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Bradyrhizobia with a distinct *nodA* gene nodulate *Lupinus polyphyllus* in New Zealand soils

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Abstract

Lupinus polyphyllus plants were heavily nodulated at ten field sites across the South Island of NZ. Nineteen bacterial isolates from these nodules formed functional nodules on *L. polyphyllus* indicating that rhizobia that nodulate *L. polyphyllus* are widespread in the South Island. 16S rRNA and *nodA* gene sequences identified all 19 isolates as *Bradyrhizobium*. These *Bradyrhizobium* had distinct *nodA* gene sequences. *Bradyrhizobium* isolates from three other exotic invasive Genisteae species (*Ulex europaeus*, *Cytisus scoparius* and *Chamaecytisus palmensis*) with similar *nodA* gene sequences nodulated *L. polyphyllus* suggesting that these four species share a common pool of rhizobia in NZ.

Keywords: Lupin, *Bradyrhizobium*, Genisteae, nodulation genes

Introduction

Wild populations of perennial, horticultural lupins (*Lupinus polyphyllus* × *Lupinus* spp. hybrids, tribe Genisteae, hereafter *L. polyphyllus*) have colonised roadsides and riverbeds throughout the South Island of New Zealand (NZ) (Scott, 1989). *Lupinus polyphyllus* has potential as a forage crop on acidic, low phosphorus high aluminium soils in extensive high country grasslands in the NZ South Island (Scott, 1989; Black *et al.*, 2014; Black *et al.*, 2015). As with most legumes, *L. polyphyllus* is capable of fixing atmospheric nitrogen (N_2) via symbiotic bacteria (rhizobia) in root nodules but their rhizobial symbionts have not been characterised. The objectives of this study were to 1) determine if *L. polyphyllus* is nodulated over a wide range of sites throughout the NZ South Island, 2) genotypically characterise rhizobia that nodulate *L. polyphyllus* in the NZ South Island on the basis of their 16S rRNA and *nodA* gene sequences and 3) determine if rhizobia from three other exotic Genisteae weeds in the NZ South Island (*Ulex europaeus*, gorse; *Cytisus scoparius*, common broom and *Chamaecytisus palmensis*, tree lucerne) can nodulate *L. polyphyllus*.

Materials and methods

Bacterial isolates

Nineteen rhizobial isolates were obtained from nodules of different *L. polyphyllus* plants sampled from ten field sites across the NZ South Island (see Ryan-Salter *et al.*, 2014 for location co-ordinates). Selected isolates are deposited in the International Collection of Microorganisms from Plants (ICMP), Landcare Research, Auckland, NZ. Their ICMP numbers are given in Figure 1. A further isolate was obtained from nodules of *L. polyphyllus* supplied 'Group G' commercial inoculant (recommended for annual lupins in NZ; BASF, Auckland, NZ) under sterile conditions. Root nodules were surface sterilised by immersion in 96% ethanol for 5 s and 0.5% sodium hypochlorite for 3 min and then were rinsed with sterile water. Surface-sterilised nodules were crushed in sterile water, and this suspension was streaked onto yeast mannitol agar (YMA) (Vincent, 1970) and incubated at 20° C in the dark for 2-4 days. A purified culture was obtained by repetitive subculture. Samples of all cultures were inoculated into a suspension of yeast

mannitol broth (YMB) (Vincent, 1970) and used for preparation of DNA or inoculum. Strains ICMP 19842, ICMP 19828 and ICMP 19825 isolated from gorse, common broom and tree lucerne respectively were obtained from a separate study (Liu, 2015).

Gene sequencing and phylogenetic analyses

DNA was extracted from the bacterial cultures using the standard Gentra PUREGENE Purification Kit (Qiagen) following the protocol for gram-negative bacteria. Two genes were sequenced: the small subunit ribosomal RNA (16S rRNA) and N-acyltransferase nodulation protein A (*nodA*) as described previously (Ryan-Salter *et al.*, 2014).

DNA sequences were aligned and Maximum Likelihood trees constructed with 500 bootstrap replications with partial deletion and 80% coverage cut off using MEGA6 software (Tamura *et al.*, 2007). Only bootstrap values ≥ 50% are shown for each tree. Type strains of the most closely related *Bradyrhizobium* spp. on the GenBank sequence database (www.ncbi.nlm.nih.gov/genbank) were included in the 16S rRNA and *nodA* trees. *Sinorhizobium meliloti* was used as an out-group on both trees. Selected gene sequences obtained in this study have been deposited in the Genbank sequence database, and their accession numbers are shown in Figure 1.

