India is endowed with diverse natural resources for which it has acquired the sobriquet - “Mineral bowl of the world”. It has the world’s largest resource of coal. In India coalmining is an important core sector industry, second to agriculture, which plays a very critical role in the country’s economic development. On the other hand, coalmining is alarmingly hazardous as far as our environment is concerned. During the excavation of coal or surface mining, the overlaying soil with vegetation is removed and rock debris get deposited in the form of overburden (OB). Mining not only visibly disrupts soil composition, structure, soil microbial populations, nutrients cycles that are crucial for sustaining a healthy ecosystem. So, it is imperative to adopt the reclamation strategies, so that a pollution free economic gain can be achieved. Reclamation strategy of mine affected areas is the promotion of microbial populations, nutrient cycling and amendment in order to restore the land as closely as possible to its pre disturbance condition that support self sustaining ecosystem (Singh et al., 2004). For such eco-restoration exercise the coalmine wasteland should be treated with beneficial microorganisms along with amendment by organic matter and other synthetic nutrient substances, plantation of different plant species that are able to withstand in adverse environmental conditions.

In general two types of environmental problems are encountered in high sulfur containing deep mine and in opencast coalmine (1) Alteration of ecology due to physical removal of top soil and accumulation of different solid wastes; (2) Generation and accumulation of highly acidic liquid wastes referred to as acid mine drainage (AMD) which pollute the surrounding area to such an extent that they become wasteland. It is estimated that every million of coal extracted out of opencast mine damages a surface area of about 4 ha (Roy et al., 2005).

The AMD is one of the main problems associated with high sulfur (2-11%) containing coalmining activities. One of the effects is the increase in metal solubility, which results in the accumulation of these toxic elements in the environment. In
consequence the site becomes unproductive and unfit for average living organisms except those microorganisms, able to tolerate the acidity and the high concentration of metals. Thus, the bioprospection of these naturally occurring microbes represents an important strategy in order to obtain agents for bioremediation process (Li, 2006). So, isolation and characterization of microorganisms from coalmining environment and evaluation of these microbial sources for bioremediation of contaminated soil and of mine wasteland is the growing concept for environmental safety. Application of beneficial microbes to coalmine pollutant affected areas as well as to mine wasteland is the first step toward making the wasteland habitable for higher flora and fauna. A great variety of microorganisms have been found in mine wasteland which can be widely used to retard the adverse impact of mine wastes of the environment (Zhenli et al., 2005; Burd et al., 2000; Glick and Pasternak, 2003).

Mine waste has been generated for several centuries after initiation of the mining industries. Earlier mining activities were limited and so environmental concerns were also limited and local issue. But with the advent of industrial revolution the problems are aggravating. To day coalmining is indispensable as industrial fuel and hence lifeline of a national economy. Therefore since 1950s coalmine generated wasteland and degradation of surrounding environment has become global problem.

The development of coalfields in Assam was initiated in the year 1870 (Akala, 1995) and is now operated in the name of North Eastern Coalfields, Coal India Limited (NECF-CIL), Margherita. There are about 1.00 billion tones coal reserves estimated in this coal bearing zone of North East (NE) India, which is 0.5% of the country’s total reserve of about 200 billion tones (Chaoji, 2002).

The generation of mine tailings in an opencast mine as waste rock to coal is approximately 1:14, which is a peculiar character of NE coals (Chaoji, 2002; Changmai, 2002). The environmental degradation as a result of the dumping of mine tailings known as overburden (OB) is enormous (Chaoji, 2002). These materials are generally dumped in an identified area as artificial hills with distinct strips. Ecological succession in such area spans 25-30 years (Deka Boruah, 2006; Weilinga et al., 1999; Gonzalez-Toril et al., 2003, Bada and Lazcane, 2002; Dopson et al., 1997). According to recent report of NECF-CIL,
Margherita, alone generate 1000 ha of OB dumping site which is a matter of serious concern.

