

Phenotypic and molecular diversity of *Lupinus mariae-josephi* endosymbiotic Bacteria

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Abstract

Lupinus mariae-josephi is a new *Lupinus* species recently described in a Southeastern area of Spain (Valencia) in soils of singularly high pH and active lime content. Bacteria from *L. mariae-josephi* have been isolated and correspond to extra-slow growing bacteria symbiotically and phylogenetically distant to endosymbiotic strains nodulating other *Lupinus* sp. native of the Iberian Peninsula and adapted to growth in acid soils. Cross-inoculation experiments revealed that the *L. mariae-josephi* endosymbiotic bacteria are unable to nodulate or efficiently fix nitrogen with well-known *Lupinus* spp. Their species affiliation was examined by a multilocus sequence analysis of four housekeeping genes (16S rDNA, *glnII*, *recA*, *atpD*) and the symbiotic *nodC* gene. Single and concatenated phylogenetic analyses of these genes consistently revealed that *L. mariae-josephi* endosymbiotic bacteria belong to a clade, within the *Bradyrhizobium* genus, highly differentiated from the *Bradyrhizobium* clade that includes currently named *Bradyrhizobium* species as well as the endosymbiotic bacteria from *Lupinus* species tested in this study. Within this new clade the *L. mariae-josephi* bacteria nested in several subgroups that may correspond to novel sister species. The phylogenetic analysis based on the *nodC* gene showed that *L. mariae-josephi* endosymbiotic bacteria define a novel branch in the *nodC* *Bradyrhizobium* tree and likely have a common unique ancestor for the symbiotic genes with nodule isolates from *Retama* spp.

At this moment two draft genome sequences belonging to a *Bradyrhizobium* isolated from *L. angustifolius* (ISLU101) and to a *Bradyrhizobium* isolated from *L. mariae-josephi* (LmjC) have been obtained. The first analysis showed that both genomes correspond to very large chromosomes (>8000 genes) with a high number of unique proteins. *nod* genes organization are highly conserved among ISLU101, LmjC and *B. japonicum* USDA110. LmjC presents a single cluster with *nod* genes from diverse origins. ISLU101 *fix* genes are found in a single cluster homologous to that of USDA110. LmjC possess a complete copy of *fix* genes homologous to that from USDA110 and an incomplete one similar to *S. meliloti*. LmjC possess TypeIII and TypeIV secretion systems. ISLU101 has a Type IV homologous to that of photosynthetic *Bradyrhizobium* BTA1, and two copies of Type VI, one homologous to that of USDA110 and the other to *R. leguminosarum*. Preliminary data indicate that ISLU101 contains a potential second replicon (~100 genes) with high homology to *Bradyrhizobium* BTA1 plasmid sequence.

Geographical distribution of *Lupinus mariae-josephi* plant in Spain



Figure 1. Map of Valencia area where *L. mariae-josephi* are located.

Results



Figure 2. *Lupinus mariae-josephi* in its natural environment

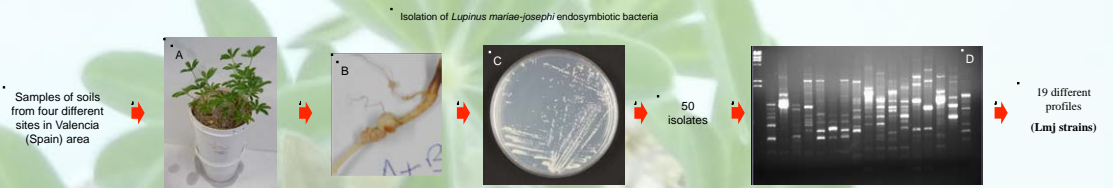


Figure 3. Isolates of *Lupinus mariae-josephi* endosymbiotic bacteria were obtained by using trap plants from soils of five spots of native plant population of Valencia province. A, Trap plants in the Leonard pots; B, example of nodule obtained from trap plants 21 days after inoculation; C, appearance of Lmj isolates in YMB after 10 days; D, PCR-RAPD profiles of Lmj strains.

Images of cross-inoculation test

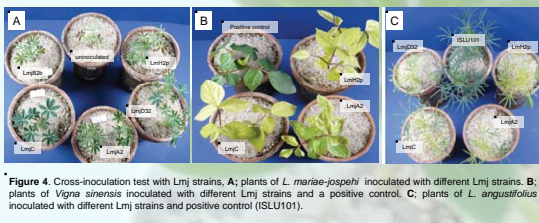


Figure 4. Cross-inoculation test with Lmj strains. A, plants of *L. mariae-josephi* inoculated with different Lmj strains. B, plants of *Vigna sinensis* inoculated with different Lmj strains and a positive control. C, plants of *L. angustifolius* inoculated with different Lmj strains and positive control (ISLU101).

