The region around the root, the rhizosphere, supports large and active microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth. The importance of rhizosphere microbial populations for maintenance of root health, nutrient uptake, and tolerance of environmental stress is now recognized (Basnayake and Birch, 1995; Benhamou *et al.* 1996)). These beneficial microorganisms can be a significant component of management practices to achieve the attainable yield, which has been defined as crop yield limited only by the natural physical environment of the crop and its innate genetic potential (Benhamou *et al.* 1996). Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation; decomposition of organic matter; toxin removal and the cycling of carbon, nitrogen, phosphorous and sulphur. In addition, microorganisms play key roles in suppressing soil borne diseases, in promoting plant growth and in changes in vegetation (Garbeva *et al.* 2004).

Rhizosphere bacteria can have a profound effect on plant health. Rhizosphere colonization is important not only as the first step in pathogenesis of soil borne microorganisms, but also is crucial in the application of microorganisms for beneficial purposes (Lugtenberg *et al.* 2001). Most significant among these applications are biofertilization, phytostimulation, biocontrol and phytoremediation (Lugtenberg, 2000). Colonizing microorganisms can be detected attached to the roots, as free organisms in the rhizosphere or as endophytes. The interactions between plants and microorganisms are immensely complex and very little is known about the sum of factors that lead to reliable biocontrol and biofertilisers applications. The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth has shown considerable promise in laboratory and greenhouse studies, but responses have been variable in the field (Basnayake and Birch, 1995). This approach has potential environmental benefits of leading to a reduction in the use of agricultural chemicals making it suitable for sustainable management practices.

The present study was undertaken in order select potential microorganisms from tea rhizosphere and make detailed studies so that one or more of such microorganisms could be used as PGPRs. At the onset, a large number of

microorganisms were isolated from the rhizosphere of tea plants growing both in the hills and the terai regions. The isolated bacteria were tested against root rot pathogens- *Poria hypobrunnea, Fomes lamaoensis, Sphaerostilbe repens* and *Sclerotium rolfsii* for determining antagonistic activity. From among all the samples tested four were initially selected which showed antagonistic activities. Of these four, two were finally selected for the present study and these were characterized and identified as *Bacillus pumilus* TRS4 and *Paenibacillus lentimorbus* TRS5. In earlier studies, Gardener (2004) described recent advances in understanding of the ecology of *Bacillus* and *Paenibacillus spp.* and how different subpopulations of these two genera can promote crop health. The abundance, diversity, and distribution of native populations and inoculants strains in agricultural fields have been characterized using a variety of methods. While native populations of these genera occur abundantly in most agriculture soils, plant tissues are differentially colonized by distinct subpopulations. Screening of rhizosphere microflora for antagonism against pathogenic fungi in order to select suitable biocontrol agents has been reported by a large number of workers. Kobayashi *et al.* (2000) isolated three bacteria showing antagonism to *Rhizoctonia solani* from the rhizospheric soil of different crops which they identified as *Pseudomonas fluorescens, Bacillus cereus* and *B. pumilus.* An evaluation of rice rhizosphere was conducted by Torres-Rubio *et al.* (2000) from which 69 bacteria were isolated including *Pseudomonas* sp. and *Azotobacter* sp. In another study, 11 *Bacillus pumilus* isolates were evaluated by Bargabus *et al.* (2004), of which 2 strains were found to be most effective against *Cercospora beticola.* The potential of various isolates of *Bacillus pumilus* has thus been recorded previously also.

In the present study, the two selected PGPRs were tested for their plant growth promoting activity and also biocontrol activity against one of the important root rot diseases of tea caused by *Poria hypobrunnea.* Several *in vitro* tests were conducted prior to the *in vivo* tests. Initially, the optimum conditions of the growth of the test pathogen and selected bacteria were determined. The fungus showed optimum growth in Potato Sucrose medium, at pH 5.0-5.5, with incubation period of 15 days, with fructose and peptone as carbon and nitrogen sources, respectively. Both the bacteria grew best between 35^0C in nutrient broth medium at pH 6. Kobayashi *et al. (2000)* observed that *B. cereus* isolate 96 and *B. pumilus* isolate 235 have an optimal

temperature for growth at 30°C but survived even at 41°C and 50°C respectively. One advantage of *Bacillus* sp. is their ability to form spores which are long lived and are resistant to heat and desiccation (Osbern *et al.* 1995). Umamaheswari *et al.* (2003) assessed the growth of different strains of fluorescent pseudomonads under different pH and temperature conditions. Optimum pH for the different strains ranged between 6 & 7.

