LITERATURE REVIEW

Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria). The search for PGPR and investigation of their modes of action are increasing at a rapid pace as efforts are made to exploit them commercially as biofertilizers. These microorganisms have important contributions toward the growth and development of plants. Plant growth promoting rhizobacteria increase plant growth directly by producing hormones, siderophores or by solubilising phosphates or indirectly either by the suppression of well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens. Considering the importance of the role of PGPRs in agriculture and understanding their mechanisms of action, several authors have reviewed this topic exhaustively (Lugtenberg *et al.*, 2001; Whipps, 2001; Haas and Keel, 2003; Morris and Monier, 2003; Morgan *et al.*, 2005). The main areas of focus of some of the recent reviews have been discussed below.

Rhizosphere colonization is one of the first steps in the parthenogenesis of soil borne microorganisms. It can also be crucial for the action of microbial inoculants used as biofertilisers, biopesticides, phytostimulators and bioremediators. *Pseudomonas*, one of the best root colonizers is therefore used as a model root colonizer. The review by Lugtenberg *et al.* (2001) focused on (a) the temporal spatial description of root colonising bacteria as visualized by confocal laser scanning microscopical analysis of autofluorescent microorganisms, and (b) bacterial genes and the traits used for the colonization of root and of animal tissues, indicating the general importance of a study. Finally, they identified several noteworthy areas for future research.

The loss of organic material from the roots provides the energy for the development of active microbial populations in the rhizosphere around the root. Generally, saproptrophs or biotrophs such as mycorrhizal fungi grow in the rhizosphere in response to this carbon loss, but plant pathogens may also develop and infect a susceptible host, resulting in disease. The review by Whipps (2001) examined the microbial interactions that can take place in the rhizosphere and that

are involved in biological disease control. The interactions of bacteria used as biocontrol agents of bacterial and fungal plant pathogens, and fungi used as biocontrol agents of protozoan, bacterial and fungal plant pathogens were considered. Whenever possible, modes of action involved in each type of interaction were assessed with particular emphasis on antibiosis, competition, parasitism, and induced resistance. The significance of plant growth promotion and rhizosphere competence in biocontrol was also considered. Multiple microbial interactions involving bacteria and fungi in the rhizosphere were shown to provide enhanced biocontrol in many cases in comparison with biocontrol agents used singly. The extreme complexity of interactions that occur in the rhizosphere was highlighted and some potential areas for future research in this area were discussed briefly.

Certain strains of fluorescent pseudomonads are important biological components of agricultural soils that are suppressive to diseases caused by pathogenic fungi on crop plants. The biocontrol abilities of such strains depend essentially on aggressive root colonization, induction of systemic resistance in the plant, and the production of diffusible or volatile antifungal antibiotics. Evidence that these compounds are produced *in situ* is based on their chemical extraction from the rhizosphere and on the expression of antibiotic biosynthetic genes in the producer strains colonizing plant roots. Well-characterized antibiotics with biocontrol properties include phenazines, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, lipopeptides, and hydrogen cyanide. *In vitro*, optimal production of these compounds occurs at high cell densities and during conditions of restricted growth, involving (*i*) a number of transcriptional regulators, which are mostly pathway-specific, and (*ii*) the GacS/GacA two-component system, which globally exerts a positive effect on the production of extracellular metabolites at a posttranscriptional level. Small untranslated RNAs have important roles in the GacS/GacA signal transduction pathway. One challenge in future biocontrol research involves development of new strategies to overcome the broad toxicity and lack of antifungal specificity displayed by most biocontrol antibiotics studied so far (Haas and Keel, 2003).

Bacteria associated with plants have been observed frequently to form assemblages referred to as aggregates, microcolonies, symplasmata, or biofilms on

leaves and on root surfaces and within intercellular spaces of plant tissues. In a wide range of habitats, biofilms are purported to be microniches of conditions markedly different from those of the ambient environment and drive microbial cells to effect functions not possible alone or outside of biofilms. The review by Morris and Monier (2003) constructed a portrait of how biofilms associated with leaves, roots and within intercellular spaces influenced the ecology of the bacteria they harbor and the relationship of bacteria with plants. They also considered how biofilms may enhance airborne dissemination, ubiquity and diversification of plant-associated bacteria and may influence strategies for biological control of plant disease and for assuring food safety.

After an initial clarification of the term biofertilizers and the nature of associations between PGPR and plants (i.e., endophytic versus rhizospheric), the review by Vessey (2003) focused on the known, the putative, and the speculative modes-of-action of PGPR. These modes of action include fixing N_2, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant-microbe symbioses. The combination of these modes of actions in PGPR was also addressed, as well as the challenges facing the more widespread utilization of PGPR as biofertilizers.

Colonization of the rhizosphere by micro-organisms results in modifications in plant growth and development. The review by Persello *et al.* (2003) examined the mechanisms involved in growth promotion by plant growth-promoting rhizobacteria which are divided into indirect and direct effects. Direct effects include enhanced provision of nutrients and the production of phytohormones. Indirect effects involve aspects of biological control: the production of antibiotics and iron-chelating siderophores and the induction of plant resistance mechanisms. The study of the molecular basis of growth promotion demonstrated the important role of bacterial traits (motility, adhesion and growth rate) for colonization. New research areas emerge from the discovery that molecular signalling occurs through plant perception of eubacterial flagellins. Recent perspectives in the molecular genetics of cross-talking mechanisms governing plant-rhizobacteria interactions were also discussed.

Soil microbial populations are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural systems and natural ecosystems. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low-input biotechnology, to help sustainable, environmentally-friendly, agro-technological practices. Much research is addressed at improving understanding of the diversity, dynamics, and significance of rhizosphere microbial populations and their co-operative activities. An analysis of the co-operative microbial activities known to affect plant development was the general aim of the review by Barea *et al.* (2005). In particular, they summarized and discussed significant aspects of this general topic, including (i) the analysis of the key activities carried out by the diverse trophic and functional groups of micro-organisms involved in co-operative rhizosphere interactions; (ii) a critical discussion of the direct microbe–microbe interactions which results in processes benefiting sustainable agro-ecosystem development; and (iii) beneficial microbial interactions involving arbuscular mycorrhiza, the omnipresent fungus–plant beneficial symbiosis. The trends of this thematic area will be outlined, from molecular biology and ecophysiological issues to the biotechnological developments for integrated management, to indicate where research is needed in the future.

The review by Morgan *et al.* (2005) looked briefly at plants and their rhizosphere microbes, the chemical communications that exist, and the biological processes they sustain. Primarily it is the loss of carbon compounds from roots that drives the development of enhanced microbial populations in the rhizosphere when compared with the bulk soil, or that sustains specific mycorrhizal or legume associations. The benefits to the plant from this carbon loss were discussed. Overall the general rhizosphere effect could help the plant by maintaining the recycling of nutrients, through the production of hormones, helping to provide resistance to microbial diseases and to aid tolerance to toxic compounds. When plants lack essential mineral elements such as P or N, symbiotic relationships can be beneficial and promote plant growth. However, this benefit may be lost in well-fertilized (agricultural) soils where nutrients are readily available to plants and symbionts reduce growth. Since these rhizosphere associations are common place and offer key

benefits to plants, these interactions would appear to be essential to their overall success.

The review presented below has been compiled on two important aspects of rhizosphere microflora related to the present work i.e. plant growth promotion and disease reduction.

Plant growth promotion by rhizobacteria

Abbass and Okon (1993) observed that treating seedling hypocotyls and roots of several plant species with cultures of *Azotobacter paspali* changed plant growth and development and significantly increased weight of shoot and roots. Morphological changes of root tips were already observed 5 days after inoculation. After 21 days the main effect was on the root surface area. Plant growth promotion was dependent on the inoculum size, indicating that for any given plant growth condition there is an optimal number of *A. paspali* for a positive effect on the plant.

Co-inoculation of plant growth promoting rhizobacteria (PGPR) with *Bradyrhizobium* has been shown to increase legume nodulation and nitrogen fixation at optimal soil temperatures. Nine rhizobacteria co-inoculated with *Bradyrhizobium japonicum* 532C were tested by Zhang *et al.* (1996) for their ability to reduce the negative effects of low root zone temperature (RZT) on soybean [*Glycine max*(L.) Merr.] nodulation and nitrogen fixation. Three RZTs were tested: 25 (optimal), 17.5 (somewhat inhibitory), and 15°C (very inhibitory). At each temperature some PGPR strains increased the number of nodules formed and the amount of fixed nitrogen when co-inoculated with *B. japonicum*, but the stimulatory strains varied with temperatures. The strains that were most stimulatory varied among temperatures and were as follows: 15°C, *Serratia proteamaculans* 1-102; 17.5°C, *S. proteamaculans* 1-102 and *Aeromonas hydrophila* P73; 25°C, *Serratia liquefaciens* 2-68.

Cook *et al.* (1998) reported that rhizobacteria, particularly *Pseudomonas* species were (i) able to colonize and maintain populations in the rhizosphere of wheat 5–10 cm and more below the seed, (ii) able to produce one or more antibiotics inhibitory to the target root pathogens, and (iii) tolerant to seed-treatment chemicals,

needed for immediate protection of germinating seeds. Their strains were from the rhizosphere of wheat growing in soil from fields where wheat had been grown continuously for many years, to help ensure that the strains are rhizosphere competent on the crop intended for protection. Initially, they concentrated on *P. fluorescens* 2-79 and *P. aureofaciens* 30-84 with ability to produce phenazine (PHZ) antibiotics. The second phase of their field work concentrated on *Bacillus* species L324-92 with antibiotic activity against three wheat root diseases, and on *P. fluorescens* Q69c-80 with no known ability to produce antibiotics, but widely effective in the field. The authors have reported that they are also now concentrating on *P. fluorescens* Q8R1-96 with ability to produce the antibiotic 2,4-diacetylphloroglucinol (PHL). The evidence is strong that PHL-producing strains like Q8R1-96 account for take-all decline. Used as a seed treatment, this strain produced the highest yields of wheat at every location where tested in 1997. They now have cultures of Q8R1-96 transformed to produce PHZ in addition to PHL.

Krebs. *et al.* (1998) isolated several *Bacillus* strains belonging to the *B. subtilis/amyloliquefaciens* group from plant-pathogen-infested soil possess plant-growth-promoting activity. Three out of the four strains investigated were identified as *B. amyloliquefaciens* and were able to degrade extracellular phytase (myo-inositol hexakisphosphate).

Lazarovits *et al.* (1998) developed a gnotobiotic bioassay, using potato plantlets derived from single-node explants grown in tubes containing solidified agar medium. Studies with this model system served to illustrate some important features that may be expected from PGPR per plant interactions. Growth-promotion of potato by PsJN (*Pseudomonas* sp. [strain PsJN]) was cultivar-specific. Inoculated plantlets of cv. 'Norchip' showed a five- to eightfold increase in root weight, cv. 'Kennebec' a two- to threefold increase, cv. 'Shepody' no response, and cv. 'Chaleur' a decrease of 50 %. PsJN was shown by other researchers to promote growth in a number of crop species, but to be cultivar-specific with them also. In their laboratory studies PsJN consistently promoted growth of potato, but in the field the response was site-specific. Bacterized plants had improved growth and yield of tubers in Alliston soils, but were reduced in Simcoe soil. This effect was reproduced under growth room conditions. Pasteurization of Simcoe soil eliminated the inhibitory impact of PsJN,

suggesting that the effect was determined due to presence of soil organisms. Therefore, they investigated the interactions of PsJN with other bacteria in the geocaulosphere. They harvested tubers at 2-day intervals from each soil, sterilized their surface, made a liquid homogenate, and plated the homogenates onto nutrient agar. A dozen bacteria isolates were recovered and identified by fatty acid profiles and biochemical tests. All genera isolated were found in tubers from both soils except *Pseudomonas acidovorans*, which was present only in tubers from Simcoe soil. The presence of PsJN significantly altered the types and populations of bacteria recovered from the tubers grown in non-pasteurized soil. However, four isolates, *Serratia proteamaculans, P. acidovorans, Alcaligenes piechaudii* and *Rahnella aquatilis*, showed antagonism to PsJN in assays on agar media. Several isolates also inhibited growth of potato nodal explants. Combinations of PsJN with each of the bacteria isolates showed that *Pantoea agglomerans*, which by itself had neutral or beneficial effects, when combined with PsJN was inhibitory to the growth of plantlets.

