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Draft Genomes of Gammaproteobacterial Methanotrophs Isolated from Terrestrial Ecosystems

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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, Bringle 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.

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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 platform | Genome assembly and annotation | Genome coverage (×) | Genome size (Mb) | No. of scaffolds (no. of contigs) | Core metabolic pathways ^a | NCBI accession number |
|-----------------------------------------------------------|---------------------|-------------------------------------|---------------------|------------------|-----------------------------------|--------------------------------------------------------------------------------------------------|-----------------------|
| <i>M. luteus</i> 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 ₄ MTP, H ₄ FP, pSC, dPPP, RuMP, EDD, EMP, TCA | ATYJ000000000 |
| <i>M. fibrata</i> AML-C10 ^T (= ATCC 700909) | Illumina | Allpaths, Velvet 1/1/05, Phrap 4.24 | 1,112 | 5 | 8 (34) | pMMO, Mxa, Xox, FDH, H ₄ MTP, H ₄ FP, pSC, dPPP, RuMP, EDD, EMP, TCA | ARCU000000000 |
| <i>M. miyakonense</i> 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 ₄ MTP, H ₄ FP, pSC, dPPP, RuMP, EDD, EMP, TCA | AQZU000000000 |
| <i>M. agile</i> ATCC 35068 | PacBio | Prodigal, GenePRIMP | 210.3 | 4.5 | 4 (4) | pMMO, Mxa, Xox, FDH, H ₄ MTP, H ₄ FP, pSC, dPPP, RuMP, EDD, EMP, TCA | JPOJ000000000 |
| <i>M. whittenburyi</i> UCM-B-3033 | PacBio | Prodigal, GenePRIMP | 209.5 | 5.4 | 7 (7) | pMMO, Mxa, Xox, FDH, H ₄ MTP, H ₄ FP, pSC, dPPP, RuMP, EDD, EMP, TCA | JQNS000000000 |

^a pMMO, membrane-bound methane monooxygenase; Mxa, PQQ-linked methanol dehydrogenases; Xox, PQQ-linked methanol and formaldehyde dehydrogenases; FDH, formate dehydrogenases; H₄MTP, methanopterin-linked C1 transfer; H₄FP, 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.

Iosarcina 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. fibrata* 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).

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