In order to determine the potential of the two bacterial isolates in plant growth promotion and disease suppression, *in vivo* experiments were next carried out. Experiments were carried out on tea plants which are perennial as well as on annuals i.e., chickpea, mungbean and marigold. Different varieties of tea at various growth stages were selected starting from young seedlings in nursery to 10 years old bushes in the field. Significant promotion of growth was obtained in the seedling, in two year old potted plants as well as in the field. When the bacteria were applied as soil drench or foliar spray, both the bacteria promoted growth to a more or less similar degree. Most of the reported works on plant growth promotion by PGPRs is on annuals with relatively few on perennials. Enebak *et al.* (1998) obtained both positive and negative result, using 12 rhizobacterial strains as seed treatment in pine. According to them loblolly pine shoot length as well as above and below ground biomass were reduced when seeds were treated with two bacterial strains, while three strains significantly increased the below ground biomass of seedling root systems. They suggested that the effect of rhizobacteria inoculation on seedling emergence and plant growth are independent and that the effects are seedling specific. In alder, Ramos *et al.* (2003) reported that *Bacillus licheniformis* increased growth when applied at the seedling stage. On studying changes in microbial communities, they suggested that increases in plant growth could also be attributed to changes in the rhizosphere microbial communities. Plant growth promotion in tea, *Camellia sinensis* (L.) O.Kuntze by *Bacillus megaterium*, *B. pumilus*, *Ochrobactrum anthropi* and *Serratia marcescens* was reported by Chakraborty *et al.* (2004). Besides tea, in the present study, both *B.pumilus* and *P.lentimorbus* could increase growth in chickpea and mungbean, as well as growth and flowering in marigold. Results thus show that plant growth is promoted by the rhizobacteria in both annuals and perennials. Though both the species had the ability for growth promotion individually, joint application with the two bacteria proved to be synergistic and enhanced growth promotion to even greater

degree. As no microorganism survives individually in the soil they would no doubt be interacting among themselves m the rhizosphere, some of which would be antagonistic and some would be synergistic. Uses of bacterial consortia have sometimes shown to be better option than single ones especially when being applied as formulations. It was reported by Chakraborty *et al.* (2007) that dual application of *Bacillus megaterium* and *Ochrobactrum anthropi* was more effective than either of the single applications in plant growth promotion. Tilak *et al.*, (2006) observed that dual inoculation of pigeon pea with PGPR including *P. fluorescens* and *B. cereus* along with *Rhizobium* sp. increased growth nodulation and nitrogenase activity by various degrees. They reported that the combination of *Rhizobium* sp. with *Azotobacter chroococcum* or *Azospirillum brasilens* registered a marginal but non significant increase over inoculation of *Rhizobium* alone. Most of the previous work in plant growth promoting rhizobacteria had focused on two genera- *Pseudomonas* and *Bacillus* and hence several reports are available regarding the PGPR activity of these two bacteria. Inoculation of sunflower seeds and soil with a strain of *Rhizobium* was observed to cause a significant increase in root dry mass, both under normal and water stress conditions. This *Rhizobium* sp. secreted an exopolysaccharide which had the capacity for soil aggregation on roots which in turn affected nitrogen uptake and plant growth promotion (Alami *et al.* 2000). In a study conducted to examine the effect of PGPR inoculation alone and in combination with three levels of mineral nitrogen fertilizer (0-56-60, 56-56-60 and 112-56-60 kg NPK/ha) on cotton (cv.MNH-552), the bacterial inoculum (50 g / kg of seed) significantly increased seed cotton yield (21%), plant height (5%) and microbial population in soil (41 %) over their respective controls while boll weight, GOT and staple length remained statistically unaffected (Anjum *et al.* 2007).

