Tea [*Camellia sinensis* (L.) O. Kuntze] is an important plantation crop of India and the most common non-alcoholic beverages of the world used since ancient times. India is the largest producer and exporter of processed tea with over 30% share in the global tea production. Thus, tea holds a key position in Indian economy generating over 660 million US dollars of foreign exchange annually. Tea is grown in more than 30 countries around the world from Georgia (CIS) 43° N latitude to Nelson (South Island) in New Zealand 42° S latitude, and altitudes varying from sea level to 2300 m above mean sea level. In India, it is cultivated over an area of 4, 28,000 ha, most of it in the states of Assam, West Bengal, Tamil Nadu and Kerala.The other states having fairly large area (>2000 ha) under tea cultivation area Tripura, Himachal Pradesh, Uttar Pradesh and Karnataka. There are three species namely *C.sinensis*, *C.assamica* and *C.assamica spp. laxicolyx* which have been cultivated in India.

Tea plants, as all other plants, are subjected to various environmental stresses. Being a perennial, the tea plant has to withstand such adverse conditions year after year and successful growth and production of the plants are influenced to a great degree by the response of the plant to the environment. The root environment is of great significance as the plants, growing in the same soil year after year, have to depend on the particular soil for all their nutrients and water requirements.

For excessive production and keeping away disease causing organisms, huge amount of chemical fertilizers and pesticides are used in tea gardens. The excessive application of chemical fertilizers causes soil sealing, fertility diminishing, and residual problems. The leftover of chemical fertilizers and pesticides have seriously affected the quality of products, people's health and caused environmental pollution. The over use of fertilizers has also damaged the soil's original micro-ecological balance and deteriorated the diseases spread by soil. These ecological, environmental and human health problems have increased public awareness and attempts are being made to search for effective alternative approaches which have minimal deleterious effects, more environmentally friendly, and will contribute to the goal of sustainability in agriculture. In this line plant growth promoting rhizobacteria (PGPR) present immense potential and promise as effective substitutes for agro-chemicals. Exploitation of soil microorganisms offers an attractive alternative to use of agro-chemicals.

The term 'Rhizosphere' was introduced by Hiltner in 1904, and refers to the area surrounding the root whose physiological and physical features are affected by the root. The rhizosphere has attracted much interest since it is a habitat in which several biologically important processes and interactions take place.Rhizosphere constitutes: internal, surface and external. Internal rhizosphere refers to the cell of the root components. Surface refers to the root surface; the external rhizosphere refers to the area around the root. Microbial population is of greater densities in rhizosphere than the non-rhizosphere zone. It is caused by secretion of root exudates and casting of sloughed off root tissue by plant in soil during growth phases(Baker and Snyder,1965).Rhizosphere micriflora,may vary greatly with the age of the plant and different localities of growth. Microorganisms always interact among themselves and with the plant. Some of these interactions are beneficial to the plant, while others are deleterious, and the growth of the plant is, in turn affected by these interactions. These microorganisms grow in close association with the plant roots and forming a cover around the root are referred to as Rhizobacteria (Bashan,1998).They select and regulate the root's function in secreting and absorbing nutrients.PGPR was first defined by Kloepper and Schroth (1978). PGPR mainly constitute species of *Azotobacter*, *Azospirillum*, *Pseudomonads*, *Acetobacter*, *Burkholderia*, *Enterobacter*, *Streptomyces* and bacilli. PGPRs have been applied in a wide range to the agricultural field for the purpose of growth enhancement, including increased seed emergence, weight, crop yield and disease control (Glick, 1995; Aino *et al.* 1997; Ait Barka *et al.* 2000; Dobbelaere *et al.* 2003)

The mechanism by which PGPR increases plant growth is not well understood. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (termed Biofertilizers), or phytohormone production (termed Biostimulants).They suppress plant disease through at least one mechanism; induction of systemic resistance, and production of siderophores or antibiotics. Exposure to the PGPR triggers a defense response by the crop as if attacked by pathogenic organisms. The crop is thus armed and prepared to mount a successful defense against eventual challenge by a pathogenic organism.Siderophores produced by some PGPRs scavenge heavy metal micronutrients in the rhizosphere (e.g. iron), starving pathogenic organisms of proper nutrition to mount an attack of the crop.Interestingky, plants seem capable to still acquire adequate micro-nutrient nutrition in presence of these PGPR.Antibiotic producing PGPRs release compounds that prevent the growth of pathogens.

