

**Draft Genome Sequence of *Methanocalculus natronophilus* sp. strain Z-7105^T, an Alkaliphilic,
Methanogenic Archaeon Isolated from a Soda Lake**

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Running title

Genome of *Methanocalculus natronophilus* Z-7105^T

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Abstract

A methanogenic archaeon was isolated from bottom sediments in the vicinity of Lake Tanatar II
(Altai, Russia), an alkaline soda lake. Here we present the draft genome sequence of
Methanocalculus natronophilus sp. strain Z-7105^T.

Announcement

Methanocalculus natronophilus sp. strain Z-7105^T is an alkaliphilic, hydrogenotrophic, and
halotolerant methanogenic archaeon isolated from bottom sediments in the vicinity of soda
lake Tanatar II (51.65189 N, 79.829406 E, Kulunda Steppe region, Altai, Russia) (1, 2). It was
originally isolated at 35°C in *Natrimonella* medium (DSMZ medium 784) with a headspace of pure
H₂ (DSMZ medium 784-a). The isolate was acquired from the DSMZ-German Collection of

Microorganisms and Cell Cultures GmbH with isolate identifier DSM 25006. Within the methanogens, little is known about genomic or physiological adaptations to high pH, thus the genome of *Methanocalculus natronophilus* sp. strain Z-7105^T was sequenced to understand how these ancient autotrophs are adapted to this pH extreme.

For DNA extraction, cells were grown anaerobically in 115 mL serum vials with 30 mL of medium. Prior to inoculation, the medium was reduced with 2.1 mL 2.5% Na₂S x 9H₂O and amended with 2.8 mL of Wolin's vitamin solution (3). Vials were topped off with a headspace of 80% H₂/20% CO₂ after inoculation. Cultures were incubated at 35°C for 12 days.

Genomic DNA was extracted from a single culture using the Qiagen DNeasy Ultraclean Microbial kit, following manufacturer's instructions. The Illumina library was prepared at the sequencing center using the Accel-NGS 2S Plus DNA library preparation kit/MiSeq nanokit v2 following manufacturer's instructions. DNA was sequenced using short-read (Illumina MiSeq, PE 250 bp) sequencing technologies with a sequencing depth of 75x, resulting in 2,409,898 reads. Post-processing bioinformatic analyses were conducted on the Department of Energy Systems Biology Knowledgebase (KBase) (4). Default parameters were used for all analysis software. Reads were trimmed using Trimmomatic v0.36 (5), resulting in 97.7% of paired reads surviving. Reads were quality-checked using FastQC v0.12.1 (6), with 0 sequences flagged as poor quality. The trimmed reads were assembled using SPAdes v3.15.3 (7), and the assembly was quality checked using Quast v4.4 (8), resulting in 179 total contigs, an N₅₀ value of 273,644 bp, a maximum contig length of 403,126 bp, a total length of 2,028,189 bp, and G+C content of 51.25%. CheckM v1.0.18 (9) was used to determine a genome completion of 100% and contamination of 3.65%. Sample metadata can be found in Table 1.

Table 1. Metadata for *Methanocalculus natronophilus* sp. strain Z-7105^T

PARAMETER	DATA
ENVIRONMENTAL DATA	
GEOGRAPHIC LOCATION	
REGION	Russian Federation Altai, Kulunda Steppe region, Tanatar soda lake II
GEOGRAPHIC COORDINATES	51.65189 N, 79.829406 E
COLLECTION DATE	06/2008
BIOME	Alkaline soda Lake
LAKE NAME	Tanatar II
SAMPLE TYPE	Bottom sediments

SAMPLING METHOD	Water collector
PH	10.4
TOTAL MINERALIZATION	60 g/L
GROWTH CONDITIONS	DSMZ 784-a
SEQUENCING	
RNA/DNA QUANTIFICATION INSTRUMENT	Nanodrop UV/visible spectrophotometer (Thermo Fisher Scientific)
ILLUMINA LIBRARY	Accel-NGS 2S Plus DNA library preparation kit/MiSeq nanokit v2
SEQUENCING TECHNOLOGIES	Illumina MiSeq
NO. OF ILLUMINA READS	2,409,898
SEQUENCING CENTER	Microbial Genome Sequencing Center, University of Pittsburgh, Pittsburgh, PA, USA
SEQUENCING DEPTH	75x
ASSEMBLER	SPAdes v3.15.3
NO. OF CONTIGS	179
LARGEST CONTIG (BP)	403,126
CONTIG N50 (BP)	273,644
ORF CALLER	Prodigal v2.6.3
GENOMIC FEATURES	
GENOME SIZE (BP)	2,028,189
G+C CONTENT (MOL%)	0.5125
NO. OF PROTEIN-CODING GENES	1,968
NO. OF RNA GENES	47
NO. OF RRNA GENES	2
NO. OF 5S RRNA GENES	1