Besides plant growth promotion, the ability of *B.pumilus* was also tested in reducing root rot disease. It could reduce Poria root rot intensity caused by *P.hypobrunnea* effectively. In a previous work, it was shown that *Bacillus megaterium* could effectively control brown root rot of tea caused by *Bacillus megaterium* (Chakraborty *et al.* 2006). *B. amyloliquefaciens, B. subtilis* and *B. pumilus* were observed to have the ability to reduce incidence of tomato mottle virus leading to a corresponding increase in fruit yield (Murphy *et al.* 2000). *Pseudomonas fluorescens* was able to induce resistance in rice leaves against *Xanthomonas oryzae*

(Vidyasekaran *et al.* 2001). Zhang *et al.* (2002) evaluated five PGPR strains, S. *marcescens, P. fluorescens,* two strains of *B. pumilus* and *B. pasteuri* for reduction in blue mould 'disease of tobacco. Of the five strains three were able to reduce severity. Field applications of biocontrol agent's *P. fluorescens, B. subtilis* and *Trichoderma viride* induced systemic resistance in banana cultivars against *Mycrosphaerella musicola* (Kannan *et al. 2003).* Radhajeyalakshmi *et al.* (2003) observed that foliar application of culture filtrate of *P. fluorescens* and *B. subtilis* when sprayed twice at boot leaf stage and at *50 %* flowering stage reduced seed infection of rice caused by *Helminthsporium oryzae, Saroclavum oryzae* and *Trichoconis padvickii.* Guo *et al.* (2004) also reported the ability of PGPRs *Serratia* sp., *Pseudomonas* sp. and *Bacillus* sp. to reduce wilt of tomato. *Paenibacillus polymyxa* was reported to protect *Arabidopsis thaliana* against both biotic and abiotic stresses (Timmusk, 2003; Timmusk *et al.* 2005).

Thus, it is clear from the present work as also those of previous workers that several rhizobacteria have the ability of plant growth promotion as well as disease suppression. PGPRs enhance plant growth by direct and indirect means, but the specific mechanisms involved have not all been well-characterized (Castillo *et al.* 2002; Chanway *et al.* 2000). Direct mechanisms of plant growth promotion by PGPRs can be demonstrated in the absence of plant pathogens or other rhizosphere microorganisms, while indirect mechanisms involve the ability of PGPR to reduce the deleterious effects of plant pathogens on crop yield. PGPRs have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Castillo *et al.* 2002). Direct enhancement of mineral uptake due to increases in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Ait Barka *et al.* 2000; Bais *et al.* 2004). PGPR strains may use one or more of these mechanisms in the rhizosphere. In the present study, among the direct mechanisms tested, both *B.pumilus* and *P.lentimorbus* could solubilize phosphate, produce siderophores and volatiles as well as sufficient amounts of IAA. However, no HCN was produced. Torres-Rubio, *et al.* (2000) also reported that all the microorganisms isolated from rice rhizosphere produced IAA in the medium. Khalid *et al.* (2004) evaluated thirty isolates from the rhizosphere soil of

