Fungi play a major role in soil ecosystems and are the principal decomposers of forest litter or dung, fruits or other organic materials (Carlile et al., 2001). Majority of the soil fungi are well known as saprobes, decomposing organic matter and contributing to nutrient cycling, while several species form mycorrhizal associations and also act as harmful agents as plant and animal pathogens (Martins et al., 2007). Several fungal species produce bioactive compounds, secondary metabolites and chemical models having pharmaceutical importance (Suay et al., 2000; Zhang et al., 2009). There are about 23,000 known secondary metabolites, 42% of which are produced by Actinobacteria, 42% by fungi (e.g. Penicillium spp.) and 16% by other bacteria (Kutzner, 1986). The Penicillium spp. is among the most commonly occurring and economically important members of them. However, documentation of microbial diversity of the virgin places of Indo Burma Biodiversity hot spot was very scanty. North east India is one of the centres of mega biodiversity region and possesses a vast potential of undiscovered organisms including Penicillium spp. (Myers et al., 2000).

Penicillium is an ascomycetous fungal genus with widespread occurrence in most terrestrial environments. About two hundred species are well described and most of them are soil inhabitants, food borne contaminants or food ingredients used in the preparation of cheese and sausages (Pitt et al., 2000; Houbraken et al., 2010). The Penicillium spp. are among the most commonly occurring and economically important members of microfungi family. Although much is known about Penicillium physiology and mycotoxin chemistry, one of the main challenges is in the area of rapid and reliable
identification of Penicillium in many settings including community health care, occupational health and food safety (Scott, 1977; Cruz-Perez et al., 2001; Meklin et al., 2004; Portnoy et al., 2004).

Many isolates produce diversified active secondary metabolites, including antibacterial (Rancic et al., 2006; Larsen and Knochel, 1997), antifungal substances (Jayashree and Sivagurunathan, 1999), immuno-suppressants and also potent mycotoxins (Frisvad et al., 2004). Although many Penicillium isolates have probably been screened in bioprospecting programs, and new bioactive metabolites continue to be discovered (Larsen and Knochel, 1997; Maskey et al., 2003), indicating their current importance as sources of high amounts of novel bioactive molecules to be used by pharmaceutical industry.

In the present study, we have isolated a number of Penicillium spp. from various locations together with its associated mycobiota from virgin soils and also screened for novel fungal natural products targeting at metabolites with biotechnological applications for the pharmaceutical industry (Bordoloi et al., 2001). The soils studied here derived from different locations in Brahmaputra Valley of Assam, India.

Soil fungal diversity depends on a large number of factors of the soil such as pH, organic contents, and moisture (Rangaswami and Bagyarat, 1998). The present study was undertaken to study the common fungal diversity from different virgin forest floors of different ecosystem specially to isolate the different Penicillium species. Diversity was found to be higher in the undisturbed land in summer seasons. Among the various genera of soil fungi of different soil collected sites, Penicillium and Aspergillus was the
most common genera that was distributed in all the types, indicating that it adapts easily
to different environment as well (Wahegaonkar et al., 2011).

It was already reported that *Penicillium* spp. are known as ubiquitous and
opportunistic saprophytes and as such, they receive their nutrition through the
decomposition of (mostly) plant material in the soil for which most of the species are
present in the soil or degraded materials. *Penicillium* is an important cellulose
decomposing fungus common in tropical forest soils (UmaDevi and Manoharachary,
1987; Mamtaz and Mishra, 1991). Limited knowledge was available on the abundance
and diversity of soil mycobiota from tropical virgin forest soils in North East India. This
study will enrich the microbial databank from this part and the availability of the major
dominant fungal flora especially in the undisturbed habitat.

