Draft Genome Sequence of *Methylovulum psychrotolerans* Sph1T, an Obligate Methanotroph from Low-Temperature Environments

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**ABSTRACT** *Methylovulum psychrotolerans* Sph1T is an aerobic, obligate methanotroph, which was isolated from cold methane seeps in West Siberia. This bacterium possesses only a particulate methane monooxygenase and is widely distributed in low-temperature environments. Strain Sph1T has the genomic potential for biosynthesis of hopanoids required for the maintenance of intracytoplasmic membranes.

*Methylovulum psychrotolerans* Sph1T is a neutrophilic aerobic methanotroph of the class *Gammaproteobacteria*, family *Methylococcaceae*. It was isolated from a cold methane seep in the Irtysh basin, West Siberia (1). This methanotroph grows well at low temperatures and is commonly detected in various low-temperature environments, such as arctic lakes, glaciers, and northern peatlands (2–4). Cells of *M. psychrotolerans* Sph1T are Gram-negative, nonmotile, encapsulated, large cocci that multiply by binary fission and utilize methane and methanol.

For the genome sequencing of strain Sph1T, a combination of fragment and mate-paired library approaches was used. Both libraries were sequenced with the Illumina MiSeq platform using 2 × 250-bp paired-end sequencing reagents. Mate-paired reads were treated with the NextClip tool (5), resulting in 1,082,354 read pairs with a mean insert size of 3,183 bp. Paired-end reads were filtered and trimmed by quality with CLC Genomics Workbench (Qiagen, Germany) using recommended parameters, resulting in 431,801 read pairs. De novo assembly was performed with the SPAdes version 3.11.0 assembler (6). The total length of the final assembly was 5,189,806 bp; it consisted of 97 genomic scaffolds with an N50 value of 334,445 bp. Final genome coverage was 40× for the mate-paired library and 23× for the paired-end library. The estimated size of the *M. psychrotolerans* Sph1T genome is 5.2 Mb (coverage, 63×), with an average G+C content of 50.8%. In total, 4,577 predicted protein-coding genes were identified.

The average nucleotide identity between the genomes of *M. psychrotolerans* Sph1T and another described member of this genus, *M. miyakonense* HT12T (7), is only 79%, which confirms their classification as two different species. The genome of *M. psychrotolerans* Sph1T contains a single *pmoCAB* operon for particulate methane monooxygenase (MMO). The presence of the homologous *pxmABC* operon, which is characteristic of many gammaproteobacterial methanotrophs (7, 8), was not detected. In contrast to *M. miyakonense* HT12T, an operon encoding soluble MMO (mmoXYBZDC) is lacking in *M. psychrotolerans* Sph1T. The ability to grow on methanol is explained by the presence of the gene operons encoding MxaFl- and XoxF-methanol dehydrogenases (9). Genes involved in tetrahydromethanopterin (H₄MTP), tetrahydrofolate (H₄-folate)-linked C1 transfer, and formate oxidation were...
identified. A complete set of genes encoding formaldehyde assimilation in the ribulose monophosphate pathway was present, while the serine cycle was incomplete.

The genome includes all genes required for glycolgen biosynthesis (glgA, glgB, and glgC) (10) and nitrogen metabolism, including genes for nitrate/nitrite reduction, ammonium and urea uptake and assimilation, as well as key genes for nitrogen fixation.

Strain Sph1T has the genomic potential for the biosynthesis of hopanoids, which is required for the maintenance of intracytoplasmic membranes (11). A complete set of genes for the nonmevalonate pathway and the ispA gene encoding farnesyl diphosphate synthase were identified. The genes responsible for squalene synthesis (12), two copies of the shc gene encoding the enzyme responsible for the cyclization of squalene into diploptene (13), as well as the hpnH and hpnG genes involved in the conversion of diploptene to bacteriohopanetetrol (14) were also present.

**Accession number(s).** The *M. psychrotolerans* Sph1T genome sequence was deposited in GenBank under the accession no. PGFZ00000000.

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**REFERENCES**

1. Oshkin IY, Belova SE, Danilova OV, Miroshnikov KK, Rijpstra WIC, Sinninghe Damsté JS, Dedysh SN. 2016. *Methyloluvum psychrotolerans* sp. nov., a cold-adapted methanotroph from terrestrial environments, and emended description of the genus *Methyloluvum*. Int J Syst Evol Microbiol 65:2417–2423. https://doi.org/10.1099/ijsem.0.001046.

