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Marine Genomics 53 (2020) 100768

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

Complete genome sequence of *Methanofervidicoccus* sp. A16, a thermophilic methanogen isolated from Mid Cayman Rise hydrothermal vent

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ARTICLE INFO

Keywords:
Thermophilic
Methanogenesis
Methanofervidicoccus sp.
Hydrothermal vent

ABSTRACT

Methanofervidicoccus sp. A16 is a novel thermophilic and obligate hydrogenotrophic methanogen isolated from a deep-sea hydrothermal vent chimney sample at the Mid Cayman spreading center, Caribbean Sea. Here we report the complete genome of strain A16, which has one circular chromosome of 1,485,358 bp with a mean G+C content of 35.01 mol%. The complete genome harbors 1442 predicted protein-encoding genes. Genes involved in hydrogenotrophic methane production and N_2 fixation were identified in this genome. This study expands our knowledge of methanogenesis at high temperatures and the involvement of these microorganisms in the carbon and nitrogen cycles of deep-sea hydrothermal environments.

1. Introduction

Methanogenic archaea are strictly anaerobic prokaryotes that are widely distributed in anoxic environments such as wetlands, paddy fields, sediments and digestive tracts (Thauer et al., 2008). They produce methane as the terminal step of organic matter fermentation. Of the 500–600 million tons of methane released into the atmosphere each year, 69% originate from microbial metabolism (Conrad, 2009). Methanogens can use carbon dioxide and H_2, simple carbon compounds (acetate, formate, etc) or methylated compounds (methylamine, dimethylsulfide, choline, etc) as energy and carbon sources, and produce methane as a metabolic waste product. Nowadays, a number of hyperthermophilic or thermophilic methanogenic archaea belonging to the order *Methanococcales* have been isolated from a variety of marine hydrothermal environments, including *Methanococcus igneus* Kol 5^T, *Methanotorris formicicus* Mc-S-70^T, *Methanothermococcus okinawensis* IH1^T, *Methanococcus jannaschii* DSM 2661^T, *Methanocaldococcus infernus* ME^T, *Methanocaldococcus indicus* $SL43^T$, *Methanocaldococcus villosus* KIN24-$T80^T$, *Methanocaldococcus bathoardescens* $JH146^T$ and further species (Jones et al., 1983; Burggraf et al., 1990; Jeanthon et al., 1998; Takai et al., 2002; L'Haridon et al., 2003; Takai et al., 2004; Bellack et al., 2011; Stewart et al., 2015).

The A16 genome sequence and its annotation is reported in the study. Strain A16 was isolated from a deep-sea hydrothermal vent chimney sample collected in a depth of 2295 m from Von Damm vent field (Hole to Hell site) (18°22.6007′ N, 81°47.8909′ W) at the Mid Cayman spreading center, Caribbean Sea. It was enriched and purified in DSM 141 medium, at 70 °C, under an atmosphere of H_2/CO_2 (80/20, 0.2 MPa). Strain A16 was identified as a potential novel species of the genus *Methanofervidicoccus*, with highest 16S rRNA sequence similarity of 98.6% to *Methanofervidicoccus abyssi* HHB^T, which was reported recently as a novel methanogen belonging to the order *Methanococcales* (Sakai et al., 2019). Strain A16 is an obligate anaerobic archaeon that can use CO_2 and H_2 as the sole carbon and energy sources to produce CH_4.

2. Data description

Genomic DNA of strain A16 was extracted with the QIAGEN Genomic-tip 20/G (QIAGEN, Düsseldorf, Germany) kit following the manufacturer's standard protocol. The complete genome sequence of strain A16 was determined by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China), using the PacBio RSII platform (Table 1). For whole genome sequencing, a 10 kb DNA fragment library was constructed and sequenced on the PacBio RSII sequencing platform, with one SMRT cell (MajorBio Co., Shanghai, China). After quality control, reads were assembled by HGAP 3.0 (Chin et al., 2013). Gene prediction was performed using Glimmer version 3.02 (http://

Abbreviations: ANI, average nucleotide identity; CDS, coding DNA sequences; COG, clusters of orthologous groups; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; ORFs, open reading frames

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https://doi.org/10.1016/j.margen.2020.100768
Received 11 September 2019; Received in revised form 21 January 2020; Accepted 17 March 2020
Available online 26 March 2020
1874-7787/

Table 1
General features of *Methanofervidicoccus* sp. A16.