Several isolated genera viz. *Fusarium, Curvulari, Alternaria* etc were involved in
strong fungal associations and have dominant adaptative features as primary colonizers
probably due to their capacity for the rapid invasion of the available substrate
(Frankland, 1981). However, seasonality is one factor that was believed to affect the
fungal community structure (Seephueak et al., 2010). Diversity of fungi communities in
forest floor varied season wise although it is still unclear how the seasons affect fungal
communities (Kennedy et al., 2006). As the occurrence of fungal species was regulated
primarily by season which may be cause and effect operates via humidity and
temperature (Nikolcheva and Bârlocher, 2005). The samples collected in the summer
season tended to be richer in species diversity and had a higher Shannon diversity index
than samples collected in the dry season. On the contrary, Kodsueb et al. (2007)
reported that the diversity of saprobic fungi on litter samples collected in the dry season
had greater species richness than samples collected in the wet season, which suggest a humidity factor. Rayner and Todd (1979) also reported a greater variety and number of fungi during the dry season. High humidity was needed for the germination and dispersal of fungi (Pinnoi et al., 2006) and hence diverse population of fungi were reported in the summer. In this study we have shown that fungal communities during the wet season were more diverse. Thus, many factors affect the changes in community structure; for instance, the microclimate of the growing area, biological interaction within leaf litter, or substrate, microhabitat preference and host preferences (Lodge, 1997).

The results obtained from the study clearly indicated that there was a marked decrease in the number of colonies with decreasing of soil micro and macro nutrients and moisture (Gomez et al., 2007) during winter. All the macro and micro nutrients are supposed to increase in the summer rain and enhance the nutrient budget in soils and hence the highest numbers of fungal colonies were isolated during these seasons. Population of soil fungi was also affected by climate (Cabello and Arambarri, 2002). Natural moisture limitation during winter drought can constitute a stress for microbial communities in soil.

The soil mycobiota of the sampling sites had a distinct pattern of fungal community structure during the study period. The percentage composition and rank abundances of different fungal species fluctuated. The majority was from the genus *Penicillium* and *Aspergillus* and then other species in order of dominance. Earlier reports have indicated that, these genera appeared abundantly in soils (Mohanty and Panda, 1994b, Panda et al., 2007). This may be due to the faster growth rate of these
fungi in addition to their better intrinsic prolific sporulating capacity to utilize the substrate.

Suhail et al. (2006) reported the distribution of *Penicillium* spp. from the river bank of Pakistan and twenty four soil samples were collected from surface and the fungi were isolated by using soil dilution and soil plate method. Of the 73 strains of fungi isolated, 10 species of *Penicillium* viz., *P. caesicolum* (1.81%), *P. commune* (1.81%), *P. chrysogenum* (14.73%), *P. funiculosum* (28.36%), *P. lilacinum* (4.33%), *P. notatum* (12.53%), *P. sclerotiorum* (2.52%), *P. tardum* (26.47%), *P. vinaceum* (5.51%) and *P. roseo-purpureum* (1.89%) were reported which is less than the present report in this study. Greater numbers of species were isolated on soil plate method than dilution plate method although the dilution plate method was used in the present study.

During this study, 32 *Penicillium* species were identified from all the sample collected sites (S1-S7) out of 252 *Penicillium* isolates. It was observed that more species of *Penicillium* were present during the rainy seasons from surface and top soil. *P. chrysogenum, P. citrinum, P. digitatum, P. oxalicum and P. italicum* were the dominant species in all the soils under study. These species have also frequently been isolated in various soils in India (Behera and Mukherji, 1985; Mamtaz and Mishra, 1991; Mohanty and Panda, 1994a; Manoharachary et al., 2005; Panda et al., 2007). Their role as decomposers has also been reported (Umadevi and Manoharachary, 1987; Mohanty and Panda, 1994a). Of the 252 species isolated, about 10 were common to all the sites while a few were restricted in their distribution. In fact, the numbers of restricted species were more in the plantation site of deep forest. It was observed that more species of *Penicillium* were present in the site with higher dense vegetation
covered area than the sand dunes site. It may be due to low competition with other categories of fungi which are less abundant in monoculture plantations. The evenness index indicated that, species were of fairly even distribution. The surface soil of the sites had the highest species richness whereas sub-surface soil showed the lower species richness.

