Complete genome sequence of Methanothermus fervidus type strain (V24S\textsuperscript{1})

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Methanothermus fervidus Stetter 1982 is the type strain of the genus Methanothermus. This hyperthermophilic genus is of a thought to be endemic in Icelandic hot springs. M. fervidus was not only the first characterized organism with a maximal growth temperature (97°C) close to the boiling point of water, but also the first archaeon in which a detailed functional analysis of its histone protein was reported and the first one in which the function of 2,3-cyclodiphosphoglycerate in thermoadaptation was characterized. Strain V24S\textsuperscript{1} is of interest because of its very low substrate ranges, it grows only on H\textsubscript{2} + CO\textsubscript{2}. This is the first completed genome sequence of the family Methanothermaceae. Here we describe the features of this organism, together with the complete genome sequence and annotation. The 1,243,342 bp long genome with its 1,311 protein-coding and 50 RNA genes is a part of the Genomic Encyclopedia of Bacteria and Archaea project.

Introduction

Strain V24S\textsuperscript{1} (= DSM 2088 = ATCC 43054 = JCM 10308) is the type strain of Methanothermurus fervidus [1]. Together with M. sociabilis, there are currently two species placed in the genus Methanothermus. The strain V24S\textsuperscript{1} was isolated from an anaerobic Icelandic spring [1,2] and M. sociabilis from a continental solfatara field in Iceland [2]. Since any attempt to isolate Methanothermus from similar places (Italy, the Azores, Yellowstone National Park) was without success, Lauerer et al. (1986) have speculated that strains of Methanothermus may exist endemically within Iceland [2]. The genus name derives from the Latin word “methanum”, methane, and from the Greek adjective “therme”, meaning heat, which refers to a methane producing organism living in a hot niche [1]. The species epithet fervidus comes from the Latin adjective “fervidus”, glowing hot, burning, fervent, because of its growth in almost-boiling water [1]. No further cultivated strains belonging to the species M. fervidus have been described so far. Here we present a summary classification and a set of features for M. fervidus strain V24S\textsuperscript{1}, together with the description of the complete genomic sequencing and annotation.
Classification and features
The original 16S rRNA gene sequence of strain V24ST (M59145) shows 92% sequence identity with the 16S rRNA gene of M. sociabilis (AF095273) [2] (Figure 1) and 88% identity with an uncultured clone, NRA12 (HM041913). The highest sequence similarities of the strain V24ST 16S rRNA to metagenomic libraries (env_nt) were 87% or less (status August 2010), indicating that members of the species, genus and even family are poorly represented in the habitats screened so far. The 16S rRNA gene sequence of strain V24ST was compared with the most recent release of the Greengenes database using BLAST [13] and the relative frequencies weighted by BLAST scores, of taxa and keywords within the 250 best hits were determined. The five most frequent genera were Methanobacterium (55.3%), Methanothermobacter (23.5%), Methanobrevibacter (12.8%), Methanothermus (5.7%) and Thermococcus (1.7%). The five most frequent keywords within the labels of environmental samples which yielded hits were 'anaerobic' (7.1%), 'sludge' (4.7%), 'microbial' (3.7%), 'archaeal' (3.5%) and 'temperature' (3.4%). Besides 'sludge', these keywords fit well to what is known from the taxonomy, ecology, and physiology of strain V24ST. Environmental samples which yielded hits of a higher score than the highest scoring species were not found. The genome of M. fervidus contains two rRNA operons. One of these operons has a closely linked 7S RNA gene, encoding the RNA component of signal recognition particle [14].

Figure 1 shows the phylogenetic neighborhood of M. fervidus V24ST in a 16S rRNA based tree. The sequences of the two 16S rRNA gene copies in the genome of Methanothermus fervidus DSM 2088 differ from each other by up to four nucleotides, and differ by up to 17 nucleotides from the previously published 16S rRNA sequence (M59145), which contains 87 ambiguous base calls.

