CHAPTER I

INTRODUCTION
Early man derived his requirements from nature like any other animal. He was a hunter of animals and gatherer of plants. With the advent of modern civilization man became moulder of environment, disturbing the living crust of the earth and changing it from self sufficient life supporting system to a mere system of resources for themselves. Life could originate and flourish on earth only because the environment of this planet provided conducive conditions for it.
The perturbances in the living crust due to "civilization" cause loss of productive land resources, a loss that a developing country like India can ill afford. It is estimated that at least one third of the geographical area of the country is degraded and about one half of the forest area has poor or no forest cover (Ghosh, 1990). The loss of top soil due to deforestation is a more serious matter since it threatens to undermine the very means of our support, the biggest threat being from wind and water action. We cannot replace the lost soil since it takes centuries for the natural process to occur.

Loss of vegetation is due to a variety of reasons, one of which is shifting cultivation. It is believed that about 250 million people, thinly scattered over 300 million hectare of forest land of tropics, still follow an ancient form of agriculture which involves slashing and burning of vegetation, followed by the cultivation of crops (Goodland, 1980). This system of agriculture, characterized by rotation of fields rather than crops, is variously called in different regions eg. Podu in Orrisa, Deppa in Bastar, Dahia in Madhya Pradesh and Jhum in North East.

In Jhum cultivation the entire vegetation, including large and small trees are felled during the dry season. Large boles and branches are removed from the site and used as firewood. The slash is allowed to dry on the ground during the winter months which are rainless and burnt in situ whereas larger logs may
be heaped up and burnt a few times. After the first shower in April/ May weeding is done followed by the sowing of a mixture of crops by dibbling the seeds using a digging stick. Eight to thirteen crop species are grown together on a single field by the Garos. The practice followed by the Khasi tribe is a modified version of the typical type in that normally only lower branches of the sparsely distributed pine trees are cut instead of the whole tree, and seeds dibbled directly into the soil-ash complex (Mishra, 1981). Fertilizers are not used and rapid depletion of the fertility of the fields, which are often too steep to hold soil, water and nutrients, compels the farmer to shift to new site. These practices, and others created due to mine spoils etc., offer a hostile environment for plant growth and revegetation at some sites is often very difficult (Carpenter and Hensley, 1979).

In most forest soils, sufficient nitrogen is not present in a form that can be taken up and metabolized by plants to support optimal tree growth. Thus nitrate, urea or ammonia are the nutrients most frequently added to forest soils to increase their productivity (Gordon and Dawson, 1970). Industrial production of fertilizers is an energy demanding process and moreover the cost of nitrogen fertilizers has increased sharply. It should be noted here that farming, especially high tech one, consumes a sizable quantity of fertilizers.

Nature provides a way out for the nitrogen deficiencies under
natural conditions. A few free living bacteria like *Clostridium pasteurianum*, *Klebsiella pneumoniae* etc. fix nitrogen present in the atmosphere. Many cyanobacteria produce a characteristic cell called as heterocyst, which has hyaline contents and a thick, refractive wall. The firm identification that heterocysts as the site of nitrogen fixation and of the nitrogenase activity was given by Stewart *et al.* (1969). Nitrogenase enzyme comprises of a molybdenum and iron containing protein (dinitrogenase-1) and an iron containing protein (dinitrogenase reductase-1). Alternative nitrogenases have also been noticed and for further reading one is referred to the review article of Bishop *et al.* (1988) and Pau (1989). The fixed nitrogen is directly utilized by higher plants and could play a substantial part in the earth's nitrogen cycle (Torrey, 1978). In fact in *Gunnera-Nostoc* symbiosis the host *Gunnera* may rely completely on the cyanobacterial partner for the supply of combined nitrogen (Silvester and Smith, 1969). Besides cyanobacteria, bacteria of the genus *Rhizobium* fix atmospheric nitrogen in symbiotic association with roots of leguminous plants. The resulting, symbiosis occurs in a specialized organ, the nodule. The nature of nodules was not understood until 1886 when Hellriegel was able to demonstrate the ability of the legumes to take up atmospheric nitrogen. This facility is actually provided by assimilating bacteria in the nodules. In another type of symbiosis, diverse woody dicotyledenous plants have established symbiosis with an actinomycete called *Frankia*. *Frankia* sp. nodulated plants have
been assigned to 25 genera distributed among eight families of dicot woody species and include over 170 species. The list continues to grow. The compatibility between host and actinomycete is evidenced by the formation of root and stem nodules. Examples of such a type of symbiosis include genera Comptonia, Elaeagnus, Casuarina, Myrica, Hippophae, Alnus etc.