PCR-based DNA fingerprinting techniques such as randomly amplified polymorphic DNA analysis (RAPD) and amplified fragment length polymorphism (AFLP) represents a very informative and cost-effective approach for assessing genetic diversity of a wide range of organisms (Savelkoul et al., 1999, Vos et al., 1995; Williams et al., 1990). The usefulness of RAPD for typing *Penicillium* species has been confirmed in several studies. Boysen et al. (1996) discovered and described *P. paneum* as a new species divided from the blue cheese starter culture *P. roqueforti* using different typing methods including RAPD analysis. Likewise, Dupont et al. (1999) used RAPD typing as a tool for identification of the cheese and sausage starter cultures of *P. camemberti* and *P. nalgiovense*. However, RAPD had also been useful for discrimination between *Penicillium* isolates below species level. Lund and Skouboe (1998) characterized the genetic diversity and relatedness of *P. commune* and *P. caseifulvum* isolates and they concluded that RAPD was a very robust and reproducible method for differentiating between isolates belonging to the same species. Similarly, Mekha et al. (1997) demonstrated that *P. marneffei* isolates from Bangkok were different from a *P. marneffei* isolate from China using RAPD and Geisen et al. (2001) characterized different genotypes within *P. roqueforti* using RAPD profiles.
It was very difficult to discriminate the *P. chrysogenum* isolates with the morphological characters in different media. Production and non-production of secondary metabolites in cultural medium could be a rough starting point for grouping the isolates. In this study, morphological and cultural characteristics together with metabolite profiles were considered which was much difficult. But the RAPD analysis was much easier with appropriate primers to distinguish the isolates. In the present study nine different *P. chrysogenum* isolates with distinct morphological characteristics were distinguished by RAPD typing although for proper discrimination of *P. chrysogenum* isolates, more primers should be used.

Rafi and Rahman (2002) isolated the indigenous *P. chrysogenum* series from different samples comprising of fruits, vegetables, bread and grains. Slide culture method was adopted for the identification of fungal isolates. Only two isolates, one from spoiled mango and other from maize were found closely related to *P. chrysogenum*. But in the present studies, 9 stains of *P. chrysogenum* were isolated from the sampling sites and characterized. Among them, a few were similar as shown in the Jaccard’s genetic dissimilarity co-efficient index. Isozyme analysis of these species was showed variation in enzyme electrophoresis for peroxidase and *α*-esterase isozymes. The technique has already been adopted and applied in various domains of microbial research. Isozymes have been applied to several taxonomic problems in mycology (Micales *et al.*, 1986; Rosendahl and Sen, 1992) and isozyme patterns have previously been used in the taxonomy of Penicillia aszymograms of extracellular enzymes (Cruickshank and Pitt, 1987; Pitt and Cruickshank, 1990).
It was observed that a total of four isozyme bands were formed after activity staining for enzyme $\alpha$-esterase and three isozyme bands for peroxidase. There were no variation in band number and position in all the samples in the activity staining of peroxidase. Thus, staining with the activity staining for peroxidase was not suitable for distinguishing the *P. chrysogenum* isolates. However, $\alpha$-esterase enzyme activity staining was efficient for typing of the *P. chrysogenum* strains which was comparatively low cost with high efficiency and reliability.

All the species of the *Penicillium* have some significant importance for their industrial, pharmaceutical, bioremediation and decomposition application. In this study, we have screened out those *Penicillium* species for their antimicrobial activity against a few clinical gram positive and gram negative bacterial species by their secondary metabolites production. About ten *Penicillium* species showed potential antimicrobial activity against the tests pathogens.

The discovery of *P. notatum* by Alexander Fleming and the production of the revolutionary drug, penicillin, was perhaps the most important finding in the history of therapeutic medicine. Efforts at improving penicillin yields have centred on growth optimization, development of available strains of *P. chrysogenum* by classical mutagenesis procedures, and the search for better strains of the organism. Other than *P. chrysogenum* a number of *Penicillium* species are known to produce antimicrobial agents against clinical bacterial pathogens. *P. chrysogenum* was present in abundance in soil, air and other terrestrial habitant including fruits sample and it was commonly isolated from the soils and other natural resources together with the fruit samples.
(Kumar and Kashyap, 2003). The species is a well know penicillin producing fungus and has clinical and industrial importance (Patnaik, 2001; Paul and Thomas, 1996).

