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Methanococcoides

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Abstract :

Me.tha.no.coc.co'i.des. Gr. adj. suff. -oides similar to; N.L. neut. n. Methanococcoides organism similar to Methanococcus. Euryarchaeota / Methanomicrobia / Methanosarcinales / Methanosarcinaceae / Methanococcoides The genus Methanococcoides comprises four species, Methanococcoides methylutens, Methanococcoides burtonii, Methanococcoides alaskense, and Methanococcoides vulcani. Cells are irregular cocci, 0.5–3 µm in diameter, occurring singly or in pairs, and may be motile. Clumps of cells can also be observed. Cells exhibit a blue-green autofluorescence under UV illumination. The cell wall consists of a very thin protein S-layer, approximately 10-nm thick. Susceptible to lysis by hypotonic or detergent shock. Eurypsychrophilic to mesophilic. Strict anaerobe. Neutrophilic. Halophilic, optimal salinity near seawater. Cells can dismutate methylamines, methanol, glycine betaine, choline, tetramethylammonium, dimethyl sulfide, methyl iodide, and N,N-dimethylethanolamine for growth, but cannot catabolize acetate, dimethylsulfide, H₂/CO₂, or formate. Methanococcoides spp. are members of the phylum Euryarchaeota, class Methanomicrobia, order Methanosarcinales, and family Methanosarcinaceae. Known habitats are deep-sea mud volcano, marine anoxic sediment, hypolimnion Ace Lake, mangrove swamp, deep hypersaline anoxic basin, and hydrothermal vents. DNA G + C content (mol%): 40.8–44. Type species: Methanococcoides methylutens Sowers and Ferry 1983, VL17.

Keywords : anaerobe, psychrophile, mesophile, methyl compound reduction, marine anoxic sediment, deep-sea mud volcano, cold seeps

31

32 **Description**

33 Irregular cocci, 0.5–3.0 µm in diameter, occurring singly or in pairs. Motility is observed, it
34 is by flagellum. Whole cells are immediately lysed by 0.01% sodium dodecyl sulfate or 0.001%
35 Triton X-100. Gram-negative. Obligate anaerobe. Neutrophilic, growth occurs in a pH range of
36 5.5-8, optimally at pH 7. Optimal salinities close to seawater. Psychrophillic to mesophilic, with
37 growth occurring from 1.7 to 37°C with an optimum ranged from 23 to 30°C.
38 Tetramethylammonium, trimethylamine, dimethylamine, methylamine, methanol, glycine
39 betaine, choline, N-N dimethylethanolamine, dimethyl sulphide, methyl iodide are substrates for
40 growth and methanogenesis; acetate, formate, and H₂/CO₂ are not utilized. Yeast extract,
41 Trypticase peptones, or rumen fluid stimulate growth. Essential trace metals include nickel, iron,
42 and cobalt. During growth, a blue-green autofluorescence is observed under UV (390-440 nm).
43 Four species belong to the genus *Methanococcoides*: *M. methylutens*, *M. burtonii*, *M. alaskense*
44 and *M. vulcani*. *Methanococcoides* spp. are members of the phylum *Euryarchaeota*, class
45 *Methanomicrobia*, order *Methanosarcinales*, family *Methanosarcinaceae*. Known habitats are
46 deep-sea mud volcano, submarine anoxic sediment, hypolimnion Ace lake, mangrove swamp,
47 deep hypersaline anoxic basin, hydrothermal vents.

48

DNA G + C content (mol %): 40.8 - 44

Type species: *Methanococcoides methylutens* Sowers and Ferry 1985b, 223 (Effective publication: Sowers and Ferry 1983, 688).

Number of species with validated names: 4.

Family classification:

Methanosarcinaceae (fbm. pub2).

Further Descriptive Information

Cell morphology and ultrastructure. All four described species *M. methylutens*, *M. burtonii*, *M. alaskense* and *M. vulcani* are irregular cocci with a diameter around 0.6 to 3.0 µm (Franzmann et al., 1992; L'Haridon et al., 2014; Singh et al., 2005; Sowers and Ferry, 1983) (**Table 1**). They frequently occur as single cells, in pairs; clump of cells are also observed. *M. burtonii* and *M. vulcani* are motile and the presence of 1 to 4 flagella are observed under TEM for *M. vulcani* (**Figure 1**) (Franzmann et al., 1992; L'Haridon et al., 2014). The presence of pili have been reported for *M. alaskense* (Singh et al., 2005). No spores are produced and cells stain Gram-negative. Cells exhibit a blue-green fluorescence under UV (390-440 nm).

Nutrition and growth conditions. *Methanococcoides* spp. must be cultivated under anaerobic conditions. For the preparation of the cultivation media, different anaerobic techniques can be used (e.g., Balch and Wolfe, 1976). **Table 1** summarizes the physiological features of the

species of the genus *Methanococcoides*. *Methanococcoides* spp. are obligate anaerobes and halophiles.