Genome properties of <i>B. canariense</i> ISLU101 and <i>B. sp.</i> LmjC strains			
Genome properties	<i>Bradyrhizobium canariense</i> ISLU101	<i>Bradyrhizobium sp.</i> LmjC	<i>Bradyrhizobium japonicum</i> USDA110
Genome size	8.3 Mb	8.3 Mb	9.1 Mb
GC content	61%	59.85%	64%
Number of genes	8900	8901	8370
Percent coding	76%	75%	86%
Number of proteins	8844	8751	8317
Replications origins	2	1	1

Comparative genomics			
	<i>B. canariense</i> ISLU101 (8,844 proteins)	<i>B. japonicum</i> USDA110 (8,317 proteins)	<i>B. sp. lupini</i> LmjC (8,751 proteins)
ISLU101 unique	3184	0	0
USDA110 unique	0	373	0
LmjC unique	0	0	3571
Shared (ISLU101 & LmjC)	3713	371	4136

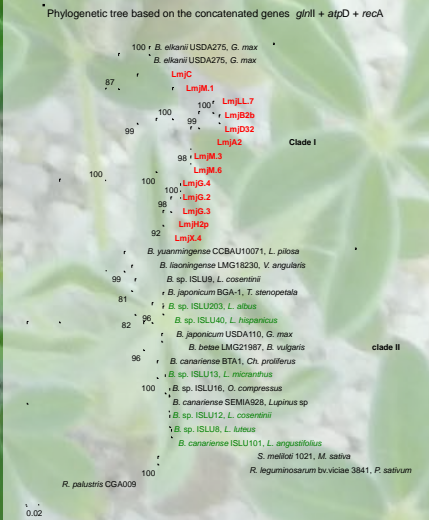


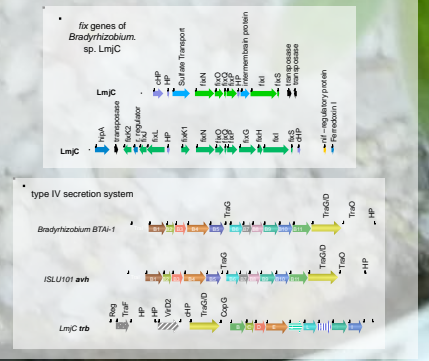
Figure 5. Phylogenetic tree based on the concatenated (*glnII*+*recA*+*atpD*) genes, obtained using the Maximum Likelihood method. *L. mariae-josephi* sequences are shown in red. The sequences of endosymbiotic bacteria from other *Lupinus* species that thrives in the Iberian Peninsula are shown in green.



Figure 6. Phylogenetic analysis based on the *nodC* gene: A, *B. japonicum* glycinisrum lineage; B, *B. canariense* lineage; C, *B. japonicum* USDA110 lineage. Lmj sequences are indicated in red, in green isolates from other *Lupinus* spp. of the Iberian Peninsula.

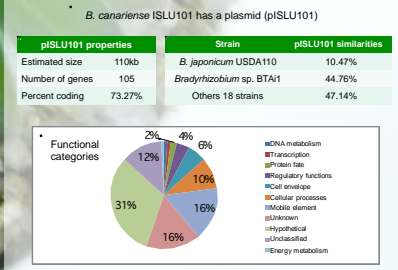
Symbiotic genes of LmjC and ISLU101 strains

strains	<i>nod</i> genes	<i>fix</i> genes	<i>nif</i> genes
<i>Bradyrhizobium sp.</i> LmjC	similar to <i>nod</i> genes of different species (<i>Mesorhizobium</i> , <i>Methylobacterium</i> , etc)	Two clusters, one similar to <i>B. japonicum</i> USDA110 <i>fix</i> genes, the other similar to <i>S. meliloti</i> <i>fix</i> genes	Similar to that from <i>B. japonicum</i> USDA110
<i>B. canariense</i> ISLU101	Similar to <i>nod</i> genes of <i>B. japonicum</i> USDA110	Similar to <i>fix</i> genes of <i>B. japonicum</i> USDA110	Similar to <i>nif</i> genes of <i>B. japonicum</i> USDA110



Secretion systems

strains	SSIII	SSIV	SSVI
<i>Bradyrhizobium sp.</i> LmjC	similar to SSIII of USDA110	unique	SSVI
<i>B. canariense</i> ISLU101		Similar to that from plasmid BTA1	1 st copy similar to the one of <i>B. japonicum</i> USDA110 2 nd copy similar to the one of from <i>R. leguminosarum</i>



Conclusions

- Endosymbiotic bacteria isolated from *Lupinus mariae-josephi* group together in a new clade within the *Bradyrhizobium* genus, and they are well differentiated from the currently named species and, singularly, from the nodule isolates from other lupins of the Iberian Peninsula.
- *B. canariense* ISLU101 and *B. sp. lupini* LmjC genomes have a very large chromosome with a high number of unique proteins, like the rest of *Bradyrhizobium* genomes sequenced to date.
- *nod* and *nif* genes are highly conserved among ISLU101, LmjC and USDA110. LmjC presents a *nod* cluster with genes from diverse origins.
- ISLU101 *fix* genes are in a single cluster homologous to the one of the USDA110. LmjC possesses a complete set of *fix* genes homologous to those from USDA110 and an incomplete cluster similar to *S. meliloti*.
- LmjC possesses Type III and Type IV secretion systems. ISLU101 has a T4SS homologous to one from BTA1, and two copies of T6SS, one homologous to the one of USDA110 and the other to *R. leguminosarum*.
- Preliminary data indicate that ISLU101 contains a potential second replicon (~100 genes) with high similarity to *Bradyrhizobium* BTA1 plasmid sequence.

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