

**Polyphasic taxonomy of rhizobia associated with
legumes occurring in South Africa**

by

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Submitted in partial fulfilment of the requirements for the degree of
PHILOSOPHIAE DOCTOR
(MICROBIOLOGY)

in the

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February 2002

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BEDANKINGS

Opgedra aan my Ouers

BEDANKINGS

Ek wil graag my opregte dank aan die volgende persone en instansies uitspreek:

Prof P L Steyn van die Departement Mikrobiologie en Plantpatologie, Universiteit van Pretoria. Baie dankie vir Prof se waardevolle leiding en ondersteuning tydens die uitvoering en afhandeling van hierdie studie. Ek waardeer die manier waarop Prof my altyd as gelyke behandel het asook die volkome vertrouwe wat u altyd in my gestel het.

Die National Research Foundation en die universiteit van Pretoria vir finansiële ondersteuning tydens hierdie studie.

Aan al die personeel en studente van die Departement Mikrobiologie en Plantpatologie vir al jul belangstelling en ondersteuning.

Al my ander familie en vriende wie my altyd met gebede en bemoedigende woorde ondersteun het. Baie dankie dat julle daar was toe ons as familie deur ons donkerste dae moes gaan. Dollie en Stiena, julle ondersteuning het vir ons baie beteken.

Aan Marinda Oosthuizen, dankie vir jou vriendskap asook jou waardevolle insette gelewer in hierdie studie.

Aan Brigitta Steyn, Marleen Kock, Aida Bosch en Boet Weyers, baie dankie vir julle ondersteuning en vriendskap.

Aan Quentin, Melanie, Gavin, Claudelle, Phillys en Nenta baie dankie vir julle volgehoue ondersteuning en liefde.

Aan my boetie en sy gesin. Boetie baie dankie vir jou liefde en volgehoue belangstelling in my werk. Aan Nicolette, Chadwick en Kayleigh baie dankie vir julle liefde.

Aan my ouers, sonder wie se hulp en liefde ek nooit tot op hierdie vlak sou kon vorder nie. Dankie is maar 'n klein woordjie, maar weet dat julle jarelange opoffering ongetwyfeld deur hierdie studie ewigheidswaardes verkry het. Dankie vir die laataande se wakkerbly om seker te maak dat ek veilig tuis kom en die vroeë-more se broodjies maak. Baie dankie vir die waardesistiem wat u vir my

daargestel het en ook geleer het hoe om met ander te leef en te deel. Mag die Here julle ryklik seën.

Aan Hom aan wie alles bekend is, die EEN wie daar was toe die fondament van die aarde gelê is, aan Hom kom toe al die lof en prys.



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DEPARTMENT: Microbiology and Plant pathology

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SUMMARY

The advantageous association between rhizobia and leguminous plants has motivated numerous studies into the diversity and identity of the associated bacterial symbionts. This, as well as developments in molecular microbiology, has led to major revisions of rhizobial taxonomy. Previous investigations of the rhizobia, associated with various leguminous plants from South Africa, concluded that most of the indigenous strains were related to the genus *Bradyrhizobium* (Dagut, 1995; Kruger, 1998). The other rhizobial genera represented, albeit to a lesser extent, were *Rhizobium*, *Sinorhizobium* and *Mesorhizobium*. The major shortcoming of these investigations was the lack of sufficient genotypic characterisation. Since the completion of these initial investigations, a new rhizobial genus and several new species have been described. These developments and the additional isolation of rhizobia, from previously uninvestigated legumes, necessitated a more detailed analysis of the indigenous rhizobia. The aim of this study was therefore to study the diversity of the indigenous strains, focusing particularly on genotypic traits.

A selection of indigenous rhizobia was characterised by partial 16S rDNA sequencing, restriction fragment length polymorphism (RFLP) of the 16S-23S intergenic spacer (IGS) region, partial *nifH* sequencing and *nodC* RFLP. Based on 16S rDNA sequencing, most of the isolates could be assigned to a specific genus, most being related to the genus *Bradyrhizobium*. A group of isolates was also related to the genus *Methylobacterium*. The IGS-RFLP analyses were sufficiently discriminatory to indicate additional variation among isolates which showed little or no 16S rDNA sequence variation. The *nifH* phylogenetic groupings correlated well with those obtained by 16S rDNA sequencing. However, *nodC* RFLP indicated that the indigenous rhizobia carry diverse range *nodC* genotypes, with only a few showing host-specific associations. In the absence of sequence data of these *nodC* genotypes, their origin and correspondence to known *nodC* genes of other rhizobial genera, remain uncertain. Considering the results obtained here and the phenotypic characteristics determined previously, several novel *Bradyrhizobium* and *Mesorhizobium* strains were identified, however, their specific status should be validated by DNA homology studies.

