

Diversity of 16S rDNA sequences of *Rhizobium* spp. Implications for species determinations



Brucella neotoma
Brucella ovis L26168
Brucella canis L3234
Brucella melitensis L26166
Brucella abortus X13695
Ochrobactrum anthropi U70978

John M. Young, Duck-Chul Park and Bevan S. Weir
Landcare Research, Private Bag 92170, Auckland, New Zealand
e-mail: youngj@LandcareResearch.co.nz



Abstract

Comparative analysis of 70 16S rDNA sequences representing 20 *Rhizobium* species showed no significant internal division of the *Rhizobium* clade to suggest that it represents more than one genus. The relationships of *R. gallicum* and *R. mongolense*, and *R. tropici* and *R. rhizogenes* require further investigation. Misidentification of some sequences may conceal one or more putative novel species.

Background

The genus *Rhizobium* has been considered to have a heterogeneous internal structure with a sub-group comprising mainly nodulating species (but including the tumorigenic *R. rhizogenes*) and the other sub-group comprising mainly tumorigenic species (but including the nodulating species, *R. galegae*, *R. giardinii*, *R. huautlense* and *R. undicola*) [Farrand et al., 2003, Young et al., 2001]. *R. galegae* and *R. huautlense* [Wei et al. 2003], and *R. giardinii* [Armager et al. 1997] have been identified as species possibly outlying the genus. *Allorhizobium* was proposed as a separate genus.

Sequences are mainly only from type strains of species have been deposited in GenBank, and therefore the extent of variability of sequences within species clades was unknown. Sequences from different species, including nodulating and tumorigenic species, could form individual species clades, or could be intermingled. Comparison of several sequences representing each species would clarify matters.

Objectives

To assess the comparative heterogeneity of 16S rDNA sequences within and between nodulating and tumorigenic *Rhizobium* spp.

Methods

16S rDNA sequences (46) for nodulating *Rhizobium* spp. were obtained from GenBank. Additional (24) sequences for *R. radiobacter*, *R. rhizogenes*, *R. rubi* and *R. vitis* strains from the International Collection of Micro-organisms from Plants were obtained by amplification using PCR, sequencing and alignment using ClustalX, and a tree obtained using Maximum Likelihood (Young et al. 2004).

Results and Discussion

The genus forms a single clade (Fig. 1). There is no support for the differentiation of *Agrobacterium* or *Allorhizobium* as distinct genera. Sequences representing particular nodulating and tumorigenic species usually form discrete clusters.

Within the generic clade, three groups of sequences are discussed.

- Group 1 comprised *R. etli*, *R. hainanense*, *R. leguminosarum*, *R. rhizogenes*, and *R. tropici*.

Within Group 1, nodulating *R. etli* sequences are heterogeneous, with tumorigenic *R. rhizogenes* sequences embedded within them.

- Group 2 comprised *R. daejeonense*, *R. galegae*, *R. giardinii*, *R. huautlense*, *R. larrymoorei*, *R. loessense*, *R. radiobacter*, *R. rubi*, *R. undicola* and *R. vitis*.

Within Group 2, most sequences of these species grouped together in individual clusters. *R. galegae* and *R. huautlense* sequences have been suggested to form a separate clade [Wei et al. 2003], with the single sequence representing *R. loessense* as a further outlier. There is no support for differentiation of *R. galegae* as a novel genus [Wei et al. 2003]. Variation between *R. galegae* sequences suggests some sequences could represent separate taxa, as subspecies of *R. galegae*, or as novel species.

- Group 3 sequences are considered in two subgroups.

Group 3a comprised sequences from *R. gallicum*, *R. indigoferae*, *R. mongolense*, *R. sullae*, and *R. yanglingense*. Sequences of *R. mongolense* are interspersed with *R. gallicum*. These species may be synonymous [van Berkum et al., 1998].

Group 3b. Sequences were received as *R. etli* AY509210 and *R. mongolense* AY509212 (from the rhizosphere of *Allysum* spp.), and *R. gallicum* AF417558, AF417561 (origins unknown) and AY509211 (from the rhizosphere of *Allysum* spp.). These appear to be unrepresentative of their species identifications and may represent a novel species.

- Other considerations

A single sequence of *R. vitis* (AY626396) is so separated from the other closely-knit sequences representing the species that its identity as *R. vitis* is in doubt. The similarity of the single sequence of the nodulating species, *R. tropici*, with the sequences of the tumorigenic species, *R. rhizogenes*, suggests the need for a comparative investigation of the pathogenic and nodulating characteristics of these two species.

A single sequence of *R. rubi* (AY626395) represents an incorrectly identified gall-forming strain of *R. rhizogenes* isolated from *Rubus*.

Relationships indicated for some strains with sequences deposited in GenBank cannot be investigated further because the strains have not been recorded as being deposited in publicly accessible collections.

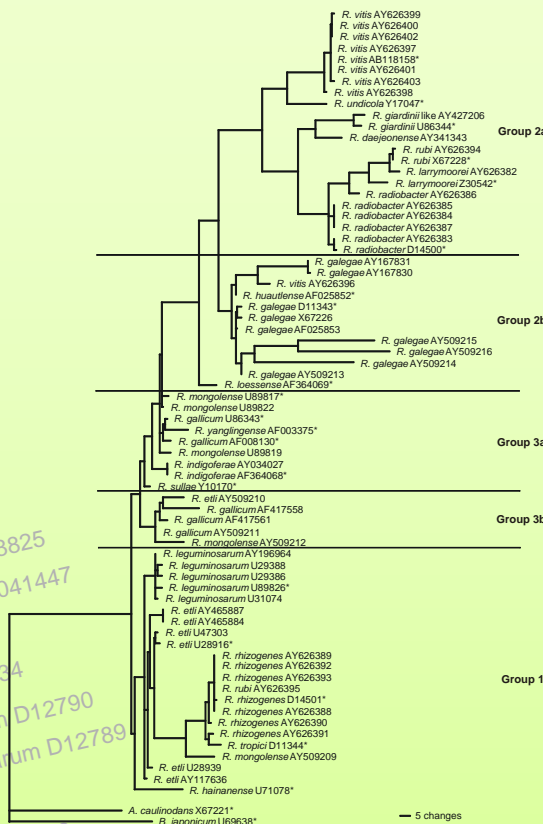


Fig. 1. Inferred relationships of species in the genus *Rhizobium* using Maximum Likelihood. Sequences from type strains are marked*.

Summary

- 16S rDNA sequences representing some individual species (e.g. *R. leguminosarum*, *R. rhizogenes*, *R. vitis*) were usually very similar, although in other cases they were more varied (e.g. *R. etli*, *R. galegae*).
- The ML tree indicates that there is no support for the differentiation of *Agrobacterium* or *Allorhizobium* as genera distinct from *Rhizobium*.
- Some sequences (Group 3b) have been misidentified and may represent novel species.

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