1.1 Bacteria in diverse environment

Bacteria are known to be the oldest living organism on earth that evolved nearly 3 billion years ago when the earth's atmosphere and environment was totally different from that of now. At that point of time the earth was not suitable for higher organisms. The bacteria were and are capable of thriving under extreme environmental conditions like very high and low temperature, lack of oxygen, very high and low pH level, salinity and chemical stress, dehydration etc. It is over a course of millions of years that the metabolic activities of the bacteria brought about changes in the atmosphere and soil thereby paving the way for higher organisms. Thus bacteria are one of the indispensable component of environment and they play a pivotal role in maintaining the earth's geochemical cycles and particularly maintenance of oxygen balance of atmosphere (Johnson, 1998).

Bacteria are the only group of living organisms which are found anywhere on earth and from atmosphere to deep under the earth's crust. Because of their ability to grow under diverse and extreme environmental conditions bacteria have enormous diversity which no other group of organism possess.

Bacterial diversity can be seen in many ways, viz., cell size and shape (morphology), metabolisms, survival strategy, motility, developmental biology, adaptation to extreme environmental conditions etc. (Madigan et al., 2003). Detailed view of the phylogenetic tree of the microbial population is shown in the Fig. 1.

Indeed, the ability of microbes to thrive under such severe conditions has given rise to the speculations and postulations that bacteria may exist outside the earth in the solar system and beyond (Goebel and Stackbrandt, 1994; Walton and Johnson, 1992; Johnson, 2003).
Some species of microbes can thrive at high temperatures once thought to be incompatible with life. For example, hot springs such as those found in Yellowstone National Park are teeming with microbes that live at near boiling temperatures. Bacteria are also found in sub-zero temperature of polar regions, in extremely salty bodies of water and in soils and waters extremely acidic or alkaline (Carey, 2005). Prokaryotes that inhabit such environment define the biological limits to physicochemical extremes. Moreover, these prokaryotes are not just tolerant to these extreme, but actually require the environmental extreme to grow. Because of this, these prokaryotes have been referred as extremophiles (Frontiers in Microbiology, Part-III, 2003).

Overall some of the microbes are heat adapted, other species have adapted to the cold. Scientists recognize two categories of microbes that live in cold environments:
psychrotolerant and psychrophilic that thrive while other known as thermotolerant or thermophilic that thrive $\sim 70^\circ\text{C}$ (Gewin, 2006; Kasting and Siefert, 2002).

1.2 Bacteria in mine environment and their role in environment build up

Coalmine wasteland represent one of the most degraded environment with extremely hostile environment. Quite obviously such wastelands make barren, sterile landscapes that can not support life. Such wastelands are normally characterized by highly acidic condition and with pH in the range of 1.1 to 2.4. This apart such wastelands contain diverse sulfur compounds, toxic heavy metals. Moreover in such wastelands organic matters are negligible to nil, making them extremely nutrient poor. In such unfavourable condition the initial inhabitants are extremophilic bacteria like acidophilic microbes (Hallberg and Johnson, 2001a), mineral oxidizing prokaryotes (Kelly and Wood, 2000), iron and sulphate reducing acidophiles (Bridge and Johnson, 1998, 2000) and other acidophilic microorganisms (Johnson, 1985). The distribution of chemolithotrophs and heterotrophs in pyretic coal spoil samples with pH 1.1 to 2.4 has been worked out by Belly and Brock (1974).

The impact of microbes on earth’s environment have been continued to be substantial. The sheer number of microbes on earth is staggering. It has been estimated that we share the planet with some $5\times10^{31}$ microbes, which weigh more than 50 quadrillion metric tons. Put another way, microbes constitute about 90 per cent of the earth’s biomass, excluding cellulose, and more than 60 per cent of the biomass when cellulose is included (ASM, 2004).

