Complete genome sequence of *Methanoculleus marisnigri* Romesser et al. 1981 type strain JR1

Iain J. Anderson¹*, Magdalena Sieprawska-Lupa², Alla Lapidus¹, Matt Nolan¹, Alex Copeland¹, Tijana Glavina Del Rio¹, Hope Tice¹, Eileen Dalin¹, Kerrie Barry¹, Elizabeth Saunders¹, Cliff Han¹, Thomas Brettin¹, John C. Detter¹, David Bruce¹, Natalia Mikhailova¹, Sam Pitluck¹, Loren Hauser¹, Miriam Land¹, Susan Lucas¹, Paul Richardson¹, William B. Whitman², and Nikos C. Kyrpides¹

¹Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, California, USA
²Microbiology Department, University of Georgia, Athens, Georgia, USA
³Los Alamos National Laboratory, Bioscience Division, Los Alamos, New Mexico, USA
⁴Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

*Corresponding author: Iain Anderson

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*Methanoculleus marisnigri* Romesser et al. 1981 is a methanogen belonging to the order *Methanomicrobiales* within the archaeal phylum *Euryarchaeota*. The type strain, JR1, was isolated from anoxic sediments of the Black Sea. *M. marisnigri* is of phylogenetic interest because at the time the sequencing project began only one genome had previously been sequenced from the order *Methanomicrobiales*. We report here the complete genome sequence of *M. marisnigri* type strain JR1 and its annotation. This is part of a Joint Genome Institute 2006 Community Sequencing Program to sequence genomes of diverse Archaea.

Introduction

*Methanoculleus marisnigri* is a methanogen belonging to the order *Methanomicrobiales*, and strain JR1 is the type strain of this species. When it was first isolated, this organism was named *Methanogenium marisnigri* [1], but then later it was transferred to the genus *Methanoculleus* [2]. The type strain was isolated from sediment of the Black Sea, while another strain was isolated from an anaerobic digestor [2]. Other species of *Methanoculleus* have been isolated from different types of anaerobic digestors and marine and freshwater sediments (reviewed in [3]).

Methanogens have been divided into two groups known as Class I and Class II based on phylogeny [4]. Class I includes the orders *Methanococcales*, *Methanobacteriales*, and *Methanopyrales*, which use H₂/CO₂ or formate as substrates for methanogenesis, although some can also use alcohols as electron donors. Class II includes the orders *Methanosarcinales* and *Methanomicrobiales*. Some of the *Methanosarcinales* are capable of using various methyl compounds as substrates for methanogenesis including acetate, methylamines, and methanol, but *Methanomicrobiales* are restricted to the same substrates as the Class I methanogens [3]. Therefore *Methanomicrobiales* are phylogenetically closer to *Methanosarcinales* but physiologically more similar to Class I methanogens, making them an interesting target for genome sequencing.

In a 2006 Community Sequencing Program (CSP) project, we proposed sequencing two members of the order *Methanomicrobiales*: *M. marisnigri* and *Methanocorpusculum labreanum*. Previously only one genome was available from this order, that of *Methanospirillum hungatei*. *M. marisnigri* and *M. labreanum* are phylogenetically distant from each other and from *M. hungatei* (Figure 1), and they represent the three phylogenetic families within the order *Methanomicrobiales*. We report here the sequence and annotation of *M. marisnigri* type strain JR1.

Classification and features

*Methanoculleus marisnigri* JR1 was isolated from Black Sea sediment at a depth of 0.5-20 cm. The enrichment medium consisted of 30% distilled
Methanoculleus marisnigri type strain JR1

