

Genome Sequence of the Arctic Methanotroph *Methylobacter tundripaludum* SV96[∇]

Mette M. Svenning,^{1*} Anne Grethe Hestnes,¹ Ingvild Wartiainen,¹ Lisa Y. Stein,² Martin G. Klotz,³ Marina G. Kalyuzhnaya,⁴ Anja Spang,⁵ Françoise Bringel,⁶ Stéphane Vuilleumier,⁶ Aurélie Lajus,⁷ Claudine Médigue,⁷ David C. Bruce,⁸ Jan-Fang Cheng,⁹ Lynne Goodwin,⁸ Natalia Ivanova,⁹ James Han,⁹ Cliff S. Han,⁸ Loren Hauser,¹⁰ Brittany Held,⁸ Miriam L. Land,¹⁰ Alla Lapidus,⁹ Susan Lucas,⁹ Matt Nolan,⁹ Sam Pitluck,⁹ and Tanja Woyke⁹

Department of Arctic and Marine Biology, University of Tromsø, 9037 Tromsø, Norway¹; Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada²; Departments of Biology and Microbiology & Immunology, University of Louisville, Louisville, Kentucky 40292³; Department of Microbiology, University of Washington, Seattle, Washington 98195⁴; Department of Genetics in Ecology, University of Vienna, Vienna, Austria⁵; Université de Strasbourg, Équipe Adaptations et Interactions Microbiennes dans l'Environnement, UMR 7156 CNRS, 67000 Strasbourg, France⁶; CEA/DSV/IG/Genoscope & CNRS UMR8030, Laboratoire d'Analyses Bioinformatiques pour la Génomique et le Métabolisme (LABGeM), Genoscope-IG-CEA, 91057 Evry, France⁷; Los Alamos National Laboratory, Joint Genome Institute, Biosciences Division, Genome Science B6, Los Alamos, New Mexico 87545⁸; U.S. DOE Joint Genome Institute, Walnut Creek, California 94598⁹; and Oak Ridge National Laboratory, Bioscience Division, Oak Ridge, Tennessee 37831¹⁰

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***Methylobacter tundripaludum* SV96^T (ATCC BAA-1195) is a psychrotolerant aerobic methane-oxidizing gammaproteobacterium (*Methylococcales*, *Methylococcaceae*) living in High Arctic wetland soil. The strain was isolated from soil harvested in July 1996 close to the settlement Ny-Ålesund, Svalbard, Norway (78°56'N, 11°53'E), and described as a novel species in 2006. The genome includes *pmo* and *pxm* operons encoding copper membrane monooxygenases (Cu-MMOs), genes required for nitrogen fixation, and the *nirS* gene implicated in dissimilatory nitrite reduction to NO but no identifiable inventory for further processing of nitrogen oxides. These genome data provide the basis to investigate *M. tundripaludum* SV96, identified as a major player in the biogeochemistry of Arctic environments.**

Arctic permafrost environments are methane (CH₄) reservoirs. Ongoing and future changes in the Arctic may contribute to increased turnover of organic carbon, resulting in higher CH₄ emissions. As the sole biological methane filter, methanotrophic bacteria (methanotrophs) are important controllers in this process. While both alpha- and gammaproteobacterial methanotrophs are present in circumpolar permafrost regions, including Canada, Siberia, and Svalbard, a dominance of the gammaproteobacterial genus *Methylobacter* was observed (8, 10, 14). *Methylobacter tundripaludum* SV96 was identified as an active methanotroph in its *locus typicus* by RNA-SIP (stable isotope probing) with ¹³C methane (5, 15).

The draft genome of *M. tundripaludum* SV96 was generated at the DOE Joint Genome Institute (JGI) using a combination of Illumina (1) and 454 technologies (9). The Illumina GAii shotgun library produced 41,446,074 reads (3,149.9 Mbp), a 454 Titanium standard library yielded 500,724 reads, and a paired-end 454 library (7-kb average insert size) delivered 271,299 reads. The 220.6 Mbp of 454 data were assembled with Newbler (version 2.3). Illumina data were assembled with

VELVET, version 1.0.13 (16). Reads were assembled after computational shredding using parallel phrap (SPS version 4.24; High Performance Software, LLC). Consed software (2, 3, 4) was used for finishing. Potential base errors and consensus quality were corrected using Illumina data and Polisher software (A. Lapidus, unpublished data). Possible misassemblies were corrected using gapResolution (C. Han, unpublished data), Dupfinisher (6), or sequencing of subcloned bridging PCR fragments. Gaps between contigs were closed by PCR or bubble PCR primer walks using Consed (J.-F. Cheng, unpublished data). Gaps were closed with 1,095 PCRs, yielding a sequence of 3 contigs (2,936,421, 367,513, and 1,544,395 bp). The final assembly represents 190.8 Mbp of 454 data and 2,930.7 Mbp of Illumina data. Sequence annotation and comparative genome analysis are under way with assistance from the MicroScope platform at Genoscope (13).