Alnus, commonly known as alder, belongs to the family Betulaceae comprising of six genera (Betula, Alnus, Corylus, Ostrya, Carpinus and Ostryopsis) (Lawrence, 1967). Of these six genera only Alnus forms symbiotic association with Frankia. The root nodules are mainly found 1-3 cm below the topsoil and correspond to "Alnus type" nodule (Zhongze and Torrey, 1985). The nodule type is specified by the host. Similar to other actinorhizal plants, the symbiont resides in the cortical cells of root nodules. Within the cortical cells the symbiont generally differentiates into hyphae and vesicles. In some cases sporangia too are seen and this is reported to be a genetic trait of the endophyte (Lumini et al., 1992).

Nitrogen fixing rate of Myrica gale was recorded to be comparable to annual legumes under similar laboratory conditions (Bond, 1951). N₂ fixation rates as high as 260 kg/ha/y have been reported for Casuarina equisetifolia (Dommergues et al., 1984). At this juncture one should note that nitrogen fixation is a highly energy requiring process. As much as 18.8 g of glucose is needed to fix 1 g of nitrogen (Gutschick, 1978). Therefore, the
plant sacrifices metabolic energy which otherwise could be utilized for potential yield (Bormann and Gordon, 1984). Nevertheless, the great contribution of Frankia – actinorhizal plants in nitrogen cycle in forests is quite obvious.

Extensive investigations on all aspects of Frankia – actinorhizal plant symbiosis have been taken up in the last fifteen years. Unfortunately very little work has been done on Frankia isolates from India. Not only this, although a great volume of information has been gathered the world over on morphological, physiological and molecular characterization of isolates, no comprehensive work has been taken up with a view to collect germplasm and screen it for better strains. If one intends to collect germplasm and screen it for genetic diversity, one should minimize, as far as possible the environmental component of total phenotypic variation. Moreover, some proven traits, that have limited or identified environmental dependence should be studied.

The fact that the genus Alnus is thought to have originated in the Indo-China region (Furlow, 1979) prompted us to hypothesize that there could be genetic diversity for alder compatible frankiae in the region. Consequently the present study was taken up with the aim of collecting and screening for alder compatible Frankia (called Frankia alni by Lalonde et al., 1988) germplasm from Meghalaya.
The investigation carried out could be grouped into two distinct classes:

1. Collection, isolation and confirmation of identity of the endophyte from *A. nepalensis* (Don.) root nodules. Specially in view of the fact that no report of isolation of *Frankia* from this species was available.

2. Screening the isolates for genetic diversity, for which certain morphological, physiological, molecular and genetic parameters listed below were chosen:

    A. Morphological parameters:
        i. Presence or absence of vesicles under different conditions
        ii. Hyphal diameter
        iii. Vesicle diameter
        iv. Presence or absence of sporangia

    B. Physiological parameters:
        i. Growth response in different culture media
        ii. Preferences for carbon sources
        iii. Responses to various nitrogen sources
        iv. Nitrogenase activity under varying culture conditions

    C. Molecular parameter:
        i. Presence or absence of plasmids
D. Genetic parameters:

i. Search for antibiotic resistance genes as markers on plasmids

ii. Possible location of \textit{nif} genes on plasmids