Phylogenetic analysis of mcrA gene of methanogenic archaea

Wannaporn Muangsuwan¹, Pattarawan Ruangsuj¹, Pichai Chaichanachaicharn¹, Kosum Chansiri² and Montri Yasawong¹

¹Department of Biochemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, and ²Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.

Keywords
mcrA gene
methyl-coenzyme M reductase
methanogens

Abstract
Methane (CH₄) is the forceful greenhouse gases, which produced from methanogens. The key enzyme for methane production is methyl-coenzyme M reductase (MCR). The α-subunit of MCR is encoded by mcrA gene. The mcrA gene was applied as a specific marker for study of methanogens diversity. The study was based on the collection of mcrA gene sequences. The gene sequences were obtained from genome database (GenBank). Phylogenetic tree was reconstructed using mcrA gene sequences. Methanopyrus kandleri (AE009439) was selected for an out group of the Bayesian analysis. The phylogeny was not only illuminated the evolution of mcrA gene but also represented the relationship between the methane production pathways and the habitats of the methanogens. Two lineages of methanogenic archaea were appeared on the consensus tree (group A and B). The group A methanogens contained two subgroups (subgroup A1 and A2). The group A methanogens possesses three methanogenesis pathways, which were hydrogenotrophic, acetoclastic and C1-pathway. The group B methanogens were mostly thermophiles or hyperthermophiles. Hydrogenotrophic pathway was the main process for methanogenesis of the methanogens group B.
Introduction

Methane is more progressive and forceful greenhouse gases than carbon dioxide unit per unit. Electrical generation used methane fundamentally flaming it as fuel. Other hydrocarbon fuels, burning creates more carbon dioxide for each unit of heat discharged than methane. Methane creates heat more than any other complex hydrocarbons. Compressed natural gas from methane is used as conveyance fuel and it's more environmentally friendly than other complex hydrocarbons. Natural gas from methane is mostly created by microorganisms and called process methanogenesis. Methanogenesis is a form of anaerobic conditions used by microorganisms. It involves in landfill, marshland, digestive tracts of animals such as ruminants and humans, in marine sediments. Biomethanation is generally confined to where sulfates are depleted, in anaerobic wastewater treatments and the guts of termites. Methanogens are constituted by methyl coenzyme M-reductase (MCR), an enzyme is extensively and wholly being in methanogens (Luton et al, 2002). One enzyme is held in the terminal step of methane bio-synthesis. Accordingly mcrA, the gene encoding the α-subunit of MCR has been applied as a specific marker for distinction assay of methanogens from dissimilar habitats. Methanogens can be calculated correctly using a specialty “functional” marker gene mcrA coding α-subunit of methyl-coenzyme M reductase (MCR), the key enzyme of methanogenesis. Distinct else enzymes in methanogenic metabolism, MCR present to be characteristic to methanogens and is interlacing in the last stage of methanogenesis begetting reduction of methyl group absorbed to coenzyme M (Fernandez et al, 1999). The owning of MCR enzyme determine a cell as a methanogen so mcrA sequences can be used for metha-nogen phylogenies (Luton et al, 2002). Therefore, the dissimilar methanogenic pathways are prominent by the method of getting the methyl group for changing to methane. The pathways are classified within three general groups based on methyl group acquisition; the first is hydrolysis. The second is acidogenesis and acetogenesis. The last is methano- genesis. This research was focus on microbial diversity of the methanogens. The study was based on the collection of mcrA gene sequences, evolutionary model selection and reconstruction of mcrA phylogenetic tree.

Materials and methods

Collection of mcrA gene sequences: The mcrA gene sequences were collected from the genome database of GenBank. Quality of the gene sequences was manually justified. Only high quality of the gene sequences was kept for further analysis.

Phylogenetic analysis: Multiple sequences alignment was performed based on iterative refinement method using MUSCLE version 3.8.31 (Edgar, 2004). Evolutionary model of mcrA gene was selected based on AIC and hLRTs criteria using Modeltest version 3.7 (Posada and Buckley, 2004). Phylogenetic tree was analyzed based on Bayesian inference method using parallel version of MrBayes (Ronquist et al, 2011). The phylogenetic analysis was performed on computer cluster (KIRI cluster). The cluster was assembled from four IBM
servers (x3250 M4), which were connected by a gigabit switch (HP ProCurve 1410-16G). The Bayesian posterior probabilities were obtained by performing two separate runs with twelve Markov chains. Each run was conducted with $1 \times 10^7$ generations and sampled every 100 generations. A consensus tree was calculated after discarding the first 25% of the iterations as burn-in.

