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Complete Genome Sequence of *Methanothermobacter marburgensis*, a Methanoarchaeon Model Organism▽

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The circular genome sequence of the chemolithoautotrophic euryarchaeon *Methanothermobacter marburgensis*, with 1,639,135 bp, was determined and compared with that of *Methanothermobacter thermautotrophicus*. The genomes of the two model methanogens differ substantially in protein coding sequences, in insertion sequence (IS)-like elements, and in clustered regularly interspaced short palindromic repeats (CRISPR) loci.

Methanothermobacter marburgensis (DSM 2133) (formerly *Methanobacterium thermoautotrophicum* strain Marburg), a member of the *Methanobacteriales* (2), was isolated in 1978 from anaerobic sewage sludge in Marburg, Germany (5). The hydrogenotrophic methanogen grows even faster (2 h versus 3 h doubling time) and to higher cell concentrations (3 g versus 1.5 g dry mass per liter) than *Methanothermobacter thermautotrophicus* (DSM 1053) (formerly *Methanobacterium thermoautotrophicum* strain ΔH) (20) (for other differences, see references 3 and 19). Both methanogens were used in the last 35 years for the elucidation of the enzymes and coenzymes involved in CO_2 reduction to methane with H_2 (4, 16–18). The genome sequence of *M. thermautotrophicus* was reported in 1997 (15); that of *M. marburgensis* is announced here.

The genome size of *M. marburgensis* is 1,639,135 bp (that of *M. thermautotrophicus* is 1,751,377 bp), the genome G+C content is 48.64% (49.54% for *M. thermautotrophicus*), and the part coding is 90.94% (91.02% for *M. thermautotrophicus*). Comparison of the sequences (13) revealed that the two genomes have 1,607 protein coding sequences (CDS) in common and 411 CDS not in common (145 CDS are found only in *M. marburgensis* and 266 CDS only in *M. thermautotrophicus*) and show a high degree of synteny. The CDS not in common could be traced back to gene splitting (15%), gene deletion (30%), gene duplication (30%), and lateral gene transfer (24%) events (percentages given are for *M. marburgensis*). Of the 1,607 CDS in common, approximately 40% show BLAST search expectation values of $>10^{-100}$ at the protein level, reflecting large differences in sequence divergence. Almost 470 CDS encode conserved hypothetical proteins.

The genome of *M. marburgensis* harbors 15 insertion sequence (IS)-like elements, whereas there is no evidence for a classically organized IS-like element in *M. thermautotrophicus*. Consistently, a CDS for a transposase is found only in *M. marburgensis.*

In the genome of *M. marburgensis* there is only one clustered regularly interspaced short palindromic repeat (CRISPR) locus with 36 repeats and only one CRISPR-associated (*cas*) gene (*csa3*), indicating that the organism is not protected from invasion by phage and plasmid DNA (7, 8, 10, 12). By comparison, in the genome of *M. thermautotrophicus* there are three CRISPR loci with 124, 4, and 47 repeats and 18 *cas* genes that encode proteins involved in adaptation and interference (http://genoweb1.irisa.fr/Serveur-GPO/outils/repeatsAnalysis/CRISPR/). The spacer sequences from locus 2 match DNA sequences found in phage ΨM1 of *M. marburgensis* (6, 11) and ΨM100 of *M. wolfei* (9), which supports the observation that *M. thermautotrophicus* is not lysed by those two phages. Unfortunately, there is no DNA sequence available for phage ΦF1, which is able to lyse *M. thermautotrophicus* (14), to compare it with the spacer sequences of the CRISPR regions. In the plasmid pM2001 (= pMTBMA4) (4,439-bp circular multicopy plasmid found only in *M. marburgensis*) (1, 19), no sequence identities for CRISPR spacer sequences of *M. thermautotrophicus* were found (14).

*A*pproximately 200 CDS were identified that are required for the synthesis of the enzymes, coenzymes, and prosthetic groups involved in CO_2 reduction to methane and in the coupling of this process with energy conservation. Some of the genes have been found only recently; others, such as those for coenzyme F_{430} biosynthesis, still remain to be discovered.

Nucleotide sequence accession number. The complete genome sequence of *M. marburgensis* was deposited in GenBank under accession numbers CP001710 (chromosome) and CP001711 (pMTBMA4).

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