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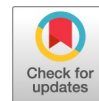
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Draft Genome Sequence of the Caffeine-Degrading Methylotroph *Methylorubrum populi* Pinkel

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ABSTRACT A pink-pigmented facultative methylotroph, *Methylorubrum populi* Pinkel, was isolated from compost by selective enrichment with caffeine (3,5,7-trimethylxanthine) as the sole carbon, nitrogen, and energy source. We report here its high-quality draft genome sequence, assembled in 35 contigs totaling 5,630,907 bp. We identified 5,681 protein-coding sequences, including those putatively involved in caffeine degradation.

Caffeine (3,5,7-trimethylxanthine) is a natural purine alkaloid synthesized by many plant species. Most of the >35 caffeine-degrading bacterial isolates that have been reported to date are members of the genus *Pseudomonas* (1). We report here the isolation and draft genome sequence of a facultative methylotroph, *Methylorubrum populi* Pinkel, which can utilize caffeine as a sole carbon, nitrogen, and energy source, and predict that it uses the *N*-demethylation pathway to convert caffeine to xanthine.

Strain Pinkel was obtained from compost that contained coffee grounds, following enrichment in a variation of BG-11 minimal medium (2) lacking citrate and sodium nitrate, with 0.006 g/liter ferric citrate substituted for ferric ammonium citrate and containing 5 mM caffeine as the sole carbon and nitrogen source. A pink-pigmented pure culture was obtained after multiple passes on plates of the same medium. The strain was identified as *Methylorubrum populi* (3, 4) based on 16S rRNA gene amplification with universal 16S primers followed by sequence homology analysis. As expected for a *Methylorubrum* isolate, the strain was a rod-shaped facultative methylotroph capable of growing on methanol as sole carbon source (3, 5). It grew on 5 mM caffeine as the sole source of carbon, nitrogen, and energy with a doubling time of approximately 6.5 h.

Genomic DNA was purified using an ArchivePure DNA kit (5 Prime, Inc., Gaithersburg, MD). The genomic DNA (100 ng) was used to create a paired-end library using the Ovation Ultralow protocol (NuGen, Redwood, CA) by Illumina. Later, the library was sequenced using the Illumina MiSeq platform, producing 1,058,337 paired-end reads (length, 300 bp). Raw reads were quality trimmed with a minimum Phred quality score of 30 from both ends using Trimmomatic v0.35 (6) with default parameters, generating 973,280 (91.96% of total) sequencing reads of good quality. *De novo* assembly and scaffolding were performed using A5-miseq (version 20150522) (7), followed by the removal of duplicate contigs and contigs containing only repeat regions. Finally, contigs were cross-checked for any putative contamination using a Kraken-based taxonomic sequence classification system (8). The final assembly consists of 35 contigs (>500 bp) with a total size of 5,630,907 bp, an N_{50} value of 589,043 bp, a G+C content of 69.2%, and ~40× coverage. The smallest and the largest contigs were 753 bp and 1,232,378 bp, respectively. Subsequently, the genome annotation was carried out with Rapid Annotations using Subsystems Technology (RAST) (9), which predicted a total of

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5,885 coding sequences, including 5,681 protein coding sequences, 52 tRNA genes, 2 rRNAs (16S and 23S), and 150 repeat regions. Out of 5,681 proteins, 1,414 (~25%) of the genes were assigned to SEED subsystems, whereas 4,267 (~75%) were predicted to have an unknown (nonhypothetical) function. Subsystems signifying the survival of the isolate in aromatic compound-contaminated soil included membrane transport (90 genes), stress response (62 genes), metabolism of aromatic compounds (29 genes), and motility and chemotaxis (93 genes). As expected for a *Methylobacterium* species, the genome contained genes for carotenoid biosynthesis, flagellar motility and chemotaxis, and methanol utilization. In addition, genes for the *N*-demethylation pathway for caffeine degradation were also identified.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [WEKV00000000](https://www.ncbi.nlm.nih.gov/nuccore/WEKV00000000). The version described in this paper is the first version, WEKV01000000. The data are under BioProject number [PRJNA514782](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA514782) and BioSample number [SAMN10160078](https://www.ncbi.nlm.nih.gov/biosample/SAMN10160078). The reads are available under SRA accession number [SRR8435744](https://www.ncbi.nlm.nih.gov/sra/SRR8435744).

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