Chapter 2
Review of Literature
REVIEW OF LITERATURE

Studies on tea rhizobacteria in respect of their pesticide degrading capabilities and its impact on growth of tea plants in the North Eastern Region of India, particularly in Assam is very limited (Mazumdar et al. 2007). The review of works on different aspects of rhizobacteria summarized below has strengthened the above mentioned statement.

**Microbial diversity in agro-ecosystems**

Zhang et. al. (1991) studied on the diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees, *Acacia senegal* and *Prosopis chilensis*.

Holguin et. al. (1992) isolated two new diazotrophic bacteria, *Listonella anguillarum* and *Vibrio cambelli*, and one non nitrogen fixing bacterium, *Staphylococcus* sp. from the rhizosphere of mangrove trees.

Hemmingser (1992) enumerated large number of polycyclic aromatic hydrocarbons (PAHs) degrading bacteria from phenanthrene polluted soil while very few were present in unpolluted soil and water (Tiwari and Sharma, 2002).

Radwan et. al. (1995) have found that oil polluted Kuwaiti desert samples were naturally rich in oil degrading micro organisms which was steadily increased
immediately after the addition of crude oil (Radwan et al. 1997) (Tiwari and Sharma, 2002).

Genus *Bacillus* was well adapted to the rhizoplane and rhizosphere of tea bushes. *B. subtilis* and *B. mycoides* appeared to be closely associated with tea roots. The two species comprised a major part of the bacterial population, even during unfavourable periods. (Pandey and Palni, 1997).

Donald et al. (1998) studied the impact of polycyclic aromatic hydrocarbon on Revarine microbial community and indicated that moderate to high PAH concentration altered microbial community structure and responded to PAH contamination at both the phenotypic and genotypic level (Tiwari and Sharma, 2002).

Zoysa et al. (1998) conducted a study to test the effect of N form (NH$_4^+$, NO$_3^-$, or both) on the transformation of soil P in the rhizosphere and its availability to tea (*Camellia sinensis* L.) plants fertilised with sparingly soluble Eppawala phosphate rock (EPR).

MacNanghton et al. (1999) reported a community shift from primarily eukaryotic biomass to a gram-negative bacterial prokaryotic biomass with time and indicated that oil treatment encouraged the growth of gram negative microorganisms within the alpha Proteobacteria and Flexibacter – cytophaga, Bacterioides phylum. Thouand et al. (1999) also found that species diversity of biodegraders changed from beginning to the end of the culture of soil from the polluted soils (Tiwari and Sharma, 2002).
Bacteria of the genus *Azospirillum* were widely distributed in the rhizosphere of tropical and subtropical grasses and sugarcane. *A. halopraeferans* was reported to occur in the rhizoplane of plants growing in saline soil in Brazil. Phosphates, distributed in nature both in organic and inorganic forms, were not readily available to plants due to its bound state. Many soil bacteria were reported to solubilize these insoluble phosphates through various processes. Reports have also indicated the P-solubilizing activity of some nitrogen fixers (Seshadri et al., 2000).

Mirza et al. (2001) reported about the isolation of nitrogen fixing, phytohormone producing bacteria from sugarcane and their beneficial effects on the growth of micropropagated sugarcane plantlets.

Ozawa et al. (2003) worked on the isolation and characterization of diazotrophic bacteria from the surface sterilized roots of some legumes.

Hameed et al. (2004) studied on isolation and characterization of rhizobial strains isolated from root nodules of cultivated legumes, i.e. chickpea, mungbean, pea and siratro.

Endophytic bacteria reside within plant tissues and have often been reported to promote plant growth. Rhizobia are particularly known for their symbiotic relationship with legumes. A bacterial strain MSSP was isolated from surface-sterilized root nodules of *Mimosa pudica*. MSSP was Gram negative, capsulated, motile, non-endospore forming rod with free nitrogen (N) fixation ability. Unlike N-fixing bacteria forming symbiotic relationship with legumes that largely exist in α-subclass of proteobacteria, MSSP belongs to β-class of proteobacteria. Phylogenetic analysis of 16S rDNA
demonstrated that MSSP belongs to the genus *Burkholderia*. This isolate secretes phytohormone, ACC deaminase, solubilizes phosphate and is antagonistic against phytopathogens (Pandey et al., 2005).

