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Genetic diversity of rhizobia associated with the common bean (*Phaseolus vulgaris* L.)

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INTRODUCTION

Rhizobia is a group of nodule-forming bacteria known for establishing symbiotic associations with legume plants. These bacteria convert atmospheric nitrogen into ammonia inside structures called radicular nodules, on a process called biological nitrogen fixation. This is an important process for agriculture, since it improves soil fertility and produces high protein grains. Rhizobial species that nodulate the common bean belong to the genera *Rhizobium*, *Ensifer*, and, recently reported, *Paraburkholderia* (Dall'Agnol, Bournaud et al. 2017) and *Pararhizobium* (Rouhrazi, Khodakaramian et al. 2016). However, the diversity of bacteria nodulating the common bean and other leguminous plants is still not well known, as well as the mechanisms of recruitment for mutualistic symbiosis of such diverse bacteria.

OBJECTIVES

SEMIA is an official rhizobial culture collection in Brazil and its registered number in the World Data Centre for Microorganisms is WDCM443. This collection was assigned as a part of government's regulation of the commercialization of rhizobial inoculants in 1975. SEMIA has entries for more than 900 strains isolated in Brazil and several countries. Considering the importance of SEMIA collection in Brazil and rhizobia-legume symbiosis relevance on agriculture, this project aims to demonstrate the nodular phenomenon of bean isolated strains from SEMIA culture collection and identify new nodulating genera.

MATERIALS AND METHODS

The genomic DNA of 107 common bean nodulating rhizobia deposited at SEMIA culture collection was extracted and the 16S rRNA was amplified using the BacPaeF and Bac1542R primers. Nucleotide sequences were determined on both amplicon strands at Macrogen Inc. (Seoul, South Korea). Sequence identity was assessed by searching SEMIA 16S rRNA sequences against EzBioCloud server database. The bacteria identified as possible nodulators for the common bean had their resistance tested to the antibiotics ampicillin, streptomycin, kanamycin, amoxicillin with clavunate, ceftazidime, cephalothin, cefoxitin, cefuroxime and meropenem, classifying them as resistant or susceptible. These results allowed a triparental mating conjugation protocol to transfer a plasmid carrying marker genes (*gfp* and *gusA*) from *Escherichia coli* into rhizobial strains. Kanamycin and a second antibiotic (ampicillin, streptomycin or cephalothin) resistance was used for selection of conjugated rhizobia. Until now, SEMIA 465 (*Ensifer*), SEMIA 436, 441, 457, 461, 462, 464, 472 and 489 (*Agrobacterium*), and SEMIA 4088 and 4080 (*R. tropici* and *R. freirei*, MAPA-recommended strains), strains were confirmed for conjugation by detection of *gusA* expression using X-Gluc reagent on YMA plates. Nodulation tests were then applied. Common bean seeds were submitted to a superficial asepsis and pre germinated for 2 days. The seeds were placed in pots after being inoculated with the conjugated rhizobia cultivated until the log phase. The plant nodules are being submitted to a fluorescence microscopy to observe bacterial presence on tissues.

CONCLUSION

The 16S rRNA sequence analysis revealed that *Bradyrhizobium*, *Ensifer*, *Mycolicibacterium*, *Skermanella*, *Stenotrophomonas*, and *Variovorax* could also represent symbionts of the common bean.

RESULTS

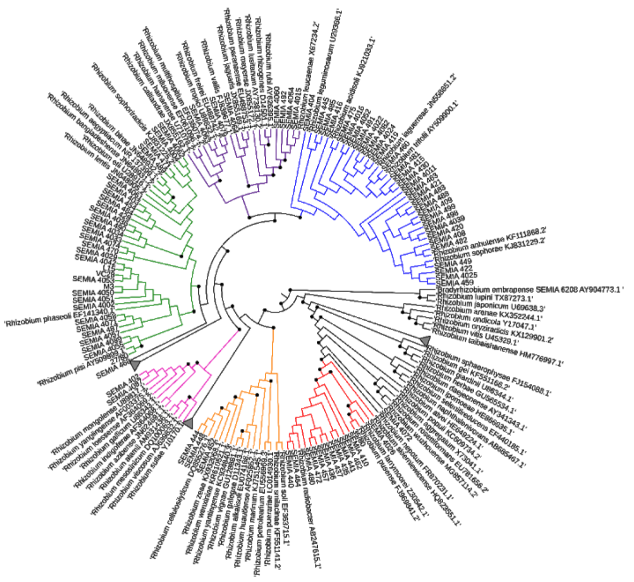
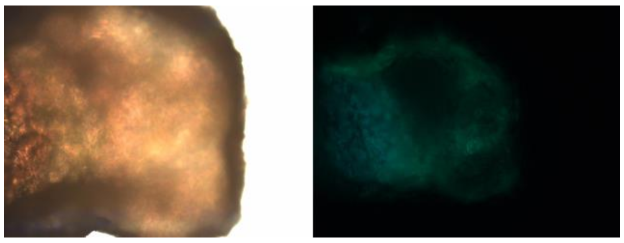


Figure 1. Phylogenetic tree of 16S rRNA gene sequences from 89 *Rhizobium* SEMIA strains and 109 *Rhizobium-type* strains, as inferred by Bayesian analysis. Circles at the branching points represents posterior probability <0.95%.

Figure 2. Fluorescence microscopy of SEMIA 4088.



16S rRNA top-hit taxon	Strains number
Rhizobium leguminosarum	29
Rhizobium esperanzae	18
Rhizobium Radiobacter	15
Rhizobium etli	8
Rhizobium aethiopicum	4
Rhizobium cellulosilyticum	4
Rhizobium lentis	4
Paraburkholderia fungorum	3
Rhizobium leucaenae	3
Rhizobium tropici	3
Bradyrhizobium centrosematis	2
Rhizobium mongolense	2
Agrobacterium fabrum	1
Bradyrhizobium japonicum	1
Ensifer meliloti	1
Mycolicibacterium obuense	1
Pararhizobium giardinii	1
Rhizobium lusitanum	1
Rhizobium paranaense	1
Rhizobium pisi	1
Rhizobium sophorae	1
Skermanella aerolata	1
Stenotrophomonas maltophilia	1
Variovorax guangxiensis	1

Table 1. 16S rRNA top-hit taxons of SEMIA strains. Sequence identity was assessed by comparing the 16S rRNA sequences of the SEMIA strains with the sequences from EzBioCloud server database.

Figure 3. Conjugated SEMIA 472 on YMA plate with X-Gluc, kanamycin and streptomycin.