CHAPTER 1
INTRODUCTION

Soil is known to be a complex microhabitat for two distinctive properties. Firstly, the microbial population in soil is very diverse and secondly soil is a structured, heterogeneous and discontinuous system, generally poor in nutrients and energy sources. The chemical, physical and biological characteristics of these microhabitats differ in both time and space. Diverse microorganisms are essential to a sustainable biosphere and the role of rhizosphere microbial populations for maintenance of root health, nutrient uptake and tolerance of environmental stress is now recognized (Zake *et al.,* 2011). Microorganisms form a vital component of all known ecosystems of earth and represent the richest repertoire of molecular and chemical diversity in nature as they underline basic ecosystem processes. Soil microflora plays the most important role in the soil region of the higher plants. The variable microflora changes the soil fertility conditions to a specific plant and in turn is dependent on the exudates of the roots in the rhizosphere. 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, phosphorus, and sulphur. In addition, microorganisms play key roles in suppressing soil borne plant diseases, in promoting plant growth and changes in vegetation (Singh *et al.,* 2011). Soil bacteria and fungi are the key players in various biochemical cycles (BGC) (Trevors, 1998) and are responsible for the cycling of organic compounds. They also influence above-ground ecosystems by contributing to plant nutrition (George *et al.,* 1995) plant health (Smith and Goodman, 1999), soil structure (Wright and Upadhya, 1998) and soil fertility (Karthick *et al*, 2011).

The study of microbial diversity is also important to solve new and emerging disease problems and to advance biotechnology. New technologies, particularly in nucleic acid analysis, computer science, analytical chemistry, and habitat sampling and characterization place the study of microbial diversity on the cutting edge of science. It is important to study microbial diversity not only for basic scientific

research, but also to understand the link between diversity and community structure and function. Human influences such as pollution, agriculture and chemical applications could adversely affect microbial diversity, and perhaps also above and belowground ecosystem functioning. In addition, a healthy rhizosphere population can help plants deal with biotic and abiotic stresses such as pathogens, drought and soil contamination. The role of rhizospheric organisms in mineral phosphate solubilization was known as early as 1903 and the ability of rhizospheric microorganisms to promote growth by phosphate solubilization is also one of the most studied mechanisms involved in plant growth promotion (Misra *et al.*, 2012). Important genera of mineral phosphate solubilizers include *Bacillus* and *Pseudomonas* (Singh, 2013) while *Aspergillus* and *Penicillium* form the important fungal genera (Nenwani *et al.*,2010)**.** In soil, phosphate-solubilizing bacteria constitute 1–50% and fungi 0.5%–0.1% of the total respective population. Generally, the phosphate-solubilizing bacteria outnumber phosphate-solubilizing fungi by 2–150 times (Kucey, 1989). The high proportion of PSM is concentrated in the rhizosphere and is known to be more metabolically active than those isolated from sources other than the rhizosphere. Species of *Aspergillus, Penicillium* and yeast have been widely reported solubilizing various forms of inorganic phosphates (Whitelaw, 2000). Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996).

The use of biological fertilizers in recent times, is receiving attention mainly on account of increased global preference for natural "organic" products. Isolation of microorganisms, screening for desirable characters, selection of efficient strains, production of inoculum and preparation of carrier-based formulation are important steps in the use of this microbe based environment friendly and sustainable technology. When these cultures are introduced into the natural environment, their individual beneficial effects are greatly magnified in a synergistic fashion. A microbial inoculant containing many kinds of naturally occurring beneficial microbes called 'Effective Microorganisms' has been used widely in nature and organic farming (Karthick *et al*, 2011).

Fig. 1. Few of the major forests of Darjeeling hills.