These diverse microbes are responsible for cycling the elements essential for life, such as oxygen, nitrogen, carbon, sulfur and hydrogen. By making these elements available in soils, microbes increase its fertility and make it suitable for plant growth. The plants, in turn, are the producers of our ecosystem, providing the energy needed by animals, including human. Microbes also cycle the gases in our atmosphere. Apart from photosynthesis by higher plants microbes also contribute substantially for generation of atmospheric oxygen. The cyanobacteria (formerly called blue-green algae) can live aerobically or anaerobically. They are responsible for the initial rise of atmospheric oxygen around 2.3 billion years ago (Kasting and Siefert, 2002). Eukaryotic algae and
land plants acquired their photosynthetic capabilities from cyanobacteria through endosymbiosis. Microbes are also major recyclers of other atmospheric gases like water vapour, carbon dioxide, nitrous oxide, methane and ozone that contribute to the earth’s “greenhouse effect". However, since 1980s accumulation of excess greenhouse gases through human activities has resulted in global warming which is a global concern. Besides these bacteria had great impact on us from food processing to paper manufacturing, oil recovery, drinking water, cooling water, biological control and in plant productivity etc.

1.3 Characterization of bacteria

Characterization or taxonomy is the theory and practice of describing, naming and classifying earth’s diverse organisms. Some people regard taxonomy as rigid and uninteresting. In fact, it is a dynamic discipline that responds to our ever-changing understanding of earth’s biodiversity. As mentioned previously, over the past several decades it has become apparent that the majority of biodiversity is found among microbes. This realization has caused taxonomists to change their classification schemes in light of new information.

Traditionally, scientists have studied microbes by obtaining samples from the environment and taking them back to the lab, where, by trial and error, they attempt to identify growth conditions that allow the microbes to be cultured indefinitely. Subsequently all the strains were allowed for gram staining, morphological characteristics, growth characteristics and biochemical characterization. All together these experiment, an isolated strain being characterized. Not only this procedure time consuming and costly, but it also excludes many species of microbes for which suitable growth conditions cannot be found (Gewin, 2006). New techniques associated with genomics are giving scientists approaches to study microbes in their natural environments and to characterize to the species level (Shreeve, 2004; Venter et al., 2004; Torsvik et al., 1990). This suggested that the sample might have contained the genomes of 7000 different taxa.

Gans et al. (2005) realized that the pattern of DNA reassociation reflects the underlying diversity of the microbial community. They applied new mathematical analyses to existing data from bacterial communities and came to a startling conclusion.
Each 10 grams of healthy soil contains 10 million different bacterial species. Most of this diversity is found among rare bacteria that are present in small numbers (Gans et al., 2005).

The overwhelming number of species found within microbial communities makes it impossible to study them using bacteria cultured in the laboratory. It is estimated that 99 per cent of the bacterial species found in the soil cannot be cultured in the laboratory (Gewin, 2006). Metagenomics is the name given to the study of gene function and interaction regardless of species within large microbial communities. Researchers today rely on methods of genomics to study the diversity and interactions that take place within these communities. The use of whole genome shotgun sequencing gives an indication of the diversity that resides within a microbial community (Venter et al., 2004). It also allows scientists to study the community in its entirety, almost as thought it (Pennisi, 2005; Torsvik et al., 1990).

1.4 Role of bacteria in bioremediation and eco-restoration

In broad sense bioremediation means improvement of an environment, mostly land and water bodies contaminated with various harmful substances with the help of some living organisms, thereby restoring it to an ecologically secured and safe state and fit for habitation by average living organisms. In practice some microbes and some higher plants have been found to be the ideal biological agents for such bioremediation. Such microbes or higher plants normally effect bioremediation by absorbing and accumulating harmful substances in their body thereby physically removing the harmful substances or transforming the harmful compounds into harmless compounds. When bioremediation is brought about by some higher plants, the phenomenon is called phytoremediation (Salt et al., 1995). Several workers reported about microbes to be useful for bioremediation (Kumar et al., 1996; Sayler and Ripp, 2000; Kloepper et al., 1989; Glick, 1995; Glick et al., 1999).