water and 70% sea water with the addition of acetate, formate, tryptophane, yeast extract, vitamin solution, trace mineral solution, and volatile fatty acid solution [1]. Cells were maintained in serum vials under an atmosphere of 80% H₂ and 20% CO₂ by a modification of the Hungate technique [1]. The physiological characteristics of M. marisnigri were described as follows [1]. The cells were irregular cocci with peritrichous flagella. The cell wall was composed of glycoprotein and lacked peptidoglycan. The optimal growth temperature was 20-25°C with growth observed between 15 and 45°C. The optimal pH for growth was 6.4 with a range of 6.0-7.5. The optimal salt concentration for growth was around 0.1 M NaCl, and growth was observed between 0.0 and 0.7 M NaCl. Neither acetate nor yeast extract was stimulatory for growth. Trypticase was required, and it could not be replaced by Casamino acids or other peptide mixtures. Coenzyme M and Coenzyme F₄₂₀ were both detected in M. marisnigri. Growth was observed with H₂/CO₂ or formate but not with acetate or methanol. M. marisnigri was subsequently shown to grow with secondary alcohols as the electron donor for methanogenesis [6]. The physiological and morphological features of M. marisnigri are presented in (Table 1).

Genome sequencing information

Genome project history

Methanoculleus marisnigri was selected for sequencing based upon its phylogenetic position relative to other methanogens of the order Methanomicrobiales. It is part of a Joint Genome Institute 2006 Community Sequencing Program project that included six archaeal genomes selected for their phylogenetic diversity. A summary of the project information is shown in Table 2. The complete genome sequence was finished in February, 2007. The GenBank accession number for the project is CP000562. The genome project is listed in the Genomes OnLine Database (GOLD) [18] as project Gc00512. Sequencing was carried out at the Joint Genome Institute (JGI) Production Genomics Facility (PGF) in Walnut Creek, California. Quality assurance using Phred [19,20] was done by JGI-Stanford. Finishing was done by JGI-Los Alamos National Laboratory (LANL). Annotation was done by JGI-Oak Ridge National Laboratory (ORNL) and by JGI-PGF.

Growth conditions and DNA isolation

The methods for DNA isolation, genome sequencing and assembly for this genome have previously been published [21].

**Figure 1.** Phylogenetic tree of selected Methanomicrobiales showing the distance between the three organisms for which complete genomes are available – Methanospirillum hungatei, Methanocorpusculum labreanum, and Methanoculleus marisnigri. The tree uses 16S ribosomal RNA sequences aligned within the Ribosomal Database Project (RDP), and the tree was constructed with the RDP Tree Builder [5]. Methanosarcina barkeri was used as the outgroup. The numbers on the branches represent bootstrap values based on 100 replicates.
### Table 1. Classification and general features of *M. marisnigri* JR1 according to the MIGS recommendations [7].

| MIGS ID | Property                     | Term                                      | Evidence Code |
|---------|------------------------------|-------------------------------------------|---------------|
|         | Current classification       | Domain *Archaea*                          | TAS [8-10]    |
|         |                              | Phylum *Euryarchaeota*                    |               |
|         |                              | Class “*Methanomicrobia*”                 | TAS [13]      |
|         |                              | Order *Methanomicrobiales*                | TAS [14]      |
|         |                              | Family *Methanomicrobiaceae*              | TAS [14]      |
|         |                              | Genus *Methanoculleus*                    | TAS [2]       |
|         |                              | Species *Methanoculleus marisnigri*       | TAS [2]       |
|         | Gram stain                   | negative                                  |               |
|         | Cell shape                   | irregular coccus                          | TAS [1]       |
|         | Motility                     | peritrichious flagella                    | TAS [1]       |
|         | Sporulation                  | nonsporulating                            | NAS           |
|         | Temperature range            | 15-45°C                                   | TAS [1]       |
| MIGS-6.3| Optimum temperature         | 20-25°C                                   | TAS [1]       |
| MIGS-22 | Salinity                    | 0.0-0.7 M NaCl                            | TAS [1]       |
|         | Oxygen requirement           | anaerobe                                  | TAS [1]       |
|         | Carbon source                | CO₂                                       | NAS           |
|         | Energy source                | H₂/CO₂, formate, secondary alcohols       | TAS [1,6]     |
|         | Habitat                      | sediment, anaerobic digestors             | TAS [1,2]     |
| MIGS-15 | Biotic relationship         | free-living                               | TAS [1]       |
| MIGS-14 | Pathogenicity                | none                                      | NAS           |
|         | Biosafety level              | 1                                         | NAS           |
|         | Isolation                    | sediment                                  | TAS [1]       |
| MIGS-4  | Geographic location         | Black Sea                                 | TAS [1]       |
| MIGS-5  | Isolation time              | 1979                                      | TAS [1]       |
| MIGS-4.1| Latitude-longitude          | not reported                              |               |
| MIGS-4.2| Depth                       | 0.5-20 cm                                 |               |
| MIGS-4.3| Altitude                    | not applicable                            |               |