**Results and Discussion**

**Collection of mcrA gene sequences**: Thirty six sequences of mcrA gene were obtained from GenBank database. The size of the complete mcrA gene sequences were ranged from 1,653 to 1,722 bases. The genes were distributed into four classes of the methanogenic archaea, which were *Methanobacteria*, *Methanococci*, *Methanomicrobia* and *Methanopyri*.

![Figure 1](image)

*Figure 1* The proportion of methanogens based on mcrA gene sequences which collected from GenBank. Names have been abbreviated in agreement with as follows: MB, *Methanobacteria*; MC, *Methanococci*; MM, *Methanomicrobia*; MP, *Methanopyri*. 

---

Bioinformatics
Figure 2 Phylogenetic tree of mcrA gene of methanogenic archaea, constructed by Bayesian inference method using the GTR+I+G model of nucleotide substitution. The values associated with nodes correspond to the clade credibility support in %.

Diversity of mcrA gene: Of the mcrA gene database, the largest group of mcrA gene was belonging to the methanogen in class Methanomicrobia (63.89%) (Figure 1). The mcrA gene of the methanogens, which were the member of class Methanobacteria and Methanococci, were shared the same proportion (16.67%) (Figure 1). There was only 2.78% of mcrA gene sequences were detected from the methanogen in class Methanopyri (Figure 1).
### Characteristics properties and habitat of methanogens groups A1, A2 and B

