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

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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
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: (1) 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; Compañet et al. 2005).

The attempted infection of a plant by a pathogen, such as a 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; Spadaro 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 the uninoculated control. The antagonistic bacteria 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 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 organisms 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 diacetil 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, antibiotic is one of the highly effective mechanisms for suppressing pathogen in the rhizosphere.

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Producing bacteria</th>
<th>Target pathogen</th>
<th>Target disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenazine-1-carboxylic acid (PCA)</td>
<td>P. fluorescens 2-79</td>
<td>G. graminis var. tritici</td>
<td>Take-all disease of wheat</td>
</tr>
<tr>
<td>2,4-Diacetyl phloroglucinol (DAPG)</td>
<td>P. aureofaciens 30-84</td>
<td>G. graminis var. tritici</td>
<td>Take-all disease of wheat</td>
</tr>
<tr>
<td></td>
<td>P. fluorescens CHA0</td>
<td>G. graminis var. tritici</td>
<td>Take-all disease of wheat</td>
</tr>
<tr>
<td></td>
<td>P. fluorescens Q-87</td>
<td>Thielaviopsis basicola</td>
<td>Black rot- rot of tobacco</td>
</tr>
<tr>
<td></td>
<td>P. fluorescens F113</td>
<td>Pythium ultimum</td>
<td>Damping-off of sugar beet</td>
</tr>
<tr>
<td></td>
<td>P. fluorescens P5</td>
<td>Rhizoctonia solani</td>
<td>Sheath blight</td>
</tr>
<tr>
<td>Pyrrolnitrin (Prn)</td>
<td>P. cepacia</td>
<td>Bipolaris maydis</td>
<td>Southern mildew leaf blight</td>
</tr>
<tr>
<td></td>
<td>P. fluorescens P5</td>
<td>Sclerotina homoeocarpa</td>
<td>Dollar spot of turf grass</td>
</tr>
<tr>
<td>Pyoluteorin (Pit)</td>
<td>P. fluorescens P5</td>
<td>Pythium ultimum</td>
<td>Damping-off of cotton</td>
</tr>
<tr>
<td>Iturin A and surfactin</td>
<td>Bacillus subtilis RB14</td>
<td>Rhizoctonia solani</td>
<td>Damping-off of tomato</td>
</tr>
</tbody>
</table>
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). Gurusidaih 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 *Gaemumannomyces graminis* var. *tritici*. The antibiotic was isolated from potato glycerol broth culture of this strain. This antibiotic was shown to be active against several fungal pathogens including *G. graminis* var. *tritici*, *Rhizoctonia solani* and *P. aris-tesporum*. Evidence for the role of phenazines includes an analysis of transposon insertion mutants that lack the ability to produce phenazine-1-carboxylic acid 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 (Mazzotta 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 RN10, which acted as biocontrol agent of take-all of wheat. They found an inverse relationship between the population size of phenazine-producing 2-79 RN10 and the number of lesions formed by *G. graminis* var. *tritici*. They also reported that the 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 phlorogluconol (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 *Thieli- viopsis basicola* (Stutz et al. 1986). It was found to produce a variety of secondary metabolites i.e. pyoluteorin, DAPG, hydrogen cyanide (HCN), salicylic acid, pyochelin and pyo-verdine, 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 housekeeping sigma factor σ7. Furthermore, a quantitative relationship between antibiotic production and disease suppressiveness is suggested by the enhancement of production of DAPG and pyoluteorin accompanied 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; Bang- gera and Thomashow 1996; Haas and Keel 2003).

(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 examples of the use of bacteriocins for control of 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 fluorescencin-BC8 and inhibited the growth of virulent *P. solanacearum* strains under in vitro conditions (Gallardo et al. 1989). The avirulent *P. solanacearum* strain lost the ability to produce fluorescencin and fails to colonize 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* P15 (Parret et al. 2005).

Schwinghamer and Brockwell (1978) reported suppression of growth of sensitive *R. trifolii* strains on inoculation with bacteriocin-producing *R. trifolii* strains in sterile broth culture and peat culture conditions. Tripplett and Barta
(1987) reported that trifolitoxin production by *P. leguminosarum* bv. *trifolii* strain T24 inhibits growth of strains of *R. leguminosarum* bvs. *trifolii*, *phaseoli* and *vicieae* as well as *R. fredi*. The genes encoding trifolitoxin production have been cloned from genomic library of strain T24 (Tripplett 1988) and the transfer of these genes into an effective *R. leguminosarum* bv. *trifolii* strain TAI conferred trifolitoxin production. The exconjugants occupied significantly more nodules on clover roots on coinoculation with trifolitoxin-sensitive reference strain (Tripplett 1990).

