum* f. sp. *cucumerinum*) on separate halves of roots of cucumber seedlings exhibited that both PGPR strains induced systemic resistance against Fusarium wilt as expressed by delayed disease symptom and reduced number of dead plants compared to the non-bacterized *F. oxysporum* f. sp. *cucumerinum*-inoculated plants (Liu *et al.* 1995). It has been shown that the biocontrol agent *P. fluorescens* strain CHA0 (Maurhofer *et al.* 1994) induces SAR-associated proteins, confers systemic resistance to a viral pathogen, and induces accumulation of salicylic acid (SA), which plays a role in signal transduction in SAR (Gaffney *et al.* 1993; Ryals *et al.* 1996). Mutants of CHA0 that do not produce the siderophore pyoverdine do not induce SAR, suggesting a novel role for bacterial metabolites in disease suppression (Maurhofer *et al.* 1994).

Induced systemic resistance triggered in some rhizobacterial strains depends on SA signaling in the plants. Induced resistance by *P. aeruginosa* 7NSK2 was found to be iron-regulated and involved three siderophores, pyoverdine, pyochelin and salicylic acid. SA is also a precursor in the production of SA-containing siderophores, such as pseudomonine in *P. fluorescens* WCS374 and pyochelin in *P. aeruginosa* 7NSK2 (Audenaert *et al.* 2002). The mutant KMPCH was derived which produces SA but is no longer able to incorporate it into pyochelin. Measuring SA levels on tobacco roots colonized by either WCS374 or KMPCH suggested that mutant KMPCH does produce SA in the rhizosphere but the wild-type strain does not. Triggering of ISR by the wild-type 7NSK2 is now postulated to depend on a combined action of pyochelin and the phenazine antibiotic pyocyanin. A mutant of 7NSK2 that lacks SA and pyochelin production no longer induces resistance, but neither does a mutant defective in pyocyanin biosynthesis trigger ISR in tomato against *B. cinerea*. A treatment with the combination of two mutants did result in significant suppression of *B. cinerea* (Audenaert *et al.* 2002). The induction of resistance

by mutant KMPCH, however, depends on SA. Additional support for induction of resistance by bacterially produced SA comes from the study in which the SA biosynthesis genes of *P. aeruginosa* PAO1 were expressed in the non-SA-producing *P. fluorescens* strain P3 and improved ISR in tobacco against tobacco necrosis virus (Maurhofer *et al.* 1998). Recently, salicylic acid has been found important in providing basal defence to *Solanum tuberosum* against *Phytophthora infestans* (Halim *et al.* 2007).

Another line of evidence for induced resistance, which may or may not involve SAR, is that some biocontrol agents suppress disease when they are applied far from the site of infection by the pathogen, and they cannot be found at the infection site (Wei *et al.* 1991; Zhou and Paulitz 1994; Liu *et al.* 1995). Furthermore, in suppression of Fusarium wilt by *P. fluorescens*, preparations of lipopolysaccharides from the bacterial cell surface induce resistance as effectively as the living bacteria, demonstrating that biocontrol is not necessarily due to transport of the bacteria or an antibiotic through the plant (Leeman *et al.* 1995). Whether or not biocontrol agents suppress disease by inducing resistance, it is essential that SAR and biocontrol strategies be compatible, because future agricultural practices are likely to require the integration of multiple pest control strategies.

The mechanism of ISR has also been studied in plant growth-promoting *Bacillus* spp. (Kloepper *et al.* 2004). Bacterial production of the volatile 2,3-butanediol is the trigger of *Bacillus*-mediated ISR in *Arabidopsis*. The signaling pathway that is activated in this case depends on ethylene but is independent of salicylic acid and jasmonic acid signaling (Ryu *et al.* 2004). Broekaert *et al.* (2006) reported that induced ethylene biosynthesis and subsequent intracellular signaling leads to a cascade of transcription factors consisting of primary EIN3-like regulators and downstream ERF-like transcription factors. The latter control the expression of various effector genes involved in various aspects of systemic induced defence responses, eventually resulting in a differential disease response. 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). One of the genes that was upregulated by WCS417r is the MYB72 transcription factor gene. An *myb72* knockout mutant of *Arabidopsis* no longer expresses WCS417r-mediated ISR, indicating that it plays an important role in signaling in the plant.

RELATION BETWEEN GROWTH PROMOTION AND BIOLOGICAL CONTROL

In recent years, there is no clear separation of growth promotion in plants and biological control induced by bacterial inoculants (Lugtenberg *et al.* 1991; Goel *et al.* 2001a). Bacterial strains selected initially for *in vitro* antibiosis as part of evaluating biological control activity frequently demonstrate growth promotion in the absence of target pathogen (Sindhu *et al.* 1999; Goel *et al.* 2002). Similarly, PGPR selected initially for growth promotion in the absence of pathogens may demonstrate biological control activity when challenged with the pathogens, presumably by controlling deleterious microorganisms or non-target pathogens (Compant *et al.* 2005).

The PGPR that enhance plant growth by controlling deleterious microorganisms can also exhibit biological control of parasitic pathogens. Inoculation of potato seed pieces with two strains of fluorescent pseudomonad PGPR, which were responsible for significant yield increases in field trials, resulted in a reduction in populations of *Erwinia carotovora* on roots, ranging from 95 to 100% fewer than controls without PGPR treatment (Kloepper 1983). Root colonization by PGPR resulted in reductions in the percentage of daughter tubers infested with *E. carotovora*, ranging from 28 to 92%, compared with controls without PGPR treatment.

Direct growth promotion occurs when a rhizobacterium produces metabolites that directly promote plant growth without interactions with native microflora (Kloepper *et al.* 1991). In contrast, antibiotics, siderophores and HCN, which decrease activities of pathogens or deleterious microorganisms and, thereby, increase plant growth, are examples of indirect growth promotion by biological control (Pierson and Weller 1994). Voisard *et al.* (1989) reported that *P. fluorescens* strain CHA0 induced increased root hair deformation on tobacco in a pathogen-free gnotobiotic assay. de Freitas and Germida (1991) described a similar increase in lateral root hairs and overall root length after seed treatment of wheat with several PGPR strains in gnotobiotic assay. Sindhu *et al.* (2002) reported plant growth promoting effects of fluorescent *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions in chick pea. The coinoculation resulted in enhanced nodulation by *Mesorhizobium* sp. and shoot dry weight was increased by 3.92 to 4.20 times in comparison to uninoculated controls.