Suliasih and Widawati (2005) undertook a study to investigate the occurrence of phosphate solubilising bacteria (PSB) and nitrogen fixing bacteria (NFB) from soil samples of Wamena Biological Garden.

Genetic diversity of five Sinorhizobia from a medicinal legume, i.e. *Mucuna pruriens* was investigated using ARDRA (Amplified ribosomal DNA restriction analysis) analysis. The study showed that there is considerable homogeneity amongst *M. pruriens* root nodule bacterial isolates (Kumar et al., 2006).

The rhizosphere of cultivated tea bushes was dominated by *Glomus* morphotypes (88.89%) along with three morphotypes of *Acaulospora*; occurrence of 35 morphotypes belonging to four genera viz. *Acaulospora* (11.43%), *Gigaspora* (11.43%), *Glomus* (68.57%) and *Scutellospora* (8.57%) was recorded in the rhizosphere of tea plants from the natural ecosite in the Kumaun region of Uttarakhand Himalaya, India (Singh et al., 2007).

Species of *Trichoderma*, *Penicillium*, *Aspergillus* and *Mucor* were found to dominate the rhizosphere of tea bushes in different regions of the Indian Himalayas. In addition, the dominant bacteria in the tea rhizosphere, i.e., *Bacillus subtilis* and *B. mycoides*, showed antagonistic activity against fungal isolates by inhibiting the growth and causing structural abnormalities in mycelium (Singh et al., 2007).
Tea rhizospheres have some specific characteristics viz lowering of soil pH, antagonistic activity among microbial communities and dominance of certain species (Lynch, 1987, Sood et al 2007). Tea rhizosphere favors the growth of microbes, which are known to produce strong antibiotics with potential biocontrol agents. Sood et al (2007) carried out a research to investigate the role of Bacillus and Pseudomonas species producing bacteriocin like compounds.

Khan et. al. (2008) reported about the isolation and identification of nitrogen fixing microorganisms during the seedling (30 days after seed sowing) stage of rice (BR 10) grown in non-calcareous Grey Flood Plain soil of Bangladesh.

Reinhardt et. al. (2008) have isolated several new bacterial strains from cassava, guinea grass, maize, sugarcane and tomato using the selective media NFF described by Hartmann et al 2006. It was found that the isolated strains from the above plants reduced acetylene on the chromatography analysis and hence indicating their N fixing ability. Further their work has been analysed by performing Dot Blot Hybridization.

Representatives of Bacillus and Pseudomonas genera were found to dominate the rhizosphere of established and abandoned tea bushes, respectively in some parts of the Indian Himalayan Region. B. subtilis and B. mycoides appeared to be closely associated with roots of established tea bushes while the rhizosphere of abandoned tea bushes was dominated by P. putida (Sood et al., 2008).

Hafez and Elbestawy (2009) investigated variations in the diversity of the indigenous bacterial and fungal population in contaminated soil which was collected
from highly contaminated agricultural soil adjacent to an industrial drain in the Nile Delta.

Parbudoss and Stella (2009) isolated Gluconacetobacter diazotrophicus strains from samples of sugar rich crops like sugarcane (root, stem, bud, leaves) sweet potato, pine apple and wild cane. The nitrogen fixing efficiency of all the native strain was compared with reference to the local isolates and was found superior.

Veenagayathri et al. (2009) isolated strains of Pseudomonas from wastewater contaminated sites, which had the ability to utilize higher concentration of phenol.

Four halotolerant rhizobial strains namely GMNW1, GMNW2, GMB2 and GMB1 of Glycine max (L.) have been isolated from saline soils of the Krishna river basin area at Satara and Sangli districts of Maharashtra (Shaikh et al. 2009).

