Phylogenetic and ecological characterisation of the root nodule bacteria from legumes in the African genus *Lessertia*

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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ABSTRACT

Legumes of the genus *Lessertia* have recently been introduced to Western Australia in an attempt to increase the diversity of perennial legumes available to help remediate effects of climate change and dryland salinity. These species were introduced along with a collection of rhizobia isolated from *Lessertia* in different agro climatic areas of the Eastern and Western Cape, South Africa.

The aim of the thesis was to perform a phylogenetic and ecological characterisation of rhizobia isolated from the herbaceous legume *Lessertia* spp. The first specific aim was to characterize 73 isolates of rhizobia associated with *Lessertia* spp. Isolates were authenticated on their original hosts and diversity at strain level was determined by ERIC- and RPO1-PCR fingerprinting analysis. Forty three distinct authenticated strains showed diverse colony morphology and growth rate.

The diversity and phylogeny of the 43 strains was examined via *dnaK,* 16srRNA and *nodA* partial sequencing. Strains were identified as *Mesorhizobium* except one strain that was identified as *Burkholderia* sp. 16s rRNA phylogeny of 17 strains was overall congruent with the *dnaK* phylogeny. The topology of the housekeeping genes phylogram was independent of the original host and geographical origin of the strains. The *nodA* sequences formed a unique cluster separate from previously known *Mesorhizobium nodA* sequences, and when compared with that of the 16s rRNA and *dnak* genes, showed a wide dispersion of nodulation genes among the different *Mesorhizobium* clades indicating possible genetic transfer.

A glasshouse experiment was set up to assess the symbiotic interaction between six *Lessertia* species (*L. diffusa, L. incana, L.excisa, L. herbacea, L. capitata* and *L. pauciflora)* and 17 rhizobial strains, selected from differerent phylogenetic clusters. The strains showed marked differences in their nodulation patterns. *L.diffusa, L.herbacea* and *L.excisa* were nodulated by

most of the strains tested, and fixed nitrogen with 10, 7 and 4 strains respectively, while the other three hosts were quite selective. Interestingly, strains belonging to the same cluster in the *nodA* phylogeny showed similar nodulation patterns in the glasshouse experiment. WSM3636, WSM3612, WSM3565 and WSM3898 were selected for field experiments.

L. capitata, *L. diffusa, L. excisa L. incana* and *L. herbacea* were sown, along with their selected rhizobial strains, at five different agroclimatic areas of the Western Australian Wheatbelt: Badgingarra, Buntine, Katanning, Newdegate and Muresk. However, surviving plant populations in the field were lower than expected and although these species are perennials, most plants showed no or little regrowth after summer. To assess whether these problems were related to the adaptation and saprophytic ability of their root nodule bacteria, two field and a glasshouse experiments were carried out. Trap plants were established around surviving *Lessertia* plants at the Badgingarra site. At Buntine and Merredin, *L. capitata*, *L.diffusa, L.excisa L. incana* and *L. herbacea* were sown and inoculated with their appropriate inoculant to assess plant establishment, summer survival and nodulation. In addition, soils were collected from *Lessertia* plots at the five sites where *Lessertia* had been sown a year earlier. The soil samples were used to set up a soil trapping experiment in the glasshouse using the same five species of *Lessertia*. Nodulation was assessed and the root nodule bacteria were re-isolated and PCR fingerprinted. None of the trap plants at Badgingarra had nodules on their roots, suggesting that the inoculant strains were not able to survive in or to colonize that soil. Plant populations at Buntine and Merredin were again very low and the plants sampled were poorly nodulated. Results from the glasshouse experiment showed that *L. herbacea* and *L. diffusa* were able to nodulate in most of the soils, *L. capitata*, and *L*. *incana* only in two of them and *L. excisa* in none. Original inoculants could not be isolated from nodules, and strains occupying the nodules were identified as *Rhizobium leguminosarum* rather than *Mesorhizobium* spp. Since *Mesorhizobium* is known to have the ability to transfer symbiotic genes through a symbiosis island, the *nodA* gene was sequenced for all the “new” strains. The *nodA* sequences were again clustered with *R. leguminosarum* sequences, thus

discarding the prospect of lateral gene transference from *Mesorhizobium,* and suggesting a competition problem with indigenous rhizobia.

Two field experiments were set up at Karridale, Western Australia, a site with more benign conditions to assess the effect of increased doses of an effective inoculant strain (WSM3565) with *L. herbacea,* and to study the competitive ability and symbiotic performance of different *Mesorhizobium* strains nodulating *L. diffusa.* Increasing the inoculation dose of *L. herbacea* with WSM3565 did not improve establishment and survival of the legume. Although WSM3565 nodule occupancy improved from 28 to 54% with higher doses of inoculation, none of the treatments increased *L. herbacea* yield over the inoculated control.

The inoculation of *L. diffusa* with strains WSM3598, 3636, 3626 and 3565 resulted in greater biomass production than the uninoculated control. These strains were able to outcompete resident rhizobia and to occupy a high (>60%) proportion of lateral root nodules.

The high numbers of resident *R. leguminosarum* in Western Australian soils, and their ability to nodulate *Lessertia spp*. represent a barrier to the successful introduction of the exotic legume genus *Lessertia* to Western Australian soils.

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Refereed Conference Abstracts:

Gerding, M., O'Hara, G.W, Bräu L., Nandasena K. and Howieson J.G. 2009. Symbiotic and taxonomic diversity of root nodule bacteria isolated from the South African forage legume *Lessertia* spp. 16th International Congress on Nitrogen Fixation. Big Sky, Montana, USA. 14–19th June, 2009.

Gerding, M., O'Hara, G.W, Bräu L., Nandasena K., Real, D. and Howieson J.G. 2009. The role of competitive inffective nodulation in the poor establishment of *Lessertia* spp. in Western Australia. 15th Australian Nitrogen Fixation Conference. Margaret River, Australia. 8-13th November, 2009.

Gerding, M., O'Hara, G.W, Bräu L., Nandasena K., Real, D. and Howieson J.G. 2011. *Mesorhizobium* strains from the South African herbaceous legume Lessertia spp. Differ in their competitive ability in Australian soils. 17th International Congress on Nitrogen Fixation. Fremantle, Australia. 27th November – 1st December, 2011.

TABLE OF CONTENTS

Pages