Under gnotobiotic conditions, fluorescent pseudomonad PGPR strains did not promote plant growth of potato (Kloepper and Schroth 1981b) whereas, growth promotion in field soils was associated with a 23-64% reduction in the population densities of indigenous rhizoplane fungi and 25-93% reduction in Gram-positive bacterial population densities (Kloepper and Schroth 1981a). Thus, the plant response was related to control of native microorganisms, rather than to direct growth promotion. Suslow and Schroth (1982) found specific strains of root-colonizing bacteria that were pathogenic on sugar beet seedlings and termed them deleterious rhizobacteria (DRB). Strains of DRB have been found in diverse genera on many crops on which they cause growth inhibition and root deformations (Fredrickson and Elliot 1985; Schippers *et al.* 1987). Specific PGPR strains reduce the population of DRB in short rotations and increase yields to levels equivalent to yields in long rotations (Schippers *et al.* 1987). Jagadeesh *et al.* (2006) studied the effect of deleterious bacteria on the growth of tomato plants in an axenic culture. Tomato bacterization with *Bacillus* DHBS demonstrated significant reduction in root and shoot length by 13.5 and 47.6%, respectively over the uninoculated control treatment. However, dual inoculation of DHBS and fluorescent *Pseudomonas* sp. RDV108 reduced the plant growth-inhibiting effect of DHBS and increased root length by 28.8%. Hence, growth promotion with such PGPR strains occurs by biological control or "indirect growth promotion".

Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) was studied by application of plant growth-promoting rhizobacteria (Dey *et al.* 2004). Nine different isolates of PGPR were selected from a pool of 233 rhizobacterial isolates obtained from the peanut rhizosphere based on ACC-deaminase activity. All the nine isolates were identified as *Pseudomonas* species. Four of these isolates, *viz.* PGPR1, PGPR2, PGPR4 and PGPR7, produced siderophore and indole acetic acid (IAA). In addition, *Pseudomonas fluorescens* PGPR1 also possessed the properties like tri-calcium phosphate solubilization, ammonification and inhibited *Aspergillus niger* and *A. flavus* under *in vitro* conditions. In addition to the traits exhibited by PGPR1, the strain PGPR4 showed strong *in vitro* inhibition to *Sclerotium rolfsii*. In field trials, however, there was wide variation in the performance of PGPR isolates in enhancing the growth and yield of peanut in different years. Plant growth-promoting fluorescent pseudomonad isolates, *viz.* PGPR1, PGPR2 and PGPR4, significantly enhanced the pod yield (23-26, 24-28 and 18-24%, respectively), haulm yield and nodule dry weight over the control in 3 years. Seed bacterization with plant growth-promoting *P. fluorescens* isolates, *viz.* PGPR1, PGPR2 and PGPR4, suppressed the soil-borne fungal diseases like collar rot of peanut caused by *A. niger* and isolate PGPR4 also suppressed stem rot caused by *S. rolfsii*.

Hynes *et al.* (2008) screened 563 bacteria obtained from the roots of pea, lentil and chickpea grown in Saskatchewan for the suppression of legume fungal pathogens and for plant growth promotion. Screening of bacteria showed that 76% isolates produced siderophore, 5% isolates showed amino-cyclopropane-1-carboxylic acid (ACC) deaminase activity and 7% isolates were capable of indole production. Twenty-six isolates (5%) suppressed the growth of *Pythium* species strain p88-p3, 40 isolates (7%) suppressed the growth of *Fusarium avenaceum* and 53 isolates (9%) suppressed the growth of *Rhizoctonia solani* CKP7. Seventeen isolates (3%) promoted canola root elongation in a growth pouch assay, and of these, 4 isolates promoted the growth of lentil and one isolate promoted the growth of pea. Fatty acid profile analysis and 16S rRNA sequencing of the isolates showed that 39-42% were the members of *Pseudomonadaceae* and 36-42% of the *Enterobacteriaceae* families. The biocontrol efficacy of three bacterial antagonists introduced into naturally *Rhizoctonia*-infested lettuce fields was assessed against *R. solani* (Scherwinski *et al.* 2008). Statistically significant biocontrol effects were observed for all applied bacterial antagonists compared with uninoculated control. Analysis of the indigenous bacterial and endophytic fungal populations revealed only negligible short-term effects resulting from the bacterial treatments, and that they were more influenced by field site, plant growth stage and microenvironment.

DEVELOPMENT OF BIOCONTROL PRODUCT AND CONSTRAINTS IN THEIR USE

Recently, various biocontrol agents have been tested in field on different crops for controlling plant diseases. Some of the antagonistic bacterial strains have led to the development of commercial biocontrol products (**Table 2**). It has been observed that these biocontrol products control relatively narrow spectrum of diseases on a particular host crop. Moreover, a company must assess many factors including demand of the product, potential market size and competition with existing chemicals or biocontrol formulation products, before approaching for commercial production.

The major disadvantages in using the PGPR as a biocontrol agent include variability of field performance and the necessity for precautions to ensure survival and delivery of the product. Also, the effectiveness of a given biocontrol agent may be restricted to a specific location, due to the effects of soil and climate. Many soil edaphic factors, including temperature, soil moisture, pH, clay content, interactions of biological-disease control microorganisms with other rhizosphere bacteria and with pathogens will also affect their viability and tolerance to adverse conditions once applied. During root colonization by introduced bacteria, introduced microorganisms have to compete with indigenous microflora for carbon source, mineral nutrients and infection sites on the roots. Sometimes, this competition is so severe that introduced microorganism fails to survive in the soil. Another factor that can contribute to inconsistent performance of PGPR is variable production or inactivation *in situ* of bacterial metabolites responsible for plant growth promotion.