Kumar et al. (2009) reported on the general microflora (bacteria, actinomycetes and fungi) in the rhizosphere and their corresponding non-rhizosphere soil samples of Ginkgo biloba L. of two age groups (Group A, <25 years-young trees; Group B, >60 years-old trees) growing under a temperate location of Indian Himalayan Region (IHR).
Functional aspects of Rhizobacteria

The discovery of Dilworth (1966) that the nitrogenase enzyme is responsible for N\textsubscript{2}-fixation which reduced C\textsubscript{2}H\textsubscript{2} (acetylene) to C\textsubscript{2}H\textsubscript{4} (ethylene) provided a useful assay for the quantification of the N\textsubscript{2}-fixation process. Keister (1975) demonstrated acetylene reduction in pure cultures of rhizobia.

DDT has a chemical structure not encountered in biological materials, and because of its low degradability (3-10 years persistence) poses a serious ecological problem. According to a report (Bumpus and Aust, 1987), the white rot fungus *Phanerochaete chrysosporium* is capable of complete degradation of DDT. *P. chrysosporium* is also capable of mineralizing the heavily chlorinated insecticides chlordane and lindane (Kennedy et al., 1990). The fungus produced a broad spectrum lignin-peroxidase that catalysed several insecticides. Bacteria dominate in biotransformation of pesticides but fungi also participate in this process. Important among them were *Aspergillus, Penicillium, Fusarium, Phanerochaete* etc, (Stolp, 1988).

It is noted that nearly 43 pesticidal compounds were degraded by a wide variety of microorganisms (Francis, 1992). Mustafa et al. (1972) reported that *Rhizobium leguminosarum* and *R. trifolii* isolated from Egyption soil can hydrolyse melathion by producing carboxyesterase. Extended studies by Francis (1992) indicated that 22 rhizobial isolates tolerated endosulfan, carbofuran, carbaryl and melathion at
the range of 25 to 125 µg/ml. Isolates from the nodules of *Indigofera echinata* and *I. duthei* tolerated melathion up to 125µg/ml. (Gangawane et al, 2007; Reddy et al., 1997).

A large number of bacteria and fungi were isolated from the rhizosphere of established tea bushes and were tested for their antifungal activity (Pandey et al., 1997).

Kothari et al. (1998) reported on the biodegradation of 2,4-D by *Penicillium citrinum* and *P. oxalicum* isolated from paint coated teak wood.

Symbiosis between rhizobia and leguminous plants leads to the formation of N2-fixing root nodules. The interaction of rhizobia and plants shows a high degree of host specificity based on the exchange of chemical signals between the symbiotic partners. The plant signals, flavonoids exuded by the roots, activate the expression of nodulation genes, resulting in the production of the rhizobial lipochitooligosaccharide signals (Nod factors). Schultze and Kondorosi (1998), reviewed on how the production of Nod factors is regulated, how these signals are perceived and transduced by the plant root, and the physiological conditions and plant factors that control the early events leading to root nodule development.

Acid and aluminum(Al) tolerant microorganisms were isolated from tea fields, from which six strains were selected and identified as *Cryptococcus humicola*, *Rhodotorula glutinis*, *Aspergillus flavus* Link, *Penicillium* sp., *Penicillium janthinellum Biourge* and *Trichoderma asperellum* (Kawai et al., 2000).
Edi Husen (2003) tested soil bacteria for indoleacetic acid (IAA) production, phosphate solubilization, dinitrogen fixation and siderophore production for their use as potential PGPR.

Metal contamination can affect the diversity of microbes in soil. Phenotypic and genetic characteristics of *Rhizobium leguminosarum* bv *trifoli* isolated from clover (*Trifolium pratense*) found at a metal contaminated and a control site, or isolated from nodules of clover was compared. Zinc and Cd tolerance of each isolate was also determined. Rhizobia isolated from the control soil compared with the metal contaminated soil differed both genetically and phenotypically. Isolates originating from the metal contaminated soil were more tolerant to Cd and Zn as compared with those observed for isolates of control soil. Rhizobia originating from the metal contaminated soil expressed a higher number of metal phenotypes as compared with isolates of control soils. Slow rates of metal accumulation over the years favored an adaptation of the rhizobia to the metal rather than elimination of metal sensitive organisms and the selection of a few pre-existing metal tolerant organisms (Delorme et al., 2003).