wheat plants for their potential to produce auxins *in vitro*. They designated four isolates as plant growth promoting rhizobacteria (PGPR) based upon auxin production and growth promoting activity. Ability of bacteria to solubilise phosphate is an important criterion when considering their use as biofertiliser. Out of 37 *Acenetobacter* sp. isolated from rhizosphere of wheat, 36 were able to solubilise phosphates under different experimental conditions (Chopade, 2003). He reported that all the phosphate solubilising *Acenetobacter* strains had zone diameter of dissolution in the range 1-5 cm. Production of volatile compound by bacteria have also been shown to be an important mechanism of plant growth promotion. In confirmation with the result obtained in the present study, Ryu *et al.* (2003) reported that *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a released two compounds into culture which they identified as 3-hydroxy-2-butanone and 2,3-butanediol. Corsea *et al. (2005)* isolated rhizobacteria with properties related to plant growth promotion from the rhizosphere of the perennial legume *Chamaecytisus proliferus* spp. *proliferus* var. *palmensis* (tagasate) growing in field conditions. Among all isolates of the species *Pseudomonas fluorescens* showed the maximum properties related to plant growth promotion, ACC deaminase activity, phytohormone production, nitrogen fixation, fungal growth inhibition and cyanogenesis and making it suitable for field testing. Siderophore production has also long been considered as one of the mechanisms of suppression of fungal growth in the rhizosphere. Siderophores are low molecular weight molecules that are secreted by microorganisms to take up iron from the environment (Hofte, 1993) and their mode of action in suppression of disease were thought to be solely based on competition of iron with the pathogens (Bakker *et al.*, 1993; Duijff, 1999). Interestingly siderophores have also been shown to induce systemic resistance (Leeman *et al.* 1996; Bakker *et al.* 2003b). Siderophore producing bacteria were also isolated from tea rhizosphere previously. Saikia and Bezbarua (1995) isolated *Azotobacter* from iron rich tea garden acid soil which was demonstrated to produce siderophore. Bezbarua *et al.* (1996) further isolated two *Pseudomonas* strains from tea rhizosphere which produced siderophore and inhibited growth of *F. lamaoensis*. *P. aeruginosa*, *P. putida* and *P. fluorescens* were shown to produce siderophores (Torres-Rubio *et al.*, 2000). Jagadeesh and Kulkarni (2003) reported that of 38 rhizobacterial strains isolated from tomato which showed antagonism to *Alstonia solanacearum*, 23 were siderophore producers.

In greenhouse experiments soybean plant growth and nodulation was obtained from the culture supernatant of *Serratia proteamaculans* strain 1-102. Authors of the study (Bai *et al.* 2002) suggested that PGPR cells produce low concentration of activator during culture process which was enhanced with the application of inducers, while the efficacy of the root activity of the activator was constant in both pouch and pot experiment. Leaf applications were not as effective as root applications. This is not in confirmatory with the present study where both soil and foliar application induced growth promotion. Ryu *et al.* (2003) also obtained growth promotion of *Arabidopsis* by *B. subtilis* and *B. amyloliquefaciens* which according to them was due to the production of volatiles by the bacteria. *B. amyloliquefaciens* was also able to promote growth in three varieties of barley (Park *et al.* 2003). Kishore *et al.* (2005) isolated 393 groundnut associated bacteria representing the geocarposphere, phylloplane and rhizosphere. Maximum increase in plant biomass was obtained following treatment with a rhizosphere isolate identified as *B. jirmis* and two phylloplane isolates *B. megaterium* and *P. aruginosa.* They concluded that identification of phylloplane bacteria as effective plant growth promoting rhizobacteria broadens the spectrum of PGPR available for field application. In this context, an interesting result obtained in the present study was that the suspension of PGPRs when applied as foliar spray was equally effective in increasing the leaf biomass of tea. As tea is cultivated mainly for its leaves, the induction of new shoots and more leaves would have great impact in considering plant growth promotion.

Since the two isolated bacteria *B.pumilus* and *P.lentimorbus* inhibited the growth of one or more fungi *in vitro,* it was expected that they would produce antifungal compounds. Keeping this in mind, active principles responsible for growth inhibition were extracted both from whole cells and cell free culture filtrates. It was observed that extract from whole cells as well as cell free culture filtrates could inhibit spore germination and growth of several test fungi. Besides, the cell free culture filtrate was also extracted with various solvents and bioassayed. Though inhibition was obtained in most of the fractions, maximum inhibition was obtained in diethyl ether fraction of both the bacteria. Results indicated that the antifungal compounds present in the bacterial cells were secreted into culture and these were solvent extractable. Kyong and Dal (2003) also obtained an antifungal antibiotic from *B. megaterium* KL 39, which was isolated from a local soil of Korea. The crude extract

