Complete Genome Sequence of a Thermophilic Methanogen, *Methanocella conradii* HZ254, Isolated from Chinese Rice Field Soil

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Members of the order *Methanocellales* play a key role in methane emissions in paddy fields. Because of their slow growth and fastidious culture conditions, pure cultures are difficult to isolate and have been unavailable until recently. Here we report the complete genome sequence of a novel isolate in this group, *Methanocella conradii* strain HZ254.

Methanocellales is a recently described order of methanogens that was initially identified in rice field soil (13). The 16S rRNA genes of members of this order were discovered in 1998 in Italian rice field soil (5). Pure cultures, however, were not available until 2007, when the first isolate was obtained from Japanese rice field soil (14). Thereafter, a second isolate was obtained in 2010 from Italian rice field soil (12). *Methanocellales* bacteria are unique among methanogens in tolerance to O₂ stress (1, 2, 18, 19) and adaptation to low H₂ partial pressures (9, 15). The genomes of two members of the order *Methanocellales* are available thus far (4, 16). Here we report the complete genome of a new isolate obtained from Chinese rice field soil, *Methanocella conradii* strain HZ254.

Strain HZ254 shows an optimum growth temperature of 50 to 55°C and a specific growth rate of up to 0.11 h⁻¹. It uses H₂/CO₂ but not formate, acetate, methanol, methylamines, or alcohols for CH₄ production (Z. Lü and Y. Lu, unpublished data). Acetate is required as a carbon source for growth. Its high growth rate makes this methanogen a potentially useful model organism for laboratory studies of *Methanocellales*.

The genome was sequenced by Illumina with a combination of 0.5-kb and 6-kb libraries. A total of 1,316.38 Mb of filtered reads were assembled with SOAPdenovo (8) into 39 contigs (N50 = 164,330) and 11 scaffolds (N50 = 2,387,204), providing 557.2× coverage of the genome. Misassemblies were corrected and gaps were closed by custom primer walks from PCR products or subclones. A total of 65 additional reactions were necessary to close gaps and raise the quality of the sequence. The error rate of the completed genome sequence is lower than 1 in 100,000 bp. Genome annotation was performed with the Integrated Microbial Genomes Expert Review (IMG/ER) annotation pipeline (10). Genes were manually evaluated using GenePRIMP (11) to correct possible anomalies. Possible frameshifts or internal stop codons present in genes were verified and corrected by sequencing PCR products of the corresponding regions. The genome is 2.4 Mbp in size, contains 52.7% G + C, and encodes two rRNA operons, a full complement of tRNA genes, and 2,512 candidate genes. Of these genes, 71.9% can be assigned a possible function. In addition, 61 pseudogenes were identified.

The genome contains all of the genes involved in hydrogenotrophic but not acetoclastic methanogenesis, as expected. Genes for formate dehydrogenase, which may be involved in biosynthesis instead of methanogenesis, are present (6). All of the genes involved in electron bifurcation (3, 7, 17) were identified. Interestingly, some membrane-associated energy components previously undetected in hydrogenotrophic methanogens were

found. The trichloroacetic acid cycle is incomplete, but a putative aconitase previously unidentified in members of the order *Methanocellales* (4, 16) was annotated. The genome contains a complete *nif* operon for nitrogen fixation, a *fla* gene cluster encoding a flagellum, and a series of genes encoding O₂ detoxification and self-repair. More-detailed comparative analyses with other methanogens will provide deeper insight into the ecophysiology of this fascinating methanogen.

Nucleotide sequence accession number. The genome sequence and annotation were deposited in GenBank under accession number [CP003243](#).

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