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Draft Genome Sequences of Gammaproteobacterial Methanotrophs Isolated from Marine Ecosystems

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This is contribution 12 from OMeGA.

The genome sequences of *Methylobacter marinus* A45, *Methylobacter* sp. strain BBA5.1, and *Methylomarinum vadi* IT-4 were obtained. These aerobic methanotrophs are typical members of coastal and hydrothermal vent marine ecosystems.

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Microbial methane oxidation is one of the key drivers of oxygen consumption in marine sediments and the overlying water column (1). Methanotrophic bacteria are the primary producers of many cold and hot seep ecosystems (2, 3). Here, we report three genome sequences of gammaproteobacterial methanotrophs isolated from three marine ecosystems. *Methylobacter*

marinus A45 (a methanol-adapted strain, formerly *Methylomonas methanica* A4, ACM 4717) was isolated from sewage outfall sediment near Los Angeles, CA (4). *Methylobacter* sp. strain BBA5.1 was isolated from the surface layer of estuary sediment collected at low tide near Newport, Bay Estuary (CA) (5). *Methylomarinum vadi* IT-4 (= JCM 13665^T = DSM 18976^T) was isolated from a

TABLE 1 General genome statistics and accession numbers

| Species | Sequencing platform(s) | Genome assembly and annotation | Genome coverage (×) | Genome size (Mb) | No. of scaffolds (no. of contigs) | Core (accessory) metabolic pathways ^a | NCBI accession no. |
|---------------------------------|------------------------|---|---------------------|------------------|-----------------------------------|---|--------------------|
| <i>M. marinus</i> A45 | Illumina | Velvet 1.1.05, AllPaths, Phrap 4.24, Prodigal 2.5 | 1,237 | 4.99 | 9 (49) | pMMO, pXmo, Mxa, XoxF1, XoxF2, H ₄ F, H ₄ MPT, FDH, RuMP, EMP, EDD, dPPP, PPP, pSC, TCA | ARVS00000000 |
| <i>Methylobacter</i> sp. BBA5.1 | Illumina, PacBio RS | AllPaths, Prodigal 2.5 | 290 | 5.07 | 87 (91) | pMMO, pXmo, Mxa, XoxF1, XoxF2, H ₄ F, H ₄ MPT, FDH, RuMP, EMP, EDD, dPPP, PPP, pSC, TCA | JQKS00000000 |
| <i>M. vadi</i> IT-4 | Illumina, PacBio RS | Prodigal 2.5 | 272 | 4.33 | 1 (1) | pMMO, Mxa, XoxF, H ₄ F, H ₄ MPT, FDH, RuMP, EMP, EDD, dPPP, PPP, pSC, TCA | JPON00000000 |

^a dPPP, dissimilatory pentose-phosphate pathway; EDD, Entner-Doudoroff pathway; EMP, Embden-Meyerhof-Parnas pathway; FDH, formate dehydrogenases; H₄F, folate-linked C₁ transfer; H₄MPT, methanopterin-linked C₁ transfer; Mxa, PQQ-linked methanol dehydrogenases; pMMO, membrane-bound methane monooxygenase; pSC, partial serine cycle; pXmo, methane/ammonia monooxygenase-related proteins of unknown function; PPP, pentose-phosphate pathway; RuMP, assimilatory ribulose monophosphate pathway; Xox, PQQ-linked methanol and formaldehyde dehydrogenases (i.e., no evidence for the glyoxylate regeneration pathway was found); TCA, tricarboxylic acid cycle.

microbial mat of a shallow submarine hydrothermal system near Taketomi Island, Okinawa, Japan (6).

DNA samples from the three strains were prepared using the standard phenol-chloroform method (7). DNA sequence data were obtained at the Joint Genome Institute using a combination of PacBio (8) and Illumina (9) technologies, and draft genome sequences were assembled. The computational tools used for genome sequencing and assembly are listed in Table 1.

All three sequenced marine methanotrophs are obligate methane and methanol utilizers. All three genomes harbor genes typical for type I methanotrophs, including genes encoding particulate methane monooxygenase (*pmoCAB*), the PQQ-dependent methanol dehydrogenases (*mxoA* and multiple copies of *soxB*), genes for tetrahydromethanopterin (H₄MPT)- and tetrahydrofolate (H₄F)-dependent C₁-transfer pathways, genes of the ribulose monophosphate pathway, including its phosphoketolase variant (10), and genes encoding a complete tricarboxylic acid (TCA) cycle and a partial serine cycle (10) (Table 1). The *pxmABC* gene clusters (11) linked to a distant homologue of the nitrate-nitrite transporter (*narK*) were found in the *Methylobacter* sp. strain BB5.1 and *M. marinus* A45 genomes. A phosphoenolpyruvate carboxylase gene (*ppc*) was found in *M. vadi* IT-4 only. Genes encoding soluble methane monooxygenase, known glyoxylate regeneration pathways, and RubisCO (*cbbL* and *cbbS*) were not detected. Genes involved in ammonium and nitrate assimilation are present in all three genomes. The genomes of strains A45 and BBA5.1 contain all genes necessary to provide for urea hydrolysis and nitrogen fixation. *M. vadi* IT-4 has the potential for dissimilatory nitrite reduction to nitric oxide, as suggested by the presence of *nir* genes. The NADH:ubiquinone reductase (H⁺)-translocating genes (*nuoABCDEFGHIJKLMN*) were identified in *M. marinus* A45 only. All strains possess genes encoding Na⁺-transporting NADH:ubiquinone oxidoreductase (*nqrABCDEF*), ubiquinol-cytochrome *bc*₁ complex, cytochrome *b*, cytochrome *c* oxidase, cytochrome P450 and P460, and cytochrome *d* ubiquinol oxidase. Cytochrome *bo*₃ quinol oxidase was found in *M. vadi* IT-4 only. Both *Methylobacter* species possess genes encoding the Na⁺-translocating ferredoxin:NAD⁺ oxidoreductase complex (*rnfABCDE*). All genomes contain genes encoding pyruvate-ferredoxin/ flavodoxin oxidoreductases, and all three strains possess ectoine biosynthesis genes.

The genome of *M. marinus* A45 includes a chromosomally integrated complete copy of a bacteriophage genome (predicted size, 65 kb) integrated in the chromosome, indicating the possibility of lysogenic infection in methanotrophic bacteria. These genomes provide a valuable resource to obtain new insights into environmental controls of fitness and diversity in methanotrophs, mechanisms of genetic exchange within methanotrophic communities, and the potential for the development of new genetic tools for methanotrophs.

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

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