

Complete genome sequences of *Methylococcus capsulatus* (Norfolk) and *Methylocaldum szegediense* (Norfolk) isolated from a landfill methane biofilter

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ABSTRACT Here we report the complete genome sequence of two moderately thermophilic methanotrophs isolated from a landfill methane biofilter, *Methylococcus capsulatus* (Norfolk) and *Methylocaldum szegediense* (Norfolk).

KEYWORDS methanotrophs, genomes, landfill, biofilter

The Strumpshaw closed landfill features a biofilter for the mitigation of the climate active gas methane, generated by the anaerobic breakdown of organic waste. This biofilter harnesses methanotrophic bacteria in a soil matrix for methane bio-oxidation. Two methanotrophs, *Methylococcus capsulatus* (Norfolk) and *Methylocaldum szegediense* (Norfolk), were isolated from this system. Biofilter soil was used to inoculate vials containing nitrate mineral salt (NMS) medium (1) and supplied with 20% (vol/vol) methane. Isolates were obtained from enrichment cultures by serial dilution and plating onto NMS agar plates, incubated in gas-tight containers supplied with 50% (vol/vol) methane. Optimal growth temperatures of the *Methylococcus* and *Methylocaldum* isolates were 45°C and 50°C, respectively. *M. capsulatus* (Norfolk) also grew on methanol (1%–5% vol/vol) as did *Methylococcus* strain MIR (2).

DNA extraction, sequencing, and genome assembly were done using a combined long- and short-read sequencing service at MicrobesNG (Birmingham, UK) as described in Fig. 1. This pipeline was used to construct genomes for *M. capsulatus* (Norfolk) and *M. szegediense* (Norfolk), producing a closed genome in both cases.

MicroScope v.3.16.0 (3) was used for automated annotation and taxonomic assignment of assembled genomes before further manual curation. Genome assembly and sequencing read summaries are shown in Table 1.

The Norfolk isolates were assigned to the *Methylococcus capsulatus* and *Methylocaldum szegediense* spp. first described by Foster and Davis (4) and Bodrossy et al. (5). Based on average nucleotide identity (ANI) scores generated using CJ Bioscience's online ANI calculator (6), the sequenced genomes with the highest similarity to *Methylococcus capsulatus* (Norfolk) and *Methylocaldum szegediense* (Norfolk) are *Methylococcus capsulatus* (Texas) (99.56%) and *Methylocaldum szegediense* (O-12) (99.64%), respectively (GenBank accession numbers [GCA_000297615.1](#) and [GCA_000427385.1](#)).

Both genomes contain genes encoding a full methane oxidation pathway. Two *pmoCAB* clusters encoding particulate methane monooxygenase were found in each genome (7), and the *Methylococcus capsulatus* (Norfolk) genome also possesses a single soluble methane monooxygenase *mmoXYBZDCGQSR* cluster (8) and a putative copper chaperone (*mopE*) gene (9). Calcium-dependent (*mxoFJGIRSACKLD*) and lanthanide-dependent (*soxFJ*) methanol dehydrogenase gene clusters (10, 11) were found in these genomes, with a clade 5 *soxF* gene present in each and an additional clade 3 *soxF* in *Methylocaldum szegediense* (Norfolk) (12). Both genomes feature complete

Editor Elinne Becket, California State University San Marcos, USA

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The authors declare no conflict of interest.

See the funding table on p. 4.

Received 23 July 2023

Accepted 10 December 2023

Published 18 January 2024

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DNA extraction

NMS media was inoculated with a single isolate colony and incubated with methane. After growth, $4\text{-}6 \times 10^9$ cells (estimated by OD_{600} measurement) were pelleted and resuspended in 500 μl of DNA/RNA shield (Zymo Research). 5–40 μl of **cell suspension lysed** with 120 μl of 0.1 mg/ml **lysozyme** and **RNase A** in TE buffer for 25 min at 37°C, then incubated for 5 min at 65°C with Proteinase K (0.1 mg/ml) and SDS (0.5% v/v). **Genomic DNA was purified** using an equal volume of solid-phase reversible immobilisation (SPRI) beads and resuspended in 10 mM Tris-HCl (pH 8.0).

Illumina short-read sequencing

DNA libraries were generated with a modified **Nextera XT Library Prep Kit** protocol (input DNA doubled, and PCR elongation time was increased to 45 seconds).