Cattelan *et al.* (1999) selected 116 isolates from bulk soil and the rhizosphere of soybean [*Glycine max* (L.) Merr.] and examined them for a wide array of traits that might increase early soybean growth in nonsterile soil (PGPR traits). A subsample of 23 isolates, all but one of which tested positive for one or more of these PGPR traits, was further screened for traits associated with biocontrol, (brady) rhizobial inhibition, and rhizosphere competence. Six of eight isolates positive for 1-aminocyclopropane-1-carboxylate (ACC, a precursor of ethylene) deaminase production, four of seven isolates positive for siderophore production, three of four isolates positive for ß-1,3-glucanase production, and two of five isolates positive for P solubilization increased at least one aspect of early soybean growth. One isolate, which did not share any of the PGPR traits tested *in vitro* except antagonism to *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, also promoted soybean growth. One of the 23 isolates changed bradyrhizobial nodule occupancy. Although the presence of a PGPR trait *in vitro* does not guarantee that a particular isolate is a PGPR, the results suggest that rhizobacteria able to produce ACC deaminase and, to a lesser extent, ß-1,3-glucanase or siderophores or those able to solubilize P *in vitro* may increase early soybean growth in nonsterile soil. A proteus strain inhibited mycelial

growth of *Fusarium oxysporum in vitro*. Seed bacterization showed significant plant growth promotion and *Fusarium*-wilt suppression activity of *Phaseolus mungo* in a gnotobiotic system. The culture filtrate of this strain exhibited three prominent bands in UV-VIS spectra between 300 and 400 nm. The growth promotion assay of the extracted compound against different indicator organisms indicated the production of a compound related to a 2-oxoacid-type siderophore. The HPLC of the purified ethyl acetate extract of the strains and standard 4-methyl-2-oxopentanoate (2-oxoisocaproate) revealed a single peak, similarly as the coinjection of the extract and the standard. The production of siderophore, probably 2-oxoisocaproate, was demonstrated by Barthakur (2000).

The studies conducted by Danuta *et al.* (2000) in the years 1997-1999 concerned the soil after cultivation of rye, winter wheat, triticale and potato. The purpose of the studies was to determine the composition of bacteria and fungi communities in the soil after the cultivation of these plant species. As a result of the microbiological analysis of the soil after rye cultivation 3.08×10^6 colonies of bacteria and 28.27×10^3 colonies of fungi were obtained. After triticale cultivation the soil contained 3.99×10^6 bacteria colonies and 38.74×10^3 fungi colonies, whereas after winter wheat cultivation there were 4.89×10^6 bacteria colonies and 26.19×10^3 fungi colonies. The soil after potato cultivation contained the greatest numbers of bacteria (5.01×10^6) and fungi (43.11×10^3) colonies. Among the obtained bacteria and fungi the greatest number of antagonistic microorganisms was found in the soil after winter wheat and rye cultivation, while the smallest number of antagonistic microorganisms was found in the soil after potato cultivation.

Experiments were conducted by Lee (2000a) to compare the germinability of pepper seeds primed by bacterial strain and solid matrix priming (SMP). Pepper seeds were soaked in the cell suspension of the bacterial strains for 1 hr and incubated at 28°C for certain period of time then dried in shade and stored. Seed priming with *Bacillus* strains showed even higher germination rate than SMP or chemical osmotic controllers. In pots experiments, pepper seeds primed by *Bacillus* sp. B2-13 showed more than 80% seedling emergency within 7 days, while SMP treatment was 11 days and untreated control was 13 days. When the bio-primed seeds were planted in pots, significant increase of shoot weight and length as well as

root weight and length were measured compare to other treatments. Bio-primed seedling revealed twofold more root biomass than untreated control.

In a further study by Lee (2000b) the effects of PGPR strains on the barley growth in Rice-Barley double cropping system was tested. The study was carried out in experimental farm of Gyeongnam Provincial RDA.The test variety was Jinyang, for brewing and PGPR strains were *Paenibacillus polymixa* E681 and *Pseudomonas fluorescens* B16. The tested barley seeds were soaked into bacterial suspension of E681 or B16 at 10^8 cells ml^{-1} for 5 hrs and dried in shade place for 12 hrs then sowed in the barely field at rate of 10kg seed $10a^{-1}$. The emergence rates of barley plants at 30 days after sowing were observed 211 m^{-2} in E681 treated plots, 233 m^{-2} in B16 treated plot and 191 m^{-2} in untreated control. The emergence rates of test plots after over-wintering were 288 m^{-2} in E681, 260 m^{-2} in B16 and 233 m^{-2} in untreated control. The plant height, average tillers and fresh weight of test plants were measured with time sequence. The dry weight of stems, leaves, and heads were also measured. The leaf dry weight of control plot was continuously reduced after heading, however, that one in bacteria treated plots were not reduced up to 7 days before harvest. Average head weight and total yield were significantly higher in bacteria treated plots.

According to Bai (2002) *Serratia proteamaculans* 1-102 (1-102) promotes soybean–bradyrhizobia nodulation and growth, but the mechanism is unknown. After adding isoflavonoid inducers to 1-102 culture, an active peak with a retention time of about 105 min in the HPLC fractionation was isolated using a bioassay based on the stimulation of soybean seed germination. The plant growth-promoting activity of this material was compared with 1-102 culture (cells) and supernatant under greenhouse conditions. The activator was applied to roots in 83, 830 and 8300 HPLC microvolts (μV) per seedling when plants were inoculated with bradyrhizobia or sprayed onto the leaves in same concentrations at 20 d after inoculation. The root-applied activator, especially at 1 ml of 830 μV per seedling, enhanced soybean nodulation and growth at the same level as 1-102 culture under both optimal and sub-optimal root zone temperatures. Thus, this activator stimulating soybean seed germination is also responsible for the plant growth-promoting activity of 1-102 culture. However, when sprayed onto the leaves, the activator did not increase growth and in higher

concentrations decreased average single leaf area. The results suggest that this inducible activator might be a lipo-chitooligosaccharide (LCO) analogue. LCOs act as rhizobia-to-legume signals stimulating root nodule formation. The activator could provide additional 'signal', increasing in the signal quality (the signal-to-noise ratio, SNR) of the plant–rhizobia signal exchange process.

The highest extracellular phytase activity was detected in strain FZB45, and diluted culture filtrates of this strain stimulated growth of maize seedlings under phosphate limitation in the presence of phytate by Idriss *et al.* (2002). The amino acid sequence deduced from the phytase *phyA* gene cloned from FZB45 displayed a high degree of similarity to known *Bacillus* phytases. Weak similarity between FZB45 phytase and *B. subtilis* alkaline phosphatase IV pointed to a possible common origin of these two enzymes. The recombinant protein expressed by *B. subtilis* MU331 displayed 3(1)-phytase activity yielding D/L-Ins(1,2,4,5,6)P5 as the first product of phytate hydrolysis. A phytase-negative mutant strain, FZB45/M2, whose *phyA* gene is disrupted, was generated by replacing the entire wild-type gene on the chromosome of FZB45 with a *km*::*phyA* fragment, and culture filtrates obtained from FZB45/M2 did not stimulate plant growth. In addition, the growth of maize seedlings was promoted in the presence of purified phytase and the absence of culture filtrate. These genetic and biochemical experiments provide strong evidence that phytase activity of *B. amyloliquefaciens* FZB45 is important for plant growth stimulation under phosphate limitation.Rhizobia form root nodules that fix nitrogen (N_2) in symbiotic legumes. Extending the ability of these bacteria to fix N_2 in non-legumes such as cereals would be a useful technology for increased crop yields among resource-poor farmers. Although some inoculation attempts have resulted in nodule formation in cereal plants, there was no evidence of N_2 fixation. However, because rhizobia naturally produce molecules (auxins, cytokinins, abscicic acids, lumichrome, rhiboflavin, lipo-chitooligosaccharides and vitamins) that promote plant growth, their colonization and infection of cereal roots would be expected to increase plant development, and grain yield.

The efficiency of 19 bacterial strains on the growth-promotion of micropropagated pineapple plantlets cv. *Perola* was tested by Marcelo *et al.* (2002) using different bacterization methods. Measurements of shoot length, leaf number,

leaf area, shoot dry weight and root dry weight at 30 days after transplanting were determined. Overall, the best bacterization methods were either root dipping or soil drenching plus root dipping. Bacterization by root dipping was chosen due to its practicability. The most efficient bacterial strains were C210, ENF16, RAB9 and ENF10. Increases as high as 163.6 %, 107.7 % and 87.0 % respectively for shoot dry weight, root dry weight and leaf area were obtained by applying the strain RAB9 by root dipping. All strains were compatible among them and combinations of ENF10 plus RAB9, ENF16 plus C210 and C210 plus RAB9 promoted root dry weight increases of 100.3%, 88.1% and 80.1%, respectively. Production of either IAA or HCN, and solubilization of phosphate by the strains were not detected under the experimental conditions used here. Only nitrogen amounts in bacterized plantlets had significantly differed from the controls. This work indicated that mixtures of the strains C210, ENF16, RAB9 and ENF10 applied by root dipping could be used to increase biomass production of micropropagated pineapple plantlets, reducing the acclimation period.

Experiments were conducted in pots to determine the growth effect of different rhizobacteria on maize under *Striga hermonthica* infestation. Babalola *et al.* (2003) selected three bacteria based on their plant growth promoting effects. Whole bacterial cells of the rhizobacteria were used to amplify 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase gene by polymerase chain reaction (PCR). Each bacterial inoculation increased agronomic characteristics of maize although not always to a statistically significant extent. The extent of growth enhancement differed between the isolates. *Enterobacter sakazakii* 8MR5 had the ability to stimulate plant growth; however in the PCR study, ACC deaminase was not amplified from this isolate, indicating that not all plant growth-promoting rhizobacteria contain the enzyme ACC deaminase. In contrast, an ACC deaminase specific product was amplified from *Pseudomonas* sp. 4MKS8 and *Klebsiella oxytoca* 10MKR7. This is the first report of ACC deaminase in *K. oxytoca*.

The inoculation effect of *Bradyrhizobium japonicum* and *Azotobacter chroococum* on soyabean (*Glycine max* (L) Merill var. Ransom) was studied by Bhattarai and Prasad (2003). Dual inoculation proved best in all the plant growth

parameters. Inoculation with *Azotobacter* alone was also little better than uninoculated control.

Three strains of plant growth promoting fluorescent Pseudomonads (HPR6, RRLJ008 and RRLJ134) were studied for their effect on growth and yield of French bean (*Phaseolus vulgaris* L.) under field conditions (Boruah *et al.*, 2003). They examined the effect of these strains on nature of root development and leaf palisade tube length. The strains induced positive response on growth and physiological parameters resulting in higher yield in P. vulgaris. Strain HPR6 produced the most promising results in thickening of leaf palisade layer, spreading of lateral roots and production of root hairs. The increase in specific leaf weight (SLW), net assimilation rate (NAR) and relative growth rate (RGR) by these strains were 68 %, 152 % and 167 %, respectively. The growth and yield parameters were also significantly improved compared to the uninoculated control. Antibiotic resistant mutant strains demonstrated that these bacteria effectively colonized the rhizosphere of French bean. The results suggest that the strains could be developed for field application on a large scale.

The effects of transplant type and soil treatment on growth and yield of strawberries (*Fragaria* x *ananassa* Duch.) produced in annual hill culture were evaluated for three years in Florida field trials. 'Sweet Charlie' and 'Camarosa' strawberry transplants were propagated by Burelle (2003) as bare root, plug, and plugs amended with a plant growth-promoting rhizobacterial (PGPR) treatment, LS213. The transplant treatments were evaluated in combination with methyl bromide, 1, 3-dichloropropene (Telone II), an unregistered iodine-based compound (Plantpro 45), and untreated soil. 'Camarosa' plugs amended with LS213 had higher overall yields than bare root transplants in all three years. Both 'Camarosa' and 'Sweet Charlie' plug and LS213 plug plants produced yields approximately two weeks earlier than bare root transplants in all years. Regardless of transplant type, and in both consecutive years of Plantpro 45 and Telone application, treatment with Plantpro 45 resulted in smaller and less healthy root systems than other soil treatments, and treatment with Telone resulted in yields comparable to methyl bromide.

Experiments were conducted during 2000 and 2001 to determine the effects of floral and foliar application of the bacterial strain *Bacillus* OSU 142 on the yield, growth and nutrient element composition of leaves of the apricot cultivar Hacihaliloglu grown in the Malatya province of Turkey. In 2000, trees were sprayed with a bacterial suspension at full bloom, and 30 and 60 days after full bloom. This experiment demonstrated significant differences in yield, shoot length and nutrient element composition of leaves only on trees treated at the full bloom stage. In view of this, the bacterial application was performed only at full bloom in 2001. The average increase in yield in 2000 and 2001 was 30 % and 90 %, respectively, compared with the untreated control. Shoot length development was significantly higher when trees were treated with OSU 142 at full bloom stage in both years. Similarly, N, P, K, Ca and Mg contents of leaves were higher on OSU 142-treated trees than on the untreated control. The results of this study by Esitken *et al.* (2003) suggest that OSU 142 has the potential to increase the yield of apricot trees.

In order to select potential Plant Growth Promoting Rhizobacteria (PGPRs), a selection of strains from the predominant genera in the rhizosphere of four lupine species, based on genetic divergence criteria, was carried out in a study by Gutierrez-Manero *et al.* (2003). This yielded 11 *Aureobacterium* (Aur), four *Cellulomonas* (Cell), two *Arthrobacter* (Arth), two *Pseudomonas* (Ps), and six *Bacillus* (Bc) strains. Cell-free culture filtrates of each bacterium were assayed for effects on germination, growth, and biological nitrogen fixation (BNF) of *Lupinus albus* L. cv. *Multolupa* seeds or seedlings. Four (Aur 6, Aur 9, Aur 11, and Cell 1) of the twenty-five strains assayed promoted germination. *Aureobacterium* 6 and Aur 9 also increased root surface, total nitrogen content, and BNF. As a result of the screening, and considering all the variables studied, authors suggested that Aur 6 can be considered a plant growth promoting rhizobacterium suitable for further field trials in other plants and in different production systems.