Biological control strategies are also emerging as promising alternatives to the use of synthetic fungicides in the preservation of fruits. Viñas (1995) reported that survivability of the antagonist is a major factor to determine its usefulness against post-harvest fruits diseases. Antagonists must survive and can be effective after their exposure to both post-harvest treatments and storage conditions. Several antagonistic microorganisms have been found that can be effective to inhibit the development of post-harvest diseases. Thus, biological control of post-harvest diseases (BCPD) has emerged as an effective alternative to the application of fungicides (Janisiewicz and Korsten 2002). Because wound-invading necrotrophic pathogens are vulnerable to biocontrol, the antagonists can be applied directly to the targeted area (fruit wounds) and a single application using existing delivery systems (drenches, line sprayers, on-line dips) can significantly reduce fruit decays. The pioneering biocontrol products BioSave and Aspire were registered by EPA in 1995 for the control of post-harvest rots of pome and citrus fruits, respectively and are commercially available. The limitations of these biocontrol products can be addressed by enhancing biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations and integration of biocontrol with other alternative methods that alone do not provide adequate protection but in combination with biocontrol agents provide additive or synergistic effects.

APPROACHES TO INCREASE THE EFFICIENCY OF BIOCONTROL AGENT

Now it has been well established that root colonization by biocontrol agent is the prerequisite to suppress the plant disease and enhance the plant growth. Root colonization by introduced bacteria could be improved by increasing the population size, distribution or survival of bacteria, along with manipulation of soil factors that may positively or negatively affect colonization. Bacterial traits such as growth rate, cell surface properties, chemotaxis to root exudates,

Table 2 List of some commercially available biological control agents.

Trade name	Biocontrol organism	Formulation	Target pathogen / disease	Crops tested	Company and countryof origin
BlightBan A506	*P. fluorescens*	Lyophilized cells / powder (Wettable)	*Erwinia amylovora* / Fire blight Frost damage control of fruits	Pear and apples Cherry, strawberry, tomato and potato	NuFarm Americas, Burr Ridge, II.
Galtrol Diegal No gall	*Agrobacterium radiobacter*	-	*Agrobacterium tumefaciens* Crown gall	Several crops	Fruit growers chemical Co, New Zealand
Epic Kodiak	*Bacillus subtilis*	Dry powder	*Rhizoctonia solani*, *Fusarium*, *Alternaria* and *Aspergillus* sp. Damping off	Cotton and legumes Cotton and legumes	Gustafsan Inc. Tx. USA
Biocoat	*P. fluorescens* WCS374r	Dust	*F. oxysporum* of *raphani* and *dianthi* Fusarium wilt and carnation wilt	-	S & G seeds, BV, Netherlands
Mycostop	*Streptomyces griseoverdis*		*A. brassicicola* Damping-off of crucifers	Crucifers	Kemira Agro, Oy, Finland
Bio-save, 10 LP and 11 LP	*P. syringae* strains ESC-10 and ESC-11	Wettable powder	*Botrytis cinerea, Mucor pyriformis, Geotrichum candidum* and *Penicillium* sp. Postharvest fungal diseases	Citrus and pome fruit	Jet Harvest Solutions, Florida
System Deny	*B. subtilis* *Burkholderia cepacia*	Dust Powder	Seedling pathogens *Pythium* sp. Damping-off	Bean, barley, cotton and peanut -	- CCT Crop, Carlsbad, USAD

production of secondary metabolites and tolerance to dehydration and temperature also contribute to rhizosphere competence. Use of green fluorescent protein (GFP) and *in situ* monitoring based on confocal laser scanning microscope (CLSM) could contribute to understanding of the rhizosphere competence and root colonization (Johri *et al.* 2003). Thus, the study of microbial communities has been facilitated by the use of combinations of the green fluorescent protein [GFP], cyan fluorescent protein [CFP], red fluorescent protein [RFP] and yellow fluorescent protein [YFP] and DsRed as a marker (Bloemberg and Lugtenberg 2001). With the help of this technique, it has been found that the *Pseudomonas* biocontrol strains colonize the seed and root surface at the same position, as do the pathogenic fungi that they control (Bloemberg *et al.* 2000).

Another promising approach that will likely broaden the array of traits considered important for colonization is to screen mutants directly for increased or decreased ability to colonize the roots. Mutants of *Pseudomonas* strains of both phenotypes have been identified and analysis of these mutants indicated that prototrophy for amino acids and vitamins, rapid growth rate, utilization of organic acids, and lipopolysaccharide properties contribute to colonization ability. Modification of the genes involved in the biocontrol activity of biological control agents also plays a key role in improving the potential, rhizosphere competence as well as antifungal activity of biological control agents, e.g. biocontrol activity of *P. fluorescens* carrying PCA coding mini-Tn5 vector was enhanced by introducing *phz*H gene from *Pseudomonas chlororaphis* PCL1391 (Timmis-Wilson *et al.* 2000). Whereas, mutational disruption of the biosynthesis genes coding for antifungal metabolite 2,4-diacetylphloroglucinol did not influence the ecological fitness of *Pseudomonas fluorescens* F113 in the rhizosphere of sugarbeets (Carroll *et al.* 1995). Recently, genes of *Pseudomonas* biocontrol strains have been identified that can be induced or repressed by the presence of phytopathogenic fungi. *In vivo* expression technology (IVET) has been used to show that the presence of *Phytopthora parasitica* can induce various genes in *P. putida*, including genes encoding diacylglycerol kinase, ABC transporters and outer membrane porins. In contrast, two ribosomal RNA operons of *P. fluorescens* were found to be repressed by *Pythium ultimum*.