Known members of the genera *Methanococcoides* are capable of methanogenesis directly from methylamine, dimethylamine, trimethylamine, tetramethylammonium, *N,N*-dimethylethanolamine, choline (*N,N,N*-trimethylethanolamine), glycine betaine (*N,N,N*-trimethylglycine), methanol, dimethyl sulfide and methyl iodide as substrates (Franzmann et al., 1992; L'Haridon et al., 2014; Lyimo et al., 2009; Singh et al., 2005; Sowers and Ferry, 1983; Tanaka, 1994; Ticak et al., 2015; Watkins, 2012a; Watkins et al., 2012b, 2014). Use of tetramethylammonium by *Methanococcoides* is broader than initially reported as it can be used by strains Nat1, Q3c, AM1, DM1, PM1, PM2, NM1) and no other known genus of methanogens (Watkins, 2012a). No *Methanococcoides* members have been described so far for the utilization of other known methanogenic substrates such as acetate, formate and H₂/CO₂, ethanol, isopropanol, cyclopentanol and pyruvate for growth (Watkins, 2012a). Interestingly, methyl iodide at low concentrations (<200 µM) can also be used as a substrate for methanogenesis by *Methanococcoides methylutens* TMA-10^T (Watkins, 2012a).

None of the known *Methanococcoides* require yeast extract, or other carbon sources beside the organic growth substrate. The temperature range for observed growth of *Methanococcoides* spp. is very broad ranging from 1.7 to 60°C. Singh and co-workers (2005) indicates that minimal estimated growth temperature for *M. alaskense* strain AK5^T and AK9 were -2.3 and -10.7 respectively based on the square-root equation (Ratkowsky et al., 1983) The average optimum temperature for growth is 31°C (± 7°C) (Watkins, 2012a). They grow between pH 6 and 9.5, with an optimum at pH 7. They are not very sensitive to salt stress as they can grow at 0.03 and 1.3 M Na⁺ concentration. Members of the genera *Methanococcoides* are therefore eurypsychrophilic, mesophilic, halophilic and neutrophilic methanogens.

Genome features. Genomes from 6 *Methanococcoides* spp. Aare so far (April 2019)

publicly available (Allen et al., 2009; Guan et al., 2014), representing 3 different species (*M. methylutens*, *M. burtonii* and *M. vulcani*) (**Table 2**). Genome size ranges from 2.31 Mb in *M. vulcani* strain SLH33^T to 2.58 Mb in *M. burtonii* DSM 6242^T, and GC content varies from 40.8 to 44%. Four of the genomes are closed: *M. burtonii* DSM 6242^T (NC_007955) (Allen et al., 2009), *M. vulcani* DSM 26966^T (NZ_FOHQ000000000), *M. methylutens* DSM2657^T (NZ_JRHO000000000) (Guan et al., 2014) and *M. methylutens* strain MM1 (NZ_CP009518).

The genome analysis of the eurypsychrophile *M. burtonii* has revealed the plasticity of the genome including codon usage, horizontal gene transfer and transposase activity that enables adaptation to cold environment such as Antarctic Lake Environment (Allen et al., 2009). The molecular mechanisms of cold adaptation have been established from proteomic studies between *M. burtonii* cells grown at 4°C and 23°C (Goodchild et al., 2005; Cavicchioli, 2006; Goodchild et al., 2004a, 2004b). These Proteomic studies revealed that 560 proteins have been identified from *M. burtonii* cells grown at 4°C, and 44 proteins differentially expressed from *M. burtonii* cells grown at 4°C and 23°C (Cavicchioli, 2006). The genome of *M. alaskense* would be of a great interest in order to compare the two species, both isolated from cold environments (*in situ* measured temperatures ranged from 1 to 6°C).

Ecology

Methanococcoides methylutens TMA-10^T was isolated from sediment collected from the Sumner Branch of Scripps Canyon, La Jolla, California, USA. *M. burtonii* ACE-M^T was isolated from water sample collected at 26 m depth in Ace Lake in Antarctica. *M. alaskense* AK-5^T was isolated from Skan Bay in Unalaska Island, Alaska. *M. vulcani* SLH33^T was isolated from deep sediment in Napoli mud volcano in the eastern Mediterranean Sea covered by a dense orange microbial mat at 1938 m water depth.

Strains members of the genus *Methanococcoides* are widespread in marine environments and were isolated from wide range of anoxic habitats including a marine lake, a mangrove swamp, a shallow water sediments, deep hypersaline anoxic basin, methane seeps, hydrothermal vents (Cynar and Yayanos, 1991; Franzmann et al., 1992; Imachi et al., 2011; Kendall and Boone, 2006; Lyimo et al., 2009; Mohanraju et al., 1997; Singh et al., 2005; Sowers and Ferry, 1983; Tanaka, 1994; Watkins et al., 2014). Although data from environmental molecular surveys of 16S rRNA and *mcrA* genes in deposited in the Genbank database (National Center for Biotechnological Information) show that the genus *Methanococcoides*, is mainly in marine environments, *Methanococcoides* gene sequences were also detected at a saline lignite mine lake (lake Wallendorf , Germany, accession number JF973601), a Greenland ice-sheet potentially deposited by marine aerosols (Miteva et al., 2009) and a terrestrial saline mud volcano in Taiwan (accession number GU553549).