The study of the effect of the root exometabolites of tomato plants on the growth and antifungal activity of the plant growth-promoting *Pseudomonas* strains showed that the antifungal activity of plant growth-promoting rhizobacteria in the plant rhizosphere may depend on the sugar and organic acid composition of root exudates (Kravchenko *et al.*, 2003).

Mamatha *et al.* (2003) performed a greenhouse experiment in which *Bacillus coagulans* and *Pseudomonas fluorescens* were inoculated either singly or dually on growth and nutrition of sandalwood tree. Parameters such as plant height, number of leaves, biomass, P content, alkaline phosphatase & dehydrogenases and microbial population of the root zone soil revealed that all these parameters were higher in plants which were inoculated dually. Bacteria and actinomycetes populations were also higher in the root zone soil of plants, inoculated dually, but fungal and *Azotobacter* populations were not affected.

A bacterial screening was carried out by Manero *et al.* (2003) in the rhizosphere of two *Digitalis* species, *D. thapsi* and *D. parviflora*, both at the vegetative stage and at flowering. A total of 480 isolates were characterised at genus level, *Bacillus* being the dominant genera in all cases. Fifty percent of the *Bacillus* strains isolated from each species were analysed by PCR-RAPDs. At 85 % similarity, 12 groups separated for *D. thapsi* and 18 for *D. parviflora*. One strain of each group was selected for biological assay on *D. lanata*, evaluating growth promotion and cardenolide content in leaves after inoculation performed in the root system. The plant parameters evaluated were leaf surface area, shoot and root dry weight and leaf number. Lanatoside C content was evaluated by HPLC. Only 17 strains caused significant increases in at least one of the parameters evaluated. The most striking result was that some strains promoted growth and increased cardenolide content at the same time. This effect was detected on leaves while inoculation was carried out on roots. Interestingly, these two parameters are not enhanced simultaneously under regular conditions in pot or in tissue cultures.

The yield response of a wheat (Kirik) and a barley (Tokak 157/37) cultivar to inoculation with *Azospirillum brasilense* Sp246 and *Bacillus* sp. OSU-142 was studied by Ozturk *et al.* (2003) in relation to three levels of N fertilization (0, 40, and 80 kg ha^{-1}) under field conditions in Erzurum, Turkey, in 1999 and 2000. Seed inoculation with *A. brasilense* Sp246 significantly affected yield and yield components, both in wheat and barley. On average of years and N doses, inoculation with *A. brasilense* Sp246 increased spike number per m^2, grain number per spike, grain yield, and crude protein content by 7.2, 5.9, 14.7, and 4.1 % in wheat and by 6.6, 8.1, 17.5, and 5.1 % in barley, respectively, as compared to control. Inoculation

with *Bacillus* sp. OSU-142 significantly increased kernel number per spike in wheat, but no significant effect was determined in the other characteristics. Grain yields and yield components were also higher at all levels of nitrogen fertilizer in the inoculated plots as compared to the control. However, these increases diminished at high fertilizer levels. These results suggest that application of the growth promoting bacteria *A. brasilense* Sp246 may have the potential to be used as a biofertilizer for spring wheat and barley cultivation in organic and low-N input agriculture.

According to Penrose and Glick (2003) one of the major mechanisms utilized by plant growth-promoting rhizobacteria (PGPR) to facilitate plant growth and development is the lowering of ethylene levels by deamination of 1-aminocyclopropane-1-carboxylic acid (ACC) the immediate precursor of ethylene in plants. The enzyme catalysing this reaction, ACC deaminase, hydrolyses ACC to alpha-ketobutyrate and ammonia. Several bacterial strains that can utilize ACC as a sole source of nitrogen were isolated from rhizosphere soil samples. All of these strains were considered to be PGPR based on the ability to promote canola seedling root elongation under gnotobiotic conditions. The treatment of plant seeds or roots with these bacteria reduced the amount of ACC in plants, thereby lowering the concentration of ethylene.

Ramos *et al.* (2003) either inoculated alder seedlings with a suspension of *Bacillus licheniformis*, or left non-inoculated (controls) which were grown in two different soils under controlled conditions. For 8 weeks after inoculation, plant shoot and root systems were measured; nodules counted, and shoot and root length and surface area determined. In addition to plant growth, changes in the bacterial rhizosphere composition and inoculum levels were determined using the phospholipid fatty acid (PLFA) profile from the rhizosphere soil and from culturable bacteria from the rhizosphere (culturable PLFAs), respectively. They showed the differential effect of *B. licheniformis* on alder growth depending on the soil used. Increases in leaf surface area were significant only when grown in Soil A, while root growth increased in both soils. Effects were more pronounced in Soil A. Changes in the rhizosphere community after inoculation with *B. licheniformis* disappeared within a short period in both soils, 6 weeks in Soil A and only 2 in Soil B. *B. licheniformis* apparently survived at least 8 weeks in the rhizosphere, as revealed

by culturable PLFA profiles. Thus, increases in plant growth could be attributed to changes in the rhizosphere microbial communities, especially in the culturable fraction, due to the presence of the inoculated bacteria in soil. Given the different composition of soils, availability of nutrients must also be considered.

Two strains of *Azospirillum brasilense*, Sp245 and Sp7, were examined by Rothballer *et al.* (2003) for their endophytic potential on German, Brazilian and Israeli wheat cultivars. Plate count and Most Probable Number (MPN) methods were applied for quantification, as well as the fluorescent in situ hybridization (FISH) technique in combination with confocal laser scanning microscopy for the species specific detection and localization of the two *Azospirillum* strains in roots. Additionally, a plasmid bearing a constitutively expressed gfp gene was transformed into both strains, which enables visualization of the bacteria omitting the fixation process during the FISH protocol. The microscopic techniques showed that the potential of strain Sp245 to grow in the roots of all analyzed wheat varieties as an endophyte was greater than of Sp7, but overall cell densities were rather low under the applied experimental conditions. A plant growth promoting effect was clearly visible in all examined inoculated plants, irrespective of the *A. brasilense* strain used as inoculum.

Ryu *et al.* (2003) showed that some PGPR release a blend of volatile components that promote growth of *Arabidopsis thaliana*. In particular, the volatile components 2,3-butanediol and acetoin were released exclusively from two bacterial strains that trigger the greatest level of growth promotion. Furthermore, pharmacological applications of 2,3-butanediol enhanced plant growth whereas bacterial mutants blocked in 2,3-butanediol and acetoin synthesis were devoid in this growth-promotion capacity. The demonstration that PGPR strains release different volatile blends and that plant growth is stimulated by differences in these volatile blends establishes an additional function for volatile organic compounds as signaling molecules mediating plant-microbe interactions.

Plant-growth-promoting rhizobacteria (PGPR) are used on crops most often as seed treatments; however, an alternative application method for transplanted vegetables is mixing PGPR into the soilless medium in which the transplants are

grown. Studies were undertaken by Yan *et al.* (2003) to compare root colonization and persistence of rifampicin-resistant mutants of PGPR strains *Bacillus pumilus* SE34 and *Pseudomonas fluorescens* 89B61, SE34r and 89B61r, on tomato as a function of application method. When the bacteria were incorporated into PromixTM soilless medium at log 6, 7, and 8 colony-forming units g^{-1}, populations of strain SE34r per gram of medium maintained the initial inoculum densities, while populations of 89B61r decreased approximately one to two orders of magnitude by 4 weeks after planting. The populations of each PGPR strain colonizing roots after application into the soilless medium showed a similar pattern at 6 weeks as that at 4 weeks after planting, with higher populations on the whole roots and lateral roots than on the taproots. Strain SE34r but not 89B61r moved upwards and colonized the phyllosphere when incorporated into the soilless medium. Following application as seed treatment, populations of SE34r were significantly higher on upper roots and on the taproot than were populations following application through the soilless medium. Conversely, populations were higher on lower roots and lateral roots following application through the soilless medium than were populations following application as seed treatment. While strain SE34 enhanced plant growth with application both to the medium and as seed treatment, the level of growth promotion was significantly greater with application in the soilless medium. The results indicate that PGPR can be successfully incorporated into soilless media in vegetable transplant production systems.

Soil microbiota communities have demonstrated their crucial role in maintaining the soil ecological balance and therefore the sustainability of either natural ecosystems or agroecosystems. Rhizospheric microbe-plant interactions have a great influence on plant health and soil quality since these root-associated microorganisms are able to help the host plant to deal with drought, nutritional and soil-borne pathogen stress conditions. Plant growth-promoting rhizobacteria (PGPR) can be considered among rhizosphere-beneficial microorganisms. In a micropropagated plant system, bacterial inoculation at the beginning of the acclimatisation phase must also be observed from the perspective of the establishment of the soil microbiota rhizosphere. The objective of the work of

Jaizme *et al.* (2004) was to evaluate the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micropropagated bananas.

A study by Khalid *et al.* (2004) focused on the screening of effective PGPR strains on the basis of their potential for *in vitro* auxin production and plant growth promoting activity under gnotobiotic conditions. A large number of bacteria were isolated from the rhizosphere soil of wheat plants grown at different sites. Thirty isolates showing prolific growth on agar medium were selected and evaluated for their potential to produce auxins *in vitro*. Colorimetric analysis showed variable amount of auxins (ranging from 1.1 to 12.1 mg L^{-1}) produced by the rhizobacteria *in vitro* and amendment of the culture media with l-tryptophan (l-TRP), further stimulated auxin biosynthesis (ranging from 1.8 to 24.8 mg L^{-1}). HPLC analysis confirmed the presence of indole acetic acid (IAA) and indole acetamide (IAM) as the major auxins in the culture filtrates of these rhizobacteria. A series of laboratory experiments conducted on two cv. of wheat under gnotobiotic (axenic) conditions demonstrated increases in root elongation (up to 17.3 %), root dry weight (up to 13.5 %), shoot elongation (up to 37.7 %) and shoot dry weight (up to 36.3 %) of inoculated wheat seedlings. Linear positive correlation ($r = 0.99$) between in vitro auxin production and increase in growth parameters of inoculated seeds was found. Based upon auxin biosynthesis and growth-promoting activity, four isolates were selected and designated as plant growth-promoting rhizobacteria (PGPR). Auxin biosynthesis in sterilized vs nonsterilized soil inoculated with selected PGPR was also monitored that revealed superiority of the selected PGPR over indigenous microflora. Peat-based seed inoculation with selected PGPR isolates exhibited stimulatory effects on grain yields of tested wheat cv. in pot (up to 14.7 % increase over control) and field experiments (up to 27.5 % increase over control); however, the response varied with cv. and PGPR strains. It was concluded that the strain, which produced the highest amount of auxins in nonsterilized soil, also caused maximum increase in growth and yield of both the wheat cv. Their study suggested that potential for auxin biosynthesis by rhizobacteria could be used as a tool for the screening of effective PGPR strains.

Matiru and Dakora (2004) used light, scanning, and transmission electron microscopy to show that roots of sorghum and millet landraces from Africa were

easily infected by rhizobial isolates from five unrelated legume genera. With sorghum, in particular, plant growth and phosphorus (P) uptake were significantly increased by rhizobial inoculation, suggesting that field selection of suitable rhizobia/cereal combinations could increase yields and produce fodder for livestock production.

In order to examine naturally occurring variation in the ability of *Triticum aestivum* L. (hexaploid wheat) to support certain strains of *P. fluorescens*, Okubara *et al.* (2004) have surveyed 27 Pacific Northwest (PNW) cultivars for the ability to undergo root colonization with the aggressive colonizer *P. fluorescens* strain Q8r1-96, and *P. fluorescens* strain Q2-87, a less effective colonizer. In seed inoculation experiments, Q8r1-86 colonized roots of all of the cultivars equally or more effectively than did Q2-87 in a non-pasteurized, non-agricultural soil. Seven cultivars supported significantly ($P<0.05$) higher rhizosphere populations of Q8r1-96 than Q2-87 within 14 days post-inoculation (dpi), two cultivars supported relatively high population densities of each bacterial strain, and three cultivars supported low population densities of the strains. Population densities normalized to root weight reached maximum steady-state levels within 4 dpi, and differential colonization was seen as early as 7 dpi. In pairwise comparisons, the bacterial treatments differentially affected the root morphology of some of the cultivars at 14 dpi. However, principal components (factor) and correlation analysis showed that preferential colonization by Q8r1-96 was independent of root fresh weight, total length, surface area, volume, and average diameter, and that differential colonization was not correlated with changes in any specific root morphometric variable. Variation in root colonization of specific cultivars suggests useful genetic stocks for mapping and identifying host genes involved in wheat–rhizosphere interactions.