In this report, a number of strains were isolated and screened for their antimicrobial production potential from the virgin forest floor of Assam. Antibacterial activity against clinical bacterial pathogens of more than hundred isolates was tested quantitatively by disc diffusion methods. Quantitative measurement and confirmation of penicillin G production was done by HPLC method. It was also reported that *P. chrysogenum* was not only species of the genus *Penicillium*, which is capable to produce the penicillin antibiotics. There were several species of *Penicillium* which were able to produce penicillin such as *P. notatum*, *P. nalgiovense*, *P. dipodomys*, *P. griseofluvum* and *P. flavigenum* (Anderson and Frisvad, 1994; Frisvad et al., 1987; Liach et al, 2002). Other than Penicillin, there were some other secondary metabolites produced by *Penicillium* species which have the antimicrobial activity against clinical pathogens. *P. verrucosum* reported to be produced antimicrobial compounds e.g. patulin and penicillic acid against bacterial pathogens (Young et al., 1998).

Laich et al. (2002) reported that three genes (*pcbAB*, *pcbC* and *penDE*) were responsible for the production of penicillin antibiotic in the genus of *Penicillium*. It was observed that all isolates of *P. chrysogenum* did not produce penicillin G because those isolates might not have penicillin G producing gene. Although, *P. chrysogenum*, *P. nalgiovense* and *P. griseofulvum* are most common penicillin producing strains, only *P. chrysogenum* is used for the bulk production of penicillin in different industries (Prescott and Dunn, 1959; Patnaik, 2001).
Hillenga et al. (1984) reported that *P. chrysogenum* was able to synthesize penicillin with specific hydrophobic side chains, when the appropriate precursor was fed to the production medium. Due to the development of drug-resistance in bacteria, several studies have been conducted with different concentrations of various chemicals to improve the quality of penicillin antibiotic (Kumar et al., 2005). The production of penicillin was also depended upon the strains of the *P. chrysogenum*, medium for production and its process parameters. Some species of *Penicillium* have been frequently isolated from food and food products (Wolf, 1997). *P. chrysogenum* and *P. nalgiovense* are known as penicillin producers; the latter has the ability to produce penicillin when it grows on the surface of meat products and secreted it into the medium (Pestka, 1995).

The recombinant strains produced higher amount of penicillin and several workers have been working to enhance the production of penicillin (Muller et al., 1992; Theilagaaed et al., 2001; Liach et al., 2002). It is already standardized the process parameters including the medium composition for production of industrial penicillin in large scale (Muniz et al., 2007). However, in the present study only laboratory based media were used for production of the bioactive metabolites as a whole and optimized biomass and pigment production parameters for a few *Penicillium* spp.

Soil is an efficient medium for improvement in the quality of penicillin G produced by the fungus, *P. chrysogenum*. On the basis of geographical distribution and isolation, it is clear that soil quality and disturbance affects the potentiality of penicillin production. The virgin forest floors had higher number of potential isolates of *P. chrysogenum* compared to the disturbed lands (Kumar et al., 2005).
A method for the HPLC determination of penicillin has been well stabilized that has several advantages. HPLC system is a rapid, sensitive and selective method for determination of penicillin antibiotic. Recently, HPLC technique for the analysis of antibiotics have been developed and also applied to the determination of residual antibiotics in foods (Terada and Sakabe, 1985; Oka et al., 1987; Muller et al., 1992; Theilagaaed et al., 2001; Liach et al., 2002). In the present studies also HPLC method was used for quantitative analysis and confirmation of penicillin G produced by soil isolates of *P. chrysogenum*.