Although the cells of the strain V24ST do not contain a typical bacterial peptidoglycan, they stain Gram-positive. Cells are curved rods, 1-3 µm long and 0.3 - 0.4 µm in width (Figure 2 and Table 1), occurring singly and in pairs, with a doubling time of 170 minutes [1]. Round, smooth, opaque, and slightly grayish colonies of 1 to 3 mm in diameter were observed on modified MM-medium plates containing trace amounts of solid sodium dithionite, sodium silicate solution and resazurin [1]. Strain V24ST is strictly anaerobic and strictly autotrophic [26]. Due to the low melting point of agar, strain V24ST could do not be grown on agar. Cells did not grow at temperatures below 61°C or above 97°C; the optimal temperature was 83°C [1]. Growth occurs at a slightly acidic pH and equal to 6.5, while no growth could be observed at pH above 7.0 [1]. In comparison, M. sociabilis grows at the temperatures ranged between 65°C and 97°C, with the optimal temperature at 88°C, its pH for growth being acidic to neutral (pH 5.5 to 7.5) [27]. Strain V24ST produces methane from H₂ + CO₂, whereas acetate and formate are not used [1,27]. The addition of 2-mercapto-ethanesulfonic acid (coenzyme M) enhances growth, especially when small inoculates are used [1]. In artificial medium, yeast extract is required as an organic factor for growth [1]. Strain V24ST gains energy by oxidizing H₂ to reduce CO₂ as the terminal electron acceptor [26,28]. At the time of isolation, strain V24ST was described to be nonmotile [1]. Later, strain V24ST as well as M. sociabilis were described to be motile via bipolar peritrichous 'flagella', which was taken to indicate motility [28]. These cell surface appendages, however, were recently determined to have a diameter of 5-6 nm, and therefore, very probably, represent not organelles used for motility, but for adhesion [R Wirth et al., unpublished]. The genome does not contain any flagellar genes. M. fervidus produces large intracellular potassium concentrations and amounts of 2,3-cyclic diphosphoglycerate, which are both thought to be involved in the thermoadaptation of M. fervidus [29,30]. Moreover, the DNA-binding protein HMf (histone M. fervidus), which binds to double stranded DNA molecules and increases their resistance to thermal denaturation, has been of interest in M. fervidus [31]. A partial amino acid sequence analysis of the D-glyceraldehyde-3-phosphate dehydrogenase of M. fervidus shows high sequence similarity to the enzymes from eubacteria and from the cytoplasm of eukaryotes [32]. This enzyme reacts with both NAD⁺ and NADP⁺ [32,33] and is not inhibited by pentalenolactone [32]. However, the enzyme activity is low at temperatures below 40°C, but it is intrinsically stable only up to 75°C [32], which is interesting as growth of M. fervidus may occur up to 97°C [1]. Also, the biochemistry of triose-phosphate isomerase, which catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP) in the reversible Embden-Meyerhof-Parnas (EMP) pathway, has been studied to some detail in M. fervidus [34].
Figure 1. Phylogenetic tree highlighting the position of *M. fervidus* V24S<sup>T</sup> relative to the other type strains within the family *Methanothermaceae*. The tree was inferred from 1,249 aligned characters [3,4] of the 16S rRNA gene sequence under the maximum likelihood criterion [5] and rooted in accordance with the current taxonomy [6]. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are *support values from 100 bootstrap replicates [7]* if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [8] are shown in blue, published genomes in bold [9-12].

Figure 2. Scanning electron micrograph of *M. fervidus* V24S<sup>T</sup>
**Chemotaxonomy**

The cell envelope of the strain V24ST consists of a double-layer of pseudomurein and protein, while the cell wall contains pseudomurein consisting of N-acetyl-glucosamine, N-acetyl-galactosamine, N-talosaminuronic acid, glutamic acid, alanine, and lysine [1,2]. *Methanothermus fervidus* contains approximately 50% diethers, 25% diglycerol tetraethers and 25% of an unknown component moving slower than the tetraethers when examined by thin layer chromatography [2]. Here, *M. fervidus* differs from *M. sociabilis*, which lacks the unknown component while its diether and tetraethers were found at about equal proportions [2]. The diethers of *M. fervidus* contain only C₂₀ phytanyl chains while the tetraethers include about 98-99% C₄₀ biphytane and only some trace of C₄₀ monocyclic biphytane [2]. Besides an unknown core lipid (FU, 31% of total core lipids, migrates slower than caldarchaeol by thin layer chromatography), other core lipids found in *M. fervidus* were caldarchaeol (60%), archaeol (4%) and others (5%) [35,36]. Interestingly, *M. fervidus* also differs from *M. sociabilis* regarding the glycolipid composition with four glycolipids and about equal proportions of five phospholipids and only three phospholipids for *M. sociabilis* [2].