Phorate [O,O-diethyl-S-(ethylthio)methyl phosphoradiothioate] degrading bacteria were isolated from agricultural soil and characterized based on their morphological and biochemical characteristics. The selected isolates PS-1, PS-2 and PS-3 were presumptively identified as *Rhizobium, Pseudomonas* and *Proteus* species, respectively. The HPLC analysis of phorate in bioaugmented soil revealed its complete disappearance within 40 days. The degradation isotherms of the isolates PS-1, PS-2 and PS-3 suggested time-dependent disappearance of phorate following the first order rate.
kinetics at the corresponding rate constants of 0.04, 0.05 and 0.04 days⁻¹. Besides, the isolates concurrently exhibited substantial phosphate solubilization, indole acetic acid, and siderophore production. The isolate PS-3 also showed anti-fungal activity against a phytopathogen Fusarium oxysporum. As a result of the multifarious biological properties, the isolates have been suggested to be important bioresource for efficient bioinoculant development (Bano and Musarrat, 2003).

The potential of rhizosphere microbes isolated from common reed [Phragmites australis (Cav.) Trin. ex Steud] plants grown in a subsurface-flow constructed wetland to biomethylate selenate or selenite was studied in liquid cultures under controlled conditions. Total mean percentages of volatilized Se from half-strength Hoagland culture solutions (low C content) supplemented with selenate or selenite and inoculated with cultured rhizosphere microbes after 15 d of incubation were 7.9 and 49.1%, respectively. There was a relative best fit (r = 0.87) between total number of rhizosphere and cultured microbes and the percentage of volatilized Se in Hoagland solution after 15 d of incubation. However, when the same microbes were cultured in tryptic soybean broth (TSB) medium (high C content), the percentages of volatilized Se from selenate and selenite were 1.3 and 1.9%, respectively. The volatilization percentages of Se from selenate or selenite in culture solutions inoculated with rhizosphere suspension instead of cultured rhizosphere microbes were very low (1.2–3.0%) in both cultivation media. In all experiments, selenite was volatilized significantly (p < 0.05) in higher amounts by cultured rhizosphere microbes after 15 d of incubation compared with selenate. Dissolved biomethylated dimethylselenide (DMSe) in water samples taken from the subsurface-flow bed was determined by
purging with helium. The DMSe in water samples was indirectly detected up to 2.4 μg Se L⁻¹, which indicates that part of the produced DMSe was dissolved in the matrix before being released into the atmosphere (Azaizeh et al. 2003).

Siddique et al. (2003) described a high performance liquid chromatographic (HPLC) method suitable for the analysis of endosulfan and its metabolites in water and soil.

The microbial transformation of (+)-catechin and (-)-epicatechin by endophytic fungi isolated from a tea plant was investigated. It was found that the endophytic filamentous fungus Diaporthe sp. transformed them into the 3,4-cis-dihydroxyflavan derivatives, (+)-(2R,3S,4S)-3,4,5,7,3',4'-hexahydroxyflavan and (-)-(2R,3R,4R)-3,4,5,7,3',4'-hexahydroxyflavan, respectively (Shibuya et al., 2005).

Pravakaran and Allenpeterson (2005) investigated on the degradation of endosulfan by a Bacillus species.

Biodegradation of endosulfan into endosulfan sulfate by a soil bacterium was reported by Shivaramaiah & Kennedy (2006). The bacterium degraded 50% of the compound within 3 days of incubation.

Plant growth promoting rhizobacteria (PGPR) have also been reported to reduce the incidence of some fungal diseases (Alagawadi et al., 2006).

Plant growth promoting rhizobacteria (PGPR) Pseudomonas fluorescens Pfl was tested for its efficacy against the powdery mildew of the grapevine caused by
Uncinula necator. Foliar application of P. fluorescens Pf1 at 2% significantly reduced the incidence of powdery mildew under glasshouse condition (Sendhilvel et al., 2006).