Rhizobacteria with properties related to plant growth-promotion were isolated from the rhizosphere of the perennial legume *Chamaecytisus proliferus* ssp. *proliferus* var. *palmensis* (tagasaste) growing in field conditions. Donate-Correa *et al.* (2005) collected samples in two localities of the Tenerife Island: La Laguna and El Tanque, NE and NW at 600 and 1000 meters above sea level, respectively, and in two seasons, winter and summer. The strains were isolated by using culture dependent procedures, and identified by phenotypic (culturable and biochemical)

and genotypic (ERIC-PCR fingerprinting) features. The rhizosphere isolates formed a diverse community of mainly Gram-negative bacteria, with members of genera *Pseudomonas*, *Burkholderia* and *Sphingomonas* being predominant. A high level of selectivity was found in the rhizosphere environment as compared to the non-rhizosphere soil where Gram-positives were more abundant. Species richness (number of species) and species abundance were related to the sampling season and the locality, thus, samples obtained in winter at both sites had larger counts than samples obtained in summer, and the higher species richness was found in La Laguna. The species *Pseudomonas fluorescens* showed the highest number of properties related to plant growth promotion (PGP): 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, phytohormone production, nitrogen fixation, fungal growth inhibition and cyanogenesis, thus it seems to be the most suitable microorganism to be tested in PGP-field experiments.

Paenibacillus polymyxa is a plant growth-promoting rhizobacterium with a broad host range, but so far the use of this organism as a biocontrol agent has not been very efficient. Timmusk *et al.* (2005) showed that this bacterium protected *Arabidopsis thaliana* against pathogens and abiotic stress. They studied colonization of plant roots by a natural isolate of *P. polymyxa* which had been tagged with a plasmid-borne *gfp* gene. Fluorescence microscopy and electron scanning microscopy indicated that the bacteria colonized predominantly the root tip, where they formed biofilms. Accumulation of bacteria was observed in the intercellular spaces outside the vascular cylinder. Systemic spreading did not occur, as indicated by the absence of bacteria in aerial tissues. Studies were performed in both a gnotobiotic system and a soil system. The fact that similar observations were made in both systems suggests that colonization by this bacterium can be studied in a more defined system. They discussed the problems associated with green fluorescent protein tagging of natural isolates and deleterious effects of the plant growth-promoting bacteria.

In search of efficient PGPR strains with multiple activities, a total of 72 bacterial isolates belonging to *Azotobacter*, fluorescent *Pseudomonas*, *Mesorhizobium* and *Bacillus* were isolated from different rhizospheric soil and plant root nodules in the vicinity of Aligarh by Ahmad *et al.* (2006). These test isolates were biochemically characterized. These isolates were screened *in vitro* for their

plant growth promoting traits like production of indoleacetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore, phosphate solubilization and antifungal activity. More than 80 % of the isolates of *Azotobacter*, fluorescent *Pseudomonas* and *Mesorhizobium ciceri* produced IAA, whereas only 20% of *Bacillus* isolates was IAA producer. Solubilization of phosphate was commonly detected in the isolates of *Bacillus* (80 %) followed by *Azotobacter* (74.47 %). *Pseudomonas* (55.56 %) and *Mesorhizobium* (16.67 %). All test isolates could produce ammonia but none of the isolates hydrolyzed chitin. Siderophore production and antifungal activity of these isolates except *Mesorhizobium* were exhibited by 10–12.77% isolates. HCN production was more common trait of *Pseudomonas* (88.89 %) and *Bacillus* (50 %). On the basis of multiple plant growth promoting activities, eleven bacterial isolates (seven *Azotobacter*, three *Pseudomonas* and one *Bacillus*) were evaluated for their quantitative IAA production, and broad-spectrum (active against ≥three test fungi) antifungal activity. Almost at all concentration of tryptophan (50–500 µg/ml), IAA production was highest in the *Pseudomonas* followed by *Azotobacter* and *Bacillus* isolates. *Azotobacter* isolates (AZT_3, AZT_{13}, AZT_{23}), *Pseudomonas* (Ps_5) and *Bacillus* (B_1) showed broad-spectrum antifungal activity on Muller-Hinton medium against *Aspergillus*, one or more species of *Fusarium* and *Rhizoctonia bataticola*. Further evaluation of the isolates exhibiting multiple plant growth promoting (PGP) traits on soil–plant system is needed to uncover their efficacy as effective PGPR.

A study was conducted by Cakmakci *et al.* (2006) with sugar beet in greenhouse and field at two soil type with different organic matter (containing 2.4 and 15.9 % OM, referred as the low- and high-OM soil) conditions in order to investigate seed inoculation of sugar beet, with five N_2-fixing and two phosphate solubilizing bacteria in comparison to control and mineral fertilizers (N and P) application. Three bacterial strains dissolved P; all bacterial strains fixed N_2 and significantly increased growth of sugar beet. In the greenhouse, inoculations with PGPR increased sugar beet root weight by 2.8–46.7 % depending on the species. Leaf, root and sugar yield were increased by the bacterial inoculation by 15.5–20.8, 12.3–16.1, and 9.8–14.7 %, respectively, in the experiment of low- and high-OM soil. Plant growth responses were variable and dependent on the inoculants strain,

soil organic matter content, growing stage, harvest date and growth parameter evaluated. The effect of PGPR was greater at early growth stages than at the later. Effective *Bacillus* species, such as OSU-142, RC07 and M-13, *Paenibacillus polymyxa* RC05, *Pseudomonas putida* RC06 and *Rhodobacter capsulatus* RC04 may be used in organic and sustainable agriculture.

In a study by Chen *et al.* (2006), isolation, screening and characterization of 36 strains of phosphate solubilizing bacteria (PSB) from Central Taiwan were carried out. Mineral phosphate solubilizing (MPS) activities of all isolates were tested on tricalcium phosphate medium by analyzing the soluble-P content after 72 h of incubation at 30°C. Identification and phylogenetic analysis of 36 isolates were carried out by 16S rDNA sequencing. Ten isolates belonged to genus *Bacillus*, nine to genus *Rhodococcus*, seven to genus *Arthrobacter*, six to genus *Serratia* and one each to genera *Chryseobacterium*, *Delftia*, *Gordonia* and *Phyllobacterium*. In addition, four strains namely, *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. were reported for the first time as phosphate solubilizing bacteria (PSB) after confirming their capacity to solubilize considerable amount of tricalcium phosphate in the medium by secreting organic acids. P-solubilizing activity of these strains was associated with the release of organic acids and a drop in the pH of the medium. HPLC analysis detected eight different kinds of organic acids, namely: citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of these isolates. An inverse relationship between pH and P solubilized was apparent from this study. Identification and characterization of soil PSB for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field application.

Seventeen rhizobacteria isolated from different ecological regions, i.e. Brazil, Indonesia, Mongolia and Pakistan were studied to develop inoculants for wheat, maize and rice. Almost all the bacterial isolates were Gram-negative, fast-growing motile rods and utilized a wide range of carbon sources. These isolates produced indole-3-acetic acid at concentrations ranging from 0.8-42.1 μg ml^{-1}, irrespective of the region. Fifteen isolates fixed N at rates ranging from 20.3-556.8 nmole C_2H_2 reduced h^{-1} $vial^{-1}$. Isolate 8N-4 from Mongolia produced the highest amount of

indole-3-acetic acid (42.1 μg ml^{-1}, produced siderophores (0.3 mg ml^{-1}) and was the only isolate that solubilized phosphate (188.7 μg P ml^{-1}). Inoculation of the wheat variety *Orkhon* with 8N-4 isolate resulted in the maximum increase in plant biomass, root length, and total N and P contents in plants. Random amplified polymorphic deoxyribonucleic acid (RAPD) analysis, conducted with 60 decamer primers, revealed a high level of polymorphism among the bacterial isolates from different geographic regions and a low level of polymorphism among isolates from the same region. The complete 16S rRNA gene sequence analysis demonstrated that 8N-4 is a *Bacillus pumilus* strain (Accession number AY548949). It was concluded that *Bacillus pumilus* 8N-4 can be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety *Orkhon* in Mongolia (Hafeez *et al.*, 2006).

Five bacterial strains with phosphate-solubilizing ability and other plant growth promoting traits increased the plant biomass (20–40 %) as tested by paper towel method. Glasshouse and field experiments were conducted using two efficient strains *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35. Increase in plant biomass (dry weight) was 99 % with EB 67 and 94 % with CDB 35 under glasshouse conditions. Increase in plant biomass at 48 and 96 days after sowing was 66 % and 50 % with EB 67 and 51 % and 18 % with CDB 35 under field conditions. Seed treatment with EB 67 and CDB 35 increased the grain yield of field-grown maize by 85 % and 64 % compared to the uninoculated control. Population of EB 67 and CDB 35 were traced back from the rhizosphere of maize on buffered rock phosphate (RP) medium and both the strains survived up to 96 days after sowing (Hameeda *et al.*, 2006).

Field trials were conducted by Kokalis-Burelle *et al.* (2006) in Florida on bell pepper (*Capsicum annuum*) to monitor the population dynamics of two plant growth-promoting rhizobacteria (PGPR) strains (*Bacillus subtilis* strain GBO3 and *Bacillus amyloliquefaciens* strain IN937a) applied in the potting media at seeding and at various times after transplanting to the field during the growing season. In-field drenches of an aqueous bacterial formulation were used for the mid-season applications. The effects of the applied PGPR and application methods on bacterial survival, rhizosphere colonization, plant growth and yield, and selected indigenous rhizosphere microorganisms were assessed. The Gram-positive PGPR applied to the

potting media established stable populations in the rhizosphere that persisted throughout the growing season. Additional aqueous applications of PGPR during the growing season did not increase the population size of applied strains compared to treatments only receiving bacteria in the potting media; however, they did increase plant growth compared to the untreated control to varying degrees in both trials. Most treatments also reduced disease incidence in a detached leaf assay, indicating that systemic resistance was induced by the PGPR treatments. However, treatments did not result in increased yield, which was highly variable. Application of the PGPR strains did not adversely affect populations of beneficial indigenous rhizosphere bacteria including fluorescent pseudomonads and siderophore-producing bacterial strains. Treatment with PGPR increased populations of fungi in the rhizosphere but did not result in increased root disease incidence. This fungal response to the PGPR product was likely due to an increase in nonpathogenic chitinolytic fungal strains resulting from the application of chitosan, which is a component of the PGPR formulation applied to the potting media.

A study was conducted by Shaharoona *et al.* (2006) to test the hypothesis that the bacterial strains possessing 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity may also promote growth of inoculated plants and could increase nodulation in legumes upon co-inoculation with rhizobia. Several rhizobacteria were isolated from maize rhizosphere through enrichment on ACC as a sole N source. Purified isolates were screened for growth promotion in maize under axenic conditions and for in vitro ACC-deaminase activity. A significant positive correlation was observed between in vitro ACC-deaminase activity of bacterial cells and root elongation. None of the isolates produced auxins. *Bradyrhizobium japonicum* produced lesser amount of auxins but did not carry ACC-deaminase activity. Results of pot experiment revealed that co-inoculation with *Bradyrhizobium* and plant growth promoting rhizobacteria (PGPR) isolates enhanced the nodulation in mung bean compared with inoculation with *Bradyrhizobium* alone. It is highly expected that inoculation with rhizobacteria containing ACC-deaminase hydrolysed endogenous ACC into ammonia and alpha-ketobutyrate instead of ethylene. Consequently, root and shoot growth as well as nodulation were promoted.

The effect of different plant-growth promoting rhizobacteria (*Azotobacter chroococcum, Azospirillum brasilense*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Bacillus cereus*) on pigeonpea (*Cajanus cajan* (L) Milsp.) cv. P-921 inoculated with *Rhizobium* sp. (AR-2–2 k) was assessed. A glasshouse experiment was carried out by Tilak *et al.* (2006) with a sandy-loam soil in which the seeds were treated with *Rhizobium* alone or in combination with several PGPR isolates. It was monitored on the basis of nodulation, N_2 fixation, shoot biomass, total N content in shoot and legume grain yield. The competitive ability of the introduced *Rhizobium* strain was assessed by calculating nodule occupancy. The PGPR isolates used did not antagonize the introduced *Rhizobium* strain and the dual inoculation with either *Pseudomonas putida*, *P. fluorescens* or *Bacillus cereus* resulted in a significant increase in plant growth, nodulation and enzyme activity over *Rhizobium*-inoculated and uninoculated control plants. The nodule occupancy of the introduced *Rhizobium* strain increased from 50 % (with *Rhizobium* alone) to 85 % in the presence of *Pseudomonas putida*. This study enabled us to select an ideal combination of efficient *Rhizobium* strain and PGPR for pigeonpea grown in the semiarid tropics.