Roberts *et al.* (2007) reported that *Enterobacter cloacae* strain 501R3 shows promise as biological control agents for *Pythium ultimum*-induced damping off disease in cucumber and other crops. Population of *Enterobacter cloacae* M59 (a mini-Tn5 Km transposon mutant of strain 501R3) was significantly lower on cucumber roots and decreased much more rapidly than those of strain 501R3 with increasing distance from the soil line. The strain M59 was deficient in growth and chemotaxis on most individual compounds detected in cucumber root exudate and on a synthetic medium supplemented with cucumber root exudate. Molecular characterization of strain M59 demonstrated that mini-Tn5 Km was inserted in *cya*A, which encodes adenylate cyclase. Adenylate cyclase catalyzes the formation of cAMP, and cAMP level in cell lysates from strain M59 was approximately 2% those of strain 501R3. Addition of exogenous, non-physiological concentrations of cAMP to strain M59 restored growth (1 mM) and chemotaxis (5 mM) on synthetic cucumber root exudate and increased cucumber seedling colonization (5 mM) by this strain without serving as a source of reduced carbon, nitrogen, or phosphorus. These results demonstrated a role for *cya*A in colonization of cucumber roots by *Enterobacter cloacae*.

Cabbage seeds were encapsulated in alginate polymer containing an antagonistic bacterium, *Pseudomonas fluorescens* strain LRB3W1 (Someya *et al.* 2007). Seed germination was not inhibited by the encapsulation. Seedlings were transplanted into soil infested with *Rhizoctonia solani*, a pathogen, which causes cabbage damping-off disease. A week after treatment, the damping-off disease in encapsulated seedlings was lower than that of untreated control. Additionally, 2 weeks after germination, the seedlings were inoculated with *Fusarium oxysporum* f. sp. *conglutinans*, a pathogen of cabbage yellows. The yellows disease was less severe with bacterial encapsulation treatment compared with the untreated control. The bacterium colonized in the cabbage rhizosphere after germination of encapsulated seeds. The bacterium survived in the alginate polymer for a prolonged period at 4°C temperature, thus, encapsulation of cabbage seed with the biocontrol bacterium was found effective for protection against cabbage soilborne diseases.

Talc-based formulated *Burkholderia cepaci*a strain Bu1 was found more effective to suppress the rapeseed damping off disease caused by *Rhizoctonia solani* than the suspension of bacteria cells in carboxymethylcellulose solution (1% w/v), in both greenhouse and field trials (Sharifi-Therani *et al.* 2007). The formulation of strain Bu1 as soil and seed treatments was the most effective treatment to increase the root dry weight in the infected greenhouse soil. The formulation of strain Bul as soil drench had the greatest effect on the enhancement of roots fresh weight and stem fresh and dry weights. The formulation of strain Bu1 stored at 4°C temperature exhibited better shelf life and efficacy in *in vitro* than its counterpart stored at 25°C temperature.

The biocontrol performance of soil pseudomonads may be improved by the introduction of antibiotic biosynthetic genes (Haas and Keel 2003). Recombinant strains with greatly increased DAPG and phenazine-1-carboxamide (PCN) production have been constructed (Mavrodi *et al.* 1998; 2001). Bacteria are being constructed which combine two of the three useful traits: production of DAPG and PCN; and ISR. The production of DAPG and PCN will be placed under the control of strong promoters or of exudate-induced or rhizosphere-induced promoters (Mavrodi *et al.* 2006). Moreover, the genes responsible for the production of secondary metabolites and involved in plant growth promotion could be transferred to other rhizobacterial strains possessing good colonizing and competitive ability. A good PGPR strain should produce the secondary metabolites under variable growth conditions. Therefore, such strains should be selected, which show a constant and medium-independent production of secondary metabolites. Further, it has been found that PGPR show greater and more consistent disease suppression when applied as mixtures of ecologically diverse strains with similar functions. Finally, combining biocontrol traits of several strains into one cell sometimes led to increase the level of biocontrol. However, in a number of cases the results were negative, presumably because of metabolic interference of biosynthetic pathways.

CONCLUSION

The studies reviewed here show that there is a large potential for sustainable biocontrol in suppression of plant diseases. However, the complex interactions between the biocontrol agent, the plant and the environment are responsible for the variability observed in disease suppression and plant growth promotion. The inconsistency in performance of these biocontrol agent strains is a major constraint to their wide spread use as biocontrol agent in commercial agriculture. Genetic manipulation of biocontrol agent bacteria has the potential to construct significantly better strains with improved biocontrol efficacy (Trevors *et al*. 1990; Weller and Thomashow 1993). Genetic manipulations could result in new biocontrol strains with increased production of toxic compounds or lytic enzymes, improved space or nutrient competence, wider host range or enhanced tolerance to abiotic stress. Genes and enzymes involved in the biocontrol mechanism could be applied directly or transferred to crop. Further, the efficacy of biocontrol bacteria can be improved by developing the better cultural practices and delivery systems that favor their establishment in the rhizosphere (Sharifi-Tehrani *et al.* 2007; Someya *et al.* 2007). The application of mixtures 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 (Pierson and

Weller 1994).

Future strategies are required to clone genes involved in the production of antibiotics, siderophores and other metabolites, and to transfer these genes into the strains having good colonization potential along with other beneficial characteristics such as nitrogen fixation. Although, production of a high level of antifungal metabolites (AFMs) poses a physiological burden on the producing cell and results in a slower growth rate. However, by using rhizosphere- or exudate-inducible promoters, the production of AFMs can be limited to the plant root, the only site where AFM is needed. In the near future, the biotechnological approaches used in manipulation of bacterial traits will lead to improved biocontrol activity and better plant growth.

ACKNOWLEDGEMENTS

We extend special thanks to our collaborators and laboratory members for help and thoughtful discussions.

