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GENOME ANNOUNCEMENT

Draft Genome Sequence of *Methanobacterium formicicum* DSM 3637, an Archaebacterium Isolated from the Methane Producer Amoeba *Pelomyxa palustris*

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Here is reported the draft genome sequence of *Methanobacterium formicicum* DSM 3637, which was isolated from the methane-producing amoeba *Pelomyxa palustris*. This bacterium was determined to be an endosymbiont living in the cytoplasm of *P. palustris* and the source of methane; however, the global characteristics of its genome suggest a free-living lifestyle rather than an endosymbiotic one.

Pelomyxa palustris Greeff 1874 is a giant, multinucleated, free-living amoeba (7). It is found living in the mud, in low-oxygen freshwater environments. *Pelomyxa* is special because of its unusual features. It lacks Golgi bodies, mitochondria, hydrogenosomes, and a contractile vacuole. The most striking feature of the *Pelomyxa* cytoplasm is the presence of three bacterial endosymbionts. The role of the endosymbiotic bacterium in *P. palustris* may be related to the mineralization of organic matter to methane by anaerobic degradation. In this scheme, the methanogenic bacterium plays a major role in the consumption of H_2, since the H_2-producing fermentation of organic matter is only possible at low pressures of H_2. Van Bruggen et al. (5) measured the methane production of a *P. palustris* specimen and isolated a methanogenic bacterium by squashing a *P. palustris* specimen over a mineral anaerobic medium aerated with H_2/CO_2 and supplemented with formate (6). The isolated bacterium was deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany) as *Methanobacterium formicicium* DSM 3637, from which all our DNA samples were obtained.

Reads were generated by 454 GS FLX sequencing (2), and raw data were assembled using the GS *de novo* assembler Newbler, version 2.5.3. The assembled contigs were submitted to the RAST annotation server for subsystem classification and functional annotation (1). Coding sequences (CDSs) were assigned using BLASTp with KEGG Orthology (KO). The NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) was employed for gene annotation in preparation for submission of sequences to GenBank (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). The draft genome sequence of *M. formicicium* DSM 3637comprises 2,684,623 bases, representing 60-fold coverage of the genome. The assembled genome consists of 31 large contigs with more than 100 bp (N_{50} contig size, approximately 100,000 bp). The G+C content is 38.02%. The genome contains 2,584 putative CDSs, and the RAST annotation server only detected 21 missing genes. The draft genome sequence contains five rRNAs (one large-subunit gene, one small-subunit gene, and three 5S genes) and 46 tRNA loci.

The SEED Viewer multigenome comparison tool (4) was used to compare the *M. formicicum* DSM 3637 predicted proteins with four representatives of the major groups of methanogens. Fifteen percent, corresponding to 401 proteins, could not be assigned to a homologous protein in any of the other methanogens. This indicated that these genes are exclusive to *M. formicicum* DSM 3637. However, most of them were annotated as encoding hypothetical proteins. On the other hand, 24% (631 proteins) showed a hit in the four compared genomes, i.e., these proteins are the conserved core of *M. formicicum* DSM 3637, which includes all genes necessary for methanogenesis. Although *M. formicicum* DSM 3637 was described as a *Pelomyxa* endosymbiont (6), its genome did not show the typical genome reduction of endosymbionts (3). The mean methanogen genome size is 2,277,055 bases, and the mean number of predicted genes in methanogen genomes is 2,130; in both cases, the sizes for *M. formicicum* DSM 3637 are above the means. These results and the fact that this bacterium can be cultivated easily *in vitro* (6) suggest a free-living lifestyle.

Nucleotide sequence accession numbers. The results of this whole-genome shotgun project have been deposited at DDBJ/EMBL/GenBank under accession number AMPO00000000. The version described in this paper is the first version, AMPO01000000.

ACKNOWLEDGMENTS

I thank sincerely the CITIUS General Biology Service team at the University of Seville: Cristina Reyes, Modesto Carballo, and especially Laura Navarro for their invaluable and selfless effort. I also thank Antonio Marín for his faith in this project and J. J. A. van Bruggen for sending me his Ph.D. thesis, in which I found a lot of unpublished information about *P. palustris* and *M. formicum*.

This research was not supported by any grant funding.

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Received 28 September 2012 **Accepted** 3 October 2012

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doi:10.1128/JB.01829-12

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