Maintaining threshold populations of inoculum microorganisms in the soil environment is important for such practical applications as biocontrol, plant growth-promotion, bioremediation, and nodulation. However, because of both technical and labour constraints in monitoring bacterial viability in nonsterile soils, few studies have reported on survival kinetics, particularly in relation to subtle alterations in soil acidity-related factors. A genetically modified strain Pseudomonas putida R20lacZY or Rhizobium leguminosarum bv. trifolii 162S7a gusA was introduced into conditioned, nonsterile Gilpin (fine loamy, mixed mesic, Typic Hapludult) silt loam soil, limed at four low levels (pH 4.71, 4.81, 4.92, and 4.99) or derivative soil solutions with highly correlated ($R^2 \geq 0.81$) chemical properties. Immediate declines in viability of both strains were found in all soils, reaching 0.1 to 1% initial colony-forming unit (CFU) g$^{-1}$ soil in 35 h for P. putida and in 68 h for R. leguminosarum bv. trifolii. Death rate constants ($k_d$) for both strains were directly related to lime level (soil pH) (Staley & Brauer, 2006).

Thirty-six strains of Bradyrhizobium japonicum and three strains of R. galegae were examined for their intrinsic resistance to different concentrations of several antibiotics. The results of this investigation indicated diversity among the strains tested for their intrinsic resistance to different concentrations of antibiotics (Milicic et al., 2006).
Batta et al. (2006) reported on detection of endosulfan residues in the soil of Western Jordan Valley. Soil samples were collected from ten different locations of the Western Jordan Valley (W. J. V.) extending from Pardala (north) to Jericho city (south). The residues of Endosulfan which is the most widely used insecticides in the W. J. V. were detected using Gas Chromatography/ Mass Spectrometry (GC/ MS).

Jayan, (2007) revealed that use of pesticides lowered the nitrogen-fixing capacity of soil bacteria. Jennifer (2007) demonstrated that certain agrochemicals bind and block connections to specific receptors inside rhizobacteria living in root nodules in the soil. This disruption leads to lower yields and/or significantly delayed growth.

A bacterium identified as Pseudomonas fluorescence isolated from Taxus baccata rhizosphere yielded an active antimicrobial compound which showed significant antimicrobial activity against two-gram positive bacteria (B. subtilis and S. aureus), four-gram negative bacteria (E. coli, K. pneumoniae, S. flexneri and P. aeruginosa), and one pathogenic fungus (Candida albicans). The minimum inhibitory concentration (MIC) of the compound ranged between 75μg to 250 μg/ml (Tayung et al., 2007).

Eupenicillium parvum was recorded for first time during isolation of phosphate solubilizing microorganisms from the tea rhizosphere. The fungus developed a phosphate solubilization zone on modified Pikovskaya agar, supplemented with tricalcium phosphate (Vyas et al., 2007).

Bacillus subtilis, B. pumilus, Pseudomonas pseudoalcaligenes and Brevibacterium halotolerans were examined for their ability to increase the availability
of water soluble Cu, Cr, Pb and Zn in soils and for their effect on metals uptake by Zea mays and Sorghum bicolor (Abou-Shanab et al., 2008).

Etesami et al. (2008) have shown that some strains of rhizobial group can also be effective in plant growth promotion due to their growth hormones production, in addition to their ability in N fixation.

Sarkar et al., (2009) studied on the degradation potential of Pseudomonas sp. isolated from tea rhizosphere towards the pesticide dicofol.

Muthuselvam and Arunkumar (2009) reported on biological degradation of herbicide (Atrazine) using Pseudomonas aeruginosa and Trichoderma viridae.

Biodegradation of triazole propiconazole fungicide by selected Pseudomonas strains isolated from tea rhizosphere was reported by Sarkar et al. (2009).

The ability of a newly isolated Pseudomonas citronellolis KHA to degrade diesel oil and to synthesize fatty acid esters has been screened in aerobic batch cultures (Sadouk et al., 2009).

