INTRO UCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the important plantation crops of India. Since tea is grown in tropical agro climates, pests, weeds and disease causing organisms are capable of causing serious damage to the crops, for which extensive use of chemicals has been implemented in the past. Besides, as most of the nutrients in the soil are not in a readily available form, extra input of chemical fertilizers has been necessary for the optimum productivity of the tea plant. But the uses of fertilizers and pesticides have caused a serious problem of pollution and loss of land fertility (Bezbaruah *et al.*, 1996). In recent years concern over pesticide load in the environment and also pesticide residue in agricultural produce has led to a reduction in use of chemical fungicides commonly used to control plant pathogens. In its place major thrust is being given to biological control as a component of integrated disease management. In case of tea, the demand of organic tea in the world market is very high, and is increasing. Excessive use of chemicals and the presence of residue in the leaves is a major concern for consumers. Hence, biofertilisers, whether it is microorganism based or a plant based have the potential to be used in tea industry on a large scale. Tea plant is is a perennial and grows in several geographical regions of India, including the plains of Assam, North Bengal as well hills of Darjeeling and South India (Plate I) its rhizosphere is expected to be a rich source of microorganisms, some of which could be exploited for use as biofertiliser/ bioprotectant capable of improving the growth of the plant, either by suppression of pathogenic soil fungi or by growth promotion through other mechanisms.

The term 'Rhizosphere' was introduced by Hiltner in 1904, and is now defined as the volume of soil surrounding the plant root in which bacterial growth is stimulated. Rhizosphere is the habitat in which several biologically important processes and interactions takes place. It is the zone of intense activity of various groups of microorganisms. The root system is known to produce exudates with a number of sugars, organic acids and amino acids, favouring the population build up of rhizosphere microorganisms. These microorganisms grow in close association with the plant and are referred to as rhizobacteria (Bashan, 1998). They live at the expense of the plant, feeding on the nutrients released from the plant roots. Some of them affect the growth and development of plant.

Plate I (A-B): Tea gardens in hills (A) and plains (B).

These plant associated microorganisms mainly belong to the bacteria, fungi and actinomycetes. These may be beneficial or deleterious to the plant, or may not have any relation to the plant at all. The beneficial groups of microbes with the capacity to enhance plant growth by increasing seed emergence, plant weight and crop yields are designated as the plant growth promoting rhizobacteria (PGPR) (Kloepper, 1992). These bacteria appear completely harmless, do not cause any symptoms and yet induce substantial resistance against different pathogens (Van Loon, 1997). PGPRs have been applied to a wide range of agricultural species for the purpose of growth enhancement, including increased seed emergence, weight, crop yields and disease control (Glick, 1995).

The mechanisms by which PGPRs can influence plant growth may differ from species to species as well as from strain to strain. Symbiotic plant colonizers such as rhizobia mostly contribute to plant growth by nitrogen fixation. Free living rhizobacteria usually do not rely on single mechanism of promoting plant growth (Glick *et al.* 1999). There are several determinants for mechanisms of growth promotion that include bacterial synthesis of plant hormones like indole-3- acetic acid (IAA), cytokinin and gibberellin, breakdown of plant induced ethylene by bacterial production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and increased mineral and N availability in the soil (Kloepper, 1992; Glick, 1995). Several indirect possible mechanisms have also been proposed that reduces the stress by limiting the damage caused by phytopathogens. These mechanisms may include suppression of diseases caused by the plant pathogens (Suslow and Schroth, 1982), competition with pathogenic microorganism by colonizing root.

Besides growth promotion, PGPRs have been tested and characterized for their activity as biocontrol agents against soil borne pathogens. It is clear that the biological control of soil borne plant pathogen by antagonistic microorganisms may offer a practical supplement or alternative to existing disease management strategies that depend heavily on chemical pesticides. Disease suppression by antagonistic bacteria depends on their ability to colonize roots and to produce substances inhibitory to pathogens. Such microorganisms can produce substances that limit the damage caused by pathogens, by producing antibiotics, siderophores and other lytic enzymes. These microorganisms can also function as competitors of pathogens for

nutrients and colonization sites. Potential biocontrol agents produce antibiotics, which play an important role in disease suppression and increase yield of the plants under greenhouse conditions. The most widely studied group of rhizobacteria with respect to production of antibiotics is that of the fluorescent pseudomonads (Haas and Keel, 2003; Dileep Kumar and Bezbaruah, 1997). Well-characterized antibiotics with biocontrol properties include phenazines, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, lipopeptides, and hydrogen cyanide. *Bacillus megaterium* have also been reported to produce antibiotics against several fungal pathogens by Jung and Kim (2003).

Interest in biological control has increased recently by public concerns over the use of chemicals in the environment in general, and the need to find the alternatives to the use of chemicals for disease control (Whipps, 2001). The key to achieving successful, reproducible biological control is the gradual appreciation that knowledge of the ecological interactions taking place in soil and root environments is required to predict the conditions under which biocontrol can be achieved (Deacon, 1994; Whipps, 1997). It is also essential to know the interactions of the microorganisms and the host. In this context, the mechanisms of plant growth promotion and induced systemic resistance (ISR) by PGPRs have been extensively studied in the past decade. ISR occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens (Van Loon *et al.*, 1998). ISR developed by non-pathogenic fluorescent *Pseudomonas* bacteria, unlike the pathogen-induced systemic acquired resistance (SAR), is independent of salicylic acid (SA) but requires jasmonic acid (JA) and ethylene (ET) (Bakker *et al.* 2003a). PGPR mediated ISR results in reinforcement of plant cell wall by lignin, callose and phenolic compounds, alteration of physiological and biological reactions of plant cells, and production of antimicrobial substances, such as pathogenesis related proteins (PR) and phytoalexins (Ramamoorthy *et al.*, 2001).PGPRs act as a biological control and protects plants from the various pathogens in several crops by activating defense genes encoding chitinases, β-1,3 glucanase, b-1,4 glucanase, peroxidase, phenylalanine ammonia lyase and other enzymes which are involved in synthesis of phytoalexin (M'Piga *et al.*, 1997). The antagonists may produce mycolytic enzymes viz. β-1,3 glucanase, β-1,4 glucanase and lipases. Since the

mechanism of action of PGPRs are highly complex , it is quite essential that this type of work would require a study not only of any potential agent *per se*, but also its interactions with the crop, the natural resident biota and environment as well.

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 objectives giving special emphasis on PGPRs and their mechanism of action:

i. Isolation of microorganisms from tea rhizosphere and their identification .
ii. Screening of isolated microorganisms against common soil fungi *Fomes lamaoensis, Sclerotium rolfsii* I, *Sclerotium rolfsii* II, *Sclerotinia sclerotiorum, Sphaerostilbe repens,* and *Poria hypobrumea in vitro.*
iii. Testing of plant growth promoting activity of the selected antagonists and selection of PGPRs; *in vivo* testing of the PGPRs for determination of disease suppressing activity; determination of population antagonists as well as pathogen in soil after definite time interval; *in vivo* determination of PGPR activity.
iv. Determination of biochemical changes in test plants induced by PGPRs.
v. Extraction of active principles from selected PGPRs and bioassay of isolated compounds and their characterization.