Biofertilizers are also available for increasing crop nutrient uptake of nitrogen from nitrogen fixing bacteria associated with roots (*Azospirillum*),iron uptake from siderophore producing bacteria(*Pseudomonas*), sulfur uptake from sulfur-oxidizing bacteria(*Thiobacillus*),and phosphorus uptake from phosphate-mineral solubilization bacteria (*Bacillus,Pseudomonas*). Species of *Pseudomonas* and *Bacillus* can produce as yet not well characterized phytohormones or growth regulators that cause crops to have greater amounts of fine roots which have the effect of increasing the absorptive surface of the plant roots of uptake of water and nutrients. These PGPRs are referred to as Biostimulants and the phytohormones they produce include indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production.

There are a large number of pathogens in the soil causing a number of root diseases. Manipulation of soil environment has become a tool for biological control of such soil borne plat pathogens. Manipulation can be done by artificial introduction of antagonists in the soil or spraying these antagonists on the aerial parts of plants.

The study of rhizosphere microflora and the exploitation of potentially antagonistic microorganisms for suppression of root diseases have been carried out by several workers. Among the most commonly used microorganisms are the fluorescent Pseudomonads, *Bacilli, Serratia* and *Trichoderma* and *Gliocladium*. It has been. reported that the saprophytic microflora of the rhizosphere includes doth deleterious and beneficial organisms that have potential to influence plant growth and crop yield significantly (Lynch,1982; Chen *et al.* 1995; Bloemberg *et al.* 2001; Compant *et al.* 2005; Gnanamanickam *et al.* 2007; Chakraborty *et al.* 2007; Christopher and West, 2007).The beneficial microorganisms affect plant growth positively by mechanisms which include promotion of the availability and uptake of mineral nutrients (Loper *et al.* 1999; Kuiper *et al.* 2001), provision of plant growth substances (Gaskins *et al.* 1984; Ping and Boland, 2004), and importantly, suppression of deleterious microorganisms (Schipper *at al.* 1997; Dekkers *et al.* 1998; Ait Barka *et al.* 2000) and modify cropping environment to enhance productivity (Lynch,1990; De Weger *et al.* 1995). The deleterious microorganisms, on the other hand, affect plant growth

Plate I: Tea garden in Hill (A) and Plain (B).

negatively. Reports on the role of fluorescent Pseudomonad in suppression of diseases in experimental conditions or in suppressive soils are well documented. According to Cook and Baker (1983), soil microflora determines two types of mechanisms of suppression: one is called general, other is specific. General suppression is achieved by the whole microflora; the higher microflora biomass and activity, higher the suppressiveness.In contrast with the biocontrol achieved by non-pathogenic *Fusarium oxysporum* or by fluorescent Pseudomonads, suppression of *Fusarium* wilts in naturally suppressive soils operates very effectively under various experimental conditions. The consistency of suppression might be related to the diversity of the microbial population present in the soils. With a view to stimulating the complex microbial interaction responsible for soil suppressiveness, several workers have used a combination of both antagonistic microorganisms in biocontrol experiments (Fuchs and Defago, 1991; Leeman *et al.* 1996; Schisler *et al.* 1997; Park and Kloepper, 2000; Quazi and Vyas, 2007).

It has also been suggested that induction of resistance in the host may also play an important role in the observed disease suppression. Van Peer *et al.* (1991) also reported experimental evidence for the inclination of bacterial induction of host resistance against *Fusarium* wilt.Siderophore production was also postulated to be an important mechanism for the biocontrol activity of PGPR (Castognetti *et al.* 1986; Bakker *et al.* 1993). Ongena *et al.* (2000) reported that protection of cucumber against *Pythium* rot by some strains of *Pseudomonas* was neither due to the production of siderophores, nor to antibiosis, but by induction of antifungal phenolics in the host root.

