

Physiological and genetic properties of key methanogenic Archaea for global methane emission from rice paddy fields.

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Rice paddy fields (RPFs) are one of the major sources of the greenhouse gas methane: it contributes 10-25% of the global methane emissions to atmosphere. Methane emission from RPFs is caused by the microbial production of methane, as the end products of anaerobic degradation of organic compounds. Cultivation-independent molecular studies suggested that one of the orders of methanogens, the order *Methanocellales*, which previously recognized as uncultured group Rice Cluster I (RC-I), is identified as the predominant methanogen in RPFs. However, despite their numerical significance and cosmopolitan dispersal, RC-I methanogens had so far escaped isolation.

Previously, we successfully isolated a novel methanogen, belonging to the RC-I lineage from a Japanese rice paddy field. To enrich the RC-I methanogens from rice paddy samples, we attempted to mimic the in situ conditions of RC-I on the basis of the idea that methanogens in such ecosystems should thrive by receiving low concentrations of substrate (H_2) continuously provided by heterotrophic H_2 -producing bacteria. For this purpose, we developed a coculture method using an indirect substrate (propionate) in defined medium and a propionate-oxidizing, H_2 -producing syntroph, *Syntrophobacter fumaroxidans*, as the H_2 supplier. By doing so, we significantly enriched the RC-I methanogens and eventually obtained a methanogen within the RC-I group in pure culture. That was the first report on the isolation of a methanogen within the RC-I group. We determined further morphological and physical properties of the isolate, and proposed the isolate as a novel mesophilic hydrogenotrophic methanogen *Methanocella paludicola*.

In addition, we reported complete genome sequence of *M. paludicola*. The genome sequence of *M. paludicola* consists of a single circular chromosome of 2,96 Mbp containing 3004 protein-coding sequences (CDS). Genes for most of the functions known in the methanogenic archaea were identified, e.g. a full complement of hydrogenases and methanogenesis enzymes. Comparative genome analysis among the previously determined methanogen genomes pointed to the genome-wide relatedness of *M. paludicola* to the orders *Methanosarcinales* and *Methanomicrobiales* methanogens in terms of the genetic repertoire. Meanwhile, the unique evolutionary history of *M. paludicola* was also traced in an aspect by the comparative genome analysis among the methanogens. The physiological and genetic properties of *M. paludicola* would provide better understanding how RC-I methanogens contribute global methane emission from RPF environments.

Keywords: methane, methanogenic archaea, rice paddy fields, cultivation