Besides penicillin, different species of *Penicillium* also produced industrially importance secondary metabolites and pigments having industrial and clinical importance. There is worldwide interest for the production of pigments from natural sources due to a serious safety problem with many artificial synthetic colorants, which have widely been used in foodstuff, cosmetic and pharmaceutical manufacturing processes (Kim et al., 1995). For industrial applications of microbial secondary metabolites and pigments, higher production of pigment yield, chemical and light stability are essential features. Isolation of new strain is still of particular interest because of necessity to obtain microorganisms with suitable characteristics for submerged cultivation (Rasheva et al., 1998). In this study, three different *Penicillium* species has been utilized having antimicrobial activity together with colour pigments production to isolate and screen the efficient pigment producers. For commercial application, optimization of fermentation condition in order to produce more yield and stability of pigment from *Penicillium* is necessary.
Other than *Penicillium* species, several microorganisms produce secondary coloured pigments although only a few are available in sufficient quantities to be useful for industry because they are usually extracted from plants (Lauro, 1991). *Penicillium* species produced a much diversified array of active secondary metabolites, including antibacterial (Rancic *et al*., 2006; Lucas *et al*., 2007), antifungal substances (Nicoletti *et al*., 2007), immunosuppressants, cholesterol-lowering agents (Kwon *et al*., 2002), and also potent mycotoxins (Frisvad and Samson, 2004). In this study, we have been screening a collection of filamentous fungi isolated from virgin forest soils of Brahmaputra valley of Indo Burma Biodiversity hotspot region for novel fungal natural products targeting at metabolites with biotechnological applications for the pharmaceutical industry. Three most active extracts producing coloured secondary metabolites was obtained from *Penicillium* isolates.

It was also reported that the growth medium, its pH and temperature had strong influences on the growth, sporulation and conidial discharge of the fungal species (Vogelgsang and Shamoun, 2002). The three *Penicillium* species produced maximum number of secondary metabolites in appropriate fermentation condition. Temperature, water activity (aW) and pH were considered to be some of the most important factors in fungal growth and differentiation (Mcmeekin and Ross, 1996). In the present study, optimal culture conditions for the production of red pigment by *Penicillium* species were investigated in shake flask and batch fermenters.

Northolt and Bullerman (1982) reported that the growth of fungi depends on the composition of the growth media, water activity (aw), pH, temperature, light, and the surrounding atmospheric gas mixture. Devi *et al*.(2009) reported that PD medium at pH
6.5 and temperature 27±2°C, *P. chrysogenum* produces citrinin as secondary metabolites under batch culture. Optimum concentration of pigment was produced during the stationary period of growth phase. It is therefore essential to monitor the growth of the organism simultaneously with the metabolic production for optimal yield of secondary metabolites. However, the results of this study revealed that both the secondary metabolites and culture characteristics including mycelial weight, colony diameter and number of spores of these three *Penicillium* isolates were significantly affected by the type of the growth medium. Cho *et al.* (2002) reported that because the production of secondary metabolites or the pigments was affected by environmental conditions and the production is restricted to certain conditions.

The effect of environmental factors on growth of fungi is generally less specific and restricted than the effect on secondary metabolite production. By optimization of culture conditions of *Penicillium* isolates, red pigment production can be improved by seven fold under submerged fermentation as reported by Gunasekaran and Poorniammal (2008). The higher concentration of pigment production by *Penicillium* spp. favours for commercial production of pigments.

From the above findings, it can be concluded that the exploration of microbial diversity from the virgin forest floors of Assam especially from the sample collected sites is not only a challenge but also a promising job in order to exploit them for pharmaceutical, industrial and agricultural importance. From the sample collected sites as much as 32 species were collected which have the economic importance and need to characterize properly for their beneficial utilization. It was also confirmed that diversity index in the sites were higher which require conservation of the species in *in situ*
condition. Different type of *P. chrysogenum* isolates were collected and had genetic diversity as revealed by RAPD and isozyme analysis and may the source of novel strain for penicillin production. Other than *P. chrysogenum*, three other species which produced coloured secondary metabolites may be useful for industrial application and industrial pigment production. Among the all isolated species, novel strains may be screened out for their pharmaceutical and industrial application.