genomeA
Journals.ASM.org

Draft Genomes of Gammaproteobacterial Methanotrophs Isolated from Terrestrial Ecosystems

Richard Hamilton,[a] K. Dimitri Kits,[b] Victoria A. Ramonovskaya,[c] Olga N. Rozova,[d] Hiroya Yurimoto,[e] Hiroyuki Iguchi,[e] Valentina N. Khmelenina,[d] Yasuyoshi Sakai,[e] Peter F. Dunfield,[f] Martin G. Klotz,[g] Claudia Knief,[h] Huub J. M. Op den Camp,[i] Mike S. M. Jetten,[i] Françoise Bringel,[j] Stéphane Vuilleumier,[j] Mette M. Svenning,[k] Nicole Shapiro,[l] Tanja Woyke,[l] Yuri A. Trotsenko,[d] Lisa Y. Stein,[b] Marina G. Kalyuzhnaya[a]

San Diego State University, San Diego, California, USA[a]; Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada[b]; Department of Biology of Extremophilic Microorganisms, Institute of Microbiology and Virology of National Academy of Science, Kyiv, Ukraine[c]; GK Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia[d]; Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa-Oiwake, Sakyo-ku, Japan[e]; Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada[f]; Department of Biological Sciences, University of North Carolina, Charlotte, North Carolina, USA[g]; Institute of Crop Science and Resource Conservation—Molecular Biology of the Rhizosphere, University of Bonn, Bonn, Germany[h]; Department of Microbiology, Faculty of Science, Radboud University, Nijmegen, The Netherlands[i]; Department of Microbiology, Genomics and the Environment, Université de Strasbourg, CNRS, Strasbourg, France[j]; Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway[k]; DOE Joint Genome Institute, Walnut Creek, California, USA[l]

Genome sequences of *Methylobacter luteus*, *Methylobacter whittenburyi*, *Methylosarcina fibrata*, *Methylomicrobium agile*, and *Methylovulum miyakonense* were generated. The strains represent aerobic methanotrophs typically isolated from various terrestrial ecosystems.

Received 17 April 2015 **Accepted** 23 April 2015 **Published** 4 June 2015

Citation Hamilton R, Kits KD, Ramonovskaya VA, Rozova ON, Yurimoto H, Iguchi H, Khmelenina VN, Sakai Y, Dunfield PF, Klotz MG, Knief C, Op den Camp HJM, Jetten MSM, Bringel F, Vuilleumier S, Svenning MM, 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. doi:10.1128/genomeA.00515-15.

Address correspondence to Marina G. Kalyuzhnaya, mkalyuzhnaya@mail.sdsu.edu.

Methane is a potent greenhouse gas (1–3). Methanotrophic bacteria of terrestrial ecosystems contribute to methane sinks not only by mitigating methane emissions but also by consuming atmospheric methane (1–6). Here we report five genomes of gammaproteobacterial methanotrophs isolated from various terrestrial ecosystems. *Methylobacter whittenburyi* (formerly "*Methylobacter capsulatus*" = UCM-B-3033), and *Methylomicrobium agile* (ATCC 35068) are methanotrophic bacteria commonly found in sediment samples from wetlands (7, 8). *Methylobacter luteus* strains (formerly *Methylobacter bovis*, represented here by the strain 98 [IMV-B-3098]) have typically been obtained from meadows, dry hay, and cow mouth samples (7–9). *Methylovulum miyakonense* HT12^T (= ATCC BAA-2070) was isolated from a forest soil (10). *Methy-*

