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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 Methylomethylum miyakonense were generated. The strains represent aerobic methanotrophs typically isolated from various terrestrial ecosystems.

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 "Methyllobacter 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). Methylomethylum miyakonense HT12T (= ATCC BAA-2070) was isolated from a forest soil (10). Methy-

TABLE 1 General genome statistics and accession numbers

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>Sequencing platform</th>
<th>Genome assembly and annotation</th>
<th>Genome coverage (×)</th>
<th>Genome size (Mb)</th>
<th>No. of scaffolds (no. of contigs)</th>
<th>Core metabolic pathways</th>
<th>NCBI accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. luteus 98 ( = IMV-B-3098)</td>
<td>Illumina, PacBio</td>
<td>Allpaths, Velvet 1/1/05, Phrap 4.24</td>
<td>1,288</td>
<td>5.1</td>
<td>4 (17)</td>
<td>pMMO, Mxa, Xox, FDH, H_{4}MTP, H_{4}FP, psC, dPPP, RuMP, EDD, EMP, TCA</td>
<td>ATYJ00000000</td>
</tr>
<tr>
<td>M. fibrata AML-C10T ( = ATCC 700909)</td>
<td>Illumina</td>
<td>Allpaths, Velvet 1/1/05, Phrap 4.24</td>
<td>1,112</td>
<td>5</td>
<td>8 (34)</td>
<td>pMMO, Mxa, Xox, FDH, H_{4}MTP, H_{4}FP, psC, dPPP, RuMP, EDD, EMP, TCA</td>
<td>ArcU00000000</td>
</tr>
<tr>
<td>M. miyakonense HT12T ( = ATCC BAA-2070)</td>
<td>Illumina</td>
<td>Allpaths, Velvet 1/1/05, Phrap 4.24</td>
<td>1,199</td>
<td>4.7</td>
<td>9 (32)</td>
<td>pMMO, Mxa, Xox, FDH, H_{4}MTP, H_{4}FP, psC, dPPP, RuMP, EDD, EMP, TCA</td>
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</tr>
<tr>
<td>M. agile ATCC 35068</td>
<td>PacBio</td>
<td>Prodigal, GenePRIMP</td>
<td>210.3</td>
<td>4.5</td>
<td>4 (4)</td>
<td>pMMO, Mxa, Xox, FDH, H_{4}MTP, H_{4}FP, psC, dPPP, RuMP, EDD, EMP, TCA</td>
<td>JPOJ00000000</td>
</tr>
<tr>
<td>M. whittenburyi UCM-B-3033</td>
<td>PacBio</td>
<td>Prodigal, GenePRIMP</td>
<td>209.5</td>
<td>5.4</td>
<td>7 (7)</td>
<td>pMMO, Mxa, Xox, FDH, H_{4}MTP, H_{4}FP, psC, dPPP, RuMP, EDD, EMP, TCA</td>
<td>JQNS00000000</td>
</tr>
</tbody>
</table>

* pMMO, membrane-bound methane monooxygenase; Mxa, PQQ-linked methanol dehydrogenase; Xox, PQQ-linked methanol and formaldehyde dehydrogenases; FDH, formate dehydrogenase; H_{4}MTP, methanotropin-linked C1 transfer; H_{4}FP, 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-C107 (= 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 monoxygenase were detected only in the M. miyakonense HT112 genome (24). A functional operon encoding methane monoxygenase was present in all genomes, and a homologous operon encoding related proteins (pxmAABC) (25) was found in all except M. miyakonense HT112. 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-hexulosoisomerase (PHI) were found. The genomes of M. miyakonense HT112 and M. fibrata AML-C107 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 hpsi-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-C107. The genome sequences indicated that all strains can import and assimilate ammonium (amtb/ghnA/gdhB/id) or urea (urrABCDE/ureABCDEFG) as the sole source of nitrogen. M. miyakonense HT112, 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. 9uteus (29, 30). Two gene clusters encoding a bacteriocin-producing peptide 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**

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**REFERENCES**