A Rhizobium sp. nodulating Sesbania aculeate L. was isolated and the sensitivity was studied against two antibiotics (Streptomycin and Rifampicin) and one antimetabolite, Sodium azide (Singh et al., 2009).

Rhizobium sp. isolated from root nodules of Trigonella foenumgraecum was tested for siderophores production by Chrome-Azurol S (CAS) assay method. The bacterium was screened for its ability to inhibit against three fungal phytopathogens.
such as *Macrophomina phaseolina*, *Fusarium oxysporum* and *Aspergillus niger* (Sharma et al, 2009).

Forty six *Rhizobium* isolates from legume root and stem nodules were examined for their phosphate solubilizing ability on Pikovskaya’s agar medium. *Rhizobium* isolates from root nodules of *Cassia absus*, *Vigna trilobata* and three strains from *Sesbania sesban* showed zone of tricalcium phosphate (TCP) solubilization (Srivedi and Mallaiah, 2009).

Ali et. al. (2009) reported on stress tolerant rhizobial isolates of wild legumes growing in dry regions of Rajasthan, India.

Interaction of forest plant *Jatropha curcas* with its microbial community was investigated for the phosphorous supplement. Phosphatase activity of all the treatments containing *J. curcas* seedlings and *Pseudomonas flourescens* isolates was measured by calorimetric method using p-nitro phenyl phosphate (Jamaluddin et al, 2010).

Rinu and Pandey (2010) reported on the temperature-dependent phosphate solubilization by cold- and pH-tolerant species of *Aspergillus* which were isolated from Himalayan soil.

Multiclass pesticide residues viz endosulphan, cypermethrin, monocrotophos and chlorpyriphos have been estimated qualitatively and quantitatively in two vegetables, tomato (*Lycopersicon esculentum*) and radish (*Raphanus sativus*) by adopting gas liquid chromatographic and high performance liquid chromatographic methods (Kumar et. al., 2011).
Balamurugan et al. (2011) reported on cellulose degrading bacteria of tea garden soil. Their cellulase activity was studied in vitro.

**Molecular approaches in microbial diversity**

Broughton et al., (1986) identified plasmid sequences of *Rhizobium* which are involved in recognition of *Psophocarpus*, *Vigna* and other legumes.

Russell et al. (1987) isolated and partially characterized the lipopolysaccharides (LPSs) from *Rhizobium trifolii* ANU843 and several transposon (Tn5) symbiotic mutants derived from ANU843. The mutant strains were unable to induce normal root hair curling (Hac- phenotype) or nodulation (Nod phenotype) in clover plants.

Mixed-phase (heterogeneous) and single-phase (homogeneous) DNA subtraction-hybridization methods were used to isolate specific DNA probes for closely related *Rhizobium loti* strains. Both methods allowed the rapid isolation of strain specific DNA fragments which were suitable for use as probes (Bjourson and Cooper, 1988).

Proton nuclear magnetic resonance ($^1$H NMR) and fast atom bombardment mass spectrometric analyses were performed on enzymatically derived oligosaccharides from the acidic excreted polysaccharides (EPS) from representative bacterial strains of the pea nodulating symbiont, *Rhizobium leguminosarum* and the clover-nodulating
symbiont, *Rhizobium trifolii*. The results revealed structural similarities and differences between EPS of these two species. EPS structure might be one of the contributing factors which determined the host range of the *R. trifolii-R. leguminosarum* complex (Saleela Philip-Hollingsworth et al., 1989).

Sessitsch et al., (1998) reported on the advantages of using marker genes in studying rhizobial competition as compared to traditional approaches. Reporter genes such as the β-glucuronidase gene (*gusA*) or a thermostable β-glucosidase gene (*celB*) allow detection of rhizobial strains in nodules when they are still attached to the root system. This detection technique was therefore highly suitable for the study of rhizobial competition and studies using *gusA*-marked strains of *Rhizobium*. By making use of *gusA* and *celB*, differentially marked strains can be produced and distinguished easily on roots.

