

Metabolic and Phylogenetic Profiling of *Rhizobium leguminosarum* Strains Isolated from NZ Soils of Varying pH

Authors : Anish Shah, Steve A. Wakelin, Derrick Moot, Aurélie Laugraud, Hayley J. Ridgway

Abstract : A mixed pasture system of ryegrass-clover is used in New Zealand, where clovers are generally inoculated with commercially available strains of rhizobia. The community of rhizobia living in the soil and the way in which they interact with the plant are affected by different biotic and abiotic factors. In general, bacterial richness and diversity in soil varies by soil pH. pH also affects cell physiology and acts as a master variable that controls the wider soil physiochemical conditions such as P availability, Al release and micronutrient availability. As such, pH can have both primary and secondary effects on soil biology and processes. The aim of this work was to investigate the effect of soil pH on the genetic diversity and metabolic profile of *Rhizobium leguminosarum* strains nodulating clover. Soils were collected from 12 farms across New Zealand which had a pH(water) range of between 4.9 and 7.5, with four acidic (pH 4.9 - 5.5), four 'neutral' (5.8 - 6.1) and four alkaline (6.5 - 7.5) soils. Bacteria were recovered from nodules of *Trifolium repens* (white clover) and *T. subterraneum* (subterranean clover) grown in the soils. The strains were cultured and screened against a range of pH-amended media to demonstrate whether they were adapted to pH levels similar to their native soils. The strains which showed high relative growth at a given pH (~20% of those isolated) were selected for metabolic and taxonomic profiling. The Omnilog (Biolog Inc., Hayward, CA) phenotype array was used to perform assays on carbon (C) utilisation for selected strains. DNA was extracted from the strains which had differing C utilisation profiles and PCR products for both forward and reverse primers were sequenced for the following genes: 16S rRNA, recA, nodC, nodD and nifH (symbiotic).

Keywords : bacterial diversity, clover, metabolic and taxonomic profiling, pH adaptation, rhizobia

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