1. INTRODUCTION

INTRODUCTION

Fertilisers have played a key role in the modernization of Indian agriculture and in making the country self sufficient in food grain production. Phosphorus is reported to be the limiting plant nutrients and is considered to be the key to crop productivity. In time the shortage of essential plant nutrient phosphate may also seriously limit crop production. Out of essential plant nutrients, nitrogen is abundant in the air, deposits of potassium are ample, but the phosphate reserves will become scarce. This may lead to conflicts for a share of phosphate fertilizers long before the exhaustion of phosphate rocks. Only strict rules to recycling and efficient use could postpone this first serious shortage of essential plant nutrient (FAO, 2006).

Most agricultural soils contain large reserve of total phosphate, commonly in the range of 200 to 5000 mg phosphate kg $^{-1}$ and a part of phosphate accumulated depends on regular application of chemical fertilizers (Gyaneswar *et. al.,* 2002). Both phosphate fixation and precipitation occur in soil because of the large reactivity of phosphate ions with numerous soil constituents (Rodriguez and Fraga, 1999).

Most soils contain insoluble inorganic phosphates but they are of no use to crops unless they are solubilised. Upto 75% of the soluble phosphate fertilizers added to crops may be converted to sparingly soluble forms by reacting with the free Ca^{2+} ions in high pH soils or with Fe^{3+} and Al^{3+} in low pH soils (Goldstein, 1986; Arpana *et. al.,* 2002). A greater part of soil phosphorus, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants (Vassileva *et. al.,* 1998). To increase the availability of phosphorus for plants, large amounts of fertilizer are used on a regular basis. But after application, a large proportion of fertilizer phosphorus is quickly transferred to the insoluble form (Omar, 1998). Therefore, very little percentage of the applied phosphorus is used, making continuous application necessary (Abd Alla, 1994). Plants can absorb phosphate in two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) ions (Glass, 1989). Interest has been focused on the inoculation of phosphate-solubilising micro-organisms into the soil so as to increase the availability of native fixed phosphate and to reduce the use of fertilizers (Illmer and Schinner, 1992).

Microorganisms are important in agriculture because of its role in promoting the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible (Cakamakci *et. al.*, 2006).

Microorganisms play key role in effecting the availability of soil phosphate to plant roots, and increasing phosphate mobilization in soil, though the development of effective microbial inoculants remains a major scientific challenge (Richardson, 2001). Phosphate solubilising microorganisms are capable of solubilising tricalcium, aluminium and iron phosphates, as well as rock phosphate making the phosphorus present in the soil available to the plants (Nowotny-Mieczynska & Golebiowska, 1956; Sperber, 1958; Muromtsev, 1958; Katznelson & Bose, 1959; Katznelson *et. al.*, 1962). Soils also contain organic phosphorus, which can be used by crops only if it is mineralized. Organisms that cause increases in plant available phosphate in the soil system belong to a diversified group including bacteria, actinomycetes and several groups of fungi. The composition and dynamics of this functional group was influenced greatly by vegetation type, soil texture, soil chemical elements, and pH in soil solution (Curl *et. al.*, 1986; Kucey 1983; Lin *et. al.*, 2000; Yi, 1988). It was reported that about 20% of microorganisms in soil can solubilise insoluble inorganic phosphate and that phosphate solubilising activity of PSM is related to the environmental conditions such as farming practices. Phosphate solubilising bacteria are common in the rhizosphere and secretion of organic acids and phosphatases are common method of facilitating conversion of insoluble forms of phosphate to plant-available forms (Kim *et. al.*, 1998). Phosphate solubilising bacteria have been used also to improve rock phosphate value because they convert insoluble rock phosphate into soluble form available for the plant growth (Nahas *et. al.*, 1990, Bojinova *et. al.*, 1997). This conversion is through acidification (Muromtsev, 1958; Louw & Webley, 1959), chelation and exchange reactions (Duff & Webley, 1959; Sperber, 1958; Gerke, 1992) and produces, in the periplasm, strong organic acids (Alexander, 1977), which have become indicators for routine isolation and selection procedures of phosphate solubilising bacteria (Illmer *et. al.*, 1995).

Many phosphate-solubilising bacteria (PSB) belonging to *Pseudomonas, Bacillus, Rhizobium, Agrobacterium, Burkholderia, Achromobacter, Microccocus, Aerobacter, Enterobacter, Flavobacterium,* and *Erwinia* genera have been isolated from soils (Rodriguez and Fraga,1999).

Among phosphate solubilising fungi, *Aspergillus niger, A. flavus, A. awamori* have been reported from the rhizosphere of maize, soybean, chilli, acidic lateritic soils and compost (Subba-Rao and Bajpai, 1965). *Penicillium digitatum, Paeciliomyces fusisporus* and species of *Cephalosporium, Alternaria, Fusarium, Rhizoctonia* are other solubilisers of insoluble phosphate. Among yeasts, *Torula thermophila, Saccharomyces cerevisiae,* and *Rhodotorula minuta* can solubilise inorganic phosphate (Varsha-Narsian *et. al.,* 1994).

It is well known that phosphate dynamics can be affected by microorganisms in different ways as follows: (a) an increase in phosphate solubilising microorganisms biomass as a result of rhizodeposition can increase solubilisation in the rhizosphere (Gyaneshwar *et. al.,* 1998) (b) the release of phosphate once microbial cells die can occur; microbial biomass can immobilize from 1 to 10% of the total soil phosphate (Richardson, 2001) and (c) mineralization of organic phosphate to inorganic phosphate can occur through phosphatase activity (Oehl *et. al.,* 2001).