Park et al (2005) worked for isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. 16S rDNA gene amplification and sequencing was done for differentiation of the isolates and productions of IAA by the cultures were also estimated.

Xue et al (2007) extracted total microbial DNA from the soils in 8, 50 and 90 years old tea orchards, adjacent wasteland, and 90 years old forestland in Meijiawu tea area of Hangzhou. The 16S rDNA V3 fragment was amplified by PCR, and the polymorphism of this fragment was analyzed by DGGE. The results indicated that both the tea orchard age and the land use type had significant effects on soil microbial genetic diversity.
16S rRNA gene-based single-strand conformation polymorphism (SSCP) analysis revealed that the predominant bacterial population of different mangrove rhizosphere soils were related to bacterial genera of root and root-free soil environments, namely Bacillus, Planococcus, Planomicrobium, low G+C gram positive bacterium, glacial ice bacterium and unidentified bacteria (Bharathkumar et al., 2008).

13 morphologically distinct strains of thermophilic bacteria isolated from a hot spring site in Garhwal region of Indian Himalaya have been characterized and identified using phenotypic and genotypic characters. Based on the 16S rRNA analysis, 11 strains showed maximum similarity with Geobacillus stereothermophilus, one strain with G. kaustophilus and one with Geobacillus sp (Sharma et al., 2009).


Md. Harun-or Rashid. et al. (2009) characterized Rhizobia strains using molecular techniques based on PCR amplification such as repetitive extra genomic palindromic (REP-PCR) sequences and restriction fragment length polymorphism (RFLP) analyses along with traditional techniques such as physiological, biochemical and intrinsic antibiotic resistance.

The nifH gene sequence of the nitrogen-fixing bacterium Azotobacter spp. isolated from marine source, was determined with the use of polymerase chain reaction (PCR). The phylogenetic tree revealed that the isolated Azotobacter spp. was distantly
related to uncultivated and uncultured organisms. They did not form any branch with other *Azotobacter* spp. in the data base (Rajeswari and Mangai Kasthuri, 2009).

Ogutcu et. al. (2009) conducted a study to determine the phenotypic and genotypic differences in *Rhizobium leguminosarum* subsp. ciceri strains isolated from perennial wild chickpeas (*Cicer anatolicum*) from high altitudes (2000-2500 m) in mountains of Erzurum, Eastern Anatolia, Turkey. Rep-PCR (ERIC-, REP- and BOX-PCR) fingerprinting methods were used for the genotypic characterization and phylogenetic analysis of *Rhizobium leguminosarum* sub-sp. Ciceri strains isolated from perennial wild chickpeas. It showed a high intra species diversity among the strains in terms of rep-PCR (ERIC-, REP- and BOX-PCR) profiles.

**Application of Rhizobial strains in plant growth**

Rhizobial inoculation increased grain yield in rice (*Oryza sativa*L.), a nonlegume plant, but little is known about the mechanism(s) involved. The study was conducted to determine whether inoculation with rhizobia could influence leaf photosynthesis of rice plants under greenhouse conditions. Grain yield and yield components determined at maturity. A significant increase in single-leaf net photosynthetic rate by rhizobial inoculation was observed. These results have suggested that certain strains of rhizobia can promote rice growth and yield through mechanisms that improve single-leaf net photosynthetic rate (Peng et al, 2002).
Inoculation of soil or seeds planted in soil was practiced for the development of plant growth promoting rhizobacteria, biological control of plant pathogens, or bioremediation of soil containing organic pollutants (Yee et al., 1998; Pletsch et al., 1999; Bloemberg and Lugtenberg, 2001; Whipps, 2001). In spite of numerous attempts to inoculate beneficial rhizobacteria, considerable variations in the efficacy of the procedures used have been reported (Kloeper and Beauchamp, 1992), mainly due to the poor development on seeds and roots (Truelove and Curl, 1985). The introduced microorganisms must colonized plant roots and demonstrate rhizosphere competence before they can exhibit a beneficial property (Lugtenberg and Dekkers, 1999).

