

# PhyMet<sup>2</sup>: phylogenetic tree

## Supplemental materials

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The 16S rRNA sequences of methanogens were extracted from GenBank (<https://www.ncbi.nlm.nih.gov/>) and Silva (<https://www.arb-silva.de/>) databases. The sequences were aligned in MAFFT using slow and accurate algorithm L-INS-i with 1,000 cycles of iterative refinement. Sites suitable for phylogenetic studies from the alignments were selected in GBlocks . The phylogenetic tree was constructed using MrBayes . We applied the mixed model of nucleotide substitutions to specify appropriate substitution models across the large parameter space . Moreover, we assumed the heterogeneity rate across sites described by the invariant model and the discrete gamma model with five category rates. Two independent runs starting from random trees, each using eight Markov chains, were applied. The trees were sampled every 100 generations for 20,000,000 generations. In the final analysis, we selected the last 85,720 trees that reached the stationary phase and convergence (i.e. when the standard deviation of split frequencies stabilized and was lower than the proposed threshold of 0.01).

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