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**Table 1. Classification and general features of *M. fervidus* V24ST according to the MIGS recommendations [15]**

| MIGS ID  | Property                  | Term                                      | Evidence code |
|----------|---------------------------|-------------------------------------------|---------------|
|          | Current classification    |                                           |               |
|          | Domain                    | Archaea                                   | TAS [16]      |
|          | Phylum                    | Euryarchaeota                              | TAS [17,18]   |
|          | Class                     | Methanobacteria                            | TAS [18,19]   |
|          | Order                     | Methanobacteriales                         | TAS [20-22]   |
|          | Family                    | Methanomethacaeae                          | TAS [1,23]    |
|          | Genus                     | Methanothermus                            | TAS [1,23]    |
|          | Species                   | Methanothermus fervidus                    | TAS [1,23]    |
|          | Type strain               | V24S                                      | TAS [1]       |
|          | Gram stain                | positive                                  | TAS [1]       |
|          | Cell shape                | straight to curved, single and in pair rods| TAS [1]       |
|          | Motility                  | non-motile                                | TAS [1]       |
|          | Sporulation               | not reported                              | NAS           |
|          | Temperature range          | 61°C–97°C                                 | TAS [1]       |
|          | Optimum temperature       | 83°C                                      | TAS [1]       |
|          | Salinity                  | not reported                              | NAS           |
| MIGS-22  | Oxygen requirement        | strict anaerobic                          | TAS [1]       |
|          | Carbon source             | CO₂                                       | TAS [1]       |
|          | Energy source             | H₂ + CO₂                                  | TAS [1]       |
| MIGS-6   | Habitat                   | solfataric fields                         | TAS [1]       |
| MIGS-15  | Biotic relationship       | not reported                              | NAS           |
| MIGS-14  | Pathogenicity             | no                                        | NAS           |
|          | Isolation                 | Icelandic hot spring                      | TAS [1]       |
| MIGS-4   | Geographic location       | Kerlingarfjöll mountains, Iceland          | TAS [1]       |
| MIGS-5   | Sample collection time    | 1979                                      | NAS           |
| MIGS-4.1 | Latitude                  | 64.65                                     | NAS           |
| MIGS-4.2 | Longitude                 | 19.25                                     | NAS           |
| MIGS-4.3 | Depth                     | surface                                   | TAS [1]       |
| MIGS-4.4 | Altitude                  | 1.477 m                                   | NAS           |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [25]. If the evidence code is IDA, then the property was directly observed by one of the authors or an expert mentioned in the acknowledgements
Genome sequencing and annotation

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position [37], and is part of the Genomic Encyclopedia of Bacteria and Archaea project [38]. The genome project is deposited in the Genome OnLine Database [8] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

| MIGS ID | Property                      | Term                                                                 |
|---------|-------------------------------|----------------------------------------------------------------------|
| MIGS-31 | Finishing quality             | Finished                                                             |
| MIGS-28 | Libraries used                | Three genomic libraries: one 454 pyrosequence standard library, one 454 PE library (17.8 kb insert size), one Illumina library |
| MIGS-29 | Sequencing platforms          | Illumina GAii, 454 GS FLX                                            |
| MIGS-31.2| Sequencing coverage           | 530 × Illumina; 75.0 × pyrosequence                                  |
| MIGS-30 | Assemblers                    | Newbler version 2.00.20-PostRelease-11-05-2008-gcc-3.4.6, phrap      |
| MIGS-32 | Gene calling method           | Prodigal 1.4, GenePRIMP                                              |
| INSDC ID|                               | CP002278                                                             |
| GOLD ID |                               | Gc01509                                                              |
| NCBI project ID |                       | 33689                                                                |
| Database: IMG-GEBA |               | 2502422313                                                           |
| MIGS-13 | Source material identifier    | DSM 2088                                                             |
| Project relevance |               | Tree of Life, GEBA                                                   |

Growth conditions and DNA isolation

*M. fervidus* V24S, DSM 2088, was grown anaerobically in culture vessels made of type III glass (alkali-rich soda lime glass) in DSMZ medium 203 (*M. fervidus* medium) [39] at 83°C. DNA was isolated from 0.5-1 g of cell paste using Qiagen Genomic 500 DNA Kit (Qiagen, Hilden, Germany) following the standard protocol as recommended by the manufacturer, with a modified cell lysis step. The modified lysis mixture contained only 100 µl lysozyme, but additional 58 µl a-chromopeptidase, lysostaphine, mutanolysin, each, for over night incubation at 35°C on a shaker. Proteinase K digestion was reduced to 200 µl for 1h 37°C.