Nodulation and N_2 fixation

All isolates were tested for nodulation and nitrogenase activity under sterile laboratory conditions. Seeds of *L. polyphyllus* were scarified then surface sterilised in 0.5% sodium hypochlorite for 15 min, rinsed in deionised water and then germinated on moist germination paper at room temperature in the dark. After germination, seedlings were transferred to polyethylene terephthalate jars (one seedling per jar) containing vermiculite and were supplied with a complete nutrient medium (pH 6.0) as described previously (Tan *et al.*, 2012). Plants were grown in a controlled environment cabinet and exposed to a 16-h photoperiod (400 μmol photons/m^2/s) at a constant 25°C. At planting, seedlings were inoculated with 5 ml of the appropriate rhizobial strain grown to log phase, ca. 1×10^8 cfu/ml. Uninoculated plants supplied with YMB only were used as controls. There were three replicate jars per treatment. At 40-50 days after inoculation, plants were tested for nitrogenase activity using the acetylene reduction assay (Cummings *et al.*, 2009).

Results and discussion

Lupinus polyphyllus plants were heavily nodulated at all ten field sites sampled across the NZ South Island. These nodules were pink inside (indicating the presence of leghaemoglobin) and were assumed to be functional. Nineteen bacterial isolates from these nodules formed functional nodules on *L. polyphyllus* indicating that rhizobia that nodulate *L. polyphyllus* are widespread in the NZ South Island. The 16S rRNA and *nodA* gene sequences identified all 19 isolates as *Bradyrhizobium* spp. (Figure 1a, b). Generally, annual lupins are nodulated by *Bradyrhizobium* sp. (Ryan-Salter *et al.*, 2014). Here, the 16S rRNA gene sequences separated into four groups and the *nodA* sequences separated into two groups. For *nodA* sequences, two of the isolates aligned closest to the isolate from the Group G inoculum, but 17 of the isolates clustered together closest to, but clearly separate from (92.89%-96.67% similarity), the *B. cytisi* type strain isolated from *C. villosus* (hairy broom) in the Moroccan Rif (Chahboune *et al.*, 2011).

Strains ICMP 19842, ICMP 19828 and ICMP 19825 isolated from gorse, broom and tree lucerne respectively showed similar *nodA* gene sequences to those of the *L. polyphyllus* (Figure 1a) and produced functional nodules on *L. polyphyllus*.

