

Bradyrhizobium sp endosymbiote bacteria nodulate several species of spontaneous leguminous plants of Genistee tribe from northeastern of Algeria

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218 bacterial isolates obtained from different legume species belonging to the Genistee tribe (*Lupinus micranthus*, *Retama sphaerocarpa*, *retama raetam* and *Cytisus villosus*) from different sampling sites in Algeria were studied. Cultivated on YMA medium these strains show a slow growth. The phylogenetic analysis based on 16S rDNA and the household genes of the representatives of these strains indicates that they belong to the genus *Bradyrhizobium*. A variety of *Bradyrhizobium* sp. (1, 2, 3) is present in this collection with the presence of strains that can lead to new species.

Introduction

The Mediterranean has a very rich flora legumes adapted to various soil and climatic conditions. The Genistee tribe includes several genera of shrubs, such as *Retama*, *Genista*, *Cytisus* and *Spartium*, with great ecological significance in Mediterranean countries. The study of rhizobia nodulating these wild-grown legume shrubs is attracting great interest because of their high potential for environmental applications (Boullila et al. 2009).

The genus *Cytisus* is widespread in the Mediterranean Basin. It comprises ca. 60 species, eight of which grow in Northern Algeria. Broom, *Cytisus villosus* is disseminated in Northern Africa, appearing from the mountains of Central and Northern Morocco to the mountains of the Cape Bon in Tunisia (Quezel and Santa 1962).

In the Central-Western region of the Moroccan Rif, endosymbiotic bacteria from *C. villosus* growing have been described. These bacteria belong to different species of the genus *Bradyrhizobium* (Chahboune et al. 2011a, 2011b, 2012). In Northeastern Algeria, Ahnia et al., 2014 have described and characterized by phylogenetic and phenotypic analyses the rhizobia isolated from nodules of *C. villosus*.

The *Lupinus* genus includes approximately 275 species classified most of them were originated from diversity centers in Southern and Western North America, the Andean areas of South America, and the Mediterranean regions and Africa (Ainouche and Bayer 1999; Kurlovich and Stankevich 2002). Shrubby legumes of the *Retama* genus are endemic to the Mediterranean Basin and distributed in the various Mediterranean climates (from humid to arid) and ecosystems since *Retama* shrubs are tolerant to extreme drought conditions. Three species, *Retama monosperma*, *Retama raetam*, and *Retama sphaerocarpa*, are recognized within the *Retama* genus. These plant species are of ecological interest for dune stabilization, soil fixation, and revegetation of semiarid ecosystems (Caravaca et al., 2003).

Our objective is to study the biodiversity and phenotypic and phylogenetic characterization of nitrogen-fixing bacteria by symbiosis at the root nodules of the different species belonging to the Genistee tribe. Subsequently the selection of rhizobia adapted to environmental conditions could be used for economic and environmental purposes. This study was supported in part by the Algéro-Espagnol AECID / PCI research project (A1 / 038234/11). This project was developed on the spontaneous legumes of Algeria by the Center of Biotechnology and Plant Genomics of the Polytechnic University of Madrid (CBGP / UPM) in Spain in collaboration with the Laboratory of Microbial Ecology of the University of Bejaia (LEM / UAMB).

