

Genome Sequence of “*Candidatus Methanomethylophilus alvus*” Mx1201, a Methanogenic Archaeon from the Human Gut Belonging to a Seventh Order of Methanogens

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We report the draft genome sequence of “*Candidatus Methanomethylophilus alvus*” Mx1201, a methanogen present in the human gut. It was enriched from human feces under anaerobic conditions with methanol as the substrate. Its circular genome, of around 1.7 Mb, contains genes needed for methylotrophic methanogenesis from methanol and tri-, di-, and monomethylamine.

Methanogenic archaea play a crucial role in the functioning of digestive ecosystems through the consumption of the hydrogen generated during anaerobic fermentations (3). The first archaeal species associated to the human gut, *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* (order *Methanobacteriales*), were cultured and described 30 years ago (12, 13). During the last decade, the diversity of the human-associated archaea was progressively enlarged by the detection of 16S rRNA genes affiliated to the *Crenarchaeota* (15), to the *Halobacteriales* (14), and to a *Thermoplasmatales*-related lineage (10, 11, 16). A species of the latter lineage, *Methanomassiliicoccus luminyensis*, was recently isolated and described as a methanogen growing on methanol and hydrogen only (5). More recently, its genome was sequenced (6).

To allow genomic characterization of novel methanogens, we performed enrichment cultures targeting methanogens in feces of elderly people (68 to 93 years old), selecting for the presence of a *Thermoplasmatales*-related lineage. Enrichment cultures carried out with methanol as the substrate led to the progressive dominance of “*Candidatus Methanomethylophilus alvus*” over the microbial community. This archaeon is distantly related to its closest related species, *M. luminyensis* and *Aciduliprofundum boonei*, with 87% and 80% 16S rRNA gene sequence identity, respectively. Sequences of 16S rRNA genes of uncultured archaea with more than 97% identity to “*Ca. Methanomethylophilus alvus*” have previously been recurrently retrieved in the digestive tract of various animals, suggesting that these archaea are specifically associated to these environments. Those sequences form a portion of the previously described rumen cluster C (8).

A 3-kb mate-paired library was constructed and sequenced from a quarter plate of a 454 GS FLX Titanium run (Macrogen, Republic of Korea). A total of 347,052 reads corresponding to 158 Mb were obtained. The reads were assembled with a GS De Novo assembler (version 2.6), first in 15 contigs (average depth coverage of 36.9-fold) and then in a unique scaffold of 1,664,410 bp. The gaps between the contigs were partially closed using GapFiller (2) and by sequencing of PCR products (4 remaining gaps). Around 1,800 bp was lacking between the two scaffold termini, as shown by PCR amplification. The open reading frames were predicted with Glimmer 3 (4) and annotated using RAST (1).

The genome of “*Ca. Methanomethylophilus alvus*” has a G+C content of 55.6%, 44 usual tRNA genes, a single copy of each of the

23S and 16S rRNA genes, and 2 noncontiguous copies of 5S rRNA genes that were distant in the genome from the 23S and 16S rRNA genes. A total of 1,785 protein coding sequences were predicted. The identification of the 56 ribosomal proteins ubiquitous in archaeal genomes (17) confirms the near completeness of the genome.

Two confidently annotated CRISPR regions (and two candidates) were identified using CRISPRFinder (7), in close association with *cas* genes (encoding CRISPR-associated proteins).

The presence of genes involved in methanogenesis, among them the highly conserved *mcrA* gene, supports the experimental observations that “*Ca. Methanomethylophilus alvus*” is a methanogen. Genes needed for methylotrophic methanogenesis from methanol and tri-, di-, and monomethylamine compounds were also identified. The amber codon in methyltransferases genes (*mtmB*, *mtbB* and *mttB*), together with genes involved in pyrrolysine (Pyl) biosynthesis (the 22nd amino acid carried by a tRNA^{Pyl} with a CUA anticodon), suggests the presence of Pyl in these proteins (9). “*Ca. Methanomethylophilus alvus*” would not only be able to reduce methanol with H₂ for methanogenesis but could probably also reduce other methylated compounds. To our knowledge, methylamines have not yet been considered substrates for methanogenesis, *in extenso* as valuable electron acceptors, in the human gut.

Nucleotide sequence accession numbers. The draft genome sequence of “*Ca. Methanomethylophilus alvus*” Mx1201 has been deposited at DDBJ/EMBL/GenBank under the accession no. [AMSE00000000](https://doi.org/10.1128/AMSE00000000). The version described in this paper is the first version, [AMSE01000000](https://doi.org/10.1128/AMSE01000000).

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