TABLE 1 General genome statistics and accession numbers

Species and strain	Sequencing plaform	Genome assembly and annotation	Genome coverage (×)	Genome size (Mb)	No. of scaffolds (no. of contigs)	Core metabolic pathways[a]	NCBI accession number
M. luteus 98 (= IMV-B-3098)	Illumina, PacBio	Allpaths, Velvet 1/1/05, Phrap 4.24	1,288	5.1	4 (17)	pMMO, Mxa, Xox, FDH, H_4MTP, H_4FP, pSC, dPPP, RuMP, EDD, EMP, TCA	ATYJ00000000
M. fibrate AML-C10^T (= ATCC 700909)	Illumina	Allpaths, Velvet 1/1/05, Phrap 4.24	1,112	5	8 (34)	pMMO, Mxa, Xox, FDH, H_4MTP, H_4FP, pSC, dPPP, RuMP, EDD, EMP, TCA	ARCU00000000
M. miyakonense HT12^T (= ATCC BAA-2070)	Illumina	Allpaths, Velvet 1/1/05, Phrap 4.24	1,199	4.7	9 (32)	pMMO, sMMO, Mxa, Xox, FDH, H_4MTP, H_4FP, pSC, dPPP, RuMP, EDD, EMP, TCA	AQZU00000000
M. agile ATCC 35068	PacBio	Prodigal, GenePRIMP	210.3	4.5	4 (4)	pMMO, Mxa, Xox, FDH, H_4MTP, H_4FP, pSC, dPPP, RuMP, EDD, EMP, TCA	JPOJ00000000
M. whittenburyi UCM-B-3033	PacBio	Prodigal, GenePRIMP	209.5	5.4	7 (7)	pMMO, Mxa, Xox, FDH, H_4MTP, H_4FP, pSC, dPPP, RuMP, EDD, EMP, TCA	JQNS00000000

[a] pMMO, membrane-bound methane monooxygenase; Mxa, PQQ-linked methanol dehydrogenases; Xox, PQQ-linked methanol and formaldehyde dehydrogenases; FDH, formate dehydrogenases; H_4MTP, methanopterin-linked C1 transfer; H_4FP, folate-linked C1 transfer; pSC, partial serine cycle (i.e., no evidence for glyoxylate regeneration pathway is found); dPPP, dissimilatory pentose phosphate cycle; RuMP, assimilatory ribulose monophosphate pathway; EDD, Entner-Doudoroff pathway, EMP, Embden-Meyerhof-Parnas pathway; TCA, tricarboxylic acid cycle; sMMO, soluble methane monooxygenase.

losarcina fibrata AML-C10^T (= ATCC 700909) was isolated from a landfill site (11).

The draft genome sequences were generated at the DOE Joint Genome Institute (JGI), using the Illumina (12) and/or PacBio technology (13) (Table 1). Raw reads were assembled using Allpaths, version 39750 (14), Velvet, version 1.1.05 (15) HGAP, version 2.1.1 (16), and/or Phrap, version 4.24 (High Performance Software, LLC). Possible misassemblies were corrected by manual editing in Consed (17–19). All general aspects of library construction and sequencing performed at the JGI can be found at http://www.jgi.doe.gov. Genome annotation was performed using Prodigal (20) and GenePRIMP (21). Additional gene prediction analyses were performed within the IMG (22) and MaGe (23) platforms.

Genome statistics and predicted core metabolic pathways are shown in Table 1. Genes encoding a soluble methane monooxygenase were detected only in the *M. miyakonense* HT12^T genome (24). A functional operon encoding methane monooxygenase was present in all genomes, and a homologous operon encoding related proteins (*pxmABC*) (25) was found in all except *M. miyakonense* HT12^T. Each genome contains at least one homologue of the large subunit of methanol dehydrogenase (26). Two types of the structural organization of the gene cluster encoding 3-hexulose-6-phosphatesynthase (HPS) and 6-phospho-3-hexuloisomerase (PHI) were found. The genomes of *M. miyakonense* HT12^T and *M. fibrata* AML-C10^T contain the *hps-phi* operon and another *hpsi* gene encoding an HPS-PHI fused protein (27). *M. luteus* 98 and *M. whittenburyi* UCM-B-3033 possess only the *hps-phi* operon. The genome of *M. agile* ATCC 35068 has only the *hpsi* gene. Genes encoding respiratory nitrate reductase (28) were identified only in the genome of *M. fibrate* AML-C10^T. The genome sequences indicated that all strains can import and assimilate ammonium (*amtB/glnA/gdhB/ald*) or urea (*urtABCDE*/*ureABCDEFG*) as the sole source of nitrogen. *M. miyakonense* HT12^T, *M. luteus* 98, and *M. whittenburyi* UCM-B-3033 possess the key genetic elements for nitrogen fixation (*nifKDHWENX*).