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Organism</th>
<th>Class</th>
<th>Habitat</th>
<th>pH</th>
<th>Condition Temp (°C)</th>
<th>Pathway</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C005000</td>
<td>Methanococcoides burtonii</td>
<td>Methanomicrobia</td>
<td>Antarctica</td>
<td>7.5</td>
<td>35</td>
<td>C1</td>
<td>Dhaunta et al, 2013</td>
</tr>
<tr>
<td>C001994</td>
<td>Methanohalophilus mohnii</td>
<td>Methanomicrobia</td>
<td>Great Salt Lake</td>
<td>7.8</td>
<td>30-40</td>
<td>C1</td>
<td>Spring et al, 2010</td>
</tr>
<tr>
<td>C002069</td>
<td>Methanocaldococcus esterigatum</td>
<td>Methanomicrobia</td>
<td>Saline lagoons</td>
<td>7-8</td>
<td>30-40</td>
<td>C1</td>
<td>Cheng et al, 2007</td>
</tr>
<tr>
<td>C002101</td>
<td>Methanocaldococcus ziliense</td>
<td>Methanomicrobia</td>
<td>Boss lake</td>
<td>7-8</td>
<td>30-40</td>
<td>C1</td>
<td>Mathrani et al, 1988</td>
</tr>
<tr>
<td>C003083</td>
<td>Methanobacterium psychrophilus</td>
<td>Methanomicrobia</td>
<td>Hot springs</td>
<td>6-8</td>
<td>50</td>
<td>C1</td>
<td>Zhang et al, 2008</td>
</tr>
<tr>
<td>C003626</td>
<td>Methanomethylophilus hollandica</td>
<td>Methanomicrobia</td>
<td>Eutrophic lake</td>
<td>6.5-7.0</td>
<td>37</td>
<td>C1</td>
<td>Jiang et al, 2005</td>
</tr>
<tr>
<td>C000099</td>
<td>Methanosarcina barkeri</td>
<td>Methanomicrobia</td>
<td>Marine mud</td>
<td>5</td>
<td>37-42</td>
<td>H</td>
<td>Anderson et al, 2012</td>
</tr>
<tr>
<td>AE008384</td>
<td>Methanosarcina mazei</td>
<td>Methanomicrobia</td>
<td>Sediments</td>
<td>7.0-7.5</td>
<td>37-40</td>
<td>A</td>
<td>Assis et al, 2013</td>
</tr>
<tr>
<td>AE010299</td>
<td>Methanosarcina acetivorans</td>
<td>Methanomicrobia</td>
<td>Sediments</td>
<td>6.5-7.0</td>
<td>35-40</td>
<td>C1</td>
<td>Soevers et al, 1984</td>
</tr>
<tr>
<td>AM114193</td>
<td>Methanocella arborae</td>
<td>Methanomicrobia</td>
<td>Rice paddy soil</td>
<td>7.0</td>
<td>45</td>
<td>H</td>
<td>Jeantot et al, 1999</td>
</tr>
<tr>
<td>AP011326</td>
<td>Methanocella paludicola</td>
<td>Methanomicrobia</td>
<td>Rice paddy soil</td>
<td>7.0</td>
<td>35-37</td>
<td>H</td>
<td>Sakai et al, 2008</td>
</tr>
<tr>
<td>C003243</td>
<td>Methanocella concava</td>
<td>Methanomicrobia</td>
<td>Rice paddy soil</td>
<td>6.8</td>
<td>55</td>
<td>H</td>
<td>Lu et al, 2012</td>
</tr>
<tr>
<td><strong>Group A2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C003117</td>
<td>Methanoseta harundinacea</td>
<td>Methanomicrobia</td>
<td>Waste water</td>
<td>7.2-7.6</td>
<td>34-37</td>
<td>A</td>
<td>Ma et al, 2006</td>
</tr>
<tr>
<td>C000477</td>
<td>Methanoseta thermophila</td>
<td>Methanomicrobia</td>
<td>Thermal lake mud</td>
<td>6.7</td>
<td>55</td>
<td>A</td>
<td>Engel et al, 2012</td>
</tr>
<tr>
<td>C000265</td>
<td>Methanoseta concava</td>
<td>Methanomicrobia</td>
<td>Mat. form</td>
<td>7.1-7.5</td>
<td>35-40</td>
<td>A</td>
<td>Anderson et al, 2009</td>
</tr>
<tr>
<td>C003167</td>
<td>Methanoregula formicicum</td>
<td>Methanomicrobia</td>
<td>Gravel sludge</td>
<td>7.4</td>
<td>30-33</td>
<td>H</td>
<td>Yashiro et al, 2011</td>
</tr>
<tr>
<td>C002117</td>
<td>Methanoplanus petrolearius</td>
<td>Methanomicrobia</td>
<td>Ore oil field</td>
<td>7.0</td>
<td>37</td>
<td>H</td>
<td>Ollivier et al, 1997</td>
</tr>
<tr>
<td>C000562</td>
<td>Methanocaldococcus marisnigri</td>
<td>Methanomicrobia</td>
<td>Pig manure digest</td>
<td>8</td>
<td>40</td>
<td>H</td>
<td>Anderson et al, 2009</td>
</tr>
<tr>
<td>HE964772</td>
<td>Methanocaldococcus bourgensis</td>
<td>Methanomicrobia</td>
<td>Sewage sludge</td>
<td>6.7</td>
<td>37</td>
<td>A</td>
<td>Ollivier et al, 1986</td>
</tr>
<tr>
<td>C000254</td>
<td>Methanospirillum hungatei</td>
<td>Methanomicrobia</td>
<td>Soil</td>
<td>7.5</td>
<td>30</td>
<td>H</td>
<td>Iino et al, 2010</td>
</tr>
<tr>
<td>C000559</td>
<td>Methanocorpusculum labreanum</td>
<td>Methanomicrobia</td>
<td>Tar Pit Lake</td>
<td>7</td>
<td>37</td>
<td>A</td>
<td>Zhao et al, 1989</td>
</tr>
<tr>
<td>C001338</td>
<td>Methanosaetae porphyra</td>
<td>Methanomicrobia</td>
<td>Peatland</td>
<td>5.5</td>
<td>28-30</td>
<td>H</td>
<td>Cadillo et al, 2009</td>
</tr>
<tr>
<td>C000780</td>
<td>Methanoregula boonei</td>
<td>Methanomicrobia</td>
<td>Oil wells</td>
<td>5.1</td>
<td>53</td>
<td>H</td>
<td>Braun et al, 2011</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C002551</td>
<td>Methanobacterium sp.</td>
<td>Methanobacteria</td>
<td>Oil field</td>
<td>-</td>
<td>60</td>
<td>H</td>
<td>Maus et al, 2013</td>
</tr>
<tr>
<td>C002278</td>
<td>Methanobacterium fervidus</td>
<td>Methanobacteria</td>
<td>Hot springs</td>
<td>6.5</td>
<td>83</td>
<td>H</td>
<td>Steet et al, 1981</td>
</tr>
<tr>
<td>C002737</td>
<td>Methanottorpus igneus</td>
<td>Methanococci</td>
<td>Hydrothermal</td>
<td>5.7</td>
<td>83</td>
<td>H</td>
<td>Takai et al, 200</td>
</tr>
<tr>
<td>L77117</td>
<td>Methanocaldococcus jannaschii</td>
<td>Methanococci</td>
<td>Sea floor surface</td>
<td>6.0</td>
<td>85</td>
<td>H</td>
<td>Mehta et al, 2006</td>
</tr>
<tr>
<td>C001787</td>
<td>Methanocaldococcus vulcanis</td>
<td>Methanococci</td>
<td>Hydrothermal</td>
<td>6.5</td>
<td>80</td>
<td>H</td>
<td>Jeantot et al, 1999</td>
</tr>
<tr>
<td>C002792</td>
<td>Methanocaldococcus okinawensis</td>
<td>Methanococci</td>
<td>Hydrothermal</td>
<td>6.7</td>
<td>60-65</td>
<td>H</td>
<td>Takai et al, 2002</td>
</tr>
<tr>
<td>C001791</td>
<td>Methanobrevibacter ruminantium</td>
<td>Methanobacteria</td>
<td>Rumen fluid</td>
<td>7.0</td>
<td>37</td>
<td>H</td>
<td>Balch et al, 1979</td>
</tr>
<tr>
<td>C004050</td>
<td>Methanobrevibacter sp.</td>
<td>Methanobacteria</td>
<td>Rumen of animal</td>
<td>38</td>
<td>38</td>
<td>H</td>
<td>Leahy et al, 2013</td>
</tr>
<tr>
<td>AE000666</td>
<td>Methanothermohacter</td>
<td>Methanobacteria</td>
<td>Hot springs</td>
<td>7.2-7.6</td>
<td>65-70</td>
<td>H</td>
<td>Wasserfallen et al, 2000</td>
</tr>
<tr>
<td>C000170</td>
<td>Methanothermohacter</td>
<td>Methanobacteria</td>
<td>Hot springs</td>
<td>6.8-7.4</td>
<td>65</td>
<td>H</td>
<td>Wasserfallen et al, 2000</td>
</tr>
</tbody>
</table>
Phylogenetic analysis: The mcrA gene sequence of Methanopyrus kandleri (AE009439) was selected for an out group for the Bayesian analysis. The mcrA gene tree was shown in Figure 2. The mcrA gene of methanogen was clearly separated in two lineages, which were group A and B. The mcrA group A was able to classify into two subgroups (group A1 and A2) based on the Bayesian tree (Figure 2). The group A of mcrA gene consisted of 23 species of methanogen. The mcrA gene group B contained 12 species of methanogen (Figure 2).