REFERENCES

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* **163**, 173-181

Arwiyanto T, Goto M, Tsuyumu S, Takikawa Y (1994) Biological control of *Pseudomonas solanacearum* isolated from sterlitziaginae. *Annual Phytopathological Society of Japan* **60**, 421-430

Asaka O, Shoda M (1996) Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Applied and Environmental Microbiology* **62**, 4081-4085

Audenaert K, Puttary T, Cornelis P, Hofte M (2002) Induction of systemic resistance by *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: Role of salicylic acid, pyochelin and pyocyanin. *Molecular Plant-Microbe Interactions* **15**, 1147-1156

Bakker PAHM, Boer-de M, Zhang-Ke C, Sluis Van-der I, Loon Van LC (2002) Dose response relationships for control of Fusarium wilt by *Pseudomonas fluorescens* RSIII. *Bulletin-OILB/Crop* **25**, 107-110

Bangara MG, Thomashow LS (1996) Identification and characterization of a gene cluster for synthesis of polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *Journal of Bacteriology* **181**, 3155-3163

Bano N, Musarrat J (2003) Isolation and characterization of phorate degrading soil bacteria of environmental and agronomic significance. *Letters in Applied Microbiology* **36**, 349-353

Bano N, Musarrat J (2004) Characterization of a novel carbofuran degrading *Pseudomonas* sp. with collateral biocontrol and plant growth promoting potential. *FEMS Microbiology Letters* **231**, 13-17

Bender CL, Rangaswamy V, Loper J (1999) Polyketide production by plant-associated pseudomonads. *Annual Review of Phytopathology* **37**, 175-196

Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhzobacteria. *Biocontrol Science and Technology* **11**, 557-574

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology* **4**, 343-350

Bloemberg GV, Wijijes AHM, Lamers GEM, Sluurman N, Lugtenberg BJJ (2000) Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different auto fluorescent proteins in the rhizosphere; new perspectives for studying microbial communities. *Molecular Plant-Microbe Interactions* **13**, 1170-1176

Borneman J, Becker JO (2007) Identifying microorganisms involved in specific pathogen suppression in soil. *Annual Review of Phytopathology* **45**, 153-172

Broekaert WF, Delaure SL, De Bolle MFC, Cammue BPA (2006) The role of ethylene in host-pathogen interactions. *Annual Review of Phytopathology* **44**, 393-416

Bull CT, Weller DM, Thomashow LS (1991) Relationship between root colonization and suppression of *Gaeumanomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. *Phytopathology* **81**, 954-959

Buyer JS, Leong J (1986) Iron transport-mediated antagonism between plant growth-promoting and plant-deleterious *Pseudomonas* strains. *The Journal of Biological Chemistry* **261**, 791-794

Buysens S, Heungens K, Poppe J, Hofte M (1996) Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. *Applied and Environmental Microbiology* **62**, 865-871

Carroll H, Moenne-Loccoz Y, Dowling DN, O'Gara F (1995) Mutational disruption of the biosynthesis genes coding for the antifungal metabolite 2,4-diacetylphloroglucinol does not influence the ecological fitness of *Pseudomonas fluorescens* F113 in the rhizosphere of sugarbeets. *Applied and Environmental Microbiology* **61**, 3002-3007

Chet I, Borak Z, Oppenheim A (1993) Genetic engineering of microorganisms for improved biocontrol activity. *Biotechnology* **27**, 211-235

Chet I, Ordentlich A, Shapira R, Oppenheim A (1990) Mechanism of biocontrol of soil borne plant pathogens by rhizobacteria. *Plant and Soil* **129**, 85-92

Compant S, Duffy B, Nowak J, Clemet C, Barka EA (2005) Use of plant growth-promoting rhizobacteria for biocontrol of plant diseases: principles, mechanism of action and future prospects. *Applied and Environmental Microbiology* **71**, 4951-4959

de Freitas JR, Germida JJ (1991) *Pseudomonas cepacia* and *Pseudomonas putida* as winter wheat inoculants for biocontrol of *Rhizoctonia solani*. *Canadian Journal of Microbiology* **37**, 780-784

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research* **159**, 371-394

Dong YH, Xu, JL, Li XJ, Zhang LH (2000) AiiA, an enzyme that inactivates the acyl-homoserine lactone quorum-sensing signal and attenuates the virulence of *E. carotovora*. *Proceedings of the National Academy of Sciences USA* **97**, 3526-3531

Dong YH, Zhang XF, Xu JL, Zhang LH (2004) Insecticidal *Bacillus thuringiensis* silences *E. carotovora* virulence by a new form of microbial antagonism, signal interference. *Applied and Environmental Microbiology* **70**, 954-960

Dowling DN, O'Gara F (1994) Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. *Trends in Biotechnology* **12**, 133-141

Downing KJ, Leslie G, Thomson JA (2000) Biocontrol of sugarcane borer *Eldana saccharina* by expression of the *Bacillus thruingiensis cry*1AC7 and *Serratia marcescens chi*A genes in the sugarcane-associated bacteria. *Applied and Environmental Microbiology* **62**, 4081-4085

Duijff BJ, de Kogel WJ, Bakker PAHM, Schippers B (1995) Influence of pseudobactin 358 on the iron nutrition of barley. *Soil Biology and Biochemistry* **26**, 1681-1688

Elad Y (1990) Production of ethylene in tissues of tomato, pepper, French-bean and cucumber in response to infection by *Botrytis cinerea*. *Physiology and Molecular Plant Patho*logy **36**, 277-287

Ferreira RB, Monteiro S, Freitas R, Santos CN, Chen Z, Batista LM, Duarte J, Borges A, Teixeira AR (2006) Fungal pathogens: the battle for plant infection. *Critical Reviews in Plant Science* **25**, 505-524

Fray RG, Throup JP, Daykin M, Wallace A, Williams P, Stewart GSAB, Grierson D (1999) Plants genetically modified to produce AHLs communicate with bacteria. *Nature Biotechnology* **17**, 1017-1020

Fredrickson JK, Elliot LF (1985) Effects of winter wheat seedling growth by toxin-producing rhizobacteria. *Plant and Soil* **83**, 399-409

Gaffeny T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kassmann H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**, 754-756

Gallardo PB, Panno LC, Guichaquelen V (1989) *In vitro* inhibition of *Pseudomonas solanacearum* by using the antagonist BC8 strain of *Pseudomonas fluorescens*. *Revista de Microbiologia* **20**, 27-33

Glick BR (1995) The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41**, 109-117

Glick BR (2004) Bacterial ACC deaminase and the alleviation of plant stress. *Advances in Applied Microbiology* **56**, 291-312

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. *Biotechnological Advances* **15**, 353-378

