

Two annual legume species and their selection of nodule occupants

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Key Words

Rhizobia, ERIC-PCR, genotypes, New Zealand

Introduction

With its intensive animal production, New Zealand relies heavily on legume and ryegrass dominated pastures. Legumes in association with their rhizobial symbionts fix nitrogen and provide high quality forage. Little is known about naturalised rhizobia strains in New Zealand soils except that they are noted to be at reasonably high levels (Lowther and Kerr 2011). It is well established that some rhizobial strains nodulate multiple genera and species of legumes, while others might nodulate only one plant species. This work sets out to determine whether naturalised rhizobia resident in a pasture soil will nodulate the two different annual legume species tested. ERIC (enterobacterial repetitive intergenic consensus) PCR is a method used to determine bacterial strain diversity (Versalovic *et al.* 1991). It can be used to distinguish even closely related *Rhizobium* strains and has been used in numerous studies to determine nodule occupancy (Nangul *et al.* 2013; Youseif *et al.* 2014). The aim of this work was to discern if two different annual legume species were nodulated by naturalised strains and whether they were selective for a particular rhizobia strain.

Methods

Soil was collected from a dryland sheep pasture in Canterbury, New Zealand (S43^o38.818, E172^o21.196, elevation 41 m). The paddock had a history of inoculation with commercial strains, but not in the last five years. Seeds of either haresfoot trefoil (*Trifolium arvense*) or striated clover (*T. striatum*) were sown into 1.5 L pots containing sieved pasture soil. There were 11 replicates arranged in a randomised block design. Plants were grown for 4-5 months. Bacteria were recovered from 45 randomly selected nodules onto yeast mannitol agar and incubated at 20°C for 48 h. To ensure purity, bacteria were subcultured twice from single colonies. Bacteria with morphologies consistent with rhizobia were genotyped and all others were discarded.

Yeast mannitol broth cultures of each isolate were incubated at 28°C for 24 h at 220 rpm (LABNET 211 DS, Labnet International, US). DNA was extracted using the PUREGENE™ (Gentra Systems,US) DNA extraction kit. Rhizobia strains were genotyped using ERIC PCR as described by Versalovic *et al.* (1991). PCR products were separated on a 1% agarose gel (Progen Biosciences) at 10 V/cm for 50 min in 1 × TAE buffer, stained with ethidium bromide and visualized under ultraviolet light using the FireReader™ gel imaging system (Uvitec, UK). Gel photographs were analysed using Phoretix 1D Pro and Phoretix 1D Database (TotalLab Ltd, Newcastle, UK). UPGMA dendrograms were constructed using Jaccard similarity.

Results and Discussion

From the dendrogram two main clades are visible. Clade 1 is a mixture of strains from both striated clover and haresfoot trefoil, of which 68% are strains specific to striated clover (n =34/50). Clade 2 is dominated by strains from haresfoot trefoil (27/31 = 87%). A total of 22 and 34 genotypes were identified in nodules of striated clover and haresfoot trefoil, respectively. This research has demonstrated that there is a resident population of rhizobia in a dryland pasture site that is genetically diverse and capable of nodulating annual legumes. Similar work has been demonstrated for lucerne (Wigley 2011) and four other annual clover species (Nangul *et al.* 2013). In that work, depending on the species, >26 ERIC-PCR fingerprints were identified from 50-60 bacteria recovered per plant species. In the work of Nangul *et al.* (2013) it was demonstrated that arrowleaf clover was preferentially nodulated by a single strain (43% of nodules) despite deliberate inoculation with an ALOSCA™ preparation of WSM1325. The presence of a dominant strain was not shown for either haresfoot trefoil or striated clover.

Despite being grown in the same soil, haresfoot trefoil and striated clover selected markedly different rhizobial genotypes with only three genotypes in common. In similar work that assessed the rhizobia present in common bean using ERIC-PCR, Vásquez-Arroyo *et al.* (1998) noted that bacterial strains from different cultivars formed distinct groups.

The results showed that there was a much stronger selection of specific isolates by haresfoot trefoil, which indicates it had more specific nodule associations. Further research is required to assess the nitrogen fixing ability of the different strains to determine if the species are selecting strains which are efficient nitrogen fixers. Both beneficial and ‘parasitic’ strains (that provide negligible benefit to the plant) can be effective nodulators and it is unknown if one or both of these annual legume species can sanction poor performing strains (Kiers and Denison 2008).

