

Genome Sequence of *Methyloversatilis universalis* FAM5^T, a Methylotrophic Representative of the Order *Rhodocyclales*[∇]

Weerayuth Kittichotirat,¹ Nathan M. Good,¹ Rob Hall,¹ Françoise Bringel,² Aurélie Lajus,³ Claudine Médigue,³ Nicole E. Smalley,¹ David Beck,^{4,5} Roger Bumgarner,¹ Stéphane Vuilleumier,² and Marina G. Kalyuzhnaya^{1*}

Department of Microbiology, University of Washington, Seattle, Washington¹; Adaptations et Interactions Microbiennes dans l'Environnement, Université de Strasbourg, UMR 7156 CNRS, 67000 Strasbourg, France²; Laboratoire d'Analyses Bioinformatiques pour la Génomique et le Métabolisme (LABGeM), Genoscope-IG-CEA, 91057 Evry, France³; and Department of Chemical Engineering⁴ and eScience Institute,⁵ University of Washington, Seattle, Washington

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***Rhodocyclales* are representative of versatile bacteria that are able to utilize a wide variety of organic compounds for growth, but only a few strains have been isolated in pure culture thus far. Here we present the genome sequence of *Methyloversatilis universalis* FAM5^T, the first cultivable methylotrophic member of the order.**

Methylotrophic bacteria are capable of utilizing single-carbon (C₁) compounds as sole sources of carbon and energy and, thus, play a significant role in controlling emissions of major greenhouse gases, such as methane and dimethylsulfide (DMS), in the environment. Recent efforts in the cultivation of methylotrophic strains have resulted in the isolation and characterization of a variety of novel bacteria that differ phylogenetically and metabolically from known methylotroph taxa (3–5, 12). We sequenced the genome of *Methyloversatilis universalis* strain FAM5, the first cultivated methylotrophic member of the order *Rhodocyclales*, isolated from Lake Washington sediment (3), to improve our understanding of the diversity and metabolic potential of this group of bacteria.

DNA preparations of *Methyloversatilis universalis* FAM5^T were subjected to direct and 3-kb paired-end 454 pyrosequencing (10), yielding a total of 169 Mbp, which were assembled with Newbler (version 2.3) into 41 large contigs (average size, 103 kb) organized in 3 scaffolds, totaling 4.2 Mb (approximately 50× coverage), with an average G+C content of 67.0 mol%. Of the predicted open reading frames (ORFs), 25% showed similarity to proteins of other organisms (>45% amino acid identity over >80% of protein length), while only 13% lacked similarity to sequences in databases so far. The strain FAM5 sequence showed a close relationship to that of the methylotroph *Methylibium petroleiphilum* PM1 (55% ORFs with bidirectional best hits [BBH] to FAM5) (5) and, to a lesser extent, to *Methylovorus* sp. strain SIP3-4 (45% BBH) (6). In total, 630 ORFs (>30% amino acid identity over >80% of protein length) are shared between strains FAM5, PM1, and SIP3-4, with 431 ORFs in syntenic clusters.

Confirming the results of previous biochemical and genetic studies, genes essential for oxidation of methanol (*mdh2*) and methylamine (the *N*-methylglutamate pathway) were identified

(4, 7). Strain FAM5 contains three formaldehyde oxidation systems: aldehyde:ferredoxin oxidoreductase (9, 14) and H₄MTP- and H₄folate-linked C₁ transfer pathways (2, 15). Two gene clusters presumably involved in formate oxidation were detected: a tungsten-dependent formate dehydrogenase (1, 8) and a homolog of the NAD-dependent molybdenum-containing enzyme from *Ralstonia eutropha* (13). Strain FAM5 harbors complete serine (*icl*⁺ variant) and Calvin-Benson-Bassham cycles for assimilation of C₁ units. It also features the complete Calvin cycle and nonoxidative pentose phosphate and Embden-Meyerhof-Parnas (with pyrophosphate-dependent phosphofructokinase [11]) pathways. Genetic evidence for additional metabolic pathways was obtained, including genes for uptake and utilization of hydrogen (*hoxALMRQTVXZ*) and nitrogen compounds (e.g., *narGHI*) for energy metabolism and for utilization of longer-chain amines (*ghpABCX*), methanesulfonic acid (*msmABCD*), alkanesulfonates (*ssuEADCB*), alkylnitronate (*npd*), urea (*ureABCREFG*), acetone (*acxABC*), cyanate (*cynS*), glycerol (*glpKD*), and phenols (*dmpLMNOP*) and for catabolism of aromatic compounds (*meta* cleavage pathway). The availability of the strain FAM5 genome sequence offers the basis for a detailed characterization of newly uncovered metabolic pathways in the strain and provides a framework for comparative and evolutionary analyses of the methylotrophic functions. Overall, preliminary analysis of the genome informs previously obtained experimental data (3, 4, 7) but also highlights that much is to be learned regarding the metabolic capabilities and lifestyle of *Methyloversatilis universalis* strain FAM5.

Nucleic acid sequence accession number. The *Methyloversatilis universalis* FAM5 genome sequence is available in GenBank under accession number AFHG00000000.

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* Corresponding author. Mailing address: Department of Microbiology, University of Washington, Seattle WA 98195. Phone: (206) 543-0355. Fax: (206) 616-5721. E-mail: mkalyuzh@uw.edu.

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