Items	Description
General features	
Superkingdom	Archaea
Strain	*Methanofervidicoccus* sp. A16
Geographic location	Atlantic Ocean: Caribbean Sea, Von Damm vent field (Hole to Hell site) at the Mid Cayman spreading center
Sample type	deep-sea hydrothermal vent chimney sample
Latitude and longitude	18°22.6007′ N, 81°47.8909′ W
Depth	−2295 m
Collection date	June 2013
Biotic relationship	Free-living
Genome characteristics	
Sequencing platform	PacBio RSII
Assembly method	HGAP 3.0
Genome coverage	650×
Finishing quality	Complete genome
NCBI accession number	CP022242
BioProject	PRJNA391986
BioSample	SAMN07281457
Size (bp)	1,485,358
DNA G+C content (%)	35.01
CDSs	1422
tRNAs	34
16S-23S-5S rRNAs	2-2-2

cbcb.umd.edu/software/glimmer/). In addition, rRNA and tRNA genes were identified using Barrnap 0.4.2 and tRNA scan-SE v1.3.1 softwares. Functional annotation was performed using NCBI prokaryotic genome annotation pipeline (Tatusova et al., 2016), the Rapid Annotation using Subsystem Technology (RAST) pipeline (http://rast.nmpdr.org/) (Overbeek et al., 2014), and the the MicroScope Microbial Genome Annotation and Analysis Platform (MaGe) (Vallenet et al., 2009). Deduced gene products were blasted against KEGG, COG, String and GO databases.

The complete genome of the strain A16 consists of a single circular chromosome with a total length of 1,485,358 bp and an average G+C content of 35.01 mol% (Fig. 1). A total of 1442 protein-coding sequences (CDSs) were predicted, with a summed up gene length of 1,178,139 bp, giving a coding intensity of 78.71%. Majority of the CDSs (1206/1422, 84.81%) could be assigned to a putative function according to COG categories, while the rest were annotated as hypothetical proteins. 34 tRNA genes for 21 amino acids, two 5S rRNAs, two 16S rRNA, two 23S rRNA, 2 miscellaneous RNAs (misc RNA) and 2 pseudogenes were also identified in the genome.

Strain A16 can use H_2 as electron donor and CO_2 as carbon source for growth and produce CH_4. Genes coding for the full pathway of hydrogenotrophic methanogenesis were identified in the genome, which included formylmethanofuran dehydrogenase (Fwd, CFE53_04850, CFE53_05015, CFE53_06085, CFE53_06090, CFE53_06095, CFE53_06100, CFE53_06105, and CFE53_06110), formylmethanofuran-tetrahydromethanopterin N-formyltransferase (Ftr, CFE53_06700), N5, N10-methenyltetrahydromethanopterin cyclohydrolase (Mch, CFE53_06735), F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase (Mtd, CFE53_05635), F_{420}-dependent N5, N10-methylenetetrahydromethanopterin reductase (Mer, CFE53_02485), N5-methyltetrahydromethanopterin: coenzyme M methyltransferase (Mtr, CFE53_01150, CFE53_01155, CFE53_01160, CFE53_01170, CFE53_01175, CFE53_01180, CFE53_01185), Methyl coenzyme M reductase (Mcr, CFE53_01190, CFE53_01195, CFE53_01200, CFE53_01205 and CFE53_01210), and heterodisulfide reductase (Hdr, CFE53_02215, CFE53_06410, CFE53_06415, CFE53_06755 and CFE53_06760) (Table S1).