The use of biocontrol agents for the protection of plants against pathogens both *in vivo* and *in vitro* have been reported by a number of authors.*T.harzianum* and *T.viride* have been widely used for biocontrol purposes especially those of root disease. Tea phyllosphere fungus *Sporothrix* sp. antagonistic to tea anthracnose fungus *Colletotricum theae-sinensis* was reported by Yamada (2004), Sain and Gour (2005) reported that *Bacillus* and *Pseudomonas sp.* could be used to control *Xanthomonas axonopodia pv. cyamopsida, X.campestris* and *X. campestris pv. glycine* causing blight in cluster bean, black rot of cabbage and leaf pustules in soybean, respectively. Meshram *et al.*(2007) were evaluated fluorescent Pseudomonas and

Bacillus subtilis isolated from cotton rhizosphere against *Fusarium* wilt (*Fusarium oxysporum* f. sp. vasinfectum) and *Macrophomina* stem break/dry root rot (*Macrophomina phaseolina/ Rhizoctonia bataticola*) disease in cotton. Singh *et al.* (2007) were also reported that *Trichoderma* strains protect tomato plants against *Fusarium* wilt .There are also reports of use of *Trichoderma* and *Gliocladium* as plant protecting agents (Sharma, 2000; Wahad, 2000). In tea, Chakraborty *et al.* (1995, 1997 and 1998) reported the use of antifungal strains of *Micrococcus luteus, Bacillus pumilus* and few other antagonistic bacteria isolated from the phyllosphere for control of foliar fungal diseases. Chakraborty *et al.* (2003, 2004, 2006 and 2007) were also reported tea rhizosphere microorganisms, *Bacillus pumilus, B. megaterium, Serratia marcescens* and *Ochrobactrum anthropi* not only control root rot pathogens but also promote plant growth.

In recent years, Indian tea is facing the prospect of losing out on tea export to traditional countries because of two major facts – the first one being that other countries like Kenya,Sri Lanka etc are increasing production rapidly and have become competitors of India; secondly countries to which orthodox Darjeeling tea were being exported are now insisting on reduced use of pesticides/fungicides for health purposes.Hence,the time has now come to look for means of reducing chemical outputs in tea, which may be achieved by increasing use of plant growth promoting or antagonistic microbes in tea phyllosphere/rhizosphere.

Poria hypobrunnea Petch. causes a widely prevalent stem-cum-root disease of tea in all the tea growing areas of Terai and Dooars. It is a wound parasite which gains its entrance into the frame of the bush through wound, especially on thick branches, caused by various agencies and gradually extends down to the roots when the affected bushes are completely killed. Thin films and small cushions of yellow-brown mycelia are produced on the root surface as well as on the wound, beneath the bark. Wound is yellowish, soft and decayed, marked with thin, irregular, light-brown lines and permeated with thin sheets of yellow brown mycelium.

Considering the importance of using biological agents for growth promotion and disease suppression in tea, to reduce the use of chemicals, the present study was undertaken with the following special objectives:

(a) Isolation and identification of rhizosphere microorganisms from different varieties of tea plants of various ages growing in different localities and determination of their microbial population.

(b) *In vitro* testing of the isolated microorganisms against the root rot pathogens causing brown root rot, violet root rot and branch canker disease of tea.

(c) Selection of microorganisms showing antagonistic activity towards the root rots pathogens.

(d) *In vivo* testing of the isolated antagonists for disease suppression.

(e) Determination of plant growth promoting activity if any in these antagonistic microorganisms

(f) Selection of such microorganism(s) showing both disease reduction and plant growth promotion.

(g) Extraction of active principles from the selected microorganisms.

(h) Bioassay of the active principles and optimization of their production in their culture

(i) Determination of the population of the antagonists as well as pathogen in the soil after definite period.