Yanni *et al.* (2006) studied the natural and intimate associations between rhizobia and rice (*Oryza sativa* L.) and assessed their impact on plant growth in order to exploit those combinations that can enhance grain yield with less dependence on inputs of nitrogen (N) fertilizer. Diverse, indigenous populations of *Rhizobium leguminosarum* bv. *trifolii* (the clover root-nodule endosymbiont) intimately colonize rice roots in the Egyptian Nile delta where this cereal has been rotated successfully with berseem clover (*Trifolium alexandrinum* L.) since antiquity. Laboratory and greenhouse studies have shown with certain rhizobial strain–rice variety combinations that the association promotes root and shoot growth thereby significantly improving seedling vigour that carries over to significant increases in grain yield at maturity. Three field inoculation trials in the Nile delta indicated that a few strain–variety combinations significantly increased rice grain yield, agronomic fertilizer N-use efficiency and harvest index. The benefits of this association leading to greater production of vegetative and reproductive biomass more likely involve rhizobial modulation of the plant's root architecture for more efficient acquisition of certain soil nutrients [e.g. N, phosphorus (P), potassium (K),

magnesium (Mg), calcium (Ca), zinc (Zn), sodium (Na) and molybdenum (Mo)] rather than biological N_2 fixation. Inoculation increased total protein quantity per hectare in field-grown grain, thereby increasing its nutritional value without altering the ratios of nutritionally important proteins. Studies using a selected rhizobial strain (E11) indicated that it produced auxin (indoleacetic acid) and gibberellin [tentatively identified as gibberellin (GA 7)] phytohormones representing two major classes of plant growth regulators. Axenically collected rice root exudate significantly enhanced E11's production of this auxin. This strain extensively colonized the rice root surface under gnotobiotic culture conditions, producing distributions of spatial patchiness that would favour their localized erosion of the epidermal surface, colonization of small crevices at epidermal junctions as a possible portal to enter into the root, and quorum sensing of diffusible signal molecules indicating that their nearest bacterial neighbours are in close proximity in situ. Studies of selected rhizobial endophytes of rice indicated that they produced cell-bound cellulase and polygalacturonase enzymes that can hydrolyze glycosidic bonds in plant cell walls, and non-trifolitoxin bacteriocin(s) that can inhibit other strains of clover rhizobia. Strain E11 was able to endophytically colonize rice roots of varieties commonly used by Filipino peasant farmers, and also to stimulate genotype-specific growth-promotion of corn (*Zea mays*, maize) under field conditions. An amalgam of these results indicate some rhizobia have evolved an additional ecological niche enabling them to form a three-component life cycle including a free-living heterotrophic phase in soil, a N_2-fixing endosymbiont phase within legume root nodules, and a beneficial growth-promoting endocolonizer phase within cereal roots in the same crop rotation. Our results further indicate the potential opportunity to exploit this newly described, plant¿rhizobia association by developing biofertilizer inoculants that may assist low-income farmers in increasing cereal production (especially rice) with less fertilizer N inputs, fully consistent with both sustainable agriculture and environmental safety.

Biological Control

Pseudomonas fluorescens strains which effectively inhibited mycelial growth of *Fusarium udum*, the pigeonpea (*Cajanus cajan*) pathogen, were isolated from the rhizoplane of different crops (Vidhyasekaran, 1997). Various powder formulations of two efficient *P. fluorescens* strains were developed. All freshly prepared powder formulations were effective in controlling the disease, but their efficacies varied depending upon the length of storage. Talc formulations were effective even after 6 months of storage, while peat formulations were effective up to 60 days of storage. The shelf life of vermiculite, lignite, and kaolinite formulations was short. Unformulated bacterial suspensions could not be stored even for 10 days, at which time their efficacy was completely lost. The bacterial strains survived in pigeonpea rhizosphere throughout the crop-growth period. The talc-based powder formulations effectively controlled pigeonpea wilt and increased yield in two field trials. Development of powder formulations of *P. fluorescens* will aid large-scale application of biological control in farmers' fields.

Selected PGPR strains belonging to diverse Gram-positive and Gram-negative genera can, upon seed treatment or soil drench treatment to plant root systems were reported by Kloepper *et al.* (1998), reduce the incidence of distally infecting pathogens. Single PGPR strains have been shown to reduce pathogen infection and symptoms of multiple diseases on cucumber and tomato. Cucumber diseases affected in both greenhouse and field studies in multiple years include foliar diseases (angular leaf spot, caused by *Pseudomonas syringae* pv. *lachrymans*; and anthracnose, caused by *Colletotrichum orbiculare*); systemic wilt diseases (cucurbit wilt, caused by *Erwinia tracheiphila*; and Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cucumerinum*), and the systemic viral disease caused by cucumber mosaic virus (CMV). In the case of cucurbit wilt, disease control is linked to PGPR-mediated reductions in plant preference by the insect vectors, the striped and spotted cucumber beetles. In field and greenhouse studies, PGPR treatments led to significant reduction in beetle feeding, which was associated with PGPR-mediated reductions in cucurbitacin C, a feeding attractant. With tomato, protection has been noted in the greenhouse or field against CMV; bacterial spot, caused by *Xanthomonas axonopodis* pv. *vesicatoria*; tomato mottle geminivirus; and bacterial

speck, caused by *P. syringae* pv. *tomato*. Mode of action studies support the conclusion that the observed systemic biocontrol results from ISR, since measurable biochemical and cytological changes occur in the plant in relation to host recognition of the inducing PGPR strains. The specific plant changes vary somewhat among PGPR strains and are the focus of intense current investigation. They have observed enhanced peroxidase activity and lignification in cucumber and induction of PR1a promoter in transgenic tobacco containing a GUS reporter gene. In this tobacco system, all PGPR strains which enhanced protection against wildfire disease – caused by *P. syringae* pv. *tabaci* – in the greenhouse induced GUS activity, whereas control strains lacking disease protecting activity did not induce GUS activity significantly relative to controls.

Plant growth-promoting rhizobacteria (PGPR) strains INR7 (*Bacillus pumilus*), GB03 (*Bacillus subtilis*), and ME1 (*Curtobacterium flaccumfaciens*) were tested singly and in combinations for biological control against multiple cucumber pathogens. Investigations under greenhouse conditions were conducted with three cucumber pathogens—*Colletotrichum orbiculare* (causing anthracnose), *Pseudomonas syringae* pv. *lachrymans* (causing angular leaf spot), and *Erwinia tracheiphila* (causing cucurbit wilt disease)—inoculated singly and in all possible combinations. There was a general trend across all experiments toward greater suppression and enhanced consistency against multiple cucumber pathogens using strain mixtures. The same three PGPR strains were evaluated as seed treatments in two field trials over two seasons, and two strains, IN26 (*Burkholderia gladioli*) and INR7 also were tested as foliar sprays in one of the trials. In the field trials, the efficacy of induced systemic resistance activity was determined against introduced cucumber pathogens naturally spread within plots through placement of infected plants into the field to provide the pathogen inoculum. PGPR-mediated disease suppression was observed against angular leaf spot in 1996 and against a mixed infection of angular leaf spot and anthracnose in 1997. The three-way mixture of PGPR strains (INR7 plus ME1 plus GB03) as a seed treatment showed intensive plant growth promotion and disease reduction to a level statistically equivalent to the synthetic elicitor Actigard applied as a spray (Raupach and Kloepper, 1998).

According to Van Loon *et al.* (1998) nonpathogenic rhizobacteria can induce a systemic resistance in plants that is phenotypically similar to pathogen-induced systemic acquired resistance (SAR). Rhizobacteria-mediated induced systemic resistance (ISR) has been demonstrated against fungi, bacteria, and viruses in *Arabidopsis*, bean, carnation, cucumber, radish, tobacco, and tomato under conditions in which the inducing bacteria and the challenging pathogen remained spatially separated. Bacterial strains differ in their ability to induce resistance in different plant species, and plants show variation in the expression of ISR upon induction by specific bacterial strains. Bacterial determinants of ISR include lipopolysaccharides, siderophores, and salicylic acid (SA). Whereas some of the rhizobacteria induce resistance through the SA-dependent SAR pathway, others do not and require jasmonic acid and ethylene perception by the plant for ISR to develop. No consistent host plant alterations are associated with the induced state, but upon challenge inoculation, resistance responses are accelerated and enhanced. ISR is effective under field conditions and offers a natural mechanism for biological control of plant disease.

According to Braun-Kiewnick *et al.* (2000) strains of *Pantoea agglomerans* (synanamorph *Erwinia herbicola*) suppressed the development of basal kernel blight of barley, caused by *Pseudomonas syringae* pv. *syringae*, when applied to heads prior to the *Pseudomonas syringae* pv. *syringae* infection window at the soft dough stage of kernel development. Field experiments in 1994 and 1995 revealed 45 to 74 % kernel blight disease reduction, whereas glasshouse studies resulted in 50 to 100 % disease control depending on the isolate used and barley cultivar screened. The efficacy of biocontrol strains was affected by time and rate of application. Percentage of kernels infected decreased significantly when *P. agglomerans* was applied before pathogen inoculation, but not when coinoculated. A single *P. agglomerans* application 3 days prior to the pathogen inoculation was sufficient to provide control since populations of about 10(^7) CFU per kernel were established consistently, while *Pseudomonas syringae* pv. *syringae* populations dropped 100-fold to 2.0 × 10(^4) CFU per kernel. An application to the flag leaf at EC 49 (before heading) also reduced kernel infection percentages significantly. Basal blight decreased with increasing concentrations (10(^3) to 10(^7) CFU ml^{-1}) of

P. agglomerans, with 10(^7) CFU/ml providing the best control. For long-term preservation and marketability, the survival of bacterial antagonists in several wettable powder formulations was tested. Over all formulations tested, the survival declined between 10- to >100-fold over a period of 1.5 years (r = –0.7; P = 0.000). Although not significant, storage of most formulations at 4°C was better for viability (90 to 93 % survival) than was storage at 22°C (73 to 79 %). However, long-term preservation had no adverse effect on biocontrol efficacy.

The efficacy of various *P. fluorescens* isolates was tested for the management of fruit rot of chilli caused by *Colletotrichum capsici*. Among the various isolates tested *P. fluorescens* isolates *viz.* Pf1 and ATR increased the plant growth and produced the maximum amout of indole acetic acid. *P. fluorescens* Pf1 effectively inhibited the mycelial growth of the pathogen under *in vitro* conditions and decreased the fruit rot incidence under greenhouse condition. Seed treatment plus soil application of talc based formulation of *P. fluorescens* isolate Pf1 effectively reduced the disease incidence. Expression of various defense related enzymes and chemicals was found involved in the induction of systemic resistance against pathogen infection. Induction of various defense related genes has been discussed for the suppression of pathogen infection by Ramamoorthy and Samiyappan (2001).

Efficacy of seven strains of *Pseudomonas fluorescens* (*Pfs*17), plant growth-promoting rhizobacteria (PGPR), were tested by Sarma *et al.* (2002) under field conditions for their ability to protect *Cicer arietinum* against *Sclerotium rolfsii* infection. Best protection was observed in strain *Pfs*3 where 23 % seedling mortality was recorded in comparison to 44 % in non-treated control. To correlate the induction of phenolic compounds by the PGPRs with disease resistance, qualitative and quantitative alterations of phenolic compounds in different parts of *C. arietinum* were estimated following PGPR application as seed treatment. High performance liquid chromatographic (HPLC) analysis of the leaves, collars and roots of the PGPR-treated and non-treated (control) plants showed the presence of gallic, ferulic, chlorogenic and cinnamic acids with varied amounts in the PGPR-treated as well as non-treated (control) plants. Maximum accumulation of cinnamic acid was observed in plants treated with *Pfs*3 strain (1660 ng g^{1} fresh wt.) which was almost 19.5 times higher than untreated control plants and also significantly high when compared to

other PGPR treatments. *Pfs*3 also caused maximum accumulation of total phenolics and gallic acid in all chickpea plant parts as compared to other treatments and untreated control. A direct relationship between the level of total phenolics and seedling survivability was observed. PGPR-mediated induction of phenolic compounds as a biochemical barrier in *C. arietinum* against *S. rolfsii* infection is envisaged.

Shternshis *et al.* (2002) tested three products based on compounds of biological origin for their ability to control the raspberry midge blight in the Siberian region of Russia. *Bacillus thuringiensis* sub sp. *israelensis* (Bacticide) and *Streptomyces avermitilis metabolies* (Phytovern) were used against *Thomasiniana theobaldi* (a general member of the midge blight) and chitinase was used against fungi (mainly *Didymella applanata*) associated with *T. theobaldi*. The Bacticide (0.2 %) and Phytoverm (0.2%) sprays caused a two fold decrease in midge blight severity and the same effect was obtained with chemical insecticides. The chitinase (1%) spray caused a four fold decrease in the severity of midge blight. In addition. Chitinase and Phytovem caused a significant suppression of the independent spur blight. These studies form the basis for further evaluation of ecologically safe control of the raspberry midge blight.

Bansal *et al.* (2003) tested the efficacy of *Azotobacter chroococcum* against tomato wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici)* during rabi 2000-01 and 2001-02 in pot house under artificial inoculum conditions. Tomato seedlings var. local, treated with *A. chroococcum* before transplanting along with soil application of nitrogen @ 60, 80 and 100 kg ha^{-1} showed complete inhibition of plant mortality (7.36%) was also observed when seedlings were reated with *A. chroococcum* only as compared to the seedlings without any treatment (17.35%). It may be attributed to the production of antifungal substances by *A. chroococcum.*

Bhatia *et al.* (2003) observed maximum colony growth inhibition due to *Pseudomonas* PS 2 (74 %) as compared to PS 1 (71 %) on trypticase soy agar (TSM) plates after 5 days of incubation. Light and scanning electron microscope examination showed hyphal coiling, vacuolation and granulation of cytoplasm resulting in lysis of hyphae of *Macrophomina phaseolina* by pseudomonads. Cell

free culture filtrates of strains PS1 and PS 2 restricted the growth of mycelium of *M. phaseolina*, PS 1 and PS 2 caused maximum colony growth inhibition by 57 and 61% respectively at 20% conc. of culture filtrate after 4 days of incubation. Volatile substances produced by PS 1 and PS 2 also inhibited the colony growth of *M. phaseolina* by 25 and 32% respectively. Inhibitory effect of volatile substances, however, decreased with advancing in incubation period. Colony growth of *M. phaseolina* was significantly decreased by PS 1 and PS 2 as compared to control both in iron sufficient and iron deficient conditions. PS 2 showed higher antagonistic activity than PS 1, as evidenced by pronounced colony growth inhibition.