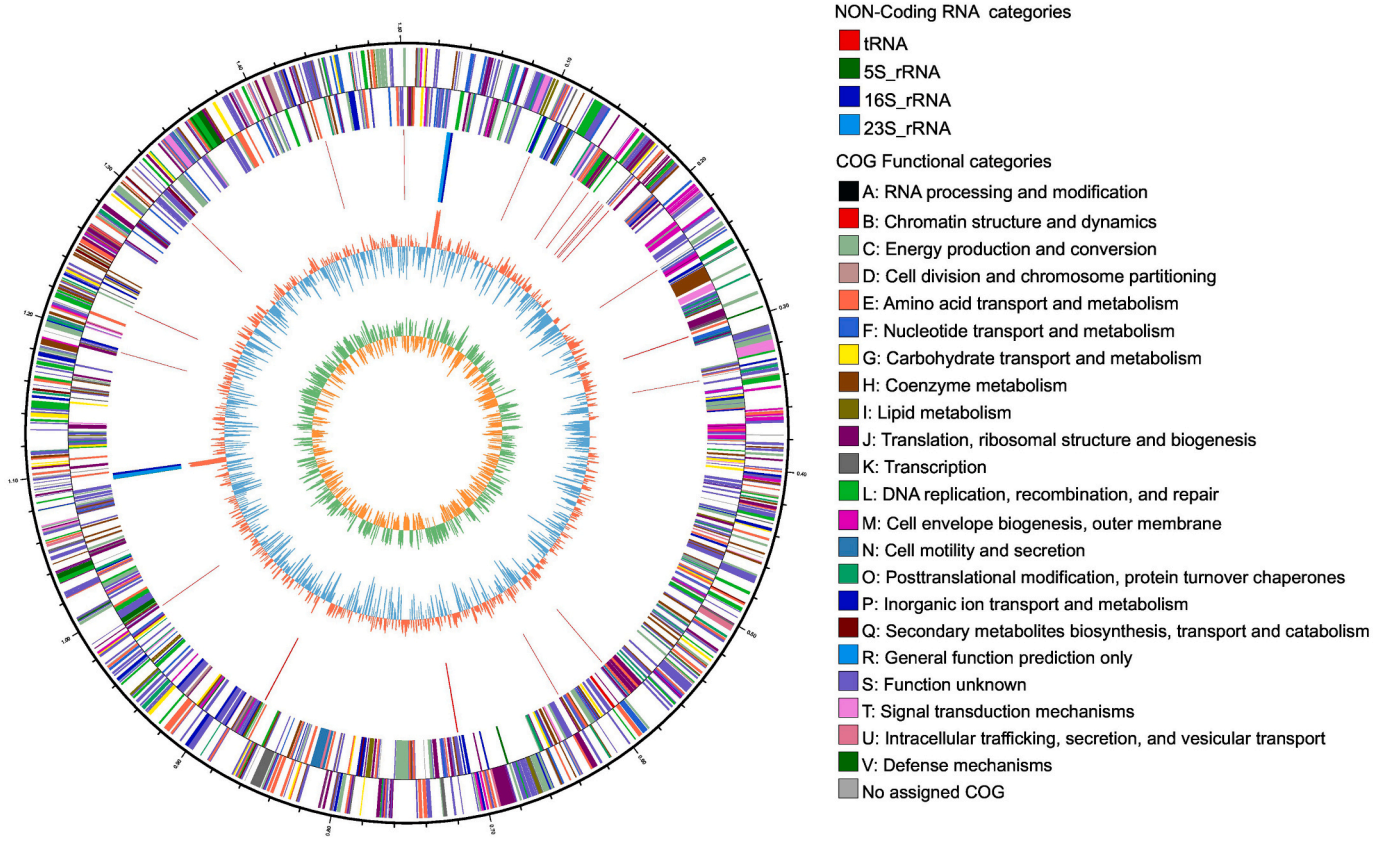

Fig. 1. Circular map of the chromosome in the genome of *Methanofervidicoccus* sp. A16. From the outside to the center: label of genome size, CDSs on forward strand (colored by COG categories), CDSs on reverse strand (colored by COG categories), RNA genes (tRNAs and rRNA operons), DNA G+C content and GC skew. CDSs are depicted in different colors according to COG categories.

