

# INTRODUCTION



## **1. INTRODUCTION**

Leguminosae is one of the largest families of Angiosperms, which include three sub-families, now raised to the rank of families namely Fabaceae, Caesalpiniaceae and Mimosaceae. They include above 600 genera and 18,000 species. Eventhough woody and tree species are represented in all the three sub-families of Leguminosae, the bulk of the tree species are found in Mimosaceae (Hutchinson, 1964; Heywood, 1971; Allen and Allen, 1981). The oldest agricultural records available indicate that leguminous trees have been cultivated for centuries and they were valued for fuel, fodder and soil enrichment. Long before, their ability to work symbiotically with bacteria was understood (Heywood, 1971). Legumes are the macrosymbionts of bacteria, the microsymbionts that form root nodules and fix atmospheric nitrogen indirectly increasing the crop yield.

The endosymbiotic association reduces the dependency of agricultural crops on fossil fuel-derived nitrogenous fertilizers. The high cost of fertilizers, rapid depletion of non-renewable energy sources, release of pollutants during fertilizer production, disruption of nutrient balance in soil, leaching of nutrients into ground water and pollution in surface water has emphasized the need of bacterization to increase productivity in legumes. Growing concerns about the environment, energy, nutrition and agriculture and forestry sustainability make the need for biological nitrogen fixation research even more compelling.

The role of tree legumes in forest management has assumed special importance in the present context of energy crisis faced acutely by the developing nations. The plantations of the tree legumes on waste lands

provide food, fodder, fuel and timber besides improving the soil fertility through the natural association with *Rhizobium*. Establishment of the *Rhizobium*-plant symbiosis is a complex process. Plant symbiosis is based on a few selected systems, which may not cover the whole scale of *Rhizobium*-plant symbiosis occurring in nature. With the isolation and characterization of new isolates, there is an urgent need to update the taxonomy of *Rhizobium*.

For many decades, the taxonomy of the Rhizobiaceae received only sporadic attention (Graham, 1964). Then the early 1980s saw the separation of the legume root nodule bacteria into two genera, fast growing *Rhizobium* and slow growing *Bradyrhizobium* (Jordan, 1982; Kreig and Holt, 1984). Index of the bacterial and year nomenclatural changes records four genera of stem and root-nodulating rhizobia. They are *Rhizobium*, *Bradyrhizobium*, *Azorhizobium* and *Sinorhizobium*. Phylogenetically, Rhizobia belong to the alpha-2 sub-class of the proteobacteria and six genera have been recognized as *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium* and *Allorhizobium* (de Lajudie *et al.*, 1998).

The cross inoculation grouping is a convenient and workable method of classifying root nodule *Rhizobium* into species. Speciation of the *Rhizobium* is historically based on host specificity (Fred *et al.*, 1932). Though cross inoculation is an important taxonomic features for identifying the species, it alone can not provide a satisfactory basis for the same. Attempts have been made from time to time to differentiate species on the basis of growth reaction on defined substrates, morphological, ecological, physiological characters and DNA base composition (Graham, 1964; Mannetje, 1967; Vincent, 1970).

The best classification schemes group organisms, that are related through evolution and separate those that are unrelated. At present, the classification schemes for bacteria is based not only on evolution, but on convenience, consisting of readily determined characteristics such as morphological, physiological, ecological and biochemical nature of the organism. The most recent and convenient method of determining the evolutionary relatedness between organisms is based on comparing the protein, plasmid and fatty acid profiles in addition to DNA sequencing. Unexpected relationship between various bacteria have been revealed by comparing their 16S ribosomal RNA sequences. The study of proteins, plasmids, fatty acids, nucleic acids are either direct gene products or the genes themselves, yield considerable information about true relatedness. These more recent molecular approaches have become increasingly important in rhizobial taxonomy. At present, an alternative and powerful approach, called numerical taxonomy is applied to classify and group the organisms based on their similarity co-efficient through computer has been emphasized (de Lajudie *et al.*, 1998).

There is an extreme interest concern on the nitrogen fixation in large scale on social forestry. Among the leguminous trees, *Pithecellobium dulce* Benth. (Mimosoideae), *Erythrina indica* Lam. and *Sesbania grandiflora* Pers. (Papilionoideae) are nitrogen fixing, root nodulating multipurpose tree legumes, widely recognized throughout the world for its potential in afforestation and rehabilitation of wastelands especially in tropical and subtropical regions of Tamil Nadu.

The perusal of the literature proved that there had been no extensive works on the tree legumes especially in relation to the

nodulation and taxonomy of rhizobia (Zhang *et al.*, 1991). Keeping these points in view, the present investigation is aimed in studying the characterization, identification and classification of rhizobia of *Pithecellobium dulce*, *Erythrina indica* and *Sesbania grandiflora*. It has been proposed to study the morphology of nodules, isolation of *Rhizobium*, conducting cultural techniques and cross inoculation tests, analysing the physiological, biochemical and ecological characteristics. Since the protein, fatty acid and plasmid profiles of various isolates are very useful in understanding the relationship and classification of isolates in the present investigation, it is also proposed to include these characteristics for the classification of *Rhizobium*. To classify the rhizobial isolates, the present work is interested in using the principles of numerical taxonomy. Finally it is also proposed to investigate the potentiality of the test *Rhizobium* to utilize as an effective biofertilizer.