Goel AK, Sindhu SS, Dadarwal KR (2001a) Application of plant growth-promoting rhizobacteria as inoculants of cereals and legumes. In: Yadav AK, Raychaudhary S, Motsara MR (Eds) *Recent Advances in Biofertilizer Technology*, Society for Promotion and Utilization of Resources and Technology, New Delhi, pp 207-256

Goel AK, Sindhu SS, Dadarwal KR (2002) Stimulation of nodulation and plant growth of chickpea (*Cicer arietinum* L.) by *Pseudomonas* spp. antagonistic to fungal pathogens. *Biology and Fertility of Soils* **36**, 391-396

Goel AK, Sindhu SS, Dadarwal KR (2001b) Seed bacterization with fluorescent *Pseudomonas* enhances the synthesis of flavonoid-like compounds in chickpea (*Cicer arietinum*). *Physiology and Molecular Biology of Plants* **6**, 195-198

Gohel V, Chaudhary T, Vyas P, Chhatpar HS (2004) Isolation and identification of marine chitinolytic bacteria and their potential in antifungal biocontrol. *Indian Journal of Experimental Biology* **42**, 715-720

Gurusiddaiah S, Weller DM, Sarkar A, Cook RJ (1986) Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. *Antimicrobial Agents and Chemotherapy* **29**, 488-495

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annual Review of Phytopathology* **41**, 117-153

Halim VA, Eschen-Lippold L, Altmann S, Birschwilks M, Scheel D, Rosahl S (2007) Salicylic acid is important for basal defense of *Solanum tuberosum* against *Phytophthora infestans*. *Molecular Plant-Microbe Interactions* **20**,

1346-1352

Hammer PE, Hill DS, Lam ST, van Pee K-H, Ligon JM (1997) Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. *Applied and Environmental Microbiology* **63**, 2147-2154

Hoffland E, Hakulinen J, van Pelt JA (1996) Comparison of systemic resistance induced by avirulent and non-pathogenic *Pseudomonas* species. *Phytopathology* **86**, 757-762

Howell CR, Stipanovic RD (1979) Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* with an antibiotic produced by the bacterium. *Phytopathology* **69**, 480-482

Howell CR, Stipanovic RD (1983) Gliovirin, a new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum*. *Canadian Journal of Microbiology* **29**, 321-324

Howell CR, Stipanovic RD, Lumsden RD (1993) Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. *Biocontrol Science and Technology* **3**, 435-441

Hynes RK, Leung GC, Hirkala DL, Nelson LM (2008) Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada. *Canadian Journal of Microbiology* **54**, 248-258

Jagadeesh KS, Krishanraj PU, Kulkarni JH (2006) Suppression of deleterious bacteria by rhizobacteria and subsequent improvement in germination and growth of tomato seedlings. *Current Science* **91**, 1458-1459

Janisiewicz WJ, Korsten L (2002) Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* **40**, 411-441

Johri BN, Sharma A, Virdi JS (2003) Rhizobacterial diversity in India and its influence on plant health In: Ghose TK, Ghosh P (Eds) *Advances in Biochemical Engineering*, Springer Verlag, Berlin, pp 84-89

Kamensky M, Ovadis M, Chet I, Cheruin L (2003) Soilborne strain IC14 of *Serratia plymuthiea* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biology and Biochemistry* **35**, 323-331

Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner P, Haas D, Defago G (1992) Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Molecular Plant-Microbe Interactions* **5**, 4-13

Keel C, Voisard C, Berling CH, Kahr G, Defago G (1989) Iron sufficiency, a prerequisite for the suppression of tobacco black root rot by *Pseudomonas fluorescens* strain CHA0 under gnotobiotic conditions. *Phytopathology* **79**, 584-589

Kende H (1993) Ethylene biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 283-207

Khot GG, Tauro P, Dadarwal KR (1996) Rhizobacteria from chickpea (*Cicer arietinum* L.) rhizosphere effective in wilt control and promote nodulation. *Indian Journal of Microbiology* **36**, 217-222

Kloepper JW (1983) Effect of seed piece inoculation with plant growth promoting rhizobacteria on population of *Erwinia carotovora* on potato roots and in daughter tubers. *Phytopathology* **73**, 217-219

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**, 883-884

Kloepper JW, Mahaffee WF, McInroy JA, Backman PA (1991) Comparative analysis of five methods for recovering rhizobacteria from cotton roots. *Canadian Journal of Microbiology* **37**, 953-957

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* **94**, 1259-1266

Kloepper JW, Schroth MN (1981a) Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria on potato plant development and yield. *Phytopathology* **70**, 1078-1082

Kloepper JW, Schroth MN (1981b) Plant growth promoting rhizobacteria and plant growth under gnotobiotic conditions. *Phytopathology* **71**, 642-644

Kohli U, Dogra RC, Kapoor KK, Sharma PK (2006) Chitinolytic *Pseudomonas maltophila* antagonistic to root rot pathogens of sunflower (*Helianthus annuus*). *Indian Journal of Microbiology* **46**, 45-49

Koster M, van de Vosenberg J, Leong J, Weisbeek PJ (1993) Identification and characterization of the *pup*B gene encoding an inducible ferric pseudobactin receptor of *Pseudomonas putida* WCS358. *Molecular Microbiology* **8**, 591-601

Kuc J (1995) Phytoalexins, stress, metabolism and disease resistance in plants. *Annual Review of Phytopathology* **33**, 275-297

Leeman M, van Pelt JA, den Ouden FM, Heinsbroek M, Bakker PAHM, Schippers B (1995) Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* **85**, 1021-1027

Leong J (1986) Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Annual Review of Phytopathology* **24**, 187-209

Lim H-S, Kim Y-S, Kim S-D (1991) *Pseudomonas stutzeri* YPL-1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot. *Applied and Environmental Microbiology* **57**, 510-516

Liu L, Kloepper JW, Tuzun S (1995) Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology* **85**, 695

Lugtenberg BJJ, de Weger LA, Bennett JW (1991) Microbial stimulation of plant growth and protection from disease. *Current Opinion in Microbiology* **2**, 457-464

Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: Influence of the *gac*A gene and of pyoverdine production. *Phytopathology* **84**, 139-146

Maurhofer M, Keel C, Schnider U, Voisard C, Haas D, Defago G (1992) Influence of enhanced antibiotic production by *Pseudomonas fluorescens* strain CHA0 on disease suppressive capacity. *Phytopathology* **82**, 190-195

Maurhofer M, Reimmann C, Schmidli-Sacherer P, Heeb S, Haas D, Defago G (1998) Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. *Phytopathology* **88**, 678-684

Mavrodi DV, Ksenzenko VN, Bonsal RF, Cook RJ, Boronin AM (1998) A seven gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *Journal of Bacteriology* **180**, 2541-2548

Mavrodi DV, Bonsal RF, Delaney SM, Soule MJ, Phillips G (2001) Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology* **183**, 6454-6465

Mavrodi DV, Blankenfeldt W, Thomashow LS (2006) Phenazine compounds in fluorescent *Pseudomonas* spp.: biosynthesis and regulation. *Annual Review of Phytopatology* **44**, 417-445

Mazzola M, Cook RJ, Thomashow LS, Weller DM, Pierson LS III (1992) Contribution of phanazine antibiotic synthesis to the ecological competence of fluorescent pseudomonads in the wheat rhizosphere. *Applied and Environmental Microbiology* **57**, 2171-2178

Milner JL, Stohl EA, Handelsman J (1996) Zwittermicin A resistance gene from *Bacillus cereus*. *Journal of Bacteriology* **178**, 4266-4272

Moores JC, Magazin M, Ditta GS, Leong J (1984) Cloning of genes involved in the biosynthesis of pseudobactin, a high-affinity iron transport agent of a plant growth-promoting *Pseudomonas* strain. *Journal of Bacteriology* **157**, 53-58

Nagaraj KM, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extra cellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiological Research* **159**, 73-81

Neilands JB (1981) Microbial iron compounds. *Annual Review of Biochemistry* **50**, 715-731

Neilands JB, Nakamura K (1991) Detection, determination, isolation, characterization and regulation of microbial iron chelators. In: Winkelmann G (Ed) *CRC Handbook of Microbial Iron Chelators*, CRC Press, London, pp 1-14

New PB, Kerr A (1972) Biological control of crown gall: Field measurements and glasshouse experiments. *Journal of Applied Bacteriology* **35**, 279-287

Osburn RM, Milner JM, Oplinger ES, Smith RS, Handelsman J (1995) Effect of *Bacillus cereus* UW85 on the yield of soybean at two field sites in Wisconsin. *Plant Disease* **79**, 551-556

Parret AH, Temmerman K, De Mot R (2005) Novel lectin-like bacteriocins of biocontrol strain *Pseudomonas fluorescens* Pf-5. *Applied and Environmental Microbiology* **71**, 5197-5207

Pierson FA, Weller DM (1994) Use of mixtures of fluorescent pseudomonad to suppress take-all and improve the growth of wheat. *Phytopathology* **84**, 940-947

Pierson LS III, Pierson EA (2007) Roles of diffusible signals in communication among plant-associated bacteria. *Phytopathology* **97**, 227-232

Pierson LS III, Thomashow LS (1992) Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. *Molecular Plant-Microbe Interactions* **5**, 330-339

Pierson LS, Gaffeny T, Lam S, Gong FC (1995) Molecular analysis of genes encoding phenazine biosynthesis in the biological control bacterium *Pseudomonas aureofaciens* 30-84. *FEMS Microbiology Letters* **134**, 299-307

Raaijmakers JM, Leeman M, van Oorschot MMP, van der Sluis I, Schippers B, Bakker PAHM (1995) Dose-response relationships in biological control of Fusarium wilt of radish by *Pseudomonas* spp. *Phytopathology* **85**, 1075-1081

Radjacommare R, Kandan A, Nandakumar R, Samiyappan R (2004) Association of the hydrolytic enzyme chitinase against *Rhizoctonia solani* is rhizobacteria-treated rice plants. *Phytopathology* **152**, 365-370

Roberts DP, McKenna LF, Hu X, Lohrke SM, Kong HS, de Souza JT, Baker CJ, Lydon J (2007) Mutation in *cya*A in *Enterobacter cloacae* decreases cucumber root colonization. *Archives of Microbiology* **187**, 101-115

Robinson MM, Shah S, Tamot B, Pauls KP, Moffatt BA, Glick BR (2001) Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase. *Molecular Plant Pathology* **2**, 135-145

Ryales JA, Neuenschwander UH, Willits MG, Mollina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. *Plant Cell* **8**, 1809-1819

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiology* **134**, 1017-1026

Scherwinski K, Grosch R, Berg G (2008) Effect of bacterial antagonists on lettuce: active biocontrol of *Rhizoctonia solani* and negligible, short-term effects on nontarget microorganisms. *FEMS Microbial Ecology* **64**, 106-116

Schippers B, Bakker AW, Bakker PAHM (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping prac-

