

CHAPTER 9

Concluding Remarks

The value of the symbiotic relationship between rhizobia and leguminous plants is widely recognised and has led to intensive characterisation of the involved bacterial symbionts. As a consequence, the taxonomy of these bacteria has changed dramatically since Skerman *et al.* published the Approved list of bacterial names in 1980. The most recent revision was suggested by Young *et al.* (2001) who proposed the emendment of the genus *Rhizobium* to include all species of the genus *Agrobacterium* (Conn, 1942) and *Allorhizobium undicola* (de Lajudie *et al.*, 1998a). The description of new genera and species of stem- and root-nodulating bacteria is, however, governed by a set of rules or criteria (Graham *et al.*, 1991). These criteria therefore compel the taxonomist to follow a polyphasic approach in the description of new genera and species and have significantly contributed towards a stable and reliable classification of the rhizobia.

Initial studies (Dagutat, 1995) have shown that a diverse range symbionts are associated with the leguminous plant species occurring in South Africa. Although this study presented a valuable first step towards determining the diversity of the indigenous rhizobia, a single method cannot be relied upon to accurately describe the complex inter- and intragenic relationships of the rhizobia. The aim of this work, which also extends that of Kruger (1998), was therefore to further investigate the diversity of the rhizobia associated with various leguminous plants.

The inferred 16S rRNA phylogeny showed that the indigenous rhizobia were related to the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Bradyrhizobium*. Most of the indigenous rhizobia, related to the *Bradyrhizobium* genus, showed little sequence divergence from the described bradyrhizobial species. However, the 16S rDNA sequence divergence is small among *Bradyrhizobium* strains, offering limited capacity to assess the diversity among closely related strains (Willems *et al.*, 2001a). Subsequently, 16S-23S IGS-RFLP analyses were performed on such homogeneous bradyrhizobial isolates. The same approach was followed for another homogeneous cluster of isolates closely related to the genus *Mesorhizobium*. In both instances the resolution of the 16S rDNA-based phylogeny of these isolates was noticeably improved and therefore analyses of the IGS region is a valuable and complementary tool to be used in combination with 16S rDNA sequencing.

The diverse nature of the indigenous rhizobia was further indicated by isolates whose generic affiliation remained indeterminate. Furthermore, isolates related to the genus *Methylobacterium* was also identified among the indigenous rhizobia. This was in agreement with a recent report of nodulating methylobacteria (Sy *et al.* 2001).

A limited investigation of the symbiotic genomes of the indigenous isolates focussed on partial *nifH* sequencing and RFLP of the *nodC* gene. The *nifH* phylogeny agreed with the 16S rDNA-based phylogeny. Conversely, only a few of the *nodC* genotypes showed a strong host-specific association. Our inability to define the other *nodC* genotypes may be attributed to insufficient representation in the number of the different legume hosts and the robustness of the RFLP method. Future investigations should therefore include more representatives of the various host plants and sequencing of the *nodC* genes.

The bacterial symbionts of a large number of indigenous legumes have not been studied systematically. The isolation of such symbionts is a promising strategy to increase our knowledge of the systematics and evolution of the rhizobia. Moreover, many of these unexamined rhizobia may have the ability to establish symbiotic relationships with legumes that are of economic and ecological importance. This can be achieved effectively only if knowledge regarding the diversity of the indigenous rhizobial population is known. This study has contributed significantly in this regard. In compliance with the minimal standards for description of the rhizobial symbionts, both phenotypic and phylogenetic traits which include the work of Dagut (1995) and Kruger (1998) have been investigated for most of these strains. The requirement of DNA homology analyses is, however, still lacking and should be investigated.