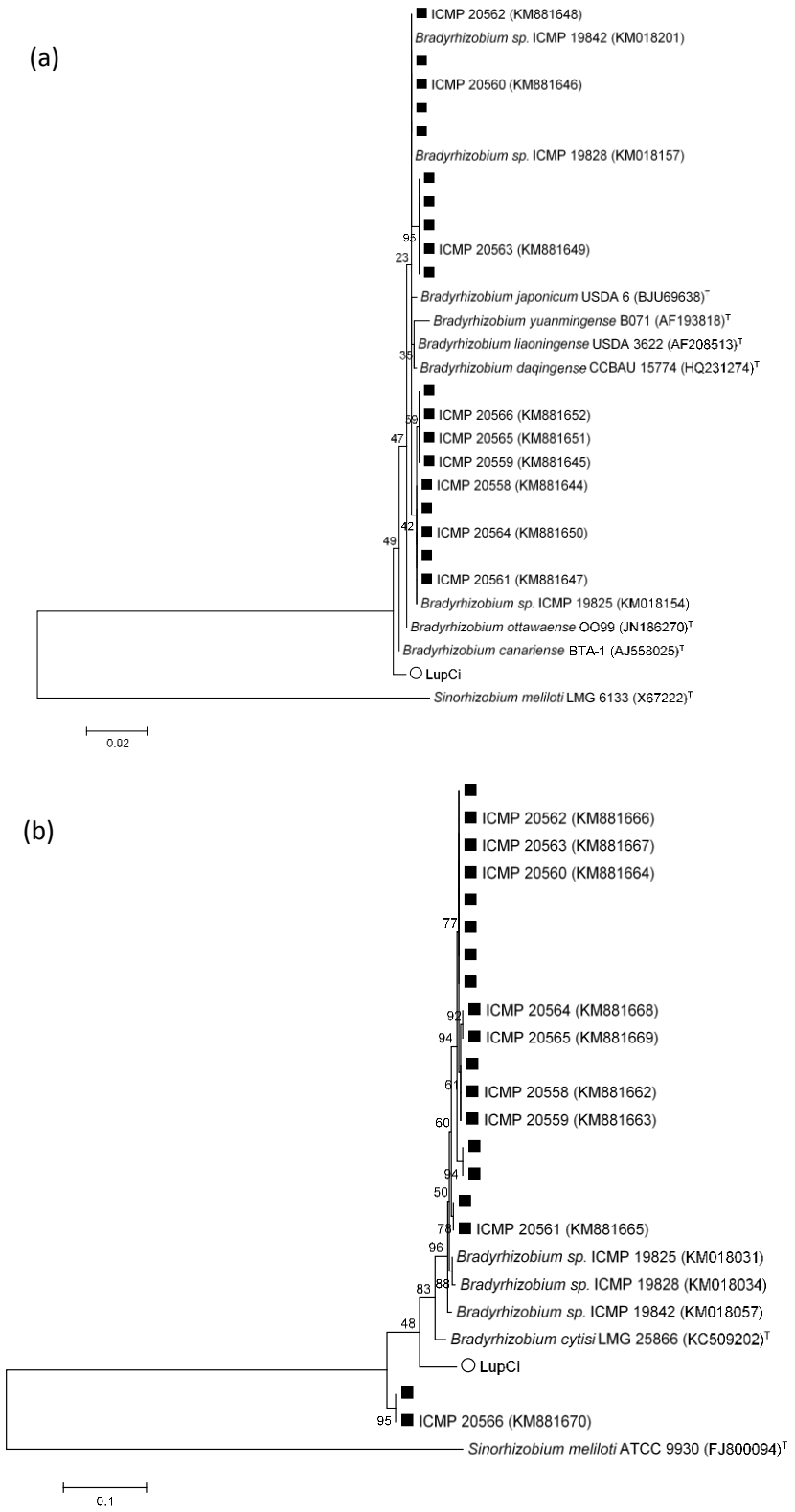

Figure 1. Phylogenetic tree of (a) 16s rRNA gene sequences (ca. 1216 bp) and (b) *nodA* gene sequences (ca. 450 bp) of 19 bacterial isolates from *Lupinus polyphyllus* (■), and one strain from each of gorse (ICMP 19842), common broom (ICMP 19828) and tree lucerne (ICMP 19825) sampled in the South Island of NZ. The commercial Group G inoculant for lupin (○) and selected *Bradyrhizobium* sp. type strains have been included. Genbank accession numbers are in parentheses. Numbers on branches are bootstrap per cent from 500 replicates. Superscript T indicates type strain.

Thus overall, the DNA sequence data indicate that bradyrhizobia with distinct *nodA* genes are of widespread occurrence in the South Island of NZ. The possible sources of these bradyrhizobia are: 1) and inoculant used in NZ in the past, 2) a strain from outside NZ that has become established with *L. polyphyllus* throughout the South Island, and 3) naturally occurring bradyrhizobia in NZ that nodulate *L. polyphyllus*. Further work is required to clarify this point. Nodulation studies suggest that *L. polyphyllus*, gorse, common broom and tree lucerne share a common pool of bradyrhizobia in the NZ South Island.

Conclusions

Bradyrhizobium strains that can nodulate *L. polyphyllus* are of widespread occurrence in the South Island of NZ. These bradyrhizobia have distinct *nodA* gene sequences. It seems likely that *L. polyphyllus,* gorse, common broom and tree lucerne share a common pool of bradyrhizobia in the NZ South Island.

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References

Black A.D., *et al.* (2014) *Proc NZ Grasslands Assoc,* 76, 35
Black A.D., *et al.* (2015) *Proc XIV Lupin Conference,* 14, X
Chahboune R., *et al.* (2011) *Int J Syst Evol Microbiol*, 61, 2922
Cummings S.P., *et al.* (2009) *Environ Microbiol*, 11, 2510
Liu W.Y.Y. (2015) PhD thesis, Lincoln University, NZ.
Ryan-Salter T.P., *et al.* (2014) *Proc NZ Grasslands Assoc,* 76, 61
Scott D. (1989) *Proc NZ Grasslands Assoc,* 50, 203
Tamura K., *et al.* (2007) *Mol Biol Evol*, 24, 1596
Tan H.W., *et al.* (2012) *PLoS One*, 7: e47677
Vincent J.M. (1970) IBP handbook 15