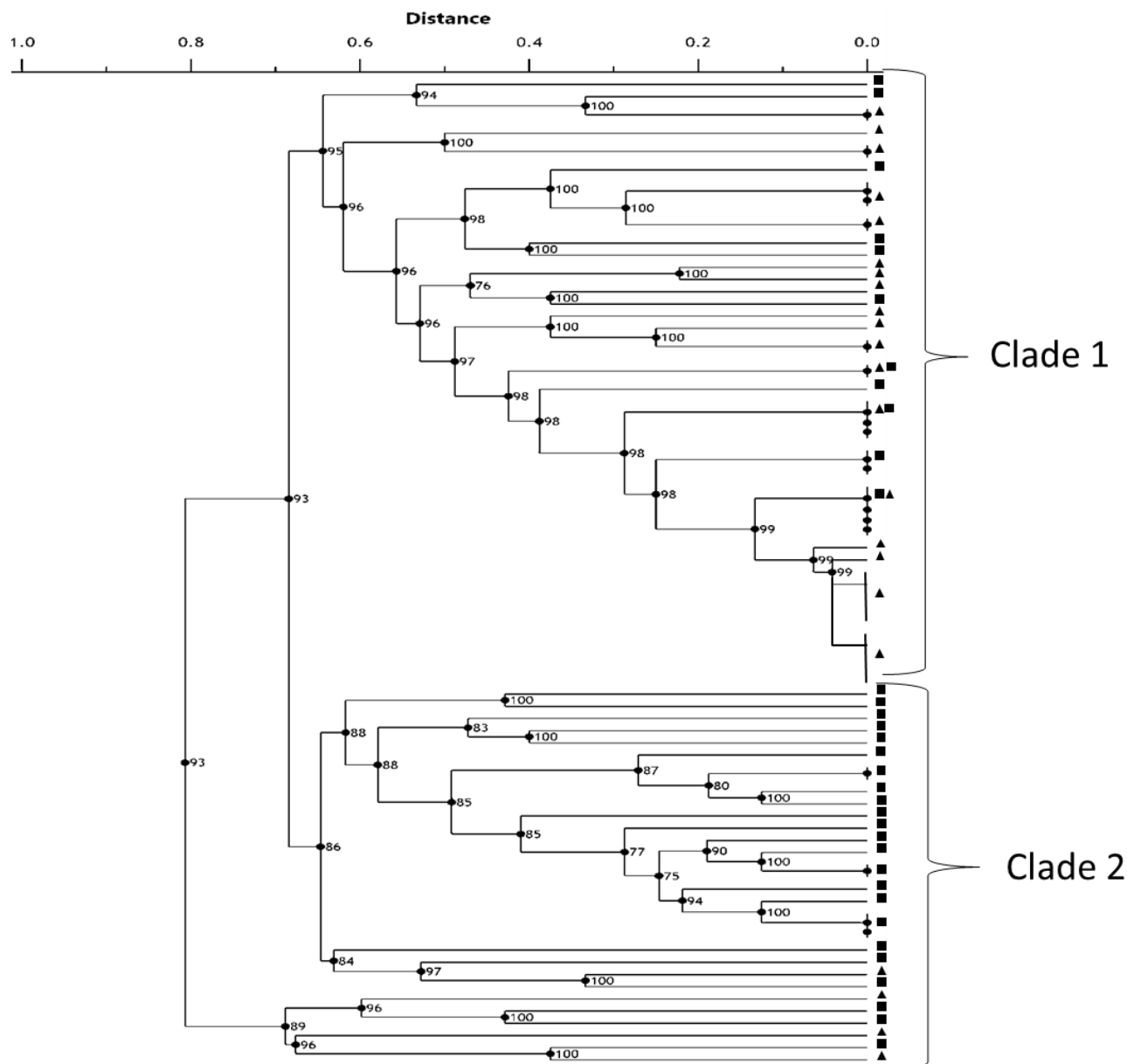


Figure 1. Dendrogram (UPGMA) showing clustering of the 81 rhizobial isolates genotyped. Haresfoot (▲) and striated (■) annotated. Branches with both ▲ and ■ indicate rhizobial genotypes found in both legume species.

Conclusion

There are diverse genotypes of rhizobia in New Zealand pastures capable of nodulating haresfoot trefoil and striated clover. These annual legumes select for different rhizobial genotypes from soil.

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