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Complete Genome Sequences of Six Strains of the Genus *Methylobacterium*

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The complete and assembled genome sequences were determined for six strains of the alphaproteobacterial genus *Methylobacterium*, chosen for their key adaptations to different plant-associated niches and environmental constraints.

Genomic and metagenomic investigations have highlighted the prevalent role of methylotrophic microorganisms in a variety of marine, freshwater, and terrestrial environments (3–4, 6). These data have propelled new understanding of the molecular intricacies of microbial methylotrophic metabolism (1) and have sparked continued interest in their potential for biotechnological applications (15). In this work, the assembled complete genome sequences of six strains of the alphaproteobacterial genus *Methylobacterium* were determined. The selected strains were chosen for key characteristics, in terms of ecology, physiology, and metabolism (Table 1), in order to investigate how such adaptive features are reflected at the level of genome composition and architecture.

Genomes were sequenced at the Joint Genome Institute (JGI) using combinations of small to medium DNA libraries (3, 6, and 8 kb), as well as fosmid libraries (35 and 40 kb), with Sanger sequencing (7.3 to 9.6× coverage) completed with 454 pyrosequencing (20× coverage). All general aspects of library construction and sequencing can be found at http://www.jgi.doe.gov/sequencing/protocols/prots_production.html. Draft assemblies and quality assessment were obtained using the Phred/Phrap/Consed software package. Possible misassemblies were corrected with Dupfinisher (8), PCR amplification, and transposon bombing of bridging clones (Epicentre Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, custom primer walking, and PCR amplification. A final assembly (7.5 to 10.5× coverage) was obtained for all 6 genomes (Table 1), and automatic annotation was performed using the JGI-Oak Ridge National Laboratory annotation pipeline (12). Additional automatic and manual sequence annotations, as well as comparative genome analysis, were performed using the MicroScope platform at Genoscope (16).

The six *Methylobacterium* strains show significant variation in chromosome size and plasmid content (Table 1), and each possesses several conserved gene clusters known to be involved in methylotrophy in *Methylobacterium* (2, 18). Five of the strains possess conserved clusters of genes associated with photosynthesis, including genes encoding the light-harvesting complex and the reaction center, and genes involved in biosynthesis of bacteriochlorophyll and carotenoids. Further analyses of these six genomes will include comparisons to the two *Methylobacterium* genomes already reported (18), i.e., *M. extorquens* AM1, a major model strain in studies of methylotrophy (2) and genome evolution (5), and the dichloromethane-degrading strain *M. extorquens* DM4 (14). This will define both core- and strain-specific features of *Methylobacterium* strains and provide new insights into the metabolic flexibility of these facultative methylotrophs and into the modes of bacterial adaptation to specific ecological niches.

Nucleotide sequence accession numbers. GenBank accession numbers for all the chromosomes and plasmids sequenced in this study are shown in Table 1.

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TABLE 1 Characteristics of the six complete *Methylobacterium* genomes sequenced in this study

		Genome analysis						
Organism	Key characteristic(s)	Size (Mb)	% GC	No. of *rrn* operons	No. of tRNAs	No. of CDS[a]	GenBank accession no.	Reference
M. extorquens strain PA1	*Arabidopsis thaliana* epiphyte	5.471	68.2	5	58	5,410	NC_010172	11
M. extorquens strain CM4	Chloromethane degrader	5.778	68.2	5	61	3,112	NC_011757	13
		0.380	66.3			388	NC_011758	
		0.023	63.9			44	NC_011760	
M. extorquens strain BJ001[b]	*Populus deltoides* x *nigra* DN34 endophyte	5.800	69.4	5	58	6,017	NC_010725	17
		0.025	64.9			30	NC_010727	
		0.023	66.8			31	NC_010721	
M. radiotolerans strain JCM 2831	Radioresistant strain	6.078	71.5	4	56	6,325	NC_010505	9
		0.586	69.6	2	1	650	NC_010510	
		0.047	62.5			66	NC_010509	
		0.043	63.2		1	75	NC_010514	
		0.038	63.7		1	60	NC_010517	
		0.036	62.0			64	NC_010518	
		0.028	61.0		1	45	NC_010502	
		0.022	61.1			38	NC_010504	
		0.021	65.1			33	NC_010507	
Methylobacterium sp. strain 4-46	*Lotononis bainesi* nodulating, photosynthetic	7.659	71.6	6	63	8,337	NC_010511	7
		0.058	65.1			108	NC_010373	
		0.020	59.2			34	NC_010374	
M. nodulans strain ORS 2060	Nonpigmented, nitrogen fixing, *Crotalaria* nodulating	7.772	68.9	7	71	8,879	NC_011894	10
		0.488	65.9		2	630	NC_011892	
		0.458	65.7			609	NC_011887	
		0.040	64.2			84	NC_011893	
		0.038	61.6			66	NC_011895	
		0.020	61.4			30	NC_011888	
		0.013	60.5			16	NC_011889	
		0.010	67.2			14	NC_011890	

[a] Number of annotated protein-coding sequences in MicroScope (16).

[b] This strain, originally reported as *M. populi* strain BJ001 (17), was assigned to the species *M. extorquens* based on 16S rRNA gene identity (99.3%) and overall genome similarity with the four other sequenced *M. extorquens* strains (~80% identity over 75% of its genome sequence) (also see reference 18).

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