Two Metagenome-Assembled Genomes of Hydrogen-Dependent *Methanomassiliicoccales* Methanogens from the Zoige Wetland of the Tibetan Plateau

Juanli Yun,a Wenbin Du,a,b

aState Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

bSavaid Medical School, University of the Chinese Academy of Sciences, Beijing, China

ABSTRACT 

Wetlands in the Tibetan Plateau play a crucial role in global carbon cycling. Here, we report the metagenome-assembled genomes (MAGs) of two hydrogen-dependent methanogens from the Zoige wetland of the Tibetan Plateau. The novel species belong to *Methanomassiliicoccales*, the seventh euryarchaeal methanogenic order.

Methanogens are a group of archaea that control methane production in wetlands. Wetlands in the Tibetan Plateau are the major methane emission center in China (1). The genome research on uncultured methanogens in this extreme environment is of great importance in explaining the methane cycle in high-altitude wetlands.

Previously, extant methanogenic organisms were thought to belong exclusively to the phylum *Euryarchaeota* (2, 3), although more recently this assertion has been challenged by reports about the *Bathyarchaeota* (4, 5), *Verstraetearchaeota* (3, 6), and newly discovered *Cyanobacteria* (7, 8) phyla. Methanogens from the *Methanomassiliicoccales* order are called the seventh order of methanogens and are widely distributed in various environments (9). Here, we announce two metagenome-assembled genomes (MAGs) of novel *Methanomassiliicoccales* species with medium completeness.

Two sediment cores from the Flower Lake National Reserve of the Zoige wetland (102°52’E, 33°56’N) were sampled using sampling equipment (10 cm in diameter). The sampling site was water saturated, and the standing water depth was about 20 cm. Sediment cores were mixed thoroughly and kept at −80°C before use. DNA was extracted from the two sediment samples using the FastDNA spin kit for soil (MP Biomedicals, Cleveland, OH, USA) following the manufacturer’s instructions. A shotgun library was prepared with the NEBNext kit. Sequencing was completed on an Illumina HiSeq 2 × 150-bp platform. The average amount of metagenomic raw data for each sample was approximately 30 Gbp.

Sequencing quality for each sample was checked with FastQC (v.0.11.8) (10), and low-quality reads were trimmed using Trimmomatic (v.2.1.7) (11). Clean data were assembled individually using MEGAHIT (v.1.0) (12). To obtain MAGs, sequencing reads for each sample were mapped to the contigs using Bowtie 2 (v.2.2.5) to obtain differential coverage of each sample (13); genome binning was conducted based on these differential coverage files with MetaBAT (14) using a 1,000-bp contig cutoff value. The completeness and contamination of the MAGs were estimated using CheckM (v.1.1.2) (15). MAGs containing *mcrA* genes were selected by GraftM (v.0.13.1) (16) and annotated using Prokka (v.1.14.6) (17). rRNA coding regions (16S and 23S) of MAGs were predicted with Barrnap (https://github.com/tseemann/barrnap). Default parameters were used for all software unless otherwise specified.


Editor Kenneth M. Stedman, Portland State University

Copyright © 2021 Yun and Du. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Wenbin Du, wenbin@im.ac.cn.

Received 24 February 2021
Accepted 8 April 2021
Published 29 April 2021

mra.asm.org

Volume 10 Issue 17 e00021-21
TABLE 1 Sequence statistics and metagenomic binning statistics for each archaeal genome

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data for:</th>
<th>Bin 47</th>
<th>Bin 107</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection site</td>
<td></td>
<td>Zoige wetland (China)</td>
<td>Zoige wetland (China)</td>
</tr>
<tr>
<td>No. of reads for assembly</td>
<td></td>
<td>75,418,769</td>
<td>93,013,493</td>
</tr>
<tr>
<td>Total length (bp)</td>
<td></td>
<td>1,057,695</td>
<td>1,674,866</td>
</tr>
<tr>
<td>No. of contigs</td>
<td></td>
<td>221</td>
<td>189</td>
</tr>
<tr>
<td>GC content (%)</td>
<td></td>
<td>58.2</td>
<td>57.3</td>
</tr>
<tr>
<td>No. of coding regions</td>
<td></td>
<td>1,139</td>
<td>1,701</td>
</tr>
<tr>
<td>Nc50 (bp)</td>
<td></td>
<td>5,726</td>
<td>10,906</td>
</tr>
<tr>
<td>No. of 16S rRNA genes</td>
<td></td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td></td>
<td>67.7</td>
<td>86.6</td>
</tr>
<tr>
<td>Contamination (%)</td>
<td></td>
<td>2.42</td>
<td>4.84</td>
</tr>
</tbody>
</table>

The completeness and contamination values are based on the CheckM estimations.

The two MAGs obtained in this study have genome sizes of 1.05 Mb (bin 47) and 1.67 Mb (bin 107), and the genome completeness values were 67.7% with 2.42% contamination and 86.6% with 4.84% contamination, respectively (Table 1). The MAGs were both identified as *Methanomassiliicoccales* strains according to the mcrA gene taxonomy assignment, with 81.3% and 83.8% similarities to the *Massiliicoccales* Lake Pavin MAG according to an online BLAST search of the NCBI nucleotide database of *mcrA* genes (18).

The two MAGs contain all genes required for hydrogen-dependent reduction of methanol to methane, as proposed for other *Methanomassiliicoccales* strains (18). This announcement provides the basis for isolating this clade from environments.

**Data availability.** The *Methanomassiliicoccales* genome sequences have been deposited in GenBank under the accession numbers JACXT0000000000 and JACXT0000000000. The versions described here are the first versions. All metagenomic data generated from this announcement are available under BioProject number PRJNA644254. Metagenomic bins can be found under BioSample numbers SAMN15455434 and SAMN15455435.

**ACKNOWLEDGMENTS**

This work was supported by the National Natural Science Foundation of China (grants 41977196 and 91951103), the China Ocean Mineral Resources R&D Association (grant DY135-B-02), and the Key Program of Frontier Sciences of the Chinese Academy of Sciences (grant QYZDB-SSW-SCMC008).

**REFERENCE**