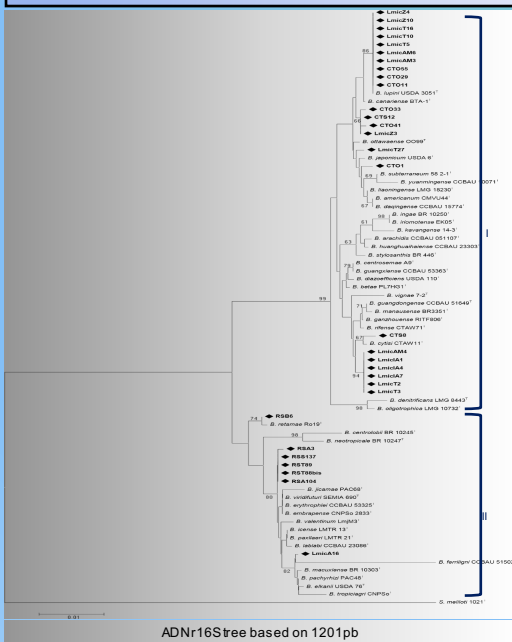
Isolation of Rhizobia

Site	Coordinates	Climate	Code des souches	Lupinus micranthus
Adzhar	36°41'35.7"N - 4°02'57.0"E	Humid	Lm16A	
Ah Maandil	36°52'48.4"N - 4°45'41.0"E	Humid	Lm16M	
Ighzer Amokrane	36°51'43.52"N - 4°36'05.91"E	Humid	Lm16A	
Talbert II	36°46'03.36"N - 4°32'02.33"E	Humid	Lm16C	
Zahli	36°42'20.66"N - 4°42'53.66"E	Humid	Lm16Z	
Oued Dass	36°51'N - 4°48' E	Humid	CT0	<i>Cytisus villosus</i> .
Saket	36°49'N - 4°56' E	Humid	CTS	
Hammam El Ebian (HEB)	36°12'20" N - 4°24'21" E	Semi-arid	RSB	<i>R. sphaerocarpa</i>
Amizour	36°58'2" N - 4°50'44" E	Sub-humid	RSA	
Stedouk	36°13'53" N - 4°41'7" E	Sub-humid	RSS	
Toudja	36°43'34" N - 4°48'12" E	Humid	RST	

Results

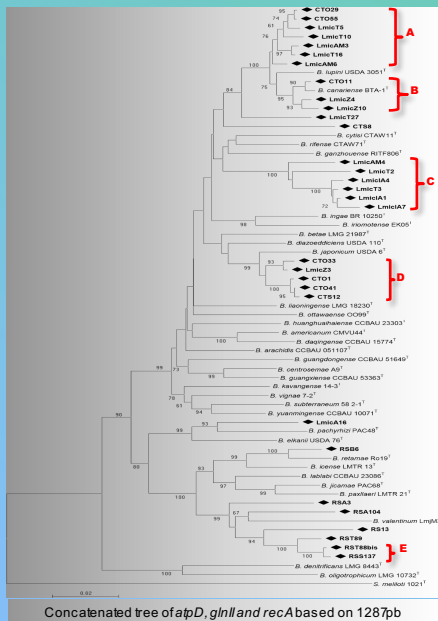
Phylogenetic analysis of 16S rRNA gene sequences

- With the exception of Lm16A, all representative strains of the *Bradyrhizobium* sp. isolated from *Lupinus micranthus* (Lm) and those isolated from *Cytisus villosus* (CT) are located on cluster I where *B. japonicum*.
- All strains representative of the *Bradyrhizobium* sp., isolated from *Retama sphaerocarpa* (RS) are located on cluster II where *B. elkanii* is located.



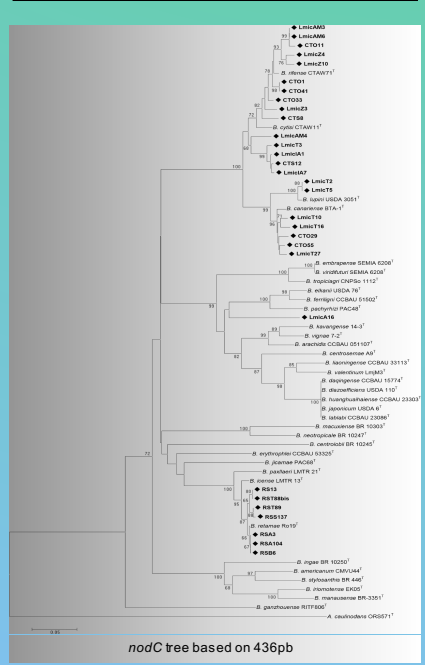
Phylogenetic analysis of the housekeeping genes atpD, glnII and recA

- These results confirm those of 16S and date:
- Group A close to *B. lupini*, group B near *B. anariensis*, Group D close to *B. japonicum*, RS B6 assigned to *B. retamae*, RSA104 isolate to *B. valentinum*, Lm16A near *B. pachytrici*, Lm16Z, CTS 8, group C, RSA3, RST3, RST8 and group D are defined from all species described in the genus *Bradyrhizobium* and could be described as new species.



Phylogenetic analysis of nodC gene

- nodC of representative strains *Bradyrhizobium* sp. isolated from root nodules of *Lupinus micranthus* and *C. villosus*, is close to the nodC of *B. affinis* or *B. cybei*, or *B. lupinus*. *B. anariensis* with the exception of Lm16A which is completely different.
- nodC of *Bradyrhizobium* sp. strains isolated from *Retama sphaerocarpa* is closely related to *B. retamae*.



Conclusion

- Strains of rhizobia isolated from root nodules of *Lupinus micranthus* and *Cytisus villosus* and *Retama sphaerocarpa* from northeastern Algeria all belong to the genus *Bradyrhizobium*.
- A very important diversity characterizes these different *Bradyrhizobium* sp.
- Lm16Z, CTS8, group C, RSA3, RST3, RST8 and group D are defined from all species described in the genus *Bradyrhizobium* and could be defined as new species.

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