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CHARACTERIZATION AND IDENTIFICATION OF SOME INDIGENOUS RHIZOBIA USING 16S rDNA SEQUENCE ANALYSIS

by

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SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MAGISTER SCIENTIAE

M. Sc (Microbiology)

DEPARTMENT: Microbiology and Plant Pathology

in the

FACULTY OF BIOLOGICAL AND AGRICULTURAL SCIENCES
UNIVERSITY OF PRETORIA
PRETORIA

December 1999

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

Signature: Mack

Date: 6/3/2000

BEDANKINGS

Ek wil graag die volgende persone opreg bedank:

Prof P L Steyn vir u hulp, leiding en al u kennis wat altyd tot my beskikking was gedurende die projek en die skryf van die verhandeling. Dankie dat Prof altyd daar was om te deel in al die vreugdes en teleurstellings van navorsing, asook van die lewe.

Marthie Ehlers, vir haar vriendskap en aanmoediging, asook haar hulp met die afronding van die verhandeling.

Raynard MacDonald, vir sy vriendskap, ondersteuning en hulp met die voltooiing van die verhandeling.

Marinda Oosthuizen, vir al haar hulp, goeie raad, waardevolle kennis en vriendskap.

Julian Jaftha, vir sy hulp, veral met die aanlering van al die nuwe molekulêre tegnieke, kennis en goeie raad.

Tolmay Kruger, vir haar kennis en hulp met die rhizobia.

Al my vriende vir hul belangstelling en ondersteuning.

My ouers, vir hul oneindige liefde, belangstelling, motivering en ondersteuning gedurende die jare van studie. **My broers en susters**, vir hul liefde en ondersteuning.

Aan my Skepper kom al die lof, eer en dank toe vir die voorreg om te kan studeer.

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SUMMARY

The use of different characteristics (the polyphasic approach) to describe bacterial taxa is a prerequisite for a stable classification. The taxonomy of root- and stem-nodulating rhizobia is in a state of transition. As more legumes are studied, new species and genera of rhizobia are described. It is important to study the indigenous South African rhizobia, as without them a complete rhizobial taxonomy is not possible. Furthermore, strains with superior nitrogen fixation abilities may be discovered. Indigenous strains better adapted to the harsh South African environment are possible candidates for commercial inoculants for cropped legumes.

Only two local studies have been done on the diversity of the indigenous rhizobia. These studies revealed the diversity of rhizobia existing in the South African context. As part of a polyphasic approach used to identify and determine the diversity of the indigenous rhizobia, 16S rDNA sequencing analysis was performed on some selected rhizobial and putative rhizobial isolates.

The aim of the study was to characterise and identify the indigenous isolates by 16S rDNA sequencing analysis and compare our data with those available in the GenBank database.

Results showed that most of the indigenous isolates were slow-growers belonging to the genus *Bradyrhizobium*. Two isolates from supposedly non-nodulating legume genera (*Cassia* and *Senna*) were found to belong to the genus *Bradyrhizobium*. Some of the isolates were shown to belong to the genera *Mesorhizobium*, *Rhizobium* and *Sinorhizobium*. The identity of five isolates was not clear and further studies need to be performed to unequivocally determine their taxonomic position. Partial sequence analysis of 16S rDNA proved a valuable tool to characterise and identify the indigenous isolates. However, the method was unable to clearly distinguish between closely related species and strains.

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KARAKTERISERING EN IDENTIFIKASIE VAN SOMMIGE INHEEMSE RHIZOBIUMS MET BEHULP VAN 16S rDNS VOLGORDE-BEPALING

deur

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OPSOMMING

'n Stabiele klassifikasiesisteem vir die beskrywing van bakteriese taksa is slegs moontlik deur verskillende eienskappe (die poli-fasiese benadering) te gebruik. Die taksonomie van die wortel- en stamnodulerende rhizobiums verander gedurig. 'n Volledige rhizobiumtaksonomie is slegs moontlik indien die inheemse Suid-Afrikaanse rhizobiums bestudeer word. Geharde inheemse rasse met voortreflike stikstofbindende vermoëns kan ontdek word. Hierdie rasse is kandidate vir kommersiële inokulums vir verboude peulplante.

Net twee plaaslike studies is gedoen om die diversiteit van die inheemse rhizobiums te bepaal. Die studies het bewys dat die inheemse rhizobiums baie divers is. As deel van die polifasiese benadering om die diversiteit van die inheemse rhizobiums te identifiseer en te bepaal, is 16S rDNS volgordebepaling gedoen op uitgesoekte rhizobia en sogenaamde rhizobia isolate.

Die doel van die studie was die karakterisering en identifisering van die inheemse isolate deur 16S rDNS volgordebepaling en die vergelyking van die data met dié beskikbaar in die GenBank databasis. Die resultate wys dat die meeste inheemse isolate stadige groeiers is en

dus behoort aan die genus *Bradyrhizobium*. Twee isolate vanaf sogenaamde nie-nodulerende peulplantgenusse (*Cassia* en *Senna*) behoort ook tot die genus *Bradyrhizobium*. Sommige isolate behoort tot die genusse *Mesorhizobium*, *Rhizobium* en *Sinorhizobium*. Die identiteit van vyf isolate was nie duidelik nie en verdere studies is nodig om hul taksonomiese posisie ondubbelsinnig te bepaal. Die gedeeltelike volgordebepaling van die 16S rDNS was 'n waardevolle hulpmiddel om die inheemse isolate mee te karakteriseer en te identifiseer, alhoewel die metode nie tussen nabyverwante spesies en rasse kon onderskei nie.

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LIST OF ABBREVIATIONS

ACCC	Agricultural Culture Collection of China
ADP	adenosine-5'-diphosphate
ATP	adenosine-5'-triphosphate
BNF	biological nitrogen fixation
bp	base pair
bv	biovar
ClustalX	cluster analysis version X
dATP	2'-deoxyadenosine-5'-triphosphate
dCTP	2'-deoxycytidine-5'-triphosphate
dGTP	2'-deoxyguanosine-5'-triphosphate
dITP	2'-deoxyinosine-5'-triphosphate
dTTP	2'-deoxytimidine-5'-triphosphate
dNTP	2'-deoxyribonucleoside-5'-triphospate
DNA	deoxyribonucleic acid
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany
DTT	dithiotreitol
e^-	electron
EDTA	ethylenediaminotetra-acetic acid
EPS	extracellular polysaccharide
Fd	ferredoxin
IAM	Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
IFO	Institute for Fermentation, Osaka, Yodogawa-ku, Osaka 532, Japan
IGS	intergenic spacer region
LMG	Laboratorium voor Mikrobiologie Gent Culture Collection, State University Gent, Belgium
MLEE	multilocus enzyme electrophoresis
ORS	ORSTOM Collection, Institut Français de Recherche Scientifique pour le Développement en Coopération, Dakar, Senegal
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PGPR	plant growth promoting rhizobacteria
RAPD	random amplified polymorhic DNA
REP-PCR	repetitive extragenic palindromic - polymerase chain reaction
RFLP	restriction fragment length polymorphism
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
T	tons

T	type strain
TY	tryptone yeast medium
U	unit
UPGMA	unweighted pair group method using arithmetic mean
UPM	Universidad Politécnica Madrid, Spain
USDA	United States Department of Agriculture, Agriculture Research Service, Beltsville, USA
US$	USA dollar
VAM	vesicular arbuscular mycorrhizae
WCP	whole cell proteins
YMA	yeast mannitol agar
YMB	yeast mannitol broth