Multistrain biofertilisers produced from nitrogen fixing, phosphate solubilising, plant promoting and plant pathogenantagonic microorganisms weretested for different crops in Vietnam. The total area of field demonstration and experiment were about 200 ha in different provinces. Field experiments showed that new biofertilisers had the positive effect on the growth and increase yield of potato 30.98-72.38%, groundnut 13.33-19.07%, tomato 20.5%, coffee 16.42% (FNCA, 2004).

Several scientists from IARI, New Delhi, brought out that by inoculation with phosphate solubilising microorganisms alongwith rock phosphate it is possible to get the same amount of yield as with superphosphate (Tilak, 1993). Potato tuber yield were increased dramatically (50-60%) due to inoculation with *Aspergillus awamori, P. striata, Bacillus polymyxa* cultures. The beneficial effect was also observed on the crops grown after harvesting the inoculated crop (Gaur, 1990). Experiments conducted in India with phosphobacterium and other phosphate solubilising microorganisms in various crops like oat, wheat, potato, peas, tomato and tobacco showed an average 10-15% increase in crop yield in about 30% of the experiments conducted (Gaur, 1990).

Various species of *Penicillium, Cladosporium, Trichoderma, and Paecilomyces* amongst fungi and *Bacillus* and *Pseudomonas* amongst bacteria have been found well adapted to higher altitudes in Sikkim. A fungal isolate *Paecilomyces lilacinus* has shown a higher degree of phosphate solubilising activity at 10°C than at 24°C. Similarly a number of *Pseudomonas* strains are found to be well adapted to higher altitude soils and have exhibited antifungal, phosphate solubilising, and plant growth promoting properties (Pandey and Palni, 1998). These species have been reported as efficient degraders of organic matter (Singh and Malik, 1993; Bhardwaj, 1995).

Darjeeling hills area is characterized by the possession of high rainfall (3212 mm), cool humid climate, sloppy land, brown forest soil, rich in undecomposed organic matter, light texture, acidic reaction. Soil is subjected to heavy erosion. Excellent quality mandarin orange grows on about 2000 ha at a height below 2000 m. Cabbages, cauliflower, radish, peas and beans, tomato, cucumbers, potato are important vegetables. Orchids, gladioli and bulbous flowers grow luxuriantly. Many ornamental and foliage plants, cacti and succulents are also grown in numerous nurseries. Many polyhouses have been erected in Kalimpong, Pokhriabong and Mirik area for commercial floriculture. Production of vegetable seeds, flower seeds and bulbs has been taken up by many. Ginger and large cardamoms are the two spices grown in this area. Some plants of plum, peach and pear are grown in small home-gardens. The hill region has special importance in the horticultural scenario of the state. Tea is of great importance in the economy of the area. This area being a component of Eastern Himalayas is also a rich source of medicinal plants.

Tea, which is one of the most important crops in Darjeeling hills and a major exportable agricultural produce has also suffered setback due to high dependence on chemical inputs. The yield and quality has fallen, and high pesticide residues in tea have reduced its exportability. In the recent past thrust is given by some of the tea estates to develop organic farming.

Multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored (Gupta *et. al.*, 1998). Field experiment with two certified nitrogen fixing biofertilisers (*A. chroococcum* and *Azospirillum brasilense)* with maize as test crop did not result in improvement of plant performance at the temperate location of Sikkim (Pandey *et. al.*, 1998). The effect of the inoculants depends on the bacterial strain, plant cultivar and environmental conditions (Fulchieri and Frioni, 1994). Previously isolated azotobacters or azospirilla from tropical and subtropical areas are useful upto certain elevation. For relatively higher elevations, emphasis should be given on the isolation and identification of native phosphate solubilising microorganisms. Native *Azospirillum* biofertiliser are found to be superior to the commercial one in the growth of nutmeg seedlings (Nair and Chandra, 2001). Due to endemicity of beneficial bacterial groups, the isolation and characterization of beneficial plant growth promoting rhizobacteria from dominant crops of a region may be necessary in development of bioinoculants for local crop production system (Asea *et. al.,* 1988; Kole and Hazra, 1998; Cho and Tiedje, 2000; Talukdar *et. al.,* 2001). Similar findings of the higher efficiency of native rhizobacteria in the plant growth promotion have been reported from different parts of the country (Chandra *et. al.,* 1995; Bhattacharya *et. al.,* 1997; Kabi and Bihari, 1999).

In view of the availability of limited information on the adaptation of commercial phosphate solubilising biofertilisers in higher altitudes, present study includes quantification , distribution, isolation, identification and characterization of rhizospheric phosphate solubilising microorganism communities in Darjeeling hill soils which could be developed as suitable location specific phosphate solubilising biofertilisers for different crops of agriculture, horticulture, forestry, tea and sericulture sector of hill areas.

On the basis of the above information the present work has been planned with the following objectives:

- Isolation of phosphate solubilising microorganisms from the rhizosphere of dominant crop plants of Darjeeling Hills
- Screening of phosphate solubilising microorganisms isolates for efficient phosphate solubilisation, production of other plant growth promoting substances *in vitro*.
- Study of survivality of superior phosphate solubilising microorganisms *in vitro* under different growth conditions
- On farm activities of superior phosphate solubilising microorganism isolates