Polluted wastelands are due to dumping of industrial wastes and effluents, contamination by toxic heavy metals, dumping of inorganic and organic toxic compounds etc. Mine OB sites are equivalent to such polluted wastelands. Mine OB sites are characterized by highly acidic condition, various toxic heavy metals, sulfur compounds,
rock debris and characterized by lack of water, organic matter and nutrients. Thus they give a barren appearance devoid of any living organisms except some stress tolerant bacteria with subdued growth. Microbes with bioremediation ability are sulphate reducing bacteria, metal transforming bacteria, metal accumulating bacteria etc. Since toxic wastelands are generally devoid of water, organic matter etc. it has been experimentally demonstrated that amendment of wastelands with chelating agents, organic nutrients etc. accelerate the bioremediation function of such bacteria (Quatraini et al., 2005; Sayler and Ripp, 2000).

Establishment of self sustaining soil plant system on mining sites and OB dumps as the first step for eco-restoration is a matter of great challenge for Indian ecologists. Eco-restoration of such sites requires understanding of the environment facilitating uptake, storage and cycling of nutrient and water by the reintroduced plant species. Apart from some bacteria and stress tolerant higher plants, it is known that some mycorrhizal fungi have bioremediation ability particularly through acquisition of P, Ca, Fe, Mg etc. (Rhodes and Gerdemsn, 1980).

In this context the interaction of stress tolerant higher plants and rhizosphere dwelling bacteria assume great significance. A stress tolerant higher plant can barely survive in mine OB wasteland on its own due to a combination of abiotic stress plus lack of water and nutrient. However it has been shown that the rhizosphere dwelling bacteria can generate nutrient and accumulate nutrients and water in the rhizosphere zone which facilitate growth of higher plants (Lilie et al., 2000). This in turn help in successful establishment of primary succession as the first step towards eco-restoration. The rhizosphere bacteria are also capable of removal of toxic heavy metals and other pollutants which indirectly help the growth of the stress tolerant higher plants (Glick and Pastnak, 2003). The plant microbe interactions are known to be symbiotic in nature. The roots of some plants secrete root exudates which help in the better growth of rhizospheric bacteria; this in turn facilitate the bioremediation process (Lilie et al., 2000).

Some arbuscular mycorrhizal fungi (AMF) can colonize profusely in extreme acidic soil contaminated with metals (Olivera et al., 2000). Effective vegetation, microbial activity and subsequent liming can influence the solubility, mobility and bioavailability of nutrients in heavy metal contaminated soil (Kairesalo, 2000). The gram
negative bacterial strain *Alcaligenes eutrophus* CH34 possess multiple resistances to heavy metals and thrive in soils heavily polluted with different metal ions. The recombinant strain *A. eutrophus* has enhanced ability to adsorb Cd$^{2+}$ from the soil and it can be effectively applied in contaminated soil. This strain showed the ability to decrease the toxic effects of heavy metal on the growth of tobacco plant (Lorenzo *et al*., 2000). Bacteria belonging to the genus *Pseudomonas*, among the other have been showed the potentiality to tackle mercury, nickel, cobalt and other heavy metal contaminations in pollutant effected soil environment (Reshawale, 2006).

Several *Pseudomonas, Bacillus* are capable of removal of metal pollutants like Cd from pollutant from polluted soil. Dense population of selected PGPR in rhizosphere enhance uptake of heavy metals from polluted soil and thereby decrease the total perturbations of pollutants’ toxicity of soil. Moreover, secretion of metal chelating molecules (phytosiderophores), solubilisation of the heavy metals by acidifying the soil environment can be performed by root colonizing bacteria and mycorrhizal fungi effectively (Salt *et al*., 1995).

Since stress tolerant higher plants can not grow well on its own in mine OB sites, it has been demonstrated that use of PGPR is very effective to facilitate plant growth as well as remediation of the polluted soil or mine wasteland (Cunnigham and Berti, 1995; Deka Boruah *et al*., 2004). The ability of bacteria and fungi to utilize a wide range of compounds make microorganisms ideal candidate for the bioremediation of pollutants (Garbisu and Alkota, 1999; Matson *et al*., 1997). Bacteria and fungi have a broad spectrum of metabolic capabilities, which have evolved to enable the microorganisms to utilize natural compounds as source of nitrogen, carbon and energy (Drobnik, 1999; Romantschuk *et al*., 2000).