NO. OF 16S RRNA GENES	0
NO. OF 23S RRNA GENES	1
NO. OF TRNA GENES	45
NO. OF OTHER RNA GENES	2
NO. OF CRISPR LOCI	2

Assembled contigs were annotated using Prodigal v2.6.3 (10) within DRAM v0.1.2 (11), resulting in 1968 protein-coding genes predicted, 2 complete rRNA genes, 45 tRNA genes, and 2 CRISPR loci. *Methanocalculus natronophilus* sp. strain Z-7105^T has genes for the complete reductive acetyl-CoA (Wood-Ljungdahl) pathway for carbon fixation, methanogenesis via hydrogen oxidation and formate utilization, and multiple genes for proton transport, including the multicomponent Na⁺/H⁺ antiporter and V/A-type H⁺-transporting ATPase, presumably to maintain cell homeostasis in its alkaline environment. A phylogenomic tree constructed with GToTree v1.4.5 (12) with all RefSeq genomes from members of the order *Methanomicrobiales* is shown in Figure 1. *Methanocalculus natronophilus* sp. Z-7105^T is most closely related to another representative of *M. natronophilus*, strain AMF5.

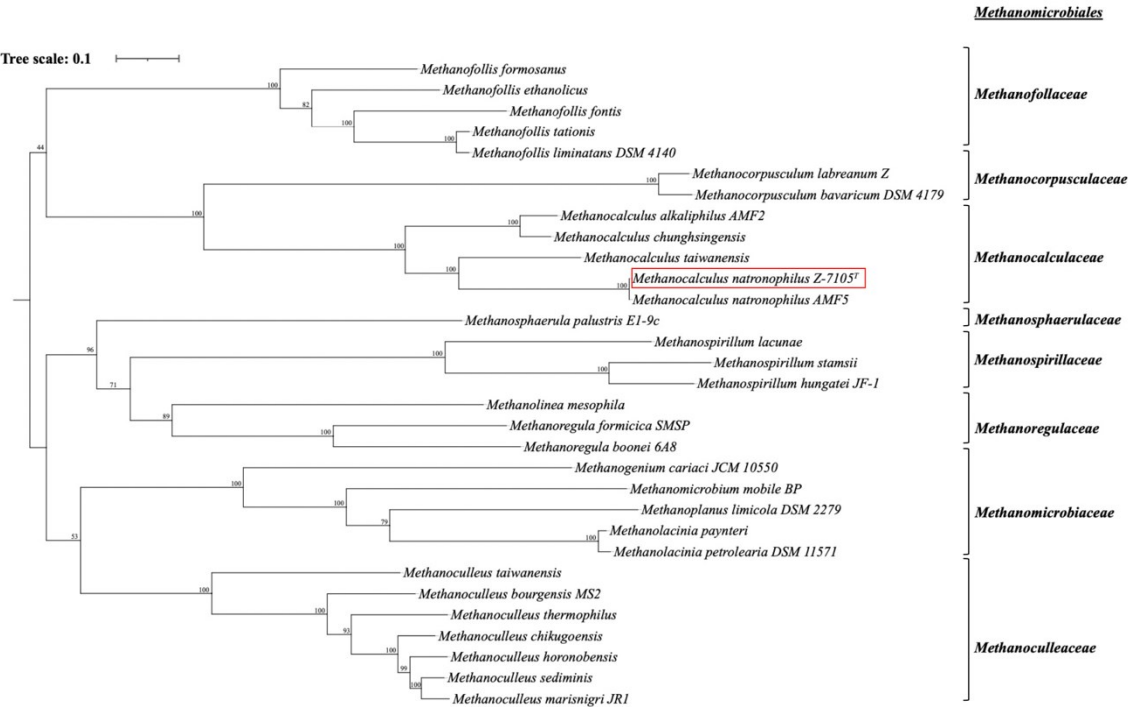


FIGURE 1 Phylogenomic tree of all RefSeq genomes of cultivated representatives of the order *Methanomicrobiales* and the position of *Methanocalculus natronophilus* sp. Strain Z-7105^T (in red box) within that order. The tree was generated using maximum-likelihood estimates based

on alignments of nucleotide sequences within GToTree (10). Bootstrap values above 40 are shown.

Data availability statement

Raw sequence reads for the genome of *Methanocalculus natronophilus* sp. Strain Z-7105^T are available at NCBI under BioProject accession number [PRJNA1089169](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1089169), BioSample accession number [SAMN40528436](https://www.ncbi.nlm.nih.gov/biosample/SAMN40528436), and SRA accession number [SRR28374704](https://www.ncbi.nlm.nih.gov/sra/SRR28374704). Assembled contigs are available under accession number JBCEXH010000000.

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