(iii) Production of siderophores

Iron is essential element for all living organisms, with the possible exceptions of certain lactobacilli. Iron is abundant in Earth's crust, but most of it is found in the insoluble form of ferric hydroxide; thus, it is only available to organisms at concentrations at or below 10^{-19} 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 (Nei1ands 1981; Nei1ands 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 endemicroorganisms, thus inhibiting their growth. The various categories of siderophores produced by PGPR include catechol, hydroxamate, pyoverdine and some other types like azotocin, anthranilic acid and azotobactin.

Klopper 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 (FeCl₃) was added to iron-deficient medium. Also, PGPR did not enhance plant growth when ferric iron was added to soil in the form of Fe-EDTA. Moores et al. (1984) constructed a gene bank from a high growth promoting *Pseudomonas* sp. strain B10. Non-fluorescent mutants of *Pseudomonas* sp. strain B10 were obtained by mutagenesis with nitrosoguanidine (NTG), ethylemethalamino-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 minimal 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 siderophore 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 (Duffy 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 (Buyens 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 chrome-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. via the antifungal activity of *Pseudomonas* was 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 blast fungal pathogen, *Rhizoctonia solani* (Nagraj et al. 2004). Production of chitinase, β-1,3-glucanase, siderophores, salicylic 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, salicylic 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 *P. 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 (laminaranase).

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 corn plants. Later old house conditions suggested that P. stutzeri strain YPL-1 produced extracellular chitinase and laminaranase 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. melliloti, 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, Pseudomonas 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 chitinolytic and proteolytic activities, produced antibiotic pyrrolnitrin as well as siderophores and secreted plant growth promoting enzymes from the parent strain in the production of other antibiotic products. Zhang and Yuen (2000) transferred cloned cry1Ac7 of Bacillus thuringiensis in the sugarcane-associated endophytic bacterium Herbaspirillum seropedicae. Expression of the genes resulted in biocontrol of sugarcane borer Eldana saccharina. Recombinant strains of Rhizobium mellioti 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. Tns-derived mutant strain CHA5 lacking HCN production was used along with parent strain CHA10 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-adenosylmethylene 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...
by the bacterial pathogen Erwinia carotovora in sealed plastic bags. The nontransformed bacterial strains did not prevent the damage to the potato slices.

**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 yelI mutant of biological control *P. chlororaphis* 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 yelI-engineered tobacco plants restored phenazine production and fungal inhibition, whereas extracts of control plants that did not express yelI 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/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.

**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 desoxyflavonoids or isoflavonoid-like compounds and their occurrence were reported (Ryals et al. 1994). 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 CHA1 (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 (Caffrey et al. 1993; Ryals et al. 1996). Mutants of CHA1 that do not produce the siderophore pyoverdin 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 in iron utilization in the rhizosphere. 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 dose siderophore 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 KMPCl, however, depends on SA. Additional support for induction of resistance by bacterial produced SA comes from the study in which the SA biosynthesis genes of P. aeruginosa PA01 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 lycopersicum against Phy- 
topthora 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 Fus- 
arium wilt by P. fluorescens, preparations of lipopolysac-
charides from the bacterial cell surface induce resistance as 
effectively as the living bacteria, demonstrating that biocon-
trol 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 resis-
tance, 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 sig-
naling 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 con-
trol 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. fluo-
rescens 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 sig-
naling in the plant.

RELATION BETWEEN GROWTH PROMOTION AND 
BIOLOGICAL CONTROL

In recent years, there is no clear separation of growth pro-
motion 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 de-
monstrate growth promotion in the absence of target patho-
gen (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 con-
trolling deleterious microorganisms or non-target pathogens 
(Compton et al. 2005).

The PGPR that enhance plant growth by controlling 
deletious or non-target pathogens 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 per-
centage of daughter tubers infested with E. carotovora, ran-
ging 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 micro-
organisms and, thereby, increase plant growth, are examples of 
direct growth promotion by biological control (Pierson 
and Weller 1994). VOIGARD et al. (1989) reported that P. fluo-
rescens strain CHA0 induced increased root hair deforma-
tion on tobacco in a pathogen-free gnotobiotic assay. de 
Freitas and Germida (1991) described a similar increase in 
radial 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 pseudomonadal 
PGPR strains did not promote plant growth of potato (Kloepper and Schrot 1981b) whereas, growth promotion 
was noticed with a 23-26% reduction of population densities of indigenous rhizoplane fungi and 25- 
93% reduction in Gram-positive bacterial population densi-
ties (Kloepper and Schrot 1981a). Thus, the plant response was related to control of native microorganisms, rather than 
to direct growth promotion. Suslow and Schrot (1982) 
found specific strains of root-colonizing bacteria that were 
pathogenic on sugar beet seedlings and termed them dele-
terious 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 in-
crease 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 unino-
culated 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 pro-
motion”.