Characteristics and properties of methanogen: Methane producing archaea is classified into three distinctions, which were mcrA group A1, mcrA group A2 and mcrA group B, as shown in Table. The members of mcrA group A1 were able to produce methane using three different pathways that were hydrogenotrophic, acetoclastic and C1 pathway. The methanogens, which were the member of mcrA group A2, produced methane via hydrogenotrophic and acetoclastic pathways. Hydro-genotrophic pathway was the main pathway for methane production of the mcrA group B.

Conclusion

This study was not only illuminated the evolutionary of mcrA gene but also represented the relationship between the methane production pathways and the habitats of the methanogens. The Bayesian tree represented two distinct lineages of methanogens. The first lineage, mcrA group A, possesses three pathways of methanogenesis that were hydrogenotrophic, acetoclastic and C1-pathway. The second lineage, mcrA group B, possessed only hydro-genotropic pathway for the methane production. Class Methanomicrobia was the major member of mcrA group A. Class Methanococci shared the same proportion of mcrA group B. Most of methanogens, which belonged to mcrA group A1, were mesophiles and isolated from sediments and rice paddy fields (Table). The mcrA group A2 was mostly mesophiles that were able to grow on acidic to neutral pH. The mcrA group A2 was isolated from several habitats such as soil, sludge, wetland and etc (Table). The deepest branch of mcrA phylogeny was the group B methanogens. This meant that the group B methanogens might have the oldest evolution among the methanogenic groups. The members of this archaean group were mostly thermophiles or hyperthermophiles. The group B methanogens were isolated from hot spring, deep-sea hydrothermal vent, rumen fluid, oil field and etc. Interestingly, methano-genesis in environments with high temperature was hydrogenotropic pathway. Cause of this evidence might come from the type of nutrients, which were found in the environ-ments. The hot environments such as hot spring had been influenced by volcanic activity. Normally, there was higher amount of inorganic com-pounds than organic compounds at the hot spring and hydrothermal vents. Aceto-clastic pathway and C1-pathway need organic compounds as substrate for methane production. These two methanogenesis pathway were mostly found from mesoplilic methanogens.

Acknowledgements

This work was financially supported by the National Research Council of Thailand (NRCT) and Graduate Studies, Mahidol University, Thailand.
References

culceus marisnigri Romesser et al. 1981 type strain JR1. Stand

Anderson KL, Apolinario EE and Sowers KR. Desiccation as a long-term survival mechanism for the archaeon Methanosarcina

mazei Strain Isolated from Sediment Samples from an Amazonian Flooded Area. Genome Announc 2013;1.

Balch WE, Fox GE, Magrum LJ, Woese CR and Wolfe RS. Methanogens: reevaluation of a unique biological group. Microl


Mehta MP and Baross JA. Nitrogen fixation at 92 degrees C by a hydrothermal vent archaeon. Sciences 2006;314: 1783-1786.