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 the Gene Ontology project [15]. If the evidence code is IDA, then the property was directly observed for a living isolate by one of the authors or another expert mentioned in the acknowledgements.

### Table 2. Genome sequencing project information

| MIGS ID | Characteristic                  | Details                                      |
|---------|---------------------------------|----------------------------------------------|
| MIGS-28 | Libraries used                  | 3kb, 6kb and 40kb (fosmid)                   |
| MIGS-29 | Sequencing platform             | Applied Biosystems 3730                     |
| MIGS-31.2| Sequencing coverage            | 11×                                          |
| MIGS-31 | Finishing quality               | Finished                                     |
|         | Sequencing quality              | less than one error per 50kb                 |
| MIGS-30 | Assembler                      | Phrap                                        |
| MIGS-32 | Gene calling method            | CRITICA [16], Glimmer [17]                  |
|         | GenBank ID                      | CP000562                                     |
|         | GenBank date of release         | October 17, 2007                             |
|         | GOLD ID                         | Gc00512                                      |
|         | NCBI project ID                 | 16330                                        |
|         | IMG Taxon ID                    | 640069318                                    |
| MIGS-13 | Source material identifier      | ATCC 35101                                   |
|         | Project relevance               | phylogenetic diversity                      |

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Methanothermobacter marisnigri type strain JR1

Genome properties

The genome of *M. marisnigri* JR1 consists of a single circular chromosome (Figure 2 and Table 3). In comparison with other methanogens, the genome size of 2.48 Mbp is larger than those of Class I methanogens, which tend to be 1.6-1.8 Mbp, but smaller than the genomes of *Methanosarcina* species and *Methanospirillum hungatei*, which range between 3.5 and 5.8 Mbp. The G+C percentage of *M. marisnigri* is 62.1%, the highest among sequenced methanogens. The genome contains 2,560 genes of which 2,506 are protein-coding genes and the remaining 54 are RNA genes. There were only 17 pseudogenes identified, constituting 0.68% of the total genes. In total, 1633 protein-coding genes (65.2%) were assigned a function, with the remaining annotated as hypothetical proteins. The percentage of genes with signal peptides (14.0%) is quite high compared to other methanogens, although several methanogens have similar percentages. The properties and statistics of the genome are summarized in Table 3 and genes belonging to COG functional categories are listed in Table 4.

![Figure 2. Graphical circular map of the chromosome. From outside to the center: Genes on forward strand (colored by COG categories), Genes on reverse strand (colored by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.](image-url)
### Table 3. Genome statistics

| Genome characteristic          | Value       | % of total  |
|-------------------------------|-------------|-------------|
| Genome size (bp)              | 2,478,10    | 100.00%     |
| DNA coding region (bp)        | 2,181,39    | 88.0%       |
| DNA G+C content (bp)          | 1,537,98    | 62.1%       |
| Number of replicons           | 1           |             |
| Extrachromosomal elements     | 0           |             |
| Total genes                   | 2560        | 100.00%     |
| RNA genes                     | 54          | 2.1%        |
| rRNA operons                  | 1           |             |
| Protein-coding genes          | 2506        | 97.9%       |
| Pseudogenes                   | 17          | 0.7%        |
| Genes with function prediction| 1633        | 65.2%       |
| Genes in paralog clusters     | 1230        | 49.1%       |
| Genes assigned to COGs        | 1985        | 79.2%       |
| Genes assigned Pfam domains   | 1790        | 71.4%       |
| Genes with signal peptides    | 352         | 14.0%       |
| Genes with transmembrane helices| 595       | 23.7%       |
| CRISPR repeats                | 0           |             |