**Polifasiese taksonomie van die rhizobia geassosieerd met inheemse
peuplante in Suid Afrika**

deur

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OPSOMMING

Op grond van die voordelige assosiasie tussen rhizobiums en peuplante, is talle studies na die diversiteit en identiteit van die endosimbionte geloods. Om hierdie rede, asook agv ontwikkelinge in molekulêre mikrobiologie, het daar grootskaalse hersienings van die taksonomie van rhizobiums plaasgevind. Vorige ondersoeke na rhizobiums, geassosieerd met verskeie peuplantspesies van Suid-Afrika, het aangetoon dat die meeste isolate aan die genus *Bradyrhizobium* verwant is (Dagutat, 1995; Kruger, 1998). Die ander rhizobium genusse, *Rhizobium*, *Sinorhizobium* en *Mesorhizobium* was wel teenwoordig, maar in mindere mate. Die grootste tekortkoming van hierdie studies was die gebrek aan voldoende genotipiese karakterisering. Sedert die voltooiing van hierdie ondersoeke is daar 'n nuwe rhizobiese genus, asook verskeie nuwe spesies beskryf. Hierdie ontwikkelinge en die isolering van rhizobiums vanuit voorheen onbestudeerde gasheerspesies, het 'n meer gedetailleerde ondersoek na die inheemse rhizobiums genoodsaak. Gevolglik was die doel van hierdie studie om die diversiteit van die inheemse rhizobia te bepaal deur spesifiek op meer genotipiese eienskappe te fokus.

'n Geselekteerde groep inheemse rhizobiums is dmv gedeeltelike 16S rDNS volgordebepaling, restriksiefragmentlengte-polimorfismes (RFLP) van die 16S-23S intergeniese skeier (IGS) gebied, gedeeltelike *nifH* volgordebepaling en *nodC*-RFLP gekarakteriseer. Op grond van 16S rDNA volgordebepaling, kon meeste van die isolate aan 'n rhizobiese genus toegewys word. Meeste was egter aan die *Bradyrhizobium* genus verwant. 'n Groep isolate was ook aan die genus *Methylobacterium* verwant. Die IGS-RFLP analise het ook 'n goeie onderskeidingsvermoë getoon en was in staat om addisionele verskille binne isolate, met geen of baie min 16S rDNS volgordevariasie, aan te toon. Die filogenetiese groepeerings, op grond van *nifH*- en 16S rRNS-geenvolgordes, het goed ooreengestem. Daarenteen het die *nodC*-RFLP aangetoon dat die *nodC*-genotipe van die inheemse isolate baie divers is. Slegs by 'n paar isolate is 'n sterk ooreenkoms tussen *nodC* genotipe en gasheerspesie aangetoon. Aangesien DNS volgorde van hierdie genotipes onbekend is, is dit moeilik om hul oorsprong en ooreenkoms met *nodC*-genotipes van ander rhizobiese genera te bepaal. Deur die genotipiese resultate van hierdie studie en fenotipiese eienskappe, soos voorheen bepaal, gesamentlik te oorweeg is verskeie nuwe *Bradyrhizobium* en *Mesorhizobium* rasse geïdentifiseer. Die spesies-status van hierdie rasse moet nog deur DNA-homologie analises bevestig word.

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ARC	Agricultural Research Council
ATP	adenosine-5'-triphosphate
bp	base pair
°C	degrees Celsius
ClustalX	cluster analysis version X
dNTP	deoxyribonucleoside-5'-triphosphate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetra-acetic acid
ERIC	enterobacterial repetitive intergenic consensus
Ha	hectar
lsu	large subunit
LMG	Laboratorium voor Microbiologie Gent Culture Collection, Gent, Belgium
M	Molar or Mega (10^6)
mg	milligram
ml	millilitre
MLEE	multilocus enzyme electrophoresis
mm	millimeter
mM	millimolar
mS	milli Siemens
m/v	mass per volume
NCBI	National Center for Biotechnology Information
ng	nanogram
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
r	Pearson product moment correlation coefficient
RAPD	Random amplified polymorphic DNA
REP	repetitive extragenic palindromic

RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	revolutions per minute
ssu	small subunit
T	type strain
T	Terra (10^{12})
U	units
UPGMA	unweighted pair group method of arithmetic averages
μg	microgram
μl	microlitre
V	volts
v/v	volume per volume

CHAPTER 1

Introduction