was reported to be active against a broad range of phytopathogenic fungi including *Rhizoctonia solani, Monilinia fructicola, Botrytis cinerea, Alternaria kikuchiana, Fusarium oxysporum* and *F. solani.* They suggested that this antibiotic had a powerful biocontrol activity against red pepper phytophthora blight disease. In an earlier study, Chakraborty *et al.* (1998) extracted an antifungal compound from *Micrococcus luteus* which was originally isolated from tea phyllosphere. This compound also showed maximum activity in diethyl ether. In a similar study, using microorganisms from tea rhizosphere, Barthakur and Bezbaruah (1997) isolated an antifungal compound from *Proteus* strain. This was shown to inhibit growth of several *Fusarium* sp. as well as tea root rot pathogens *F. lamaoensis* and *U. zonata.* It was also reported by Kobayashi *et al. (2000)* that inhibition of *R. solani* by *P.fluorescens, B. cereus* and *B. pumilus* was due to production and secretion of at least one antibiotic. In another study isolates of *B. subtilis* and *B. lentimorbus* which were antagonistic to *R. solani* were also reported to produce diffusible and volatile antibiotics (Montealegre *et al.* 2003). New antifungal compounds were isolated from *P. fluorescens* by Bajsa *el al. (2003)* which inhibited *R. solani.* Antifungal metabolites were also extracted from *P. fluorescens* and *B. subtilis* which inhibited growth of *Pythium aphanidermatum* and had maximum peak absorption of 200 nm (Kabita *et al.* 2003). It is clear from the results of the present study and that of previous workers that different species of *Bacillus* produce various antifungal metabolites in culture.

PGPRs that indirectly enhance plant growth via suppression of phytopathogens do so by a variety of mechanisms. These include the ability to produce siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root; and the ability to induce systemic resistance (Bashan and Holguin, 1998; Castillo *et al.*, 2000; Cornelis and Matthijs 2002). Biochemical and molecular approaches are providing new insight into the genetic basis of these traits, the biosynthetic pathways involved, their regulation, and importance for biological control in laboratory and field studies (Bashan and Holguin, 1998; Basnayake and Birch, 1995; Castillo *et al.*, 2000; Cornelis and Matthijs, 2002).

Biopriming plants with some PGPRs can also provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin, and in some instances even damage caused by insects and nematodes, can be reduced after application of PGPR (Kerry, 2000; Sturz, 2000; Ramamoorthy *et al.*, 2001; Ping and Boland, 2004; Ryu *et al.* 2004). Certain bacteria trigger a phenomenon known as ISR phenotypically similar to systemic acquired resistance (SAR). SAR develops when plants successfully activate their defense mechanism in response to primary infection by a pathogen. ISR is effective against different types of pathogens but differs from SAR in that the inducing PGPR does not because visible symptoms on the host plant (Van Loon *et al.*, 1998). PGPR-elicited ISR was first observed on carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by *Fusarium* sp. (Van Peer *et al.*, 1991) and on cucumber (*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare* (Wei *et al.*, 1991). Manifestation of ISR is dependent on the combination of host plant and bacterial strain (Van Loon *et al.*, 1998; Kilic-Ekici *et al.*2004). Most reports of PGPB-mediated ISR involve free-living rhizobacterial strains, but endophytic bacteria have also been observed to have ISR activity. For example, ISR was triggered by *P. fluorescens* EP1 against red rot caused by *Colletotrichum falcatum* on sugarcane (Viswanathan and Samiyappan, 1999), *Burkholderia phytofirmans* PsJN against *Botrytis cinerea* on grapevine (Ait Barka *et al.* 2000; Ait Barka *et al.* 2002) and *Verticllium dahliae* on tomato (Sharma and Nowak, 1998), *P. denitrificans* 1-15 and *P. putida* 5-48 against *Ceratocystis fagacearum* on oak (Brooks *et al.* 1994), *P. fluorescens* 63-28 against *F. oxysporum* f. sp. *radicis-lycopersici* on tomato (M'Piga et al., 1997) and *Pythium ultimum* and *F. oxysporum* f. sp. *pisi* on pea roots (Benhamou *et al.* 1994), and *Bacillus pumilus* SE34 against *F. oxysporum* f. sp. *pisi* on pea roots (Benhamou *et al.*, 1996) and *F. oxysporum* f. sp. *vasinfectum* on cotton roots (Conn *et al.*, 1997).

