

Genome Sequence of the 1,4-Dioxane-Degrading *Pseudonocardia dioxanivorans* Strain CB1190[∇]

Christopher M. Sales,^{1†} Shaily Mahendra,^{2†*} Ariel Grostern,¹ Rebecca E. Parales,³
Lynne A. Goodwin,⁴ Tanja Woyke,⁵ Matt Nolan,⁵ Alla Lapidus,⁵ Olga Chertkov,⁴
Galina Ovchinnikova,⁵ Alexander Sczyrba,⁵ and Lisa Alvarez-Cohen^{1,6}

Department of Civil and Environmental Engineering, University of California, Berkeley, California 94720-1710¹; Department of Civil and Environmental Engineering, University of California, Los Angeles, California 90095²; Department of Microbiology, University of California, Davis, California 95616³; Los Alamos National Laboratory, Joint Genome Institute, Biosciences Division Genome Science B6, Los Alamos, New Mexico 87545⁴; U.S. DOE Joint Genome Institute, 2800 Mitchell Drive B310, Walnut Creek, California 94598-1698⁵; and Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720⁶

Received 25 March 2011/Accepted 10 June 2011

***Pseudonocardia dioxanivorans* CB1190 is the first bacterium reported to be capable of growth on the environmental contaminant 1,4-dioxane and the first member of the genus *Pseudonocardia* for which there is an annotated genome sequence. Preliminary analysis of the genome (chromosome and three plasmids) indicates that strain CB1190 possesses several multicomponent monooxygenases that could be involved in the aerobic degradation of 1,4-dioxane and other environmental contaminants.**

Here we report the genome sequence of the Gram-positive actinomycete *Pseudonocardia dioxanivorans* strain CB1190. *P. dioxanivorans* strain CB1190 (NCBI taxonomy ID 675635) was isolated from 1,4-dioxane-contaminated sludge and displays the rare ability to use 1,4-dioxane, an emerging groundwater contaminant, as a sole carbon and energy source (9, 10, 11, 14). This strain can also grow on other ethers (e.g., 2-methyl-1,3-dioxolane and butyl methyl ether), alcohols, and benzene as the sole carbon and energy source, and it can grow autotrophically with CO₂ by using H₂ as the electron donor (9, 14).

Pseudonocardia represents a genus of bacteria with diverse niches, from free-living microorganisms capable of degrading xenobiotic compounds (5–8, 15, 17) to the antibiotic-producing symbionts of fungus-farming ants (1, 2, 13, 20). Strain CB1190 was chosen for sequencing by the U.S. Department of Energy Joint Genome Institute (JGI) to gain insight into 1,4-dioxane metabolism, as well as to learn about general carbon and nitrogen metabolism in the genus.

Sequencing of strain CB1190 genomic DNA was carried out by the 454 and Illumina techniques. All techniques for DNA isolation, library construction, and sequencing were performed according to JGI standards and protocols (<http://www.jgi.doe.gov>). 454 reads (67-fold coverage) were assembled with Newbler (12). Illumina reads (136-fold coverage) were assembled with Velvet 0.7.63 (19), and then contigs of >800 bp were shredded into 1.5-kb pseudoreads. The pseudoreads were combined with the 454 reads into a hybrid 454/Illumina data set with parallel phrap, version SPS-4.24 (High Performance Software, LLC). dup454Finisher was used to correct misassembled

repeats (4), and gapResolution (<http://www.jgi.doe.gov>) was used to resolve gaps in the assembly. Gaps between contigs were closed by editing in Consed, by PCR, and by Bubble PCR primer walks (J.-F. Cheng, unpublished data). The genome consists of 4 repicons, including the chromosome (7.1 Mb), plasmid 1 (circular, 192 kb), plasmid 2 (circular, 137 kb with gaps), and plasmid 3 (linear, 15 kb), and has an average G+C content of 73.1%. Coding sequences were predicted with Prodigal (<http://prodigal.ornl.gov>) and were automatically annotated by comparisons to the Pfam, KEGG, and COG databases. A total of 6,799 candidate protein-encoding genes were predicted in this manner. The genome contains 46 tRNA genes and three copies of the rRNA genes.

An analysis of the strain CB1190 genome revealed the presence of eight putative gene clusters encoding bacterial multicomponent monooxygenases, including a cluster with high similarity to a gene cluster associated with tetrahydrofuran degradation in *Pseudonocardia tetrahydrofuranoxydans* strain K1 (16). Discovery of these monooxygenase genes may indicate that strain CB1190 is capable of using a diversity of organic compounds as sole carbon and energy sources. Genes encoding complete citric acid and pentose phosphate pathways are present, indicating a heterotrophic mode of metabolism, and genes encoding the complete biosynthesis pathways for all 20 amino acids have been identified. The autotrophic capability of strain CB1190 (14) is explained by the presence of the typical Calvin-Benson-Bassham cycle for CO₂ fixation.

Nucleotide sequence accession numbers. The chromosome and plasmid sequences of *Pseudonocardia dioxanivorans* strain CB1190 have been deposited in GenBank with accession numbers NC_015312-4 and CP002595-7.

