ISOLATION AND CHARACTERIZATION OF RHIZOBIUM STRAINS FROM CENTRAL HIGHLANDS OF MEXICO, AND ANALYSIS OF THEIR ABILITY TO IMPROVE DROUGHT TOLERANCE TO *Phaseolus vulgaris*

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Keywords: Rhizobium, Phaseolus vulgaris, Drought Tolerance.

Introduction. Agriculture is one of the human activities most affected by drought (1). The 60% of world production of beans is obtained in regions subject to water deficit (2). Food demand and high environmental costs has led to seek alternatives for sustainable agriculture (3). One such alternative could be the use of bacteria of the genus Rhizobium, they have the ability to associate symbiotically with legumes to fix atmospheric nitrogen and thereby help increase yields.
Our objective is to isolate and identify Rhizobium strains able to confer drought tolerance to bean plants.

Methodology. To isolate rhizobia from soils collected in Tlaxcala and Hidalgo States, *P. vulgaris* plants were used as trap plants. Induced root nodules were disinfected superficially, macerated, and inoculated on selective medium for rhizobia. Osmotic or saline tolerant bacterial isolates were selected. Also, isolates were classified using Eckhardt's and *16S* sequence analysis. Presence of the genes *nifH*, *nodC*, *rpoB*, *recA* was also analyzed by PCR. Finally, some strains were selected to analyze their ability to confer drought tolerance to beans plants.

Results. Tested soil from Tlaxcala (2260 m, 19 ° 16 'N, 98 ° 22' W) has a franco-sandy texture and pH 7.4. Moreover, the soil from Hidalgo (2480 m 19 ° 42 'N, 98 ° 27' W) has a sandy-loam texture and pH 6.9. Using the trap plants strategy, 92 isolates of Rhizobium were obtained from four independent experiments. Most strains exhibit related characteristics, colonies are usually milky white, glossy, raised, round, with a creamy consistency and growth varies between 1 and 8 days. Some strains were able to grow in extreme stressing conditions (0.5 M NaCl or 1.0 M sorbitol). Analysis of the 16S rRNA gene showed that the isolates have a great genetic diversity, ranging from Agrobacterium to Mesorhizobium (Table 1). Nine strains were selected in relation to molecular characteristics and osmotolerance, which were tested in their ability to induce growth, nodulation, nitrogen fixation, leghemoglobin. Today, the strains are been analyzed for their ability to improve drought tolerance to *P. vulgaris* under greenhouse conditions.

Table 1. Analysis of 16S sequences.

Wild strains	Similarity to Rhizobaceae (%)
5	*98% Agrobacterium sp.*
6	*97% Mesorhizobium sp. W39*
7	*97% Mesorhizobium sp. W39*
8	*98% R. leguminosarum bv. Trifolii*
10	*99% R. etli bv. Phaseoli*
19	*99% R. etli UFLA4-42*
20	*99% Mesorhizobium sp. W39*
24	*99% R. etli Mim1*
35	*99% R. etli Mim1*

Conclusions. 1) Soils from Tlaxcala and Hidalgo showed abundance of free-living Rhizobia with a great biodiversity. 2) We identified strains with high tolerance to osmotic stress and salinity. 3) Some of the strains tested in greenhouse showed a high efficiency of nitrogen fixation.

Acknowledgements. MAVL and AAB thank CONACYT and SIP-IPN (Common Bean Megaproject-Red de Biotecnolgía-IPN) for funding. LJGG and ASM thank CONACYT for fellow.

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