JB Journals.ASM.org

GENOME ANNOUNCEMENT

Genome Sequence of *Rhizobium* sp. Strain CCGE510, a Symbiont Isolated from Nodules of the Endangered Wild Bean *Phaseolus albescens*

Luis E. Servín-Garcidueñas,[a] Marco A. Rogel,[a] Ernesto Ormeño-Orrillo,[a] Alfonso Delgado-Salinas,[b] Julio Martínez-Romero,[a] Federico Sánchez,[c] and Esperanza Martínez-Romero[a]

Centro de Ciencias Genómicas, UNAM, Cuernavaca, Morelos, México[a]; Instituto de Biología, UNAM, México D.F., México[b]; and Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, México[c]

We present the genome sequence of *Rhizobium* sp. strain CCGE510, a nitrogen fixing bacterium taxonomically affiliated with the *R. leguminosarum-R. etli* group, isolated from wild *Phaseolus albescens* nodules grown in native pine forests in western Mexico. *P. albescens* is an endangered bean species phylogenetically related to *P. vulgaris*. In spite of the close host relatedness, *Rhizobium* sp. CCGE510 does not establish an efficient symbiosis with *P. vulgaris*. This is the first genome of a *Rhizobium* symbiont from a *Phaseolus* species other than *P. vulgaris*, and it will provide valuable new insights about symbiont-host specificity.

Phaseolus albescens R. Ram. & A. Delgado is a nondomesticated species phylogenetically related to *Phaseolus vulgaris* (1, 2, 8), and its symbiotic bacteria have not been described. *P. albescens* is at risk (2, 8) because it is distributed in a restricted area and threatened by changing land use, and few seeds are safeguarded. We isolated novel rhizobia from field-collected *P. albescens* nodules, including a representative strain designated CCGE510. Strain CCGE510 established an effective symbiosis with *P. albescens*, as inoculated plants were green and nodules were pink and reduced acetylene. Interestingly, *P. vulgaris* plants inoculated with this strain were yellow and nodules turned green soon after appearance.

The genome of *Rhizobium* sp. strain CCGE510 was sequenced with the Illumina GAIIx platform, producing 6,329,550 36-bp reads (~32-fold coverage), and with the Roche 454 GS-FLX Titanium technology, generating 91.16 Mbp (~13-fold coverage) from a mate-paired library. Illumina paired reads were assembled *de novo* using Velvet 1.2.03 (11). Contigs were fragmented by using the EMBOSS splitter (9) and were assembled with 454 mate-reads using Newbler Assembler 2.3 (454 Life Science). Reads were mapped to gap-surrounding sequences using Maq 0.7.1 (5) and the Newbler runMapping option. Mapping contigs and PCR amplifications were used to eliminate gaps. The final assembly produced 142 contigs with an N_{50} size of 270.2 kb. Genome annotation was performed using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). Plasmids were detected using a modified Eckhardt procedure (4), and their sizes were estimated using regression equations comparing them with *R. etli* CFN42 plasmids.

The genome (6.9 Mbp, 60.8% G+C content) consisted of a chromosome and four plasmids and coded for 6,642 predicted open reading frames. The chromosome (5.04 Mb) was distributed in one scaffold. Plasmid pRspCCGE510a (61.69 kb) seems to be unstable or recently acquired, as it is commonly absent in other *P. albescens* nodule strains. Plasmid pRspCCGE510b (270.27 kb), the symbiotic plasmid pRspCCGE510c (579.35 kb), and plasmid pRspCCGE510d (923.84 kb) were recovered as single scaffolds.

Small-subunit rRNA gene phylogeny indicated that *Rhizobium* sp. CCGE510 is related to *R. leguminosarum* strains; however, calculated average nucleotide identity (ANI) (10) separated it from that species. Symbiotic genes were related to those found in symbiovar (where "symbiovar" [sv.] means symbiotic variant) phaseoli, but overall symbiotic plasmid synteny was not as maintained as in phaseoli symbiotic plasmids (3). Some genes required for Nod factor synthesis were divergent from those of *R. etli* CFN42. Differences were also observed in secretion systems, including type III effector proteins and a transporter for the uptake of bean exudates. Strain CCGE510 may metabolize pine compounds, as *P. albescens* roots are intertwined with pine roots. *R. etli* sv. phaseoli strains are very competitive for *P. vulgaris* nodulation (6), and the evolution of the phaseoli plasmid could have been driven by host selective pressures (6, 7). Similarly, *P. albescens* bacteria seem to be better adapted to their host and not to *P. vulgaris*. *P. vulgaris* and *P. albescens* diverged about 1 to 2 million years ago (1); it is plausible that in the corresponding symbiotic plasmids (from *P. vulgaris* and *P. albescens* bacteria) differences have accumulated since host divergence.

Nucleotide sequence accession number. The genome sequence has been deposited in DDBJ/EMBL/GenBank under the accession number AEYF00000000.

ACKNOWLEDGMENTS

We are thankful to UUSM, UNAM, especially Ricardo Grande Cano and Verónica Jiménez Jacinto; to Paul Gaytán and Eugenio López for synthesis of oligonucleotides; and to Michael Dunn for critically reading the paper.

This work was supported by PAPIIT IN205412 from DGAPA, UNAM. L.E.S.-G. was supported by a CONACyT scholarship as a Ph.D.

Received 22 August 2012 **Accepted** 29 August 2012

Address correspondence to Esperanza Martínez-Romero, emartine@ccg.unam.mx.

This work is dedicated to the memory of Raymundo Ramírez Delgadillo; his enthusiastic work allowed the recovery of field nodules and conservation of *Phaseolus albescens* seeds.

doi:10.1128/JB.01536-12

student at the Programa de Doctorado en Ciencias Biomédicas from UNAM.

REFERENCES

1. **Delgado-Salinas A, Bibler R, Lavin M.** 2006. Phylogeny of the genus *Phaseolus* (Leguminosae): a recent diversification in an ancient landscape. Syst. Bot. **31**(4):779–791.
2. **Freytag GF, Debouck DG.** 2002. Sida botanical miscellany, vol 23. Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. Botanical Research Institute of Texas, Fort Worth, TX.
3. **González V, et al.** 2010. Conserved symbiotic plasmid DNA sequences in the multireplicon pangenomic structure of *Rhizobium etli*. Appl. Environ. Microbiol. **76**:1604–1614.
4. **Hynes MF, McGregor NF.** 1990. Two plasmids other than the nodulation plasmid are necessary for formation of nitrogen-fixing nodules by *Rhizobium leguminosarum*. Mol. Microbiol. **4**:567–574.
5. **Li H, Ruan J, Durbin R.** 2008. Mapping short DNA sequencing reads and calling variants using mapping quality scores. Genome Res. **18**:1851–1858.
6. **Martinez-Romero E, Rosenblueth M.** 1990. Increased bean (*Phaseolus vulgaris* L.) nodulation competitiveness of genetically modified *Rhizobium* strains. Appl. Environ. Microbiol. **56**:2384–2388.
7. **Martínez-Romero E.** 2009. Coevolution in *Rhizobium*-legume symbiosis? DNA Cell Biol. **28**:361–370.
8. **Ramírez-Delgadillo R, Delgado-Salinas A.** 1999. A new species of *Phaseolus* (Fabaceae) from West-Central México. Sida **18**(3):637–645.
9. **Rice P, Longden I, Bleasby A.** 2000. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet. **16**:276–277.
10. **Richter M, Rossello-Mora R.** 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. U. S. A. **106**:19126–19131.
11. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. **18**:821–829.