

**Diversity of root nodulating bacteria associated with
Cyclopi species**

by

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I certify that the thesis hereby submitted to the University of Pretoria for the degree PhD (Microbiology) has not previously been submitted by me in respect of a degree at any other university.

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OPERARA AAN MY OUCERS

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DIVERSITY OF ROOT NODULATING BACTERIA ASSOCIATED WITH *CYCLOPIA* SPECIES

by

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SUMMARY

In recent years, the rhizobial taxonomy changed significantly with the discovery of novel symbiotic associations between legumes and nodulating bacteria. This was aided by the focus shift from studying only agricultural crops to legumes indigenous to certain regions, ultimately to discover new inoculant strains and to uncover the secrets of the rhizobium-legume symbiosis. In previous studies on the diversity of South African rhizobia, it has become clear that our country has a wealth of rhizobia.

Cyclopia is a legume genus, which belongs to the fynbos biome of South Africa. Honeybush tea is a herbal infusion manufactured from the leaves and stems of certain *Cyclopia* spp. Commercial cultivation of this potentially new agricultural crop is now developed to protect the natural *Cyclopia* spp. populations from harvesting and ultimately extinction. Superior inoculant strains are necessary for these commercial seedlings.

The diversity of root-nodulating strains isolated from 14 *Cyclopia* spp. was determined using 16S-23S IGS-RFLP and partial 16S rDNA base sequencing. Based on 16S-23S IGS-RFLP and partial 16S rDNA base sequencing most of the isolates, with the exception of seven strains, were found to belong to the genus *Burkholderia*. More extensive phylogenetic,

symbiotic and phenotypic studies of selected strains were performed using near full-length 16S rDNA base sequencing, *nodA* base sequencing and substrate utilisation analysis. In the genus *Burkholderia*, the isolates belonged to the novel root-nodulating species *Burkholderia tuberum* and several novel, undescribed *Burkholderia* genotypes. However, no new *Burkholderia* species could formally be proposed, since DNA-DNA hybridisation analysis, which is a prerequisite for the description of new species could not be performed in our laboratory. The seven strains not affiliated with the *Burkholderia* genus belonged to two *Bradyrhizobium* genospecies, *R. tropici* and a possibly new genus in the α -*Proteobacteria*. The *nodA* sequences of all the *Cyclopi*a isolates corresponded to a large extent, indicating that different chromosomal genotypes harbour the same symbiotic genotype. All the isolates of the *Cyclopi*a genus appear to be acid-tolerant, which is in agreement with the acidic nature of the soil from which the strains were isolated.

DIVERSITEIT VAN WORTELNODULERENDE BAKTERIEË GEASSOSIEER MET *CYCLOPIA* SPESIES

deur

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PROMOTOR: Prof PL Steyn

DEPARTEMENT: Mikrobiologie en Plantpatologie

GRAAD: PhD (Mikrobiologie)

OPSOMMING

Die rhizobium-taksonomie het ingrypend oor die afgelope jare verander met die ontdekking van nuwe simbiotiese assosiasies tussen peulplante en nodulerende bakterieë. Hierdie ontdekkings is aangehelp deur die klemverskuiwing van die studie van slegs landbougewasse na inheemse peulplante. Hierdie verskuiwing was om nuwe entstofrasse te ontdek asook om die geheime van die rhizobium-peulplant simbiose te ontrafel. In die vorige diversiteit studies van Suid-Afrikaanse rhizobiums het dit duidelik geword dat ons land ryk is aan 'n verskeidenheid rhizobiums.

Cyclopia is 'n genus wat deel maak van die fynbos bioom van Suid-Afrika. Heuningbostee is 'n kruietee wat vervaardig word van die blare en stamme van sekere *Cyclopia* spp. Die kommersiële verbouing van hierdie potensiële landbougewas word ontwikkel om die natuurlike *Cyclopia* spp. populasies te beskerm teen uitwissing agv die oes van natuurlike populasies. Effektiewe entstofrasse is noodsaaklik vir hierdie kommersiële saailinge.

Die diversiteit van wortelnodulerende isolate afkomstig vanaf 14 *Cyclopia* spp. is ondersoek deur 16S-23S IGS-RFLP en gedeeltelike 16S rDNS basis volgorde-bepaling. Al die isolate,