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 sidero-
phore and induce acetic acid (IAA). In addition, Pseudo-
onasfluorescens 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 Sclero-
tium rolfsii. In field trials, however, there was wide varia-
tion in the performance of PGPR isolates in enhancing the 
growth and yield of peanuts in different years. Plant growth-
promoting fluorescent pseudomonadal 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 bacteri-
zation 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 KCP7. 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 Pseudomonadaeceae 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 controls. Analysis of the indigenous bacterial and endophytic fungal populations revealed only negligible short-term 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 controls. Analysis of the indigenous bacterial and endophytic fungal populations revealed only negligible short-term biocontrol 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.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Biocontrol organism</th>
<th>Formulation</th>
<th>Target pathogen / disease</th>
<th>Crops tested</th>
<th>Company and country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BlightBan A506</td>
<td>P. fluorescens</td>
<td>Lyophilized cells / powder (Wettable)</td>
<td>Erwinia amylovora / Fire blight, Frost damage control of fruits</td>
<td>Pear and apples, Cherry, strawberry, tomato and potato</td>
<td>NuFarm Americas, Burr Ridge, II.</td>
</tr>
<tr>
<td>Galtrol</td>
<td>Agrobacterium</td>
<td>-</td>
<td>Agrobacterium tumefaciens, Crown gall</td>
<td>Several crops, Chemical Co, New Zealand</td>
<td>Fruit growers, Zeeland</td>
</tr>
<tr>
<td>No gall</td>
<td>Bacillus subtilis</td>
<td>Dry powder</td>
<td>Rhizoctonia solani, Fusarium, Alternaria and Aspergillus sp.</td>
<td>Cotton and legumes, Cotton and legumes</td>
<td>Gustafsan Inc. Tcx, USA</td>
</tr>
<tr>
<td>Epic Kodiak</td>
<td>P. fluorescens</td>
<td>Dust</td>
<td>F. oxysporum of raphani and dianthic vation, A. brassicicola</td>
<td>-</td>
<td>S &amp; G seeds, BV, Netherlands</td>
</tr>
<tr>
<td>Meycopest</td>
<td>Streptomyces</td>
<td>Damping-off</td>
<td>Damping-off of crucifers</td>
<td>Crucifers</td>
<td>Kemira Agro, Oy, Finland</td>
</tr>
<tr>
<td>Bio-save, 10 LP and 11 LP</td>
<td>P. syringae strains ESC-10 and ESC-11</td>
<td>Wetable powder</td>
<td>Botrytis cinerea, Macor pyrifromis, Geotrichum candidum and Penicillium sp.</td>
<td>Citrus and pome fruit</td>
<td>Jet Harvest Solutions, Florida</td>
</tr>
<tr>
<td>System Deny</td>
<td>B. subtilis</td>
<td>Dust Powder</td>
<td>Postharvest fungal diseases, Seedling pathogens, Pythium sp.</td>
<td>Bean, barley, cotton and peanut</td>
<td>CCT Crop, Carbasl, USAJAD</td>
</tr>
</tbody>
</table>
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] and yellow fluorescent protein [YFP] and DSRed as a marker (Bloomberg 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 (Bloomberg 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 biocontrol agents, which delay the infection, has demonstrated that 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 plzH gene from Pseudomonas chlororaphis PCL1391 (Timmis-Wilson et al. 2000). Whereas, mutational disruption of the biosynthesis genes coding for antifungal metabolite 2,4-diacylgllycerol glucinol 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 Phytophthora 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 MS9 (a mini-Tn5 Km transposon mutant of strain 501R3) was significantly lower on cucumber roots and decreased much more rapidly (50%) in strain 501R3 with increasing distance from the soil line. The strain MS9 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 MS9 demonstrated that mini-Tn5 Km was inserted in cyaA, which encodes adenylate cyclase. Adenylate cyclase catalyzes the formation of cAMP, and cAMP level in cell lysates of strain MS9 was ap- approximately 2% of those of strain 501R3. Addition of exogenous, non-physiological concentrations of cAMP to strain MS9 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 cyaA in colonization of the rhizosphere by Enterobacter cloacae.

Cabbage seeds were encapsulated in alginate polymer containing an antagonistic bacterium, Pseudomonas fluorescens strain LR3B3W1 (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 of cabbage from bacterial pathogen disease.

Talc-based formulated Burkholderia cepacia 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-Thehani 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 Bu1 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 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, e.g. Bacillus subtilis, which encodes the aminoglycoside phosphotransferase (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 widespread use at the field level. Therefore, it is essential to develop new biocontrol strains with increased production of toxic compounds or lytic enzymes, improved space or nutrient competition, 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-Thehani 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.
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