

Genome Announcement

Genome Sequence of the Chemolithoautotrophic Bacterium *Oligotropha carboxidovorans* OM5^{TV}

Debarati Paul,¹ Susan Bridges,^{2,3} Shane C. Burgess,^{1,3,4} Yoginder Dandass,^{2,3} and Mark L. Lawrence^{1,3*}

College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi 39762¹; Department of Computer Science and Engineering, Mississippi State University, Mississippi State, Mississippi 39762²; Institute for Digital Biology, Mississippi State University, Mississippi State, Mississippi 39762³; and Life Sciences and Biotechnology Institute, Mississippi State University, Mississippi State, Mississippi 39762⁴

Received 2 May 2008/Accepted 22 May 2008

***Oligotropha carboxidovorans* OM5^T (DSM 1227, ATCC 49405) is a chemolithoautotrophic bacterium with the capability to utilize carbon monoxide, carbon dioxide, and hydrogen. It is also capable of heterotrophic growth under appropriate environmental conditions. Here we report the annotated genome sequence of the circular chromosome of this organism.**

Oligotropha carboxidovorans strain OM5 was originally isolated from wastewater by enrichment culture studies that were conducted to isolate CO-utilizing bacteria. It was originally identified as *Pseudomonas carboxidovorans* (4) and later reclassified as *Oligotropha carboxidovorans* (5). It belongs to the gram-negative family *Bradyrhizobiaceae*, which includes plant-associated species (e.g., *Bradyrhizobium*), animal-associated species (e.g., *Afiplia*), and free-living bacteria (e.g., *Rhodopseudomonas*).

The complete genome sequence of *Oligotropha carboxidovorans* strain OM5 was determined using a combination of standard 454 pyrosequencing and paired-end 454 sequencing at 454 Life Sciences (Roche) to generate a scaffolded assembly. A total of 1,242,155 reads from standard sequencing and 536,302 reads from paired-end sequencing were assembled using Newbler assembler (Roche). Five scaffolds containing 62 contigs were obtained after paired-end sequencing, two of which were comprised of sequences belonging to the megaplasmid pHCG3, and the remaining three formed the chromosome. The gaps between contigs were closed by PCR amplification followed by Sanger sequencing with primer walking (Eurofins MWG Operon). Sanger reads were incorporated into the assembly using Sequencher (Gene Codes) and SeqManPro (Lasergene). Bases in the assembled contigs carried a Phred-equivalent quality score of 40 or above, which means that the level of accuracy was 99.99%. After gap closure, the circular chromosome was comprised of two contigs.

The *O. carboxidovorans* OM5 chromosome sequence contains 3,745,772 bp, had an average sequencing depth of ~27-fold, and contained 3,754 putative open reading frames. The sequence was

annotated using The Institute for Genomic Research Annotation Engine, which used an interleaved Markov model (Glimmer) (1) for gene prediction, and it was manually curated using Manatee (The Institute for Genomic Research) for accuracy. The genome has been deposited in DDBJ/EMBL/GenBank under the project accession ABKN00000000.

The genome contains 49 tRNA genes and a single rRNA operon. The 3.7-Mb circular chromosome has a G+C content of 62.4%. No plasmids were found except the megaplasmid pHCG3 (2). Genes encoding metabolic enzymes involved in energy metabolism, biosynthesis of amino acids/fatty acids, nucleotide metabolism, transcription, and DNA metabolism, protein synthesis and degradation, and signal transduction were identified on the chromosome. The chromosome also contains genes encoding enzymes that allow heterotrophic growth on organic acids, such as acetate, pyruvate, lactate, crotonate, malate, succinate, formate, and glyoxylate as substrates. The megaplasmid pHCG3 contains genes for chemolithoautotrophic utilization of CO and CO₂ (carboxidotrophy), H₂ and CO₂ (hydrogenotrophy), and CO₂ fixation under aerobic conditions (2).

Syngas is a mixture of CO, CO₂, and H₂ that results from gasification of organic wastes, and microbial fermentation of this gas mixture could serve as a source for biofuels (3). *O. carboxidovorans* is capable of utilizing these gases for chemolithoautotrophic growth. Therefore, the *O. carboxidovorans* genome sequence will allow future investigations into how it is able to assimilate carbon that is fixed by chemolithoautotrophy into its metabolism, which could benefit the future use of syngas for biofuel production. These studies would not be possible from the sequence of the megaplasmid alone. In addition, this genome sequence will enable future studies to determine how the metabolism of *O. carboxidovorans* functions under heterotrophic conditions versus chemolithoautotrophic conditions and how it is able to switch between these two contrasting lifestyles.

* Corresponding author. Mailing address: College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762. Phone: (662) 325-1195. Fax: (662) 325-1031. E-mail: lawrence@cvm.msstate.edu.

[†] Published ahead of print on 6 June 2008.

This research was supported by the U.S. Department of Energy under award number DE-FG3606GO86025.

We are grateful to the Mississippi State University Life Sciences and Biotechnology Institute for hosting Manatee. We thank Todd French and Michele Williams for technical advice and Michelle Banes for technical assistance. We thank Ranjit Kumar and Tony Arick for bioinformatics support.

REFERENCES

1. **Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg.** 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* **27**:4636–4641.
2. **Fuhrmann, S., M. Ferner, T. Jeffke, A. Henne, G. Gottschalk, and O. Meyer.** 2003. Complete nucleotide sequence of the circular megaplasmid pHCG3 of *Oligotropha carboxydovorans*: function in the chemolithoautotrophic utilization of CO, H₂ and CO₂. *Gene* **322**:67–75.
3. **Henstra, A. M., J. Sipma, A. Rinzema, and A. J. Stams.** 2007. Microbiology of synthesis gas fermentation for biofuel production. *Curr. Opin. Biotechnol.* **18**:200–206.
4. **Meyer, O., and H. G. Schlegel.** 1978. Reisolation of the carbon monoxide utilizing hydrogen bacterium *Pseudomonas carboxydovorans* (Kistner) comb. nov. *Arch. Microbiol.* **118**:35–43.
5. **Meyer, O., E. Stackebrandt, and G. Auling.** 1993. Reclassification of ubiquinone Q-10 containing carboxidotrophic bacteria: transfer of “[*Pseudomonas*] *carboxydovorans*” OM5T to *Oligotropha*, gen. nov., as *Oligotropha carboxydovorans*, comb. nov., transfer of “[*Alcaligenes*] *carboxydus*” DSM 1086T to *Carbophilus*, gen. nov., as *Carbophilus carboxidus*, comb. nov., transfer of “[*Pseudomonas*] *compransoris*” DSM 1231T to *Zavarzinia*, gen. nov., as *Zavarzinia compransoris*, comb. nov., and amended descriptions of the new genera. *Syst. Appl. Microbiol.* **16**:390–395.