Fourteen plant growth promoting rhizobacteria (PGPR) isolated from rhizotic zones of field-grown green gram (*Vigna radiata* (L.) Wilczek) plants were examined by Gupta *et al.* (2003) for their growth-promoting attributes and ability to affect the growth *in vitro* of 10 strains of *Bradyrhizobium* sp. (*Vigna*). None of the rhizosphere bacteria was found to repress or stimulate the growth of any of the *Bradyrhizobium* strains tested. However, they produced antibiotics and siderophores and plant growth promoting substances. *Ex planta* and plant nitrogen fixation and phosphate solubilization was not detected by any of the isolates. Under *in-vitro* conditions, nine isolates inhibited growth of soil-borne fungal pathogens; one of them identified as *Bacillus* sp. antagonized all the fungi tested on two different media. All PGPR isolates were tested both in sterile and unsterile soil for their ability to promote nodulation, nitrogen fixation, growth, and yield of green gram in the presence of two *Bradyrhizobium* sp. (*Vigna*) strains S 24 and Cog 15. In sterile soil, all PGPR isolates had a positive effect on shoot biomass development, acetylene reduction assay (ARA), and N content when co-inoculated with *Bradyrhizobium* strain Cog 15, but could influence only shoot biomass development in the presence of strain S 24. In unsterile soil, PGPR isolates had a nodule-stimulatory effect on strain Cog 15 and a plant growth promoting effect on strain S 24, after 50 and 90 days of plant growth. Five isolates EG-RS-3, EG-RS-4, and NG-ER-7 (*Bacillus* spp), and KG-ER-1 and EG-ER-2 (*Enterobacter* spp) significantly increased yield of green gram in unsterile soil.

Murphy *et al.* (2003) evaluated combinations of two strains of plant growth-promoting rhizobacteria (PGPR) formulated with the carrier chitosan for the ability

to induce growth promotion of tomato plants and resistance to infection by Cucumber mosaic virus (CMV). Each PGPR combination included GB03 (*Bacillus subtilis*) and one of the following PGPR strains: SE34 (*B. pumilus*), IN937a (*B. amyloliquefaciens*), IN937b (*B. subtilis*), INR7 (*B. pumilus*), or T4 (*B. pumilus*). The PGPR combinations formulated with chitosan are referred to as biopreparations. Tomato plants treated with each of the biopreparations appeared phenotypically and developmentally similar to nonbacterized control plants that were 10 days older (referred to as the older control). When plants were challenged with CMV, all plants in the biopreparation treatments and the older control treatment had significantly greater height, fresh weight, and flower and fruit numbers than that of plants in the CMV-inoculated same age control treatment. CMV disease severity ratings were significantly lower for biopreparation-treated and older control tomato plants than for that of same age control plants at 14 and 28 days postinoculation (dpi). CMV accumulation in young noninoculated leaves was significantly less for all biopreparation-treated plants and those in the older control than for the same age control plants at 14 dpi and for four of the five biopreparation treatments at 28 dpi. In those tomato plants shown to be infected, the amount of CMV in noninoculated leaves was significantly lower for three of the biopreparation treatments and the older control treatment at 14 dpi and biopreparation G/INR7 treatment at 28 dpi when compared with the control treatment. These data show that treatment of tomato plants with biopreparations results in significant enhancement of growth and protection against infection by CMV.

A series of laboratory, greenhouse and field experiments were conducted by Niranjan, *et al.* (2003) on the strains of plant growth promoting rhizobacteria (PGPR).The PGPR were tested as suspensions of fresh cultures and talc-based powder formulations. Evaluations were conducted on pearl millet (*Pennisetum glaucum*) for growth promotion and management of downy mildew caused by *Sclerospora graminicola.* All treatments with fresh suspensions and powdered formulations showed enhancement in germination and vigor index over the respective untreated controls. With fresh suspensions, maximum vigor index resulted from treatments by *Bacillus pumilus* strain INR7 followed by *B. subtilis* strain IN937b (64 and 38% higher than the untreated control, respectively). With powdered

formulation, treatment with strain INR7 also resulted in the highest germination and vigor indexes, which were 10 and 63%, respectively, over the untreated control. Under experimental plot conditions, prominent enhancement in growth also was observed in the disease tests. Yield was enhanced 40 and 37% over the untreated control by seed treatment with powdered formulations of strains INR7 and SE34, respectively. The same strains also increased yield by 36 and 33%, respectively, when applied as fresh suspensions. Studies on downy mildew management resulted in varied degrees of protection by the PGPR both under greenhouse and field conditions. With fresh suspensions, treatment with INR7 resulted in the highest protection (57%), followed by *B. pumilus* strain SE34 and B. subtilis strain GBO3, which resulted in 50 and 43% protection, respectively, compared with the untreated control. With powdered formulation, PGPR strain INR7 suppressed downy mildew effectively, resulting in 67% protection, while SE34 resulted in 58% protection, followed by GBO3 with 56% protection. Treatment with Apron (Metalaxyl) resulted in the highest protection against downy mildew under both greenhouse and field conditions. Thus, the present study suggests that the tested PGPR, both as powdered formulations and fresh suspensions, can be used within pearl millet downy mildew management strategies and for plant growth promotion.

Five plant growth promoting rhizobacterial formulations, each consisting of two *Bacilli* strains with chitosan as a carrier were tested for their capacity to promote growth and induce resistance against downy mildew in pearl millet under both greenhouse and field conditions. Three modes of applications were tested: seed treatment, soil amendment, and seed treatment+soil amendment. In general, irrespective of application method, most of the formulations, in comparison with the control, increased plant growth and vigor as measured by seed germination, seedling vigour, plant height, fresh and dry weight, leaf area, tillering capacity, number of earheads, length and girth of earhead, 1000 seed weight and yield. The time of flowering was also advanced by 4-5 days over the control. Likewise all the formulations significantly reduced downy mildew incidence relative to the nontreated control. However, the rate of growth enhancement and disease suppression varied considerably with the formulations. Formulations LS256 and LS257 besides being the best growth promoters were also the most efficient

resistance inducers. None of the formulations matched the level of the fungicide metalaxyl in offering protection against downy mildew. Among the application methods tested, soil amendment was found to be the most suitable and desirable way of delivering the formulations. Combination of seed treatment and soil amendment produced the same effect that was produced by soil amendment alone. This study by Raj *et al.* (2003) demonstrates a potential role for plant growth promoting rhizobacterial formulations in downy mildew management.

Greenhouse experiments showed that four mixtures of plant growth-promoting rhizobacteria (PGPR) strains (all *Bacillus* spp.) elicited induced systemic resistance in several plants against different plant pathogens. Based on these findings, Jetiyanon *et al.* (2003) sought to determine if systemic resistance induced by these PGPR would lead to broad-spectrum protection against several pathogens under field conditions in Thailand. Experiments were conducted during the rainy season (July to October 2001) and winter season (November 2001 to February 2002) on the campus of Naresuan University, Phitsanulok, Thailand. The specific diseases and hosts tested were southern blight of tomato (*Lycopersicon esculentum*) caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper (*Capsicum annuum* var. *acuminatum*) caused by *Colletotrichum gloeosporioides*, and mosaic disease of cucumber (*Cucumis sativus*) caused by Cucumber mosaic virus (CMV). Results showed that some PGPR mixtures suppressed disease more consistently than the individual PGPR strain IN937a. One PGPR mixture, *Bacillus amyloliquefaciens* strain IN937a+*B. pumilus* strain IN937b, significantly protected (P=0.05) plants against all tested diseases in both seasons. Further, cumulative marketable yields were positively correlated with some treatments.

Biochemical changes in banded leaf and sheath blight affected maize plants caused by *Rhizoctonia solani* f. sp. *sasakii* grown out of seeds treated with *Pseudomonas fluorescens* were studied by Shivakumar and Sharma (2003). There was an increase in phenolic content in maize leaf sheaths inoculated with *R. solani* or in those of maize plants raised from *P. fluorescens* treated seeds. Increase in phenolic content was observed, in leaf sheaths of plants raised from *P. fluorescens* treated seeds when inoculated with *R. solani*. Peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities increased when leaf

sheaths were inoculated with the pathogen and plants raised from *P. fluorescens* treated seeds showed higher activity. However leaves from *P. fluorescens* treated seeds did not show any further increase in PO and PPO activities after inoculation with *R. solani*. The bacterized seed with *P. fluorescens* led to accumulation of higher phenolic compounds and higher activity of PO, PPO and PAL, that may play a role in defense mechanism in plants against pathogen.

Root colonization by certain non-pathogenic bacteria can induce systemic resistance to pathogen infections in plants. In a split-root assay with tomato plants, Siddiqui and Shaukat (2003) investigated which determinants of the rhizobacterium *Pseudomonas aeruginosa* IE-6S+ were important for induction of resistance to the root-knot nematode *Meloidogyne javanica*. *P. aeruginosa* IE-6S+ produced 3.9+-1.1 μg ml^{-1} salicylic acid (SA) in a liquid casamino acid medium under laboratory conditions. The bacterial inoculant induced resistance equivalent to the application of 10 mM synthetic SA. However, SA at this concentration did not produce significant mortality of *M. javanica* juveniles *in vitro*. Soil iron (2.4 mM FeCl3cntdot6H2O) did not markedly alter the resistance that *P. aeruginosa* IE-6S+ induced in tomato roots, which suggested that *P. aeruginosa* IE-6S+ activity was not iron-regulated. However, the resistance reaction was greatly enhanced when IE-6S+ and SA were co-inoculated with 0.5% Tween-20. While IE-6S+ colonized the tomato rhizosphere at 6.38 log cfu g^{-1} fresh weight of root during the first 3 days after inoculation, the bacterial populations declined steadily, reaching a mean population density of 4.73 log cfu g^{-1} fresh weight of root at 21 days. The bacterium was not isolated from the unbacterized half of the split root system.

The ability to colonize roots is a sine qua non condition for a rhizobacteria to be considered a true plant growth-promoting rhizobacteria (PGPR). A simple screening method to detect such a potential ability of PGPR is described by Silva *et al.* (2003). Tomato seeds were surface sterilized for 30 s in 50% ethanol and this was followed by 3 min dipping in 2% NACIO. They were then washed three times in sterile water, left immersed in a propagule suspension of the rhizobacteria for 24 h, and transferred onto sterile 0.6% water-agar in tubes. The young, developing root system showed a tendency to grow downwards in the agar-gel column. Testing 500

rhizobacteria isolated from tomato rhizosphere for their ability to induce systemic resistance against *Pseudomonas syringae* pv. *tomato*, 28 of them did reduce infection to less than 40% and all 28 colonized roots according to the described bioassay.

Two plant growth-promoting rhizobacteria (PGPR), viz., *Pseudomonas fluorescens* strain Pf4 and *P. aeruginosa* strain Pag, protected chickpea (*Cicer arietinum*) plants from *Sclerotium rolfsii* infection when applied singly or in combination as seed treatment (Singh *et al.*, 2003). Pag gave the best protection to the seedlings, applied either singly (mortality 16%) or in combination with Pf4 (mortality 17%) compared with 44% and 24% mortality in control and Pf4 treatment, respectively. The two PGPR strains induced the synthesis of specific phenolic acids, salicylic acid (SA), as well as total phenolics at different growth stages of chickpea seedlings with varied amount. The maximum amount of total phenolics was recorded in all the aerial parts of 4-week-old plants. Gallic, ferulic, chlorogenic, and cinnamic acids were the major phenolic acids detected in high-performance liquid chromatography (HPLC) analysis. Induction of such phenolic acids in the seedlings was observed up to 6 weeks in comparison with control. Salicylic acid (SA) was induced frequently during the first 3 weeks of growth only. Between the two strains, Pag was more effective in inducing phenolic acid synthesis applied either singly or in combination with strain Pf4 during the entire 6 weeks of growth of chickpea. In the presence of a culture filtrate of *S. rolfsii*, the two *Pseudomonas* strains induced more phenolic acids in treated than in non-treated and control plants. The occurrence of salicylic acid was frequent in the first 24 h, but infrequent at 48 and 96 h. Foliar spray of *Pseudomonas* strains also enhanced the phenolic acid content as well as total phenolics within 24 h of application. Gallic, chlorogenic, and cinnamic acids were consistently discerned in the treated leaves, whereas SA was absent even up to 96 h of application. Resistance in chickpea plants by *Pseudomonas* strains through induction of phenolic compounds as well as induced systemic resistance via SA-dependent pathway was evident.