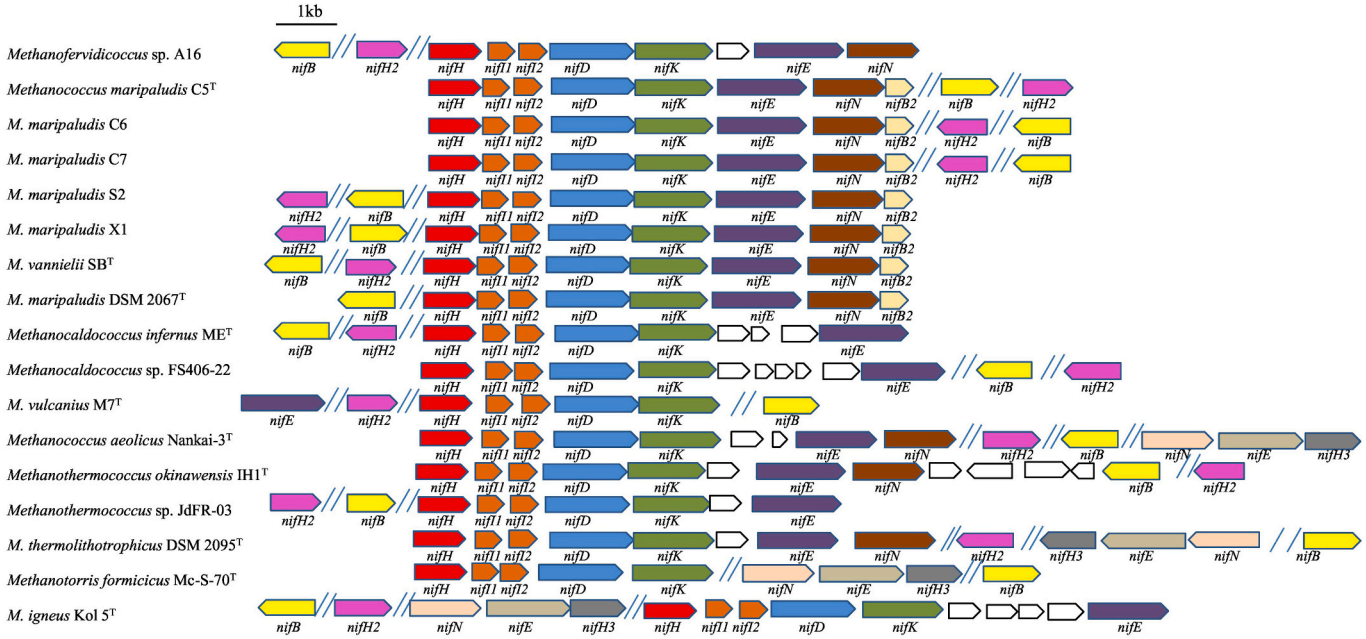

Fig. 2. Comparison analysis of the nitrogen fixation gene arrangement based on the genomes of *Methanofervidicoccus* sp. A16 and other sixteen methanogens within the order *Methanococcales*.

At the time of writing, twenty-three genomes belonging to the order *Methanococcales* were available in the NCBI and JGI databases. Based on core genome analysis, strain A16 featured the closest phylogenetic relationship to *Methanofervidicoccus abyssi* HHB^T (Fig. S1). The average nucleotide identity (ANI) value between strain A16 and HHB^T were 85.2%, which was below standard ANI criteria for species identity (95–96%) (Richter and Rosselló-Mora, 2009). Further genome sequence analysis of strain A16 revealed 116 unique genes mainly involved in Energy production and conversion, Amino acid transport and metabolism, Replication, recombination and repair, Cell wall, membrane and envelope biogenesis and Inorganic ion transport and metabolism, when compared with HHB^T (Table S2). A minimum set of six genes, *nifH* (CFE53_01675), *nifD* (CFE53_01660), *nifK* (CFE53_01655), *nifE* (CFE53_01645), *nifN* (CFE53_01640) and *nifB* (CFE53_05660), coding for structural and biosynthetic components of nitrogenase (Santos et al., 2012) are present in the A16 genome, while these are absent in strain HHB^T. In additon, two genes (CFE53_01665 and CFE53_01670) coding for nitrogen-fixation associated regulatory protein P-II are also located in this gene cluster. Comparative genome analyses further showed that the gene cluster for nitrogen fixation also exists in other sixteen strains of the order *Methanococcales*, although they show some differences in gene synteny, as shown in Fig. 2 and Fig. S1. Among these strains, *Methanothermococcus thermolithotrophicus* DSM 2095^T (Belay et al., 1984; Leigh, 2000), *Methanococcus maripaludis* DSM 2067^T (Blank et al., 1995), *M. maripaludis* S2 (Kessler et al., 1998; Santos et al., 2012), *Methanococcus aeolicus* Nankai-3^T (Kendall et al., 2006), *Methanotorris formicicus* Mc-S-70^T (Takai et al., 2004) and *Methanocaldococcus* sp. FS406-22 (Mehta and Baross, 2006), have been reported as diazotrophic methanogens. In this study, the genome analysis of strain A16 will help to understand the ecological role of this species in carbon and nitrogen cycle in submarine hydrothermal environments.

3. Nucleotide sequence accession number

The complete genome sequence of *Methanofervidicoccus* sp. A16 has been deposited in the GenBank database under number CP022242. The strain is available from the Université de Bretagne Occidentale Culture Collection under number UBOCC-M-3304.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are very grateful to the YK13-05 cruise operation teams for helping us to collect the hydrothermal samples and to the chief scientist Ken Takai. We thank Stéphane L'Haridon for the isolation of the strain. This work was supported by the PHC Cai Yuanpei Provirvent (N° 34634WE), the program MERLIN Abyss, the LIA 1211 MicrobSea and the “Laboratoire d'Excellence” LabexMER (ANR-10-LABX-1), National Key R&D Program of China (No. 2018YFC0310701), National Natural Science Foundation of China (No. 41506152).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2020.100768.

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