The *Methanococcoides* strains are characterized by eurypsychrophilic and mesophilic lifestyles. Environmental surveys have revealed gene sequences affiliated to *Methanococcoides* from habitats characterized by low temperatures such as submarine permafrost sediments (Koch et al., 2009), cold anoxic brine (Perreault et al., 2007) and sediment at a seasonally frozen day (Purdy et al., 2003). *M. burtonii* is cosmopolitan in cold environments, with a close relative *M. alaskense*; (99.8% 16S rRNA identity) isolated from Skan Bay, Alaska and closely related strains identified from an Antarctic bay (>99% 16S rRNA identity) and deep-sea sediment (98.8% 16S rRNA identity) (Li et al., 1999; Purdy et al., 2003).

The known upper temperature limit of cultured *Methanococcoides* spp. can reach 60°C (strains PM1, and TM1; Watkins, 2012). Moreover, *mcrA* gene sequences affiliated to the genus *Methanococcoides* were also detected in warm sediments of the Guaymas Basin (*in situ* temperature from 40 to 60°C; Lever and Teske, 2015), suggesting this genus might also be

involved in thermophilic methanogenesis. , It would therefore be interesting to isolate thermophilic *Methanococcoides* strains from warm environments.

Enrichment and isolation procedures.

Anaerobic culture media are prepared under a N₂/CO₂ (80/20) atmosphere (Sowers and Noll, 1995). A medium with salinity close to seawater, at neutral pH, supplemented with vitamins, trace elements solutions and a methanogenic substrate is prepared. Usually, trimethylamine (20 mM), dimethylamine (20 mM) or monomethylamine (20 mM) are used as the catabolic substrate for the enrichment of *Methanococcoides* relatives but substrates such as glycine betaine, choline, tetramethylamonium, dimethylsulfide or methyl iodide could be used to selectively enrich *Methanococcoides* strains. The media are inoculated with the original sampling material (1-5%) and are incubated at a temperature close to the *in situ* temperature with a headspace (40%) of N₂ or N₂/CO₂ (80/20; v/v). The enrichment bottles are examined for growth over a period of six months using a phase-contrast microscope, under UV (390-440 nm) *Methanococcoides* cells exhibit a blue-green fluorescence. The detection of methane by gas chromatography in the gas phase of the enrichment bottle indicates the presence of methanogenic microorganisms. The enrichments can be serially diluted three times in sequence. Pure culture of *Methanococcoides* can be obtained under anaerobic conditions by plating with the addition of 2% purified agar (BBL Microbiology Systems) or Noble agar (Difco) and a stainless steel anaerobic jar (L'Haridon et al., 2014) or anaerobic bag (AnaeroCult A mini, Merck; (John Parkes et al., 2010), by serial dilutions (Franzmann et al., 1992), or serial dilutions are added to molten agar and poured into Petri plates (Apolinario and Sowers, 1996) or agar roll tubes (Sowers and Ferry, 1983). Yellow colonies are observed within 5 to 45 days and are differentiated from non-methanogens by their blue-green fluorescence in long-wave UV light (Mink and Dugan, 1977).

Chemotaxonomic characteristics.

Nichols and co-workers (2004) revealed the major phospholipids in *Methanococcoides burtonii* strain ACE-M^T. The predominant phospholipids were archaeol phosphatidylglycerol, archaeol phosphatidylinositol, hydroxyarchaeol phosphatidylglycerol, and hydroxyarchaeol phosphatidylinositol. The authors observed the presence of unsaturated phospholipids analogues. The proportion of unsaturated lipids from cells grown at 4°C was significantly higher than for cells grown at 23°C indicating that cold adaptation in *M. burtonii* involves specific changes in membrane lipid unsaturation (Nichols et al., 2004).

Maintenance procedures.

Strains are maintained by transfer every 3 months on agar slants or in liquid medium stored at room temperature in the dark. For long-term storage, cultures are maintained by freezing in liquid growth medium and glycerol (3:1) as described by Tumbula et al. (1995) or in DMSO (5%) (L'Haridon et al., 2014). Cultures stored in glycerol at –80°C have remained viable for over 10 years.

Differentiation of the genus *Methanococcoides* from the genus *Methanosarcina*, *Methanolobus*, *Methanohalobium*, *Methanohalophilus*, *Methanosalsum*, *Methanomicrococcus*.

The genus *Methanococcoides* is distinguished from *Methanosarcina* based on its inability to grow on acetate or H₂/CO₂. Among the obligately methylotrophic genera, *Methanococcoides* and *Methanolobus* are slightly halophilic, growing optimally in 0.2 M NaCl, which distinguishes them from moderately halophilic *Methanohalophilus* and extremely halophilic *Methanohalobium*, which grow optimally in 0.5–2.5 M and >