* Corresponding author. Mailing address: Department of Civil and Environmental Engineering, University of California, Los Angeles, CA 90095. Phone: (310) 794-9850. Fax: (310) 206-2222. E-mail: mahendra@seas.ucla.edu.

† C. M. Sales and S. Mahendra contributed equally to this work.

[∇] Published ahead of print on 1 July 2011.

This project was funded by the Strategic Environmental Research and Development Program (SERDP ER-1417). The work conducted by the U.S. Department of Energy Joint Genome Institute was sup-

ported by the Office of Science of the U.S. Department of Energy under contract number DE-AC02-05CH11231.

We thank Helene Feil for help with genomic DNA extractions.

REFERENCES

1. **Barke, J., et al.** 2010. A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*. *BMC Biol.* **8**:109.
2. **Cafaro, M. J., et al.** 2011. Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. *Proc. R. Soc. B Biol. Sci.* **278**:1814–1822.
3. Reference deleted.
4. **Han, C., and P. Chain.** 2006. Finishing repeat regions automatically with Dupfinisher, p. 141. *In* H. R. Arabnia and H. Valafar (ed.), *Proc. 2006 Int. Conf. Bioinform. Comput. Biol.* CSREA Press, Las Vegas, NV.
5. **Juteau, P., R. Larocque, D. Rho, and A. LeDuy.** 1999. Analysis of the relative abundance of different types of bacteria capable of toluene degradation in a compost biofilter. *Appl. Microbiol. Biotechnol.* **52**:863–868.
6. **Kohlweyer, U., B. Thiemer, T. Schrader, and J. R. Andreessen.** 2000. Tetrahydrofuran degradation by a newly isolated culture of *Pseudonocardia* sp. strain K1. *FEMS Microbiol. Lett.* **186**:301–306.
7. **Lechevalier, M. P., H. Prauser, D. P. Labeda, and J. S. Ruan.** 1986. Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. *Int. J. Syst. Bacteriol.* **36**:29–37.
8. **Lee, S., S. E. Strand, H. D. Stensel, and R. P. Herwig.** 2004. *Pseudonocardia chloroethenivorans* sp. nov., a chloroethene degrading actinomycete. *Int. J. Syst. Evol. Microbiol.* **54**:131–139.
9. **Mahendra, S., and L. Alvarez-Cohen.** 2005. *Pseudonocardia dioxanivorans* sp. nov., a novel actinomycete that grows on 1,4-dioxane. *Int. J. Syst. Evol. Microbiol.* **55**:593–598.
10. **Mahendra, S., and L. Alvarez-Cohen.** 2006. Kinetics of 1,4-dioxane biodegradation by monoxygenase-expressing bacteria. *Environ. Sci. Technol.* **40**:5435–5442.
11. **Mahendra, S., C. J. Petzold, E. E. Baidoo, J. D. Keasling, and L. Alvarez-Cohen.** 2007. Identification of the intermediates of *in vivo* oxidation of 1,4-dioxane by monoxygenase-containing bacteria. *Environ. Sci. Technol.* **41**:7330–7336.
12. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
13. **Oh, D., M. Poulsen, C. R. Currie, and J. Clardy.** 2009. Dentigerumycin: a bacterial mediator of an ant-fungus symbiosis. *Nat. Chem. Biol.* **5**:391–393.
14. **Parales, R. E., J. E. Adamus, N. White, and H. D. May.** 1994. Degradation of 1,4-dioxane by an actinomycete in pure culture. *Appl. Environ. Microbiol.* **60**:4527–4530.
15. **Reichert, K., A. Lipski, S. Pradella, E. Stackebrandt, and K. Altendorf.** 1998. *Pseudonocardia asaccharolytica* sp. nov. and *Pseudonocardia sulfidoxydans* sp. nov., two new dimethyl disulfide-degrading actinomycetes and emended description of the genus *Pseudonocardia*. *Int. J. Syst. Bacteriol.* **48**:441–449.
16. **Thiemer, B., J. R. Andreessen, and T. Schrader.** 2003. Cloning and characterization of a gene cluster involved in tetrahydrofuran degradation in *Pseudonocardia* sp. strain K1. *Arch. Microbiol.* **179**:266–277.
17. **Vainberg, S., et al.** 2006. Biodegradation of ether pollutants by *Pseudonocardia* sp. strain ENV478. *Appl. Environ. Microbiol.* **72**:5218–5224.
18. **Zenker, M. J., R. C. Borden, and M. A. Barlaz.** 2003. Occurrence and treatment of 1,4-dioxane in aqueous environments. *Environ. Eng. Sci.* **20**:423–432.
19. **Zerbino, D. R., and E. Birney.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* **18**:821–829.
20. **Zucchi, T. D., A. S. Guidolin, and F. L. Cônsoli.** 2011. Isolation and characterization of actinobacteria ectosymbionts from *Acromyrmex subterraneus brunneus* (Hymenoptera, Formicidae). *Microbiol. Res.* **166**:68–76.