

# Classification of Tropical Tree Rhizobia Based on Phenotypic Characters Forms Nested Clusters of Phylogenetic Groups

A. Bala<sup>1,2\*</sup>, P.J. Murphy<sup>1</sup>, and K.E. Giller<sup>1,3</sup>

<sup>1</sup>Department of Biology, Imperial College at Wye; University of London, Wye, Ashford, Kent TN25 5AH, UK; <sup>2</sup>Department of Soil Science, Federal University of Technology, Minna, Nigeria; <sup>3</sup>Plant Production Systems, Department of Plant Sciences, Wageningen University, P.O. Box 430, 6700AK Wageningen, The Netherlands.

\*Corresponding author

## Abstract

Phenotypic variables were used to characterize 97 rhizobial isolates of *Calliandra calothyrsus*, *Gliricidia sepium*, *Leucaena leucocephala*, and *Sesbania sesban* sampled from several tropical soils, and had earlier been characterized using 16S rRNA analysis. Isolates were evaluated based on 40 variables, including colony morphology, growth rate, acidification of culture media, and the ability to utilize a wide range of carbon and nitrogen substrates. There was an overall agreement between phenotypic and phylogenetic classifications, with many of the phylogenetic groups nesting within different phenotypic clusters. In general, isolates did not cluster either according to host or geographical origin, suggesting that the ability to utilize certain substrates may not be an adaptive response. Although no one substrate was in itself distinctly diagnostic of any of the rhizobial groups, members of the *Agrobacterium* spp. sub-cluster exhibited poor growth on mono- and disaccharides, while the mesorhizobia and sinorhizobia failed to utilize dulcitol. All the rhizobial groups utilized fructose, arabinose, fucose, succinate, maltose, trehalose, and cellobiose as good energy sources and either grew poorly on polyethylene glycol (PEG), cyclodextrin, oxalate, and soluble starch or failed entirely to utilize them.

## Introduction

In addition to symbiotic traits, a wide range of morphological and cultural properties has traditionally been used in the characterization and identification of rhizobia. The phenotypic characters that are routinely used for this purpose are growth rate, colony characteristics on YEM media, and the utilization of carbon and nitrogen substrates as sole sources of nutrition. Different laboratories also use additional methods to characterize rhizobia, such as analyses of cell lipopolysaccharides or protein banding patterns (de Lajudie *et al.*, 1994), multilocus enzyme electrophoresis (Martínez-Romero *et al.*, 1991) and tolerance to stresses such as

acidity, salinity, heavy metals and high temperatures (Zhang *et al.*, 1991; Mpeperekí *et al.*, 1997; Odee *et al.*, 1997). Soon after Jordan (1982) separated rhizobia into two genera, *Rhizobium* and *Bradyrhizobium* on the basis of growth rate, it became evident that the use of phenotypic characters as the primary basis for rhizobial species classification was fraught with inconsistencies.

Although molecular phylogeny has now been established as the primary basis for species classification, most laboratories in developing countries, especially in sub-Saharan Africa, do not have the tools nor the skills to undertake recombinant DNA-based studies.