CHAPTER 1

Introduction

Our knowledge of the beneficial association of rhizobia with leguminous plants in utilising atmospheric nitrogen (biological nitrogen fixation) is very recent when compared to the very long period that leguminous crops have been cultured and valued for food and soil enrichment (Lim *et al.*, 1986). Soil nitrogen, originating from decomposing plant residues and microorganisms, is normally deficient for intensive crop production. This is the compelling reason to increase our understanding of biological nitrogen fixation as brought about by organisms such as rhizobia. Research has shown that the biological nitrogen fixation process is the most efficient way to supply the large amounts of nitrogen needed by legumes to produce high-yielding crops with high protein content. As an example, most grain legumes can obtain between 50% and 80% of their total nitrogen requirements from biological nitrogen fixation, while some like fababean will fix up to 90% (Peoples *et al.*, 1995).

The projected doubling of the world's population over the next fifty years is expected to increase pressure for more effective food production and the need for fixed nitrogen. Supplying this demand by industrial sources will increase the required nitrogen. However, the expanded use of the biological nitrogen fixation could reduce and replace the need for industrially produced fertiliser nitrogen. In South Africa this demand for available proteinaceous food is particularly current and can be met by establishing nodulated legumes, which are able to effectively fix nitrogen, on poor agricultural soils. Effective nitrogen fixation has been effectively achieved by inoculation of legume seeds with appropriate rhizobial inoculants before planting (Jansen van Rensburg *et al.*, 1969 & 1983). According to J.B. Skeen (Fertilizer Society of South Africa, president's report, 1996) the effective introduction of suitable legumes on 75% of natural veld could lead to the enrichment of soil with an estimated 400 000 tons of nitrogen, corresponding closely to the amount sold annually in the country. The need to select strains that are more effective and competitive for application in southern African agriculture should therefore be evident.

In recent years the classification of legume root-nodulating bacteria has undergone major revisions and improvements (Jordan, 1984). This has been the result of the application of polyphasic taxonomy, a term coined by Colwell (1970), and is used for the delineation of taxa at all levels (Murray, 1990). Polyphasic taxonomy involves techniques, which have various discriminatory powers, to resolve the complex intra- and intergenic relationships of different bacterium species (de Lajudie *et al.*, 1994). The transient nature of rhizobial taxonomy is mainly due to the application of molecular techniques within a polyphasic approach. Consequently, major revisions of the taxonomic outline of the root nodulating bacteria have taken place as shown in the second edition of Bergey's Manual of Determinative Bacteriology (Garrity, 2001).

South Africa has approximately 1400 legume species, growing under diverse geographical and climatological conditions. It is therefore expected that this diversity will be reflected in their symbiotic partners. Systematic analyses of these symbionts from diverse environmental conditions will also increase the chances of finding inoculant strains suited for effective application in South African agriculture.

Previous studies (Dagutat, 1995; Kruger, 1998) into the taxonomy of the indigenous South African rhizobia focussed on isolates from a range of leguminous plants. However, this research has been limited to the analysis of growth rate characteristics, colony morphology, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of total cellular proteins, substrate utilisation patterns and 16S rDNA-RFLP. According to Graham *et al.* (1991) both phylogenetic and phenotypic (symbiotic, cultural, morphological, and physiological) traits of a relatively large number of strains should be considered when proposing new addition to the taxonomy of the rhizobia. Clearly not all of these criteria were met and a more detailed analysis of the indigenous rhizobia was needed. The aim of this study was therefore to further investigate the diversity of the indigenous rhizobia by focussing mainly on molecular genetic techniques and morphological traits (on a limited number of new additions to the existing culture collections).