Aflatoxin contamination of groundnut, caused by *Aspergillus flavus* (Af) group of fungi, is a major problem in the rain fed agriculture in the seni arid tropics. Biological control could be one of the components of integrated management to reduce pre harvest kernel investment in the field. Thakur *et al.* (2003) evaluated six

Trichoderma and three *Pseudomonas* strains that were identified as highly antagonistic to Af 11-4 (a highly toxigenic strain) *in vitro*, in field to determine their biocontrol potential. The antagonists were applid as seed dressing and soil application in flowering in Af-sick pots. All the antagonists significantly reduced as seed infection in all three field experiments. Two *T. viridae*(Tv 17 and Tv 23), one *T. harzianum* (Th 23) and one *Pseudomonas* (pf 2) isolates provided greater protection to seed infection by Af 11-4 than others. The reduced seed contamination occurred due to significant reduction in Af population in the rhizosphere of groundnut.

Greenhouse experiments were conducted by Anith *et al.* ((2004) to study the effect of plant growth promoting rhizobacteria (PGPR; *Bacillus pumilus* SE 34, *Pseudomonas putida* 89B61, BioYield, and Equity), acibenzolar-S-methyl (Actigard), and a soil amendment with S-H mixture (contains agricultural and industrial wastes such as bagasse, rice husk, oyster shell powder, urea, potassium nitrate, calcium super phosphate, and mineral ash) on bacterial wilt incidence caused by *Ralstonia solana-cearum* (race 1, biovar 1) in susceptible tomato (*Lycopersicon esculentum* cv. Solar Set). In experiments with PGPR, *Pseudomonas putida* 89B61 significantly reduced bacterial wilt incidence when applied to the transplants at the time of seeding and 1 week prior to inoculation with *Ralstonia solanacearum*. BioYield, a formulated PGPR that contained two *Bacillus* strains, decreased disease significantly in three experiments. Equity, a formulation containing more than 40 different microbial strains, did not reduced wilt incidence compared with the untreated control. With inoculum at low pathogen densities of 1×10^5 and 1×10^6 CFU m^{-1}, disease incidence of Actigard-treated plants was significantly less than with nontreated plants. This is the first report of Actigard-mediated reduction of bacterial wilt incidence in a susceptible tomato cultivar. When PGPR and Actigard applications were combined, Actigard plus *P. putida* 89B61 or BioYield reduced bacterial wilt incidence compared with the untreated control. Incorporation of S-H mixture into infested soil 2 weeks before transplanting reduced the bacterial wilt incidence in one experiment. Combination of Actigard with the S-H mixture significantly reduced bacterial wilt incidence in tomato in two experiments.

Endophytic actinobacteria isolated from healthy cereal plants were assessed for their ability to control fungal root pathogens of cereal crops both *in vitro* and in planta. Thirty eight strains belonging to the genera *Streptomyces*, *Microbispora*, *Micromonospora*, and *Nocardioidies* were assayed by Coombs *et al.* (2004) for their ability to produce antifungal compounds *in vitro* against *Gaeumannomyces graminis* var. *tritici* (*Ggt*), the causal agent of take-all disease in wheat, *Rhizoctonia solani* and *Pythium* spp. Spores of these strains were applied as coatings to wheat seed, with five replicates (25 plants), and assayed for the control of take-all disease in planta in steamed soil. The biocontrol activity of the 17 most active actinobacterial strains was tested further in a field soil naturally infested with take-all and *Rhizoctonia*. Sixty-four percent of this group of microorganisms exhibited antifungal activity *in vitro*, which is not unexpected as actinobacteria are recognized as prolific producers of bioactive secondary metabolites. Seventeen of the actinobacteria displayed statistically significant activity in planta against *Ggt* in the steamed soil bioassay. The active endophytes included a number of *Streptomyces*, as well as *Microbispora* and *Nocardioides* spp. and were also able to control the development of disease symptoms in treated plants exposed to *Ggt* and *Rhizoctonia* in the field soil. The results of this study indicate that endophytic actinobacteria may provide an advantage as biological control agents for use in the field, where others have failed, due to their ability to colonize the internal tissues of the host plant.

A pool of 11 randomly selected, uncharacterized *Bacillus pumilus* isolates from sugar beet were evaluated by Bargabus, *et al.*(2004) using a high-throughput screen that utilized laboratory-based tests for 2 pathogenesis-related proteins, chitinase and β-1,3-glucanase, and biphasic hydrogen peroxide production. The screen was followed by a glasshouse test for induction of systemic acquired resistance for control of Cercospora leaf spot in sugar beet. These isolates were compared to the known biological control agent, *Bacillus mycoides* isolate Bac J, and a chemical inducer of resistance, acibenzolar-*S*-methyl. All laboratory-based screens identified *B. pumilus* isolates 203-6 and 203-7, which reduced Cercospora leaf spot symptoms by approximately 70%, even when spatially separated from the casual agent, *Cercospora beticola*. This level of control was similar to *B. mycoides* isolate Bac J and acibenzolar-*S*-methyl. In all cases, systemic resistance elicitation

was marked by an increase in 2 pathogenesis-related proteins, chitinase and β -1,3-glucanase, and was preceded by biphasic hydrogen peroxide production, also found in incompatible plant–pathogen interactions in which systemic resistance is induced. A combination of glycol chitin and aniline blue plate assays correctly identified all in planta inducers of systemic resistance as measured by control of Cercospora leaf spot in classical challenge assays for systemic acquired resistance without the inclusion of false positive identifications, reducing the workload in subsequent disease challenge assays by nearly 70%.

Jeun *et al.* (2004) cytologically compared the expression of induced resistance between cucumber plants induced with either plant growth-promoting rhizobacteria (PGPR) or chemicals. Inoculation with PGPR strains *Serratia marcescens* (90–166) and *Pseudomonas fluorescens* (89B61) induced systemic protection in the aerial part of cucumber plants against the anthracnose pathogen *Colletotrichum orbiculare*. Disease development was significantly reduced in these plants compared to control plants that were not inoculated with the PGPR strains. Inoculation with the PGPR strains caused no visible toxicity, necrosis, or other morphological changes. Induction with DL DL-3-aminobutyric acid (BABA) or amino salicylic acid (ASA) also significantly reduced disease development. Soil drenched with 10mM BABA and 1.0mM ASA-induced resistance in cucumber leaves without any toxicity to the plants. Higher concentrations of ASA (up to 10mM) were phytotoxic, resulting in plant stunting and blighted appearance of leaves. Cytological studies using fluorescent microscopy revealed a higher frequency of autofluorescent epidermal cells, which are related to accumulation of phenolic compounds, at the sites of fungal penetration in plants induced with PGPR and challenged by the pathogen. Neither spore-germination rate nor formation of appressoria was affected by PGPR treatments. In contrast, both BABA and ASA significantly reduced spore-germination rate and appressoria formation, while there were no differences from controls in the frequency of autofluorescent epidermal cells at the sites of fungal penetration. Their findings suggest that PGPR and chemical inducers cause different plant responses during induced resistance.

Lysobacter enzymogenes C3, the only biocontrol agent previously known to induce resistance in tall fescue against *Bipolaris sorokiniana*, was compared in growth chamber experiments with other strains of *L. enzymogenes*, strains of plant growth promoting rhizobacteria (PGPR) that induce systemic resistance in dicot plants, and the synthetic elicitor 1,2,3-benzothiadiazole-7-thiocarboxylic acid-S-methyl-ester (BTH). The treatments were evaluated for induction of localized or systemic resistance against *B. sorokiniana*, in an experiment conducted by Kific-Ekici and Yuen (2004) when applied to leaves and roots. In addition, the effects of induced resistance on pathogen conidial germination on the phylloplane were assessed. None of the bacterial or chemical treatments induced systemic resistance when applied to a leaf. Strains of *L. enzymogenes* differed in their ability to cause localized disease inhibition following foliage treatment and to induce systemic resistance in leaves when applied to roots. In contrast to C3, two other strains of *L. enzymogenes* were ineffective in inducing systemic resistance. PGPR strains varied in effectiveness in causing localized disease inhibition when applied to leaves. Most of the bacterial strains increased peroxidase activity in the treated leaves, providing evidence that localized disease inhibition may have been plant mediated. The involvement of localized induced resistance was confirmed in *P. fluorescens* WCS417r, which did inhibit *B. sorokiniana* conidial germination or hyphal growth in vitro. Soil drenches with nearly all PGPR strains resulted in systemic resistance in leaves, but the treatments varied as to the timing and strength of induced systemic resistance. BTH induced localized resistance when applied to leaves but did not activate resistance in leaves when applied to roots. All cases of induced resistance were associated with an inhibition of conidial germination on leaf surfaces and, thus, this reaction appears to be a hallmark of induced resistance in the *B. sorokiniana*-tall fescue pathosystem.

Salicylic acid (SA)-mediated induction of systemic resistance by *Pseudomonas aeruginosa* strain 7NSK2 and *P. fluorescens* strain CHA0 against soil-borne fungi and viruses have been reported by Siddiqui and Shaukat (2004). The role of SA biosynthesis in the enhancement of defence mechanism against plant-parasitic nematodes by these bacterial strains in tomato is not known. To better understand the importance of SA in rhizobacteria-mediated suppression of root-knot nematodes,

biocontrol potential of SA-negative or SA-overproducing mutants against *Meloidogyne javanica* was evaluated with their respective wild type counter parts. Culture supernatant of 7NSK2, CHA0 and their respective mutants caused significant mortality of *M. javanica* juveniles *in vitro*. SA deletion in 7NSK2 and SA overproduction in CHA0 did not influence bacterial efficacy to cause nematode deaths. Similarly, culture supernatants resulting from King's B liquid medium amended with $FeCl_3$ did not influence nematicidal activity of the bacterial strains. Strain CHA0 induced juvenile deaths more than 7NSK2 did. In pot experiments, the bacterial strains applied in unsterilized sandy loam soil markedly reduced final nematode population densities in roots and subsequent root-knot infection in tomato seedlings. SA-negative or overproducing derivatives prevented tomato roots in kinetics similar to those with their respective wild types. When soil iron concentration was lowered by the addition of ethylenediamine di(o-hydroxyphenylacetic acid), nematode biocontrol by the bacterial strains (both wild type and mutants) remained unaltered. To understand the mechanism involved in rhizobacteria mediated suppression of root-knot nematode in tomato, bacterial performance was assessed in a split root trial in which one-half of the root system was treated with bacterium while the other inoculated with nematode. Compared with the controls, application of the bacterial cell suspension to one-half of the root system lowered the populations of root-knot nematode in non-bacterized nematode-treated sections indicating enhanced defence in the non-bacterized half. With respect to nematode infection, mutants induced systemic resistance to a similar extent as that caused by the wild types in both wild type tomato and *NahG* tomato plants. It is concluded that fluorescent pseudomonads induce systemic resistance against root-knot nematode via a signal transduction pathway, which is independent of SA accumulation in roots.

Talc-based bioformulations containing cells of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Saccharomyces cerevisiae* were evaluated for their potential to attack the mango (*Mangifera indica* L.) anthracnose pathogen *Colletotrichum gloeosporioides* Penz. under endemic conditions by Vivekananthan *et al.* (2004). The preharvest aerial spray was given at fortnightly and monthly intervals. The plant growth-promoting rhizobacteria *Pseudomonas fluorescens* (FP7) amended with

chitin sprayed at fortnightly intervals gave the maximum induction of flowering, a yield attribute in the preharvest stage, consequently reduced latent symptoms were recorded at the postharvest stage. An enormous induction of the defence-mediating lytic enzymes chitinase and β-1,3-glucanase was recorded in colorimetric assay and the expression of discrete bands in native PAGE analysis after FP7 + chitin treatment. The enhanced expression of defence-mediating enzymes may collectively contribute to suppress the anthracnose pathogen, leading to improved yield attributes.

Bhatia *et al.* (2005) isolated ten isolates of fluorescent pseudomonads from rhizosphere of sunflower, potato, maize and groundnut. All the isolates produced fluorescent pigment in succinate broth and displayed siderophore production. Production of hydrocyanic acid (HCN) and indole acetic acid (IAA) by all the isolates was reduced besides phosphate solubilisation. Out of the ten strains, *Pseudomonas* PS I and PS II were found most potential. Bacterisation of sunflower seeds with fluorescent *Pseudomonas* PS I and PS II resulted in increased seed germination, root length, shoot length, fresh and dry weight of roots ans shoots, and yield of sunflower. Seed bacterisation with strains of fluorescent *Pseudomonas* PS I and PS II reduced incidence of collar rot by 69.8% and 56.9% respectively, in *Sclerotium rolfsii*-infested soil, making the organism a potential biocontrol agent against collar rot of the sunflower.

Patterns of colonization of *Vitis vinifera* L. cv. Chardonnay plantlets by a plant growth-promoting bacterium, *Burkholderia* sp. strain PsJN, were studied under gnotobiotic conditions. Wild-type strain PsJN and genetically engineered derivatives of this strain tagged with *gfp* (PsJN::*gfp*2x) or *gusA* (PsJN::*gusA*11) genes were used to enumerate and visualize tissue colonization. The rhizospheres of 4- to 5-week-old plantlets with five developed leaves were inoculated with bacterial suspensions. Epiphytic and endophytic colonization patterns were then monitored by dilution plating assays and microscopic observation of organ sections. Bacteria were chronologically detected first on root surfaces, then in root internal tissues, and finally in the fifth internode and the tissues of the fifth leaf. Analysis of the PsJN colonization patterns showed that this strain colonizes grapevine root surfaces, as

well as cell walls and the whole surface of some rhizodermal cells. Cells were also abundant at lateral root emergence sites and root tips. Furthermore, cell wall-degrading endoglucanase and endopolygalacturonase secreted by PsJN explained how the bacterium gains entry into root internal tissues. Host defense reactions were observed in the exodermis and in several cortical cell layers. Bacteria were not observed on stem and leaf surfaces but were found in xylem vessels of the fifth internode and the fifth leaf of plantlets. Moreover, bacteria were more abundant in the fifth leaf than in the fifth internode and were found in substomatal chambers. Thus, it seems that *Burkholderia* sp. strain PsJN induces a local host defense reaction and systemically spreads to aerial parts through the transpiration stream (Compant *et al.*, 2005).