tices. *Annual Review of Phytopathology* **25**, 339-358
Schmidli-Sacherer P, Keel C, Defago G (1997) The global regulator GacA of *Pseudomonas fluorescens* CHA0 is required for suppression of root diseases in dicotyledons but not in gramineae. *Plant Pathology* **46**, 80-90
Schwinghamer EA, Brockwell J (1978) Competitive advantage of bacteriocin and phage producing strains of *R. trifolii* in mixed culture. *Soil Biology and Biochemistry* **10**, 383-387
Shanahan P, O'Sullivan DJ, Simpson P, Glennon JD, O'Gara F (1992) Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Applied and Environmental Microbiology* **58**, 353-358
Sharifi-Tehrani A, Ahmadzadeh M, Sarani S, Farzaneh M (2007) Powder formulation of *Burkholderia cepacia* for control of rape seed damping-off caused by *Rhizoctonia solani*. *Communication in Agriculture and Applied Biological Sciences* **72**, 129-136
Silo-Suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, Handelsman J (1994) Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. *Applied and Environmental Microbiology* **60**, 2023-2030
Sindhu SS, Gupta SK, Dadarwal KR (1999) Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of plant growth in green gram (*Vigna radiata*). *Biology and Fertility of Soils* **29**, 62-68
Sindhu SS, Suneja S, Dadarwal KR (1997) Plant growth-promoting rhizobacteria and their role in improving crop productivity. In: Dadarwal KR (Ed) *Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production*, Scientific Publishers, Jodhpur, pp 149-191
Sindhu SS, Sunita Suneja, Goel AK, Parmar N, Dadarwal KR (2002) Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions. *Applied Soil Ecology* **19**, 57-64
Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* **89**, 92-99
Sitrit Y, Barak Z, Kapulnik Y, Oppenheim AB, Chet I (1993) Expression of *Serratia marcescens* chitinase gene in *Rhizobium meliloti* during symbiosis on alfalfa roots. *Molecular Plant-Microbe Interactions* **6**, 293-298
Someya N, Tsuchiya K, Yoshida T, Noguchi MT, Swada H (2007) Encapsulation of cabbage seeds in alginate polymer containing the biocontrol bacterium *Pseudomonas fluorescens* strain LRB3W1 for the control of cabbage soilborne diseases. *Seed Science and Technology* **35**, 371-379
Spadro D, Cullino ML (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. *Crop Protection* **24**, 601-613
Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. *Critical Reviews in Plant Science* **19**, 1-30
Stutz EG, Defago G, Kern H (1986) Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. *Phytopathology* **76**, 181-185
Sundaresan P, Ubalthoose Raja RN, Gunasekaran P (1993) Induction and accumulation of phytoalexins in cowpea roots infected with a mycorrhizal fungus *Glomus fasciculatum* and their resistance to Fusarium wilt disease. *Journal of Biosciences* **18**, 289-301
Suslow TV, Schroth MN (1982) Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. *Phytopathology* **72**, 111-115
Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *Journal of Bacteriology* **170**, 3499-3508
Thomashow LS, Weller DM (1996) Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Stacey G, Keen N (Eds) *Plant-Microbe Interactions* (Vol 1), Chapman and Hall, New York, pp 187-235
Timmis-Wilson TM, Ellis RJ, Renwick A, Rhodes DJ, Mavrodl DV, Weller DM, Thomashow LS, Bailey MJ (2000) Chromosomal insertion of phenazine-1-carboxylic acid biosynthesis pathway enhances efficacy of damping off disease control by *Pseudomonas fluorescens*. *Molecular Plant-Microbe Interactions* **13**, 1293-1300
Trevors JT, van Elsas JD, van Overbeek LS, Starodub ME (1990) Transport of genetically engineered *Pseudomonas fluorescens* strain through a soil microcosm. *Applied and Environmental Microbiology* **56**, 401-408
Triplett EW (1988) Isolation of genes involved in nodulation competitiveness from *Rhizobium leguminosarum* bv. *trifolii* T24. *Proceedings of the National Academy of Sciences USA* **85**, 3810-3814
Triplett EW (1990) Construction of a symbiotically effective strain of *Rhizobium leguminosarum* bv. *trifolii* with increased nodulation competitiveness. *Applied and Environonmental Microbiology* **56**, 98-103
Triplett EW, Barta TM (1987) Trifolitoxin production and nodulation are necessary for the expression of superior nodulation competitiveness by *R. leguminosarum* bv. *trifolii* strain T24 of clover. *Plant Physiology* **85**, 335-342
Turner JT, Backman PA (1991) Factors relating to peanut yield increased following *Bacillus subtilis* seed treatment. *Plant Disease* **75**, 347-353
Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J (1992) Acquired resistance in *Arabidopsis*. *Plant Cell* **4**, 645-656
van Loon LC, Geraats BPJ, Linthoprst HJM (2006) Ethylene as a modulator of disease resistance in plants. *Trends in Plant Science* **11**, 184-191
van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology* **81**, 728-734
Verhagen BWM, Glazebrook J, Zhu T, Chang HS, van Loon LC, Pieterse CMJ (2004) The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis. Molecular Plant-Microbe Interactions* **17**, 895-908
Viñas I (1995) Biological control in the preservation of pome fruits. *Microbiologia* **11**, 115-124
Voisard C, Keel C, Hass D, Defago G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *The EMBO Journal* **8**, 351-358
Wang C, Knill E, Glick BR, Defago G (2000) Effect of transferring 1-aminocyclopropane-1-carboxylate (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its *gac*A derivative CHA96 on their growth-promoting and disease-suppressive capacities. *Canadian Journal of Microbiology* **46**, 898-907
Wei G, Kloepper JW, Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare*, by select strains of plant growth-promoting rhizobacteria. *Phytopathology* **81**, 1508-1512
Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* **26**, 379-407
Weller DM (2007) *Pseudomonas* biocontrol agents of soilborne pathogens: Looking back over 30 years. *Phytopathology* **97**, 250-256
Weller DM, Cook RJ (1983) Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* **73**, 463-469
Weller DM, Thomashow LS (1993) Advances in rhizobacteria for biocontrol. *Current Opinion in Biotechnology* **4**, 306-311
Wilhite SE, Lumaden RD, Straney DC (1994) Mutational analysis of gliotoxin production by the biocontrol fungus *Gliocladium virens* in relation to suppression of Pythium damping off. *Phytopathology* **84**, 816-821
Wood DW, Pierson LS III (1996) The *phz*I gene of *Pseudomonas aureofaciens* 30-84 is responsible for the production of a diffusible signal required for phenazine antibiotic production. *Gene* **168**, 49-53
Zachow C, Tilcher R, Berg G (2008) Sugar beet-associated bacterial and fungal communities show a high indigenous antagonistic potential against plant pathogens. *Microbial Ecology* **55**, 119-129
Zhang Z, Yuen GY (2000) The role of chitinase production by *Stenotrophomonas maltophila* strain C_3 in biological control of *Bipolaris sorokiniana. Phytopathology* **90**, 384-489
Zhou T, Paulitz TC (1994) Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. in cucumber. *Journal of Phytopathology* **142**, 51-63