Rhizobacteria can be used for biological control and environmental restoration. Hosoda et al. (2002), performed enrichment culture of rhizobacteria, identified isolates, and investigated the physiological properties of the bacterial isolates and suggested that enrichment culture might be useful for isolating bacteria with a high root colonizing ability.

Nodulation and subsequent nitrogen fixation by soybean [Glycine(L.) Merr.] plants were inhibited by low root zone temperatures (RZTs). Plant growth promoting bacteria can help overcome these deleterious effects. Three Bacillus strains, B. subtilis NEB4 and NEB5 and B. thuringiensis NEB17, were isolated from inside the nodules of vigorous field-grown soybean plants in 1998, and were shown to have plant growth promoting activity on pouch-grown soybean plants under greenhouse conditions. To test their ability to improve soybean nodulation and growth under low RZTs, these strains were coinoculated onto soybean plants, with Bradyrhizobium japonicum, under greenhouse conditions at RZTs of 25, 17, and 15_C, and under field conditions in a
short growing season area. In all cases, the experiments were conducted with soybean cultivar OAC Bayfield. All the three *Bacillus* strains enhanced soybean nodulation and growth in greenhouse and field experiments. Coinoculation with NEB17 provided the largest and most consistent increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen, and grain yield. The other two strains provided positive responses in only 1 of the 2 yr of field-testing. Thus, *B. thuringiensis* NEB17 would be suitable for use as a plant growth promoting bacterial strain in soybean production systems in short growing season regions (Bai et al. 2003).

Three strains of plant growth promoting fluorescent Pseudomonas were studied for their effect on growth and yield of French bean (*Phaseolus vulgaris* L.) under field conditions. The effect of these strains on nature of root development and leaf palisade tube length were also examined. The strains induced positive response on growth and physiological parameters resulting in higher yield in *P. vulgaris* (Deka Boruah et al., 2003).

A chitinolytic plant growth promoting rhizobacterium, *Bacillus subtilis* AF1 was evaluated for growth promotion of pigeon pea in the field. Alginate, peat, chitin/*Aspergillus niger* mycelium supplemented peat, and spent compost formulations of *B. subtilis* AF1 effectively increased the seedling emergence, plant height and dry weight in the field, with chitin supplemented peat formulation being the most effective (Manjula et al., 2005).

Nine florescent Pseudomonas isolates obtained on King's B agar from rhizosphere of tea plant were studied for their biochemical and functional
characteristics. They were also tested for their ability to promote growth of tea seedlings. The isolates produced IAA like substances, siderophores and soluble phosphate in the range of 8.7-32.1, 13.6-196.3 and 1.4-15.7 μg/ml culture filtrate, respectively. Four isolates were able to utilized cellulose as carbon source and another four were capable of inhibiting growth of the saprophytic Rhizoctonia solani in laboratory bioassay. The growth parameters of tea seedlings in fertilizer P added pot was statistically at par with those of superior strain inoculated seedlings (Mazumder et al. 2007).

The effect of bacterial inoculations on growth and yield related parameters of maize were investigated by Kumar et al., (2007). Three bacterial inoculants, viz, Bacillus megaterium, Bacillus subtilis and Pseudomonas corrugate showed good rhizosphere competence giving high inoculum numbers.

Inoculation of Pseudomonas fluorescence and P. aeruginosa degraded 78 and 85% of chlorpyrifos in plots without cotton plants whereas 99% degradation of chlorpyrifos was observed in soil, where cotton plants were inoculated with either P. fluorescence or P. aeruginosa as compared to un-inoculated control soil (Vidya Lakshmi et al., 2009). Ochrobactrum anthropi, isolated from the rhizosphere of healthy tea plants growing at the foothills of Darjeeling and Dooars, was found to be antagonistic to several root rot pathogens of tea plant. The cell free culture filtrates of the bacterium was assayed for antagonistic effects. Further, a series of in vivo experiments were conducted on tea plants. Treating the different varieties of tea plants with O. anthropi significantly increased the growth and development of the plant. The
bacterium was able to colonize and maintain population in the rhizosphere and was able to control the root rot disease incidence in tea plants (Anonymous, 2010).