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Systematics, Specificity, and Ecology of New Zealand Rhizobia

**A thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy
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Bevan Weir

School of Biological Sciences
The University of Auckland
New Zealand

Abstract

This research investigated the rhizobia that are associated with New Zealand legume plants. Rhizobia are a diverse group of bacteria that live in symbiosis with legumes in root nodules. Rhizobia fix Nitrogen from the atmosphere and provide this nutrient to the plant.

The objectives of this research were to: 1) Determine the identity of the rhizobial species nodulating the native legumes of New Zealand: *Sophora* (kowhai), *Carmichaelia* (NZ broom), and *Clialthus* (kakabeak); and the identity and origin of rhizobial species nodulating invasive exotic legumes in New Zealand: *Ulex* (gorse), *Cytisus* (broom), and *Acacia* (wattles). 2) Determine the specificity and nitrogen fixing capacity of both groups of rhizobia. 3) Investigate the possible exchange of transmissible symbiotic genetic elements.

A polyphasic strategy was used to determine the identity of bacterial isolates. The 16S rRNA, *atpD*, *recA*, and *glnII* genes were PCR amplified and sequenced, then analysed by maximum likelihood and Bayesian methods. Phenotypic characters were also assessed by use of the Biolog and FAME techniques. Nodulation and fixation ability was assessed by inoculating legume seedlings with rhizobial strains, then determining nitrogenase activity after ten weeks by gas chromatography, and examining roots for nodules. A gene involved in symbiosis, *nodA*, was sequenced from rhizobial strains to determine if transmission between strains had occurred.

The results of the experiments showed that the native legumes were predominately nodulated by diverse *Mesorhizobium* spp. that contain three different *nodA* genotypes (two of which are novel) that have transferred between rhizobial strains. The *Mesorhizobium* spp. showed little nodulation

specificity and could nodulate an exotic legume *Astragalus* (milk vetch), but not the invasive weed legumes. *Rhizobium leguminosarum* was also found to nodulate native legumes, albeit ineffectively. The exotic invasive woody legumes of this study were nodulated by diverse *Bradyrhizobium* spp. that had *nodA* genotypes typical of Australian and European species.

The origins of these bacteria can not be categorically determined. However the evidence is presented to suggest that nodulating *Mesorhizobium* spp. arrived with the ancestors of the native legumes, while *Bradyrhizobium* spp. nodulating *Ulex* and *Cytisus* arrived recently from Europe. *Bradyrhizobium* spp. nodulating *Acacia* may be recently introduced, possibly from Australia, although further work is required to confirm these hypotheses.

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Glossary

aa	Amino acids
ATP	Adenosine triphosphate
BLAST	Basic local alignment search tool
bp	Nucleotide base pairs
bv.	Biological variant (infrasubspecific level)
cfu	Colony forming units
CTAB	Cetyl trimethyl ammonium bromide
CSV file	Comma separated values
DNA	Deoxyribose nucleic acid
dNTPs	Deoxynucleoside triphosphates
EDTA	Ethylenediaminetetra-acetic acid disodium salt
FAME	Fatty acid methyl ester
g	Grams
GC	Gas chromatograph
GN2	Gram negative, version 2
ICMP	International collection of micro-organisms from plants
indel	Insertion or deletion
ITS	Internal transcribed spacer
kb	Thousands (kilo) of base pairs
km	Kilometre
L	Litre
Meso-NZL	<i>Mesorhizobium</i> species isolated from native legumes
ML	Maximum likelihood
mm	Millimetre
mQ H₂O	Microfiltered (18.2 M Ω resistance) deionised water, autoclaved
mya	Million years ago

NJ	Neighbour joining
PCR	Polymerase chain reaction
pmol	Picomole = 1×10^{-12} M
R2A	R2A agar (commercial agar from Difco)
RCF	Relative centrifugal force
RFLP	Restriction fragment length polymorphism
<i>R.leg</i>-NZL	<i>Rhizobium leguminosarum</i> strains isolated from native legumes
RO	Reverse osmosis
SDS	Sodium dodecyl sulfate
T	Type strain
TAE	Tris-acetate-EDTA
TE	10 mM Tris (pH 8.0) : 1 mM EDTA (pH 8.0)
Tris	Tris(hydroxymethyl)methylamine
UARR	Universal amplified ribosomal repeat
UV	Ultra-violet
YMA	Yeast mannitol agar