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [40]. Pyrosequencing reads were assembled using the Newbler assembler version 2.00.20-PostRelease-11-05-2008-gcc-3.4.6 (Roche). The initial Newbler assembly consisting of 24 contigs in one scaffold was converted into a phrap assembly [41] by making fake reads from the consensus, collecting the read pairs in the 454 paired end library. Illumina GAii sequencing data (636 Mb) was assembled with Velvet [42] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. 454 Draft assembly was based on 96.5.0 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [41] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [40], Dupfinisher, or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Epigenome Biotechnologies, Madison, WI) [43]. Gaps between contigs were closed by editing in CONSED and additional sequencing reactions were necessary to close gaps.
and/or to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [44]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 605 × coverage of the genome. The final assembly contained 267,328 pyrosequence and 17,666,667 Illumina reads.

Genome annotation
Genes were identified using Prodigal [45] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [46]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [47].

Genome properties
The genome consists of a 1,243,342 bp long chromosome with a 31.6% GC content (Table 3 and Figure 3). Of the 1,361 genes predicted, 1,311 were protein-coding genes, and 50 RNAs; twenty eight pseudogenes were also identified. The majority of the protein-coding genes (74.8%) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

| Attribute                              | Value         | % of Total |
|----------------------------------------|---------------|------------|
| Genome size (bp)                       | 1,243,342     | 100.00%    |
| DNA coding region (bp)                 | 1,163,294     | 93.56%     |
| DNA G+C content (bp)                   | 393,356       | 31.64%     |
| Number of replicons                    | 1             | 100.00%    |
| Extrachromosomal elements              | 0             |            |
| Total genes                            | 1,361         | 100.00%    |
| RNA genes                              | 50            | 3.67%      |
| rRNA operons                           | 2             | 0.15%      |
| Protein-coding genes                   | 1,311         | 96.33%     |
| Pseudo genes                           | 28            | 2.06%      |
| Genes with function prediction         | 1,018         | 74.80%     |
| Genes in paralog clusters              | 100           | 7.35%      |
| Genes assigned to COGs                 | 1,126         | 82.73%     |
| Genes assigned Pfam domains            | 1,144         | 84.06%     |
| Genes with signal peptides             | 109           | 8.01%      |
| Genes with transmembrane helices       | 248           | 18.22%     |
| CRISPR repeats                         | 0             |            |
Figure 3. Graphical circular map of the genome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

Table 4. Number of genes associated with the general COG functional categories

| Code | Value | % | Description                                               |
|------|-------|---|-----------------------------------------------------------|
| J    | 144   | 12.2 | Translation, ribosomal structure and biogenesis           |
| A    | 2     | 0.2 | RNA processing and modification                           |
| K    | 47    | 4.0 | Transcription                                             |
| L    | 49    | 4.2 | Replication, recombination and repair                     |
| B    | 4     | 0.3 | Chromatin structure and dynamics                          |
| D    | 12    | 1.0 | Cell cycle control, cell division, chromosome partitioning|
| Y    | 0     | 0.0 | Nuclear structure                                         |
| V    | 6     | 0.5 | Defense mechanisms                                        |
| T    | 11    | 0.9 | Signal transduction mechanisms                            |
| M    | 52    | 4.4 | Cell wall/membrane/envelope biogenesis                    |
| N    | 1     | 0.1 | Cell motility                                             |
Methanothermus fervidus type strain (V24ST)

Table 4 (cont.). Number of genes associated with the general COG functional categories

| Code | value | % age | Description                                                                 |
|------|-------|-------|-----------------------------------------------------------------------------|
| Z    | 0     | 0.0   | Cytoskeleton                                                                |
| W    | 0     | 0.0   | Extracellular structures                                                    |
| U    | 14    | 1.2   | Intracellular trafficking and secretion, and vesicular transport             |
| O    | 46    | 3.9   | Posttranslational modification, protein turnover, chaperones                 |
| C    | 111   | 9.4   | Energy production and conversion                                            |
| G    | 39    | 3.3   | Carbohydrate transport and metabolism                                        |
| E    | 88    | 7.5   | Amino acid transport and metabolism                                          |
| F    | 47    | 4.0   | Nucleotide transport and metabolism                                         |
| H    | 115   | 9.8   | Coenzyme transport and metabolism                                           |
| I    | 17    | 1.4   | Lipid transport and metabolism                                              |
| P    | 50    | 4.2   | Inorganic ion transport and metabolism                                       |
| Q    | 5     | 0.4   | Secondary metabolites biosynthesis, transport and catabolism                 |
| R    | 172   | 14.6  | General function prediction only                                            |
| S    | 147   | 12.5  | Function unknown                                                             |
| -    | 235   | 17.3  | Not in COGs                                                                  |

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