In order to determine whether *B.pumilus* elicits ISR in tea plants, several biochemical analyses were done. The major components analysed for tea leaves in the present study included defense enzymes, polyphenolics, proteins, chlorophyll and catechins. In all tested varieties defense related enzymes *viz.* chitinase (CHT), β-1,3-glucanase (GLU), peroxidase (PO), phenylalanine ammonia lyase (PAL), as well as phenolics increased significantly, especially in presence of the pathogen. In a similar study with two isolates of *B. pumilus* were reported to be best plant growth promoters

and biocontrol agents against downy mildew disease in pearl millet (Niranjan *et al.* 2003). They also reported increased activities of PAL, PO and □ -1, 3-GLU but not of CHT activity. Ramanathan *et al.* (2003) and Bargabus *et al,* (2004) also obtained systemic resistance elicitation by *B. pumilus* in sugar beet which was marked by increase in CHT, 3-1 ,3-GLU activities which was preceded by biphasic H_2O_2 production. Chakraborty *et al.* (2006) quoted increase in defense enzymes PO, CHT, β-1,3 GLU and PAL during plant growth promotion of tea and induction of resistance by *B. megaterium.*

In a study involving the induction of systemic resistance in rice leaves by *P. fluorescens* (Vidyashekaran *et al.* 2001), increased activities of PO, PAL,4-coumarate: 5 CO ligase and increased accumulation of lignin were observed. This was observed in the resistant reactions and not in the susceptible ones. *P. fluorescens* which induced systemic resistance in chickpea against *S. rolfsii* was found to induce accumulation of several phenolics and defense enzymes were observed to be enhanced in chickpea. Increased activity of chitinase, β-1 ,3-glucanase and peroxidase were obtained in sugar beet which was induced by treatment with *B. mycoides* (Bargabus *et al.* 2002). Induction of defense related enzymes by *P. fluorescens* in balck pepper and *Phytophthora capsici* pathosystem was reported by Paul and Sharma (2003). They obtained increased level of PO, PAL, PPO and catalase in leaves apart from root of treated plants indicating the systemic protection offered to black pepper by PGPR strains. The systemic nature of protection and growth promotion in the present study is also evident as the responses were analyzed in the leaves even when the application was in the rhizosphere. In another study, Radjacommare *et al.* (2005) reported the induction of defense enzymes, phenols and lignin in rice by *P. fluorescens* against *R. solani.* Treatment of finger millet with *P. fluorescens* induced systemic resistance against *Pyricularia grisea* and increased activities of defense enzymes.

Chlorophyll content however increased with the application. Catechins are major flavor flavonoid components of tea and their quantitative changes with respect to different isomeric forms were analysed by HPLC. It was observed that the treatment with the bacteria induced some new isomeric forms. Since tea leaves are produced for their flavor enhancement of catechins isomers point to the fact that these

are also enhanced during plant growth promotion.

Since both *B.pumilus* applied either as soil drench or foliar spray could promote growth in all the tested plants, the next question was to determine whether these could be applied as suitable formulations in the rhizosphere. This information would be invaluable for commercial preparation of PGPRs. For this, formulations of the two PGPRs were prepared using carboxymethyl cellulose with talcum powder as carrier and tested under greenhouse conditions for their effect on growth promotion of tea seedlings. Observations were recorded after 2 and 4 months of application which revealed that significant growth promotion was accorded by application of bacterial suspensions. Selection of talcum as carrier was done because of it being cost effective, commonly available and inert. With increase in the focus of application of biofertilisers and biocontrol agents research on production of formulations of PGPR have also increased. The application of five commercial chitosan based *Bacillus* formulations were found to be effective in increasing the growth and grain yield of rice. A formulation Elexa 1M was also reported to induce resistance to downy mildew disease and growth promotion in pearl millet (Sharathchandra *et al.* 2004). In a further study talc based formulation of *B. subtilis* and *P. fluorescens* either singly or mixed along with or without chitin and neem amendments for reducing root rot incidence of chillies along with plant growth promotion were evaluated by Bharati *et al.* (2004). According to them the PGPR mixed bioformulation of *P. fluorescens* + *B. subtilis* + neem +chitin was found to be the best one. New formulations of *B. subtilis* for management of tomato damping off caused by *Pythium aphanidermatum* were developed by Jayraj *et al. (2005).* Their formulation included a talc based powder, lignite based powder. lignite + fly ash based powder, wettable powder, bentonite paste and polyethylene glycol paste. All of these formulations were found to be effective and enhanced plant biomass in the glass house and field condition. Viability of propagules was maintained upto one year of storage. *B. subtilis* was also shown to survive in glucose and talcum powders at 8.6 and 7.6 log10 cfu g^{-1} respectively for 1year of storage compared to 3.5 log10 cfu g^{-1} on a peat formulation (El-Hassan and Gowen 2006). Seed treatments with talc formulations of *B. subtilis* in glucose, talc and peat significantly enhanced its biocontrol activity against *F. oxysporum* causing vascular wilt of lentil. It was also shown that application of talc formulation of *P. fluorescens* along with *T. viride* either singly or combined decreased the sheath blight