Many methanotrophic species (including *Methylobacter* spp.) produce cysts (7). We were not able to identify homologues of known cyst formation genes in any of the sequenced genomes, suggesting that this stage in the life cycle of some methanotrophs might be unique. Production of bacteriocins has been reported for *M. luteus* 98 (29, 30). Two gene clusters encoding a bacteriocin-producing peptidase C39 and a putative precursor (31) were identified in this strain. The contribution of these genes to the production of the biologically active bacteriocin will require experimental validation by mutagenesis studies.

Nucleotide sequence accession numbers. The genome sequences have been deposited in GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

We thank all members of the Organization for Methanotroph Genome Analysis for collaboration (OMeGA) and Genoscope for access to its MicroScope platform for comparative genome analysis. This report is based upon work supported by the National Science Foundation under award MCB-0842686 and by faculty startup funds to M. G. Kalyuzhnaya from San Diego State University. Work conducted by the U.S. Department of Energy Joint Genome Institute was supported by the Office of Science of the U.S. Department of Energy under contract DE-AC02-05CH11231.

This is contribution 11 from the Organization for Methanotroph Genome Analysis (OMeGA).

REFERENCES

1. **Conrad R**. 1989. Control of methane production in terrestrial ecosystems, p 39–58. *In* Andreae MO, Schimel DS (ed), Exchange of trace gases between terrestrial ecosystems and the atmosphere: Dahlem Workshop Reports, Life Sciences Research Report 47. Wiley, New York, NY.
2. **Smith TJ, Murrell JC**. 2009. Methanotrophy, p 173–178. *In* Schmidt T, Schaechter M (ed), Topics in ecological and environmental microbiology. Academic Press, Elsevier, Inc., Waltham, MA.
3. **Holmes AJ, Roslev P, McDonald IR, Iversen N, Henriksen K, Murrell JC**. 1999. Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Appl Environ Microbiol **65:** 3312–3318.
4. **Le Mer J, Roger P**. 2001. Production, oxidation, emission and consumption of methane by soils: a review. Eur J Soil Biol **37:**25–50. http://dx.doi.org/10.1016/S1164-5563(01)01067-6.
5. **Kolb S, Knief C, Stubner S, Conrad R**. 2003. Quantitative detection of methanotrophs in soil by novel *pmoA*-targeted real-time PCR assays. Appl Environ Microbiol **69:**2423–2429. http://dx.doi.org/10.1128/AEM.69.5.2423-2429.2003.
6. **Knief C, Lipski A, Dunfield PF**. 2003. Diversity and activity of methanotrophic bacteria in different upland soils. Appl Environ Microbiol **69:** 6703–6714. http://dx.doi.org/10.1128/AEM.69.11.6703-6714.2003.
7. **Whittenbury R, Phillips KC, Wilkinson JF**. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J Gen Microbiol **61:** 205–218. http://dx.doi.org/10.1099/00221287-61-2-205.
8. **Bowman JP, Sly LI, Nichols PD, Hayward AC**. 1993. Revised taxonomy of the methanotrophs: description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. Int J Syst Bacteriol **43:**735–753. http://dx.doi.org/10.1099/00207713-43-4-735.
9. **Romanovskaya VA**. 1991. Taxonomy of methylotrophic bacteria, p 3–23. *In* Goldberg I, Rokem JS (ed), Biology of methylotrophs. Reed Elsevier, New York, NY.
10. **Iguchi H, Yurimoto H, Sakai Y**. 2011. *Methylovulum miyakonense* gen. nov., sp. nov., a type I methanotroph isolated from forest soil. Int J Syst Evol Microbiol **61:**810–815. http://dx.doi.org/10.1099/ijs.0.019604-0.
11. **Wise MG, McArthur JV, Shimkets LJ**. 1999. Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. Appl Environ Microbiol **65:**4887–4897.
12. **Bennett S**. 2004. Solexa Ltd. Pharmacogenomics **5:**433–438. http://dx.doi.org/10.1517/14622416.5.4.433.
13. **Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M**. 2009. Real-time DNA sequencing from single polymerase molecules. Science **323:**133–138. http://dx.doi.org/10.1126/science.1162986.
14. **Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB**. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci U S A **108:**1513–1518. http://dx.doi.org/10.1073/pnas.1017351108.
15. **Zerbino DR, Birney E**. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res **18:**821–829. http://dx.doi.org/10.1101/gr.074492.107.
16. **Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J**. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods **10:**563–569. http://dx.doi.org/10.1038/nmeth.2474.
17. **Ewing B, Green P**. 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. Genome Res **8:**186–194.
18. **Ewing B, Hillier L, Wendl MC, Green P**. 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res **8:**175–185. http://dx.doi.org/10.1101/gr.8.3.175.
19. **Gordon D, Abajian C, Green P**. 1998. Consed: a graphical tool for se-

