

Draft Genome Sequence of Plant Growth-Promoting *Rhizobium Mesorhizobium amorphae*, Isolated from Zinc-Lead Mine Tailings

Xiuli Hao,^{a,b} Yanbing Lin,^{a,b} Laurel Johnstone,^c David A. Baltrus,^d Susan J. Miller,^e Gehong Wei,^a and Christopher Rensing^b

College of Life Sciences, State Key Laboratory of Crop Stress Biology in Arid Areas, Northwest A & F University, Yangling, Shaanxi, China^a; Department of Soil, Water and Environmental Science, University of Arizona, Tucson, Arizona, USA^b; University of Arizona Genetics Core, Tucson, Arizona, USA^c; Department of Plant Science, University of Arizona, Tucson, Arizona, USA^d; and Biotechnology Computing Facility, Arizona Research Laboratories, University of Arizona, Tucson, Arizona, USA^e

Here, we describe the draft genome sequence of *Mesorhizobium amorphae* strain CCNWGS0123, isolated from nodules of *Robinia pseudoacacia* growing on zinc-lead mine tailings. A large number of metal(loid) resistance genes, as well as genes reported to promote plant growth, were identified, presenting a great future potential for aiding phytoremediation in metal(loid)-contaminated soil.

The alphaproteobacterium *Mesorhizobium amorphae* is of profound scientific and agronomic significance due to its ability to establish a beneficial nitrogen-fixing symbiosis with leguminous plants such as *Robinia pseudoacacia* (10). Copper-resistant *Mesorhizobium amorphae* strain CCNWGS0123, isolated from root nodules of *Robinia pseudoacacia* in a zinc-lead mine tailing, was found to help its host plant to survive in copper-, zinc-, and chromium-contaminated environments. This mutualistic symbiosis has been used to maximize the effectiveness of bioremediation of contaminated soil (2, 5, 11). This is the first genome sequence of a strain of *Mesorhizobium amorphae*; only three other *Mesorhizobium* strains, representing three other *Mesorhizobium* species (*Mesorhizobium ciceri* biovar *biserrulae* WSM1271, *Mesorhizobium opportunistum* WSM2075, and *Mesorhizobium loti* MAFF303099), have been sequenced in the last 10 years (4).

The genome of *M. amorphae* strain CCNWGS0123 was sequenced using a 454 GS FLX sequencer (6) and assembled with GS *De novo* Assembler (“Newbler”) version 2.3, producing 257 large contigs (>500 bp), with 7,287,282 bp in total (62.88% G+C content) and an average contig size of 28,355 bp. The draft genome sequence comprises 7,296,463 bases, with an 18-fold coverage of the *M. amorphae* strain CCNWGS0123 genome. Functional annotation was performed using the Rapid Annotation Subsystem Technology (RAST) server (1), with at least one copy each of the 5S, 23S, and 16S rRNAs, 51 tRNA genes, and 7,004 protein-coding sequences (CDSs) annotated. Of 7,004 CDSs, 1,954 were assigned to hypothetical or unknown proteins according to the current database. Moreover, based on BLASTp with KEGG (Kyoto Encyclopedia of Genes and Genomes) orthology (KO), 2,765 proteins have orthologs (bit score, >60) in five reference strains, i.e., a *Mesorhizobium loti* strain, *Sinorhizobium meliloti* 1021, *Agrobacterium tumefaciens* C58, *Mesorhizobium* sp. strain BNC1, and a *Rhizobium leguminosarum* strain, which are the closest neighbors listed in RAST (<http://www.genome.jp/kegg>). A total of 5,483 proteins could be assigned to COG families (clusters of orthologous groups) through the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>).

The *M. amorphae* CCNWGS0123 genome carries multiple genes potentially involved in copper resistance (*cusAB*, encoding an RND-type (resistance-nodulation-cell division protein family) copper efflux system; four genes encoding putative heavy/transi-

tion metal-translocating P_{1B}-type ATPases with a typical CPX metal binding motif in the sixth transmembrane helix; and two operons encoding putative copper resistance determinants, including a multicopper oxidase [MCO], an outer membrane protein, and a blue copper oxidase [copper tolerance protein]). Phylogenetic analysis of the three MCOs with known MCOs shows that they are most closely related to CopA/PcoA of previously studied copper resistance systems (3, 9).

Genes related to plant growth promotion, including those coding for nitrogen fixation (*nif*), nodulation (*nod*), siderophores, 1-aminocyclopropane-1-carboxylate deaminase (ACC), the root-promoting hormone acetoin, and antimicrobial compounds (e.g., 4-hydroxybenzoate), were also identified. Fifteen type III secretion system (TTSS)-like genes, which could influence the extent of the symbiosis, depending on the host plants, encode proteins that could form a complex across the inner and outer membranes to the external environment (7, 8). Furthermore, 25 genes were predicted to be involved in streptomycin, novobiocin, penicillin-cephalosporin, puromycin, butirosin, and neomycin biosynthesis. Three putative chloramphenicol acetyltransferase genes were identified in the genome, which provides chloramphenicol resistance at concentrations of up to 1,000 μg/ml on tryptone-yeast extract (TY) agar.

Nucleotide sequence accession numbers. The draft genome sequence has been deposited in GenBank under the accession number AGSN00000000. The version described in this paper is the first version, AGSN01000000.

ACKNOWLEDGEMENTS

This work was supported by projects from National Science Foundation of China (31125007, 30970003, and 30900215), the 973 Project of China (2010CB126502), and the International Science & Technology Cooperation Program of China (2010DFA91930).

Sequencing was performed at the University of Arizona Genetics Core.

Received 6 November 2011 Accepted 11 November 2011

Address correspondence to Gehong Wei, weigehong@yahoo.com.cn, or Christopher Rensing, rensingc@ag.arizona.edu.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.06475-11

REFERENCES

1. Aziz R, et al. 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
2. Chen WM, Wu CH, James EK, Chang JS. 2008. Metal biosorption capability of *Cupriavidus taiwanensis* and its effects on heavy metal removal by nodulated *Mimosa pudica*. *J. Hazard Mater.* 151:364–371.
3. Hoegger PJ, Kilaru S, James TY, Thacker JR, Kües U. 2006. Phylogenetic comparison and classification of laccase and related multicopper oxidase protein sequences. *FEBS J.* 273:2308–2326.
4. Kaneko T, et al. 2000. Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. *DNA Res.* 7:331.
5. Ma Y, Prasad MN, Rajkumar M, Freitas H. 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metal-liferous soils. *Biotechnol. Adv.* 29:248–258.
6. Margulies M, et al. 2005. Genome sequencing in open microfabricated high density picoliter reactors. *Nature* 437:376.
7. Marie C, Broughton WJ, Deakin WJ. 2001. Rhizobium type III secretion systems: legume charmers or alarmers? *Curr. Opin. Plant Biol.* 4:336–342.
8. Marie C, et al. 2003. Characterization of Nops, nodulation outer proteins, secreted via the type III secretion system of NGR234. *Mol. Plant Microbe Interact.* 16:743–751.
9. Roberts SA, et al. 2002. Crystal structure and electron transfer kinetics of CueO, a multicopper oxidase required for copper homeostasis in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 99:2766.
10. Wei G, et al. 2009. Invasive *Robinia pseudoacacia* in China is nodulated by *Mesorhizobium* and *Sinorhizobium* species that share similar nodulation genes with native American symbionts. *FEMS Microbiol. Ecol.* 68:320–328.
11. Wu CH, Wood TK, Mulchandani A, Chen W. 2006. Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. *Appl. Environ. Microbiol.* 72:1129–1134.