Integration of foliar bacterial biological control agents and plant growth promoting rhizobacteria (PGPR) was investigated by Ji *et al.* (2005) to determine whether biological control of bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato*, and bacterial spot of tomato, caused by *Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas vesicatoria*, could be improved. Three foliar biological control agents and two selected PGPR strains were employed in pairwise combinations. The foliar biological control agents had previously demonstrated moderate control of bacterial speck or bacterial spot when applied as foliar sprays. The PGPR strains were selected in this study based on their capacity to induce resistance against bacterial speck when applied as seed and soil treatments in the greenhouse. Field trials were conducted in Alabama, Florida, and California for evaluation of the efficacy in control of bacterial speck and in Alabama and Florida for control of bacterial spot. The foliar biological control agent *P. syringae* strain Cit7 was the most effective of the three foliar biological control agents, providing significant suppression of bacterial speck in all field trials and bacterial spot in two out of three field trials. When applied as a seed treatment and soil drench, PGPR strain *Pseudomonas fluorescens* 89B-61 significantly reduced foliar severity of bacterial speck in the field trial in California and in three of six disease ratings in the field trials in Alabama. PGPR strains 89B-61 and *Bacillus pumilus* SE34 both provided significant suppression of bacterial spot in the two field trials conducted in Alabama. Combined use of foliar biological control agent Cit7 and PGPR strain

89B-61 provided significant control of bacterial speck and spot of tomato in each trial. In one field trial, control was enhanced significantly with combined biological control agents compared to single agent inoculations. These results suggested that some PGPR strains may induce plant resistance under field conditions, providing effective suppression of bacterial speck and spot of tomato, and that there may be some benefit to the integration of rhizosphere-applied PGPR and foliar-applied biological control agents.

The deliberate targeting of the stigma with the biocontrol agent in this pathosystem prompted Ngugi *et al.* (2005) to evaluate potential negative impacts on pollination and pollination-related fruit characteristics. Application of Serenade to the stigmatic surface of detached blueberry flowers in the laboratory had no effect ($P > 0.05$) on the number of pollen tubes entering the style or their growth rates within the stylar canal. There was also no reciprocal effect, i.e., population dynamics of *B. subtilis* were unaltered by the presence of pollen. Application of the biocontrol product to open flowers, regardless of whether it was done 1 day before or immediately prior to pollination, did not impact fruit set or the number of seeds per berry, but marginally ($P = 0.048$) affected fruit weight in one of two experimental runs in the greenhouse; fruit weights in the two Serenade timing treatments were significantly different from each other but neither was different from that of the control that received pollen only. In a field experiment in which honey bees were utilized to vector the biocontrol product to open flowers, application of Serenade did not affect fruit weight but significantly reduced fruit set from 49.1 to 38.1% ($P = 0.0382$) and seed number to about half of that of the untreated control ($P = 0.0109$). However, fruit weights and seed numbers in the experiment were low even in treatments receiving no Serenade, indicative of poor pollination overall. Taken together, these results indicate that application of Serenade has no inherently adverse effects on pollination and associated fruit characteristics, but caution should be exercised in applying this product in conditions otherwise unfavorable for adequate pollination.

The *Pseudomonas fluorescens* isolate 1 (Pf1) was found to protect the ragi [*Eleusine coracana* (L.) Gaertner] blast fungus, *Pyricularia grisea*. Induction of

defense proteins *viz.* chitinase, β-1,3 glucanase, peroxidase (PO) and polyphenol oxidase (PPO) by the Pf1 isolate was studied against *P. grisea* by Radjacommare (2005). Chitinase in a resistant, susceptible and commonly used cultivar with and without challenge inoculation of *P. grisea*, revealed changes in the isoform pattern by UV illumination after staining the gel with fluorescent brightner 28. Native PAGE (polyacrylamide gel electrophoresis) of PO showed the single isoform in all the treatments including the control and a significant increase in the intensity of the band in the inoculated control and Pf1 treatment in all the varieties. Isoform analysis of PPO showed the induction of PPO in *P. fluorescens* treated plants challenged with *P. grisea*. Application of Serenade, a commercial biofungicide formulation containing the bacterium *Bacillus subtilis*, to the stigmatic surface of open blueberry flowers suppresses floral infection by the mummy berry fungus *Monilinia vaccinii-corymbosi*.

One of 500 rhizobacteria isolated from soil, rhizosphere and rhizoplane of healthy tomato plants was previously selected by Romeiro *et al.* (2005) in laboratory, greenhouse and field tests as a good inducer of systemic resistance. This plant growth-promoting rhizobacterium (PGPR) was identified as *Bacillus cereus* by fatty-acid analysis. *Bacillus cereus* bacterial cells were removed from liquid culture by centrifugation and the supernatant repeatedly dialyzed (cut-off = 12 000 daltons) against distilled water. Dialysates applied to roots protected tomato plants against leaf fungal and bacterial pathogens, evidence that macromolecules synthesized by the PGPR and released into the environment act as elicitors of systemic resistance.

The aim of a study by Demoz and Korsten (2006) was therefore to determine the ability of *Bacillus subtilis* B246, commercially registered as Avogreen and used as a biocontrol agent against avocado pre- and postharvest diseases, to attach, colonize, and survive on avocado flowers and to study the interaction of the SER pathogens and the antagonist on avocado flowers. Avocado flowers inoculated with a liquid commercial formulation of the antagonist were observed at different time intervals under the scanning electron microscope (SEM). Population dynamics of the antagonist on the flowers were determined by means of total viable counts using reference cultures and background counts from the control. Flowers were also

inoculated with antagonist-pathogen (*Dothiorella aromatica* and *Phomopsis perseae*) combinations to determine in vivo interactions. The SEM observations and population dynamics study confirmed that the antagonist could effectively attach, colonize, and survive on avocado flowers. It could also attach to conidia and hyphae of the pathogens and cause cell degradation. These modes of action can give new insights into the control of pathogens by *B. subtilis*.

Different formulations of *Bacillus licheniformis* were evaluated on their own and in combination with prochloraz and stroburilin for their ability to reduce mango post-harvest fruit diseases [anthracnose and stem-end rot (SR)] when applied as a dip treatment in a mango pack house. Untreated fruit and fruit treated with either prochloraz or stroburilin alone served as controls. In these trials treatments integrating chemical pesticides with *B. licheniformis* controlled anthracnose and SR as effectively as the chemical control. The antagonist was more effective especially in the control of post-harvest diseases when fruit were kept in cold storage to simulate export conditions. In two of the three trials, results obtained when fruit was treated with the antagonist in combination with the commercial chemical were comparable to that obtained with the commercial chemical control. In this study by Govender and Korsten (2006), it was found that the antagonist when used in mango pack house treatments could provide an effective alternative to fungicides. Furthermore, the powder formulation of the antagonist can be successfully incorporated into the existing pack line.

In greenhouse experiments, plant growth promoting rhizobacteria (PGPR) *Serratia marceseens* NBRI1213 was evaluated for plant growth promotion and biologic control of foot and root rot of betelvine caused by *Phytophthora nicotianae* (Lavania *et al.*, 2006) . Bacterization of betelvine (*Piper betle* L.) cuttings with *S. marcescens* NBRI1213 induced phenylalanine ammonia-lyase, peroxidase, and polyphenoloxidase activities in leaf and root. Qualitative and quantitative estimation of phenolic compounds was done through high-performance liquid chromatography (HPLC) in leaf and root of betelvine after treatment with *S. marcescens* NBRI1213 and infection by *P. nicotianae*. Major phenolics detected were gallic, protocatechuic, chlorogenic, caffeic, ferulic, and ellagic acids by comparison of their retention time with standards through HPLC. In all of the treated plants, synthesis of phenolic

compounds was enhanced compared with control. Maximum accumulation of phenolics was increased in *S. marcescens* NBRI1213-treated plants infected with *P. nicotianae*. In a greenhouse test, bacterization using *S. marcescens* NBRI1213 decreased the number of diseased plants compared with nonbacterized controls. There were significant growth increases in shoot length, shoot dry weight, root length, and root dry weight, averaging 81 %, 68 %, 152 %, and 290 %, respectively, greater than untreated controls. This is the first report of PGPR-mediated induction of phenolics for biologic control and their probable role in protecting betelvine against *P. nicotianae*, an important soil-borne phytopathogenic fungus.

Bacillus licheniformis N1, which has previously exhibited potential as a biological control agent, was investigated to develop a biofungicide to control the gray mold of tomato caused by *Botrytis cinerea*. Various formulations of *B. licheniformis* N1 were developed by Lee *et al.* (2006) using fermentation cultures of the bacteria in Biji medium, and their ability to control gray mold on tomato plants was evaluated. The results of pot experiments led to the selection of the wettable powder formulation N1E, based on corn starch and olive oil, for evaluation of the disease control activity of this bacterium after both artificial infection of the pathogen and natural disease occurrence under production conditions. In plastic-house artificial infection experiments, a 100-fold diluted N1E treatment was found to be the optimum biofungicide spray formulation. This treatment resulted in the significant reduction of symptom development when N1E was applied before *Bo. cinerea* infection, but not after the infection. Both artificial infection experiments in a plastic house and natural infection experiments under production conditions revealed that the N1E significantly reduced disease severity on tomato plants and flowers. The disease control value of N1E on tomato plants was 90.5 % under production conditions, as compared to the 77 % conferred by a chemical fungicide, the mixture of carbendazim and diethofencarb (1:1). The prevention of flower infection by N1E resulted in increased numbers of tomato fruits on each plant. N1E treatment also had growth promotion activity, which showed the increased number of tomato fruits compared to fungicide treatment and non-treated control and the increased fruit size compared the non-treated control under production conditions. This study suggests that the corn starch-based formulation of *B. licheniformis*

developed using liquid fermentation will be an effective tool in the biological control of tomato gray mold.

Pieterse *et al.* (2006) developed an *Arabidopsis*-based model system using *Fusarium oxysporum f sp raphani* and *Pseudomonas syringae pv tomato* as challenging pathogens, in order to study the molecular basis underlying the systemic resistance. Colonization of the rhizosphere by the biological control strain WCS417r of *P. fluorescens* resulted in a plant-mediated resistance response that significantly reduced symptoms elicited by both challenging pathogens. Moreover, growth of *P. syringae* in infected leaves was strongly inhibited in *P. fluorescens* WCS417r-treated plants. Transgenic *Arabidopsis* NahG plants, unable to accumulate SA, and wild-type plants were equally responsive to *P. fluorescens* WCS417r-mediated induction of resistance. Furthermore, *P. fluorescens* WCS417r-mediated systemic resistance did not coincide with the accumulation of PR mRNAs before challenge inoculation.. The result indicated that *P. fluorescens* WCS417r induces a pathway different from the one that controls classic systemic acquired resistance and that this pathway leads to a form of systemic resistance independent of SA accumulation and PR gene expression.

Plant growth-promoting rhizobacteria (PGPR) bioformulations (*Pseudomonas* and *Bacillus)* were tested for their efficacy against blister blight (*Exobasidium vexans*) disease in tea (*Camellia sinensis*) under field conditions for two seasons. Among the bioformulations tested, foliar application of *Pseudomonas fluorescens* Pf1 at 7-d intervals consistently reduced the disease incidence of blister blight for two seasons, almost comparable with that of chemical fungicide. In addition to disease control, it also increased tea yield significantly compared to the untreated control. Induction of defense enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase, β-1,3-glucanase and phenolics were studied. The enzyme accumulation was greater in *P. fluorescens* Pf1-treated plants compared to control. The study revealed the probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance in tea plants against blister disease by PGPR bioformulations (Saravanakumar *et al.*, 2006).

Pseudomonas corrugata, a soil bacterium originally isolated from a temperate site of Indian Himalayan Region (IHR) was examined by Trivedi *et al.*, (2006) for its antagonistic activities against two phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*. Although the bacterium did not show inhibition zones due to production of diffusible antifungal metabolites, a reduction in growth between 58 % and 49 % in both test fungi, *A. alternata* and *F. oxysporum*, was observed in sealed petri plates after 120 h of incubation due to production of volatile antifungal metabolites. Reduction in biomass of *A. alternata* (93.8) and *F. oxysporum* (76.9) in Kings B broth was recorded after 48 h of incubation in dual culture. The antagonism was observed to be affected by growth medium, pH and temperature. The reduction in fungal biomass due to antagonism of bacteria was recorded maximum in the middle of the stationary phase after 21 h of inoculation. The production of siderophore, ammonia, lipase and chitinase in growth medium by *P. corrugata* were considered contributing to the antagonistic activities of the bacterium.