Our understanding of microbial diversity, and concomitantly of species composition, of microbial communities is hampered by the inability to classify microorganisms (Sherriff *et al*., 2007). Microbes are small and, in general, without conspicuous external characters to classify them morphologically. In addition, classification based on physiological or biochemical features is often not possible because an estimated percentage of 99% of all microorganisms in nature can not be isolated. So, to obtain a better understanding of the role of microbial diversity in ecosystem functioning, other techniques, which complement the traditional microbiological methods are necessary. Pace and co-workers (1986) were the first to realize that this phylogenetic framework of rRNA sequences could be used to design primers and probes. Therefore, approaches detecting the diversity of directly extracted signature molecules of microorganisms, such as fatty acids (Frostegård, *et al.,* 1996) or DNA (Zhou *et al.,* 1997), have been developed. DNA-based characterization techniques have the advantage that specific genes can be amplified from a community mixture or pure culture by PCR and that products of such amplifications can be further characterized, e.g., by subcloning and DNA sequencing. Such data can be directly compared to DNA sequence databases and thus provide information about similarity to already-known genes (Ueda *et al*., 1995). Genetic fingerprinting techniques provide a pattern or profile of the community diversity based upon the physical separation of unique nucleic acid species (Madhavan *et al.,* 2010). The methods are rapid and relatively easy to perform, but more importantly, they allow the simultaneous analysis of multiple samples, which makes it possible to compare the genetic diversity of microbial communities from different habitats or to study the behaviour of individual communities over time. Application of these in molecular biological techniques allows us to detect and enumerate microorganisms in their natural habitat and so to determine the structure, function and dynamics of selective microbial commuinities (Jeewon *et al.,* 2013). Genetic fingerprinting techniques to study the diversity and dynamics of microbial communities can be divided into *direct methods*, whereby nucleic acids are extracted and directly analyzed, such as low-molecular-weight (LMW) RNA profiling, or into *indirect methods*, whereby the molecular marker first has to be amplified, which is the case for denaturing gradient gel electrophoresis

Fig. 2. Major riverine soil sample collection sites of Darjeeling Hills.

(DGGE) or temperature gradient gel electrophoresis (TGGE), singlestranded-conformation polymorphism (SSCP), randomly amplified polymorphic DNA (RAPD) or DNA amplification fingerprinting (DAF), bisbenzimide-polyethyleneglycol (Bb- PEG) electrophoresis, restriction fragment length polymorphism (RFLP) or amplified ribosomal DNA restriction analysis (ARDRA), and terminal RFLP (T-RFLP) or fluorescent RFLP (Flu-RFLP). Molecular analysis of genomic DNA of the organism is therefore useful for distinguishing the microbial strains better at intra-species level and these techniques provide valuable information on the magnitude of genetic variability within and between organisms of different species It has been suggested that molecular fingerprinting techniques are not only helpful in knowing un-cultured communities but also helps to track the populations of known organisms with the help of reference sequences (Sun *et al.,* 2013).

Himalayan region represents a unique combination of plant and soil types that changes drastically with altitude (Kumar *et al.,* 2011). We have till date a very poor record of the Beneficial Microorganisms of the Himalayas especially of the Eastern Himalayan region lying between the latitudes 26 o 40' - 29 o 30'N and longitudes 88 o 5’- 97o 5'E and covering a total area of 93,988 km2 comprising Arunachal Pradesh, Sikkim and Darjeeling hills of West Bengal with 83,743, 7,096 and 3,149 km2 of area respectively.). In this context the need for survey of efficient microorganisms from the Himalayan belt becomes necessary so as to use them more efficiently as “organic” products in this areas.

India is among the world’s twelve mega-diversity countries and immensely rich in bio-resources. West Bengal represents a good slice of biodiversity of the nation and is commendably bestowed with at least five ecological zones representing not only a variety of ecosystems but also remarkable diversity in its biological resource arena. As such, avenues in tapping upon the state's biodiversity resources and associated knowledge are undoubtedly immense, in order to consequently promote biodiversity-based enterprises in the modern, as well as, traditional sectors, to develop biotechnology industries in the State and also to encourage local level value addition to biodiversity resource. Darjeeling Himalayan hill region is situated on the North-Western side of the state. This region belongs to the Eastern Himalaya range.

Fig. 3. Agricultural crop fields of Darjeeling hills.

The whole of the Darjeeling district except the Siliguri division and a narrow part in the Northern part of Jalpaiguri district constitutes the region. It starts abruptly up from the Terai region. The deep gorge of Teesta has divided this mountainous region into two parts; the Singalila and Darjeeling Ranges run from north to south in the western part. The Singalila range is located along the border of Darjeeling and Nepal; it has four important peaks – Sandakphu, Phalut, Sabargam and Tangu. Isolation of microorganisms useful to improve the plant health from this region of varying altitude and vegetation will be a new attempt to explore the microbial mines of Darjeeling hills.

Keeping this in view, the following major objectives were undertaken to generate the possible information for utilization of microorganisms isolated from the different ecological regions of Darjeeling hills.

- Isolation of microorganisms from rhizosphere soil of Darjeeling hills, their characterization and identification
- Screening of isolates and characterization as phosphate solubilizers, chitin, cellulose and lignin degraders
- Selection of the isolates for their utilization as biocontrol agents against fungal pathogens
- Evaluation of the selected microorganism for plant growth promoting activities
- Molecular diversity analysis of the selected isolates using relevant tools.