disease and increased plant growth and yield in rice (Mathivanan *et al. 2005).* However, the joint application did not have any additive effect.

In the present study, it was felt necessary to determine the sustainability of PGPRs in the soil as this would be important in the field. Hence the survival of the bacteria applied either as aqueous solution in the soil or in the form of bioformulations was determined. The periods of survival of bacteria in the formulations were also determined. Determination of bacterial survival in soil and formulations was done by immunological techniques using antibodies raised against the two bacteria. These techniques i.e. ELISA and Dot blot gave very specific and accurate results, as the antibodies specifically reacted only with the specific bacteria. Results of both ELISA and Dot blot showed that bacteria survived at high concentration even after six months of inoculation when the analysis was performed. Viability of the bacteria as determined by ELISA was evident in the bioformulations even after 4 months.

Population of *P.hypobrunnea* was also determined in the soil using dot using PAbs raised against *P.hypobrunnea* the causal agent of root-stem rot of tea. It was shown that the population of the pathogen reduced significantly in the bacteria treated soil, as detected on the analysis by ELISA and Dot blot. Thus these bacteria probably secreted antifungal metabolites into the soil which caused reduction in growth of the pathogen. In an earlier study, Mohandas *et al.* (2005) reported immunodetection of *Phytophthora parasitica* var. *nicotianae* in papaya root pretreated with biocontrol agents. In plant treated with biocontrol agents and *Phytophthora* there was a considerable decrease in the *Phytophthora* population as seen by the number of fluorescent colonies as compared to plants treated with *Phytophthora* alone. A maximum reduction in *Phytophthora* population was observed in VAM+ *Trichoderma* (89.6%) treated plants followed by *Trichoderma* (86.2%) and *Pseudomonas* (79.3%) treated plants

The overall results of the present study have shown that two rhizobacteria isolated from tea rhizosphere, *B.pumilus* and *P.lentimorbus* could induce plant growth promotion and disease reduction in tea as well as in other crops. Different species of *Bacilli* are now widely used in other crops as plant growth promoting and biocontrol agents. Though both soil drench and foliar spray gave experimentally good result, soil

drench is preferable mode of application. This is because tea being cultivated for its beverage produced from its leaves and soil drench induced systemic response which transmitted to the leaves; treatment of leaves can be avoided. Regarding the mechanism of action of the bacteria it seems probable that these bacteria act through a combination of methods. It is difficult to predict the actual happening in the soil environment but probably the PGPRs secrete metabolites into the soil which in turn elicit responses in the host. The relative importance of importance of the metabolites in inducing plant growth promotion, as well as disease suppression is not yet clear.

In conclusion, it may be stated that the application of PGPRs for control of fungal pathogens in greenhouse systems shows considerable promise (Corbell and Loper, 1995), due in part to the consistent environmental conditions and high incidence of fungal disease in greenhouses. Achieving consistent performance in the field where there is heterogeneity of abiotic and biotic factors and competition with indigenous organisms is more difficult. Knowledge of these factors can aid in determination of optimal concentration, timing and placement of inoculant, and of soil and crop management strategies to enhance survival and proliferation of the inoculant (Basnayake and Birch, 1995; Conn *et al.* 1997). The concept of engineering or managing the rhizosphere to enhance PGPR function by manipulation of the host plant, substrates for PGPR, or through agronomic practices, is gaining increasing attention (Basnayake and Birch, 1995; Chin-A-Woeng *et al.*2001). Development of better formulations to ensure survival and activity in the field and compatibility with chemical and biological seed treatments is another area of focus; approaches include optimization of growth conditions prior to formulation and development of improved carriers and application technology.