The *M. tundripaludum* SV96 genome contains one operon for particulate methane monooxygenase (*pmoCAB*) and the recently discovered *pxm* operon (*pxmABC*), encoding a copper membrane monooxygenase of unknown function (12). Genes involved in methanol oxidation (*mxoA*), H₄folate (*mtdA-fch*) and H4MTP-linked (*fae-mtdB-mch-fhcABCD*) C₁ transfer pathways, membrane-associated quinoprotein formaldehyde dehydrogenase (*ald*), and formate oxidation (*fdsGBACD*) were identified. Genes for a complete RuMP pathway for carbon

* Corresponding author. Mailing address: Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, University of Tromsø, 9037 Tromsø, Norway. Phone: 4777644432. Fax: 4777646333. E-mail: mette.svenning@uit.no.

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assimilation and the oxidative TCA cycle were present, while the serine cycle was incomplete.

Genes encoding necessary inventory for nitrogen fixation, nitrate transport (*nark*) and assimilation (*narGHJI*), nitrite assimilation (*nirBD*), and ammonia transport (*amtB*) and assimilation (glutamine synthetase/glutamate synthase but not alanine dehydrogenase) were found. Unique thus far to methanotrophic bacteria, a complete urea cycle was detected, as well as a cytochrome *cd₁*, NirS nitrite reductase and associated cofactors for reduction of nitrite to nitric oxide. Genes encoding nitric oxide reductases were not found. Genes encoding hydroxylamine reductase and its electron-donating partner were identified, suggesting a hydroxylamine detoxification mechanism similar to that found in *Methylosinus trichosporium* OB3b (11). At least 20 homologs of cold shock-related proteins were detected, including 6 homologs of CspD (7).

Nucleotide sequence accession number. The *Methylobacter tundripaludum* SV96 genome sequence is available in GenBank under accession number AEGW00000000.

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REFERENCES

- Bennett, S. 2004. Solexa Ltd. Pharmacogenomics. *5*:433–438.
- Ewing, B., and P. Green. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* **8**:186–194.
- Ewing, B., L. Hillier, M. C. Wendl, and P. Green. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* **8**:175–185.
- Gordon, D., C. Abajian, and P. Green. 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* **8**:195–202.
- Graef, C., A. G. Hestnes, M. M. Svenning, and P. Frenzel. 2011. The active methanotrophic community in a wetland from the High Arctic. *Environ. Microbiol. Rep.* doi:10.1111/j.1758-2229.2010.00237.x.
- Han, C., and P. Chain. 2006. Finishing repeat regions automatically with Dupfinisher, p. 141–147. *In* H. R. Arabnia and H. Valafar (ed.), *Proceedings of the 2006 International Conference on Bioinformatics & Computational Biology*, CSREA Press, Las Vegas, NV.
- Horn, G., R. Hofweber, W. Kremer, and H. R. Kalbitzer. 2007. Structure and function of bacterial cold shock proteins. *Cell. Mol. Life Sci.* **64**:1457–1470.
- Liebner, S., K. Rublack, T. Stuehrmann, and D. Wagner. 2009. Diversity of aerobic methanotrophic bacteria in a permafrost active layer soil of the Lena Delta, Siberia. *Microb. Ecol.* **57**:25–35.
- Margulies, M., et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
- Martineau, C., L. G. Whyte, and C. W. Greer. 2010. Stable isotope probing analysis of the diversity and activity of methanotrophic bacteria in soils from the Canadian High Arctic. *Appl. Environ. Microbiol.* **76**:5773–5784.
- Stein, L. Y., et al. 2010. Genome sequence of the obligate methanotroph *Methylosinus trichosporium* strain OB3b. *J. Bacteriol.* **192**:6497–6498.
- Tavormina, P. L., V. J. Orphan, M. G. Kalyuzhnaya, M. S. M. Jetten, and M. G. Klotz. 2011. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. *Environ. Microbiol. Rep.* **3**:91–100.
- Vallenet, D., et al. 2009. MicroScope: a platform for microbial genome annotation and comparative genomics. *Database (Oxford)* **2009**:bap021. doi: 10.1093/database/bap021.
- Wartiainen, I., A. G. Hestnes, and M. M. Svenning. 2003. Methanotrophic diversity in High Arctic wetland on the islands of Svalbard (Norway)—denaturing gradient gel electrophoresis analysis of soil DNA and enrichment cultures. *Can. J. Microbiol.* **49**:602–612.
- Wartiainen, I., A. G. Hestnes, I. R. McDonald, and M. M. Svenning. 2006. *Methylobacter tundripaludum* sp. nov., a methane-oxidising bacterium from Arctic wetland soil on the Svalbard islands, Norway (78°N). *Int. J. Syst. Evol. Microbiol.* **56**:109–113.
- Zerbino, D., and E. Birney. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* **18**:821–829.