### Table 4. Numbers of genes associated with general COG functional categories.

| Code | Value | % age | Description                                                                 |
|------|-------|-------|----------------------------------------------------------------------------|
| E    | 139   | 5.5   | Amino acid transport and metabolism                                          |
| G    | 77    | 3.1   | Carbohydrate transport and metabolism                                         |
| D    | 17    | 0.7   | Cell cycle control, cell division, chromosome partitioning                   |
| N    | 23    | 0.9   | Cell motility                                                               |
| M    | 104   | 4.2   | Cell wall/membrane/envelope biogenesis                                        |
| B    | 5     | 0.2   | Chromatin structure and dynamics                                             |
| H    | 152   | 6.1   | Coenzyme transport and metabolism                                            |
| Z    | 0     | 0.0   | Cytoskeleton                                                                |
| V    | 23    | 0.9   | Defense mechanisms                                                           |
| C    | 174   | 6.9   | Energy production and conversion                                             |
| W    | 0     | 0.0   | Extracellular structures                                                     |
| S    | 255   | 10.2  | Function unknown                                                             |
| R    | 286   | 11.4  | General function prediction only                                             |
| P    | 94    | 3.8   | Inorganic ion transport and metabolism                                        |
| U    | 22    | 0.9   | Intracellular trafficking, secretion, and vesicular transport                 |
| I    | 30    | 1.2   | Lipid transport and metabolism                                               |
| Y    | 0     | 0.0   | Nuclear structure                                                            |
| F    | 63    | 2.5   | Nucleotide transport and metabolism                                           |
| O    | 84    | 3.4   | Posttranslational modification, protein turnover, chaperones                 |
| A    | 1     | 0.0   | RNA processing and modification                                              |
| L    | 84    | 3.4   | Replication, recombination and repair                                         |
| Q    | 15    | 0.6   | Secondary metabolites biosynthesis, transport and catabolism                 |
| T    | 87    | 3.5   | Signal transduction mechanisms                                               |
| K    | 97    | 3.9   | Transcription                                                               |
| J    | 153   | 6.1   | Translation, ribosomal structure and biogenesis                              |
| -    | 521   | 20.8  | Not in COGs                                                                  |

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Insights from the genome sequence

The genome sequence of *M. marisnigri* JR1 shows some similarities to Class I methanogens and some to *Methanosarcinales* but also has some unique features. In common with Class I methanogens, *M. marisnigri* uses a partial reductive TCA cycle to synthesize 2-oxoglutarate, and it has the Eha membrane-bound hydrogenase. Similar to *Methanosarcinales*, *M. marisnigri* has the Ech membrane-bound hydrogenase. A unique feature of *M. marisnigri* and the other *Methanomicrobiales* is the presence of anti- and anti-anti-sigma factors, which is surprising as *Archaea* do not use such factors. These anti- and anti-anti-sigma factors must have developed a new function in the *Archaea*. Phylogenetic analysis of methanogenesis and cofactor biosynthesis enzymes suggests that *Methanomicrobiales* form a group distinct from other methanogens, and therefore methanogens can be split into three classes [21].

There are also differences among the *Methanomicrobiales*. For instance, *M. marisnigri* is the only one of the three to have the F_{420}-nonreducing hydrogenase, and it is the only one of the three to lack the 14-subunit Mbh membrane-bound hydrogenase. This has implications for the mechanism of methanogenesis: *M. marisnigri* may couple Coenzyme M-Coenzyme B heterodisulfide reduction to the first step of methanogenesis in the cytoplasm, similar to Class I methanogens [35], while the other *Methanomicrobiales* may couple heterodisulfide reduction to generation of a membrane ion gradient [21].

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