Plant growth promoting activity of a particular microbes can affect plant growth either directly or indirectly. The direct promotion of plant growth usually entails providing the plant with a compound that is synthesized by the bacterium, such as fixed nitrogen or plant hormone, as well as these bacteria can facilitate the uptake of certain nutrients from the environment (Deka Boruah *et al*., 2003; Glick *et al*., 1999). However, such enhanced nutrition through nitrogen fixation is confined to only leguminous group of plant. The indirect promotion of plants growth occurs when plant growth promoting
bacteria lessen or prevent the deleterious effects of one or more biotic and abiotic stress factors (Deka Borua and Dileep Kumar, 2002; Deka Boruah et al., 2003).

Direct stimulation of plant growth and development by plant growth promoting bacteria can occur in several ways. The bacteria can (1) fix atmospheric nitrogen that is used by the plant. However, only Rhizobium group of bacteria are capable of doing this. (2) Synthesis of siderophores that solubilize and sequesters iron from the soil and provide it to plant cells; (3) synthesize phytohormones such as auxin, cytokinin or gibberellin, that promotes various stages of plant growth; (5) synthesizes enzymes that can modulate the level of plant hormone ethylene. Any particular plant growth promoting bacterium may utilize one or more of these mechanisms.

It has been found that the most important pathway of the PGPR promote plant growth is via synthesis of phytohormones particularly auxin and that is why this particular aspect has been the subject matter of intense research (Shah et al., 1997; Glick et al., 1998). Moreover, it has been found that some PGPR can synthesize enzyme ACC (1-aminocyclopropane 1-carboxylate deaminase) which is the key enzyme in the synthesis of phytohormone ethylene which is released to the rhizosphere zone and maintain the level of ethylene (Burd et al., 2000; Glick and Pastrnak, 1995). It is understood that better root growth means better plant growth. It has been found that the ethylene synthesis by some PGPR occur in response to abiotic stress and the ethylene in turn help in overcoming the stress by the plants. Therefore, supplementation of ethylene producing PGPR for eco-restoration purpose is gaining importance and utility (Glick and Pasternak, 2003).

Some filamentous fungi and yeasts also show high level of metals and metalloids resistance, and can accumulate the same elements. This metal tolerance by the filamentous fungi and yeast is comparable to that of metal tolerant bacteria except for cadmium (Duran et al., 1999).

Metal scavenging microbes from mine OB sites, both bacteria and fungi can be used to develop a semisolid matrix by co-cultivating with some cyanobacteria which can produce a gelatinous layer where the bacteria and fungi are entrapped. Silva et al. (2003) fixed such biofilm matrix on glass wool and it was found that when it was in contact with a solution containing toxic heavy metals like Cd, Pb, Cr, Se and As (up to
350 mg L\textsuperscript{−1} the metal were adsorbed on the surface of the biofilm due to interaction with the microbes. Use of such immobilized biofilm is emerging as a new trend in managing toxic wastes. The mine OB sites are generally a small to medium hillock. During rainy season rain water wash down the toxic compounds to downstream and pollute the adjoining fertile areas. It is possible that by erecting such immobilized biofilm in the downstream of mine OB sites that pollutants can be trapped and thereby saving the adjoining fertile areas.

In the present investigation of isolation and characterization of bacterial strains of coalmine OB sites of North Eastern Coalfields, Coal India Limited (NECF-CIL), Margherita was taken with an aim to investigate the potentiality of the bacterial strains for bioremediation of the OB sites. The objectives of the investigations are:

1.5 Objectives of the present study

1. Isolation, screening and characterization of the coalmine pollutant tolerant rhizobacteria strains.

2. \textit{In situ} assessment of the plant growth promotion activity of the potential rhizobacteria strains both in coalmine stress condition and normal condition.

3. Assessment of the collected strains for mine OB tolerance.

4. Study the biochemical changes of the plant due to mine OB and other stress.

5. Molecular characterization of selected strains and study of plant microbe interaction.