Sequenced using an **Illumina NovaSeq6000** to produce **250bp paired-end reads**.

Trimmomatic version 0.30 was used for Nextera adapter removal and read trimming with a **Q15 sliding window cut-off**.

Oxford Nanopore long-read sequencing

DNA libraries were generated with **SQK-LSK109** Oxford Nanopore kits and native barcoding expansions **EXP-NBD104** or **EXP-NBD114** using 400–500 ng of HMW DNA (no shearing/size selection).

Sequenced using an **Oxford Nanopore GridION** platform and **FLO-MIN106 (R.9.4.1)** flow cell.

Basecalling (**high accuracy basecalling model**) and barcode trimming were performed using the GridION deployment of **Guppy ver3.2.8+bd67289** (with no further filtering).

Hybrid genome assembly

Unicycler version 0.4.0 was used for hybrid genome assembly, circularisation and rotation. Assembled contigs were rotated in between rounds of polishing, the final assembly was rotated to an appropriate start gene (*dnaA*).

Note - Default parameters were used except where otherwise noted

FIG 1 Sequencing and assembly pipeline.

TABLE 1 *Methylocaldum szegediense* (Norfolk) and *Methylococcus capsulatus* (Norfolk) genome summaries

DNA sequencing reads									
Isolate	Illumina total reads	Illumina read length (bp)	Nanopore total reads	Nanopore N ₅₀ (bp)	Illumina reads ENA accession no.			Nanopore reads ENA accession no.	
<i>Methylocaldum</i>	936,436	250	184,537	4,370	ERR11151912			ERR11151913	
<i>Methylococcus</i>	891,006	250	15,738	13,497	ERR11151914			ERR11151915	
<i>Methylocaldum szegediense</i> (Norfolk) assembly									
Replicon	Sequence length (bp)	Assembly coverage	% GC	No. of CDS	Ribosomal RNA genes			MicroScope assigned taxonomy	GenBank accession no.
Chromosome	4,869,648	173×	57	5,038	16S rRNA	23S rRNA	5S rRNA	<i>Methylocaldum szegediense</i>	GCA_949769195.1
Plasmid	25,724	481×	58	38	0	0	0		
<i>Methylococcus capsulatus</i> (Norfolk) assembly									
Replicon	Sequence length (bp)	Assembly coverage	% GC	No. of CDS	Ribosomal RNA genes			MicroScope assigned taxonomy	GenBank accession no.
Chromosome	3,398,174	85×	63.5	3318	16S rRNA	23S rRNA	5S rRNA	<i>Methylococcus capsulatus</i>	GCA_949769275.1

gene inventories for tetrahydromethanopterin and tetrahydrofolate-linked formaldehyde oxidation, in addition to formate dehydrogenase genes (13). Carbon is presumed to be assimilated primarily via the ribulose monophosphate pathway as in *Methylococcus capsulatus* (Bath), although genes for a partial serine cycle and complete Calvin-Benson-Bassham pathway were detected (14). Alanine dehydrogenase and GS/GOGAT cycle genes for ammonia assimilation were present (15).

In addition to the 4.87 Mbp chromosome, *Methylocaldum szegediense* (Norfolk) also contained a ~25-kbp plasmid, encoding a plasmid replication initiator protein (TrfA), replication protein (RepA) and a toxin anti-toxin plasmid retention mechanism. A gene encoding a putative siphovirus Gp157 protein was also found, which may confer increased bacteriophage resistance (16, 17).

ACKNOWLEDGMENTS

Genome sequencing was provided by MicrobesNG (<http://www.microbesng.com>). This work was funded by the EnvEast DTP (NERC NE/L002582/1) and Norfolk County Council. The LABGeM (CEA/Genoscope and CNRS UMR8030), the France Génomique, and French Bioinformatics Institute National Infrastructures (funded as part of Investissement d'Avenir program managed by Agence Nationale pour la Recherche, contracts ANR-10-INBS-09 and ANR-11-INBS-0013) are acknowledged for support within the MicroScope annotation platform.

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FUNDING

Funder	Grant(s)	Author(s)
UK Research and Innovation (UKRI)	NE/L002582/1	David Pearce
Government of the United Kingdom (UK Government)		David Pearce

DATA AVAILABILITY

Genome assembly and raw read accession numbers are listed in Table 1.

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