2. He R, Wooller MJ, Pohlmayer JW, Catanis C, Quensen J, Leigh MB. 2012. Identification of functionally active aerobic methanotrophs in sediments from an arctic lake using stable isotope probing. Environ Microbiol 14:1403–1419. https://doi.org/10.1111/j.1462-2920.2012.02725.x.

3. Dieser M, Broemser EL, Cameron KA, King GM, Achberger A, Choquette K, Hagedorn B, Sletten R, Junge K, Christner BC. 2014. Molecular and biogeochemical evidence for methane cycling beneath the western margin of the Greenland ice sheet. ISME J 8:2305–2316. https://doi.org/10.1038/ismej.2014.59.

4. Putkenin A, Larmola T, Tuomivirta T, Sillanen HMP, Bodrossy L, Tuittila E-S, Fritze H. 2014. Peatland succession induces a shift in the community composition of *Sphagnum*-associated active methanotrophs. FEMS Microbiol Ecol 88:596–611. https://doi.org/10.1111/1574-6941.12327.

5. Leggett RM, Clavijo BJ, Clissold L, Clark MD, Caccamo M. 2014. NextClip: an analysis and read preparation tool for Nextera long mate pair libraries. Bioinformatics 30:566–568. https://doi.org/10.1093/bioinformatics/btt702.

6. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Pjribselis AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Wowk VN, McLean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembly of single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/cmb.2013.0084.

7. Hamilton R, Kits KD, Ramonovskaya VA, Rozova ON, Yurimoto H, Iguchi H, Khmelenina VN, Sakai Y, Dunfield PF, Klotz MG, Kniec C, Op den Camp HJM, Jetten MSM, Bringel F, Vuilleumier S, Svenning M, Shapiro N, Woyke T, Trotsenko YA, Stein LY, Kalyuzhnaya MG. 2015. Draft genomes of gammaproteobacterial methanotrophs isolated from terrestrial ecosystems. Genome Announc 3(3):e00515-15. https://doi.org/10.1128/genomeA.00515-15.

8. Tavormina PL, Orphan VJ, Kalyuzhnaya MG, Jetten MSM, Klotz MG. 2011. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. Environ Microbiol Rep 3:99–101. https://doi.org/10.1111/j.1758-5766.2010.00192.x.

9. Keltjens JT, Pol A, Reimann J, Op den Camp HJM. 2014. PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. Appl Microbiol Biotechnol 98:6163–6183. https://doi.org/10.1007/s00253-014-7566-6.

10. Khadem AF, van Teeseling MCF, van Niftrik L, Jetten MSM, Op den Camp HJM, Pol A. 2012. Genomic and physiological analysis of carbon storage in the verrucomicrobial methanotroph “Ca. Methylacidiphilum fumarico-licum” SolV. Front Microbiol 3:345. https://doi.org/10.3389/fmicb.2012.00345.

11. Welander PV, Summons RE. 2012. Discovery, taxonomic distribution, and phenotypic characterization of a gene required for 3-methylhopanoid production. Proc Natl Acad Sci USA 109:12905–12910. https://doi.org/10.1073/pnas.1208255109.

12. Pan JJ, Solbiati JO, Ramamoorthy G, Hillerich BS, Seidel RD, Cronan JE, Almo SC, Poulter CD. 2015. Biosynthesis of squalene from farnesyl diposphate in bacteria: three steps catalyzed by three enzymes. ACS Cent Sci 1:77–82. https://doi.org/10.1021/acscentsci.5b00115.

13. Sinninghe Damsté JS, Rijpstra WIC, Dedysh SN, Foesel BU, Villanueva L. 2017. Pheno- and genotyping of hopanoid production in Acidobacteria. Front Microbiol 8:968. https://doi.org/10.3389/fmicb.2017.00968.

14. Schmerk CL, Welander PV, Hamad MA, Bain KL, Bernards MA, Summons RE, Valvano MA. 2015. Elucidation of the *Burkholderia cenocepacia* hopanoid biosynthesis pathway uncovers functions for conserved proteins in hopanoid-producing bacteria. Environ Microbiol 17:735–750. https://doi.org/10.1111/1462-2920.12509.