quence finishing. Genome Res **8:**195–202. http://dx.doi.org/10.1101/gr.8.3.195.

20. **Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ**. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics **11:** http://dx.doi.org/10.1186/1471-2105-11-119.
21. **Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC**. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods **7:**455–457. http://dx.doi.org/10.1038/nmeth.1457.
22. **Markowitz VM, Chen IM, Palaniappan K, Chu K, Szeto E, Pillay M, Ratner A, Huang J, Woyke T, Huntemann M, Anderson I, Billis K, Varghese N, Mavromatis K, Pati A, Ivanova NN, Kyrpides NC**. 2014. IMG 4 version of the integrated microbial genomes comparative analysis system. Nucleic Acids Res **42:**D560–D567. http://dx.doi.org/10.1093/nar/gkt963.
23. **Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, Le Fèvre F, Longin C, Mornico D, Roche D, Rouy Z, Salvignol G, Scarpelli C, Thil Smith AA, Weiman M, Médigue C**. 2013. MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. Nucleic Acids Res **41:**D636–D647. http://dx.doi.org/10.1093/nar/gks1194.
24. **Iguchi H, Yurimoto H, Sakai Y**. 2010. Soluble and particulate methane monooxygenase gene clusters of the type I methanotroph *Methylovulum miyakonense* HT12. FEMS Microbiol Lett **312:**71–76. http://dx.doi.org/10.1111/j.1574-6968.2010.02101.x.
25. **Tavormina PL, Orphan VJ, Kalyuzhnaya MG, Jetten MS, Klotz MG**. 2011. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. Environ Microbiol Rep **3:**91–100. http://dx.doi.org/10.1111/j.1758-2229.2010.00192.x.
26. **Chistoserdova L**. 2011. Modularity of methylotrophy, revisited. Environ Microbiol **13:**2603–2622. http://dx.doi.org/10.1111/j.1462-2920.2011.02464.x.
27. **Yurimoto H, Kato N, Sakai Y**. 2009. Genomic organization and biochemistry of the ribulose monophosphate pathway and its application in biotechnology. Appl Microbiol Biotechnol **84:**407–416. http://dx.doi.org/10.1007/s00253-009-2120-7.
28. **Kits KD, Klotz MG, Stein LY**. 12 January 2015. Methane oxidation coupled to nitrate reduction under hypoxia by the gammaproteobacterium *Methylomonas denitrificans*, sp. nov. type strain FJG1. Environ Microbiol. http://dx.doi.org/10.1111/1462-2920.12772.
29. **Pashkova NI, Starostina NG, Tsiomenko AB**. 1997. A secretory protein involved in the antagonistic interactions between methanotrophic bacteria. Biochemistry (Mosc) **62:**386–390.
30. **Starostina NG, Pashkova NI, Tsiomenko AB**. 1998. Detection and partial characterization of bacteriocin in the methanotrophic bacterium *Methylobacter bovis*. Biochemistry (Mosc) **63:**1122–1125.
31. **De Jong A, van Heel AJ, Kok J, Kuipers OP**. 2010. BAGEL2: mining for bacteriocins in genomic data. Nucleic Acids Res **38:**W647–W651. http://dx.doi.org/10.1093/nar/gkq365.