We report the isolation of Rhizobium and Sinorhizobium strains from bean seeds (Phaseolus vulgaris) that are able to fix nitrogen. The strains were initially isolated from nodules of non-inoculated plants, but later, directly from seeds. This finding represents an exciting new research area given that nitrogen-fixing bacteria can be vertically inherited in the legume seeds.

INTRODUCTION

Rhizobia are soil bacteria that can interact with the Legumes in a symbiotic relationship. In the plant nodules, bacteria perform the biological nitrogen fixation, a key process to incorporate atmospheric nitrogen into the living organisms. Given its long time relationship, it is previsible a close interaction. Some rhizobia strains have been found in diverse plant tissues (other than the nodules) such as roots, stems, leaves and seeds. However, to date, there is no report of rhizobia strains able to fix nitrogen isolated from the seeds. Possibly they are very scarce and this precluded its isolation.

RESULTS

Presence of Rhizobia in seeds from non-inoculated plants. Positive signal of PCR for rpoB (RNA-polymerase beta unit) of rhizobia was found in seeds from non-inoculated plants cropped in years 2011 and 2012 in our greenhouse.

Rhizobia in seeds from inoculated plants. To test that Rhizobia strains were able to migrate to the seeds, we inoculated CCGM1 and CCGM7 in bean plantlets. After symbiosis, we looked for them in the seeds. Positive signals for both strains were found. A control strain, also inoculated, was not found in seeds.

Plasmid profiles. The isolated strains presented several plasmids of high molecular size, with one exception: the CCGM7 strain (arrow). The asterisks denote the symbiotic plasmids (found with nifH hybridization).

Phylogeny. We sequenced the rpoB gene of the isolated strains. They were found related to diverse rhizobial species, from the Rhizobium and Sinorhizobium groups.

Stress sensitivity. The strain CCGM1 showed unexpected sensitivity even in normal storage conditions. We assessed the growth response with diverse stresses as salt, temperature and pH, and found strong sensitivity of CCGM1 and slight for CCGM7 (not shown).

Effective symbiosis. The nitrogen fixing phenotype of the R. phaseoli CCGM1 and S. americanum CCGM7 strains was analyzed in greenhouse assays, inoculating as usual. In the panels at right can be observed the symbiotic parameters. Asterisks denote significant difference with p<0.05. Both strains were very effective to induce plant growth as observed in the photograph (greenhouse).

The genomes of CCGM1 and CCGM7 strains. We obtained the genomic sequences of CCGM1 and CCGM7 strains. The first was assembled using the genome of highly related R. phaseoli strain CIAT652 as backbone; for the second, that of S. fredii USDA 257 was used. The CIAT652 chromosome, and the plasmids A and C were well represented in the CCGM1 genome, but not the plasmid B. An interesting feature was the presence of three prophages (bottom, gene structure prediction). CCGM7 had remarkable gene homology (red lines) and synteny (dark and light blue segments) with the USDA257 chromosome.

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CONCLUSIONS

Nine strains were isolated from nodules of non-inoculated bean plants and their plasmid content and phylogeny was determined. Two were tested and found present in the seeds: the R. phaseoli CCGM1 and S. americanum CCGM7 strains. They were able to fix nitrogen in greenhouse plants. Their genomes are under analysis to find specific features regarding the occupancy of this new niche. We are searching similar strains in other Legume species. More details: App Environ Microbiol. 80:5644-5654.

SIGNIFICANCE. These endophytic strains could be used to formulate self-inoculated legume seeds ready to sow in the fields. Also, they possibly present genomic changes related to the close evolution with seeds.

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