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 (Epiconcept 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.
ACKNOWLEDGMENTS

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We are grateful to the JGI personnel who participated in the sequencing, assembly, and automated annotation processes.

REFERENCES

1. Anthony C. 2011. How half a century of research was required to understand bacterial growth on C1 and C2 compounds; the story of the serine cycle and the ethylmalonyl-CoA pathway. Science Prog. 94:109 – 137.

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Key characteristic(s)</th>
<th>Genome analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Size (Mb) % GC</td>
</tr>
<tr>
<td>M. extorquens strain PA1</td>
<td>Arabidopsis thaliana epiphyte</td>
<td>5.471 68.2 5</td>
</tr>
<tr>
<td>M. extorquens strain CM4</td>
<td>Chloromethane degrader</td>
<td>5.778 68.2 5</td>
</tr>
<tr>
<td>M. extorquens strain BJ001b</td>
<td>Populus deltoides x nigra DN34 endophyte</td>
<td>5.800 69.4 5</td>
</tr>
<tr>
<td>M. radiotolerans strain JCM 2831</td>
<td>Radioreistant strain</td>
<td>6.078 71.5 4</td>
</tr>
<tr>
<td>Methylobacterium sp. strain 4-46</td>
<td>Lotononis bainesi nodulating, photosynthetic</td>
<td>7.659 71.6 6</td>
</tr>
<tr>
<td>M. nodulans strain ORS 2060</td>
<td>Nonpigmented, nitrogen fixing, Crotalaria nodulating</td>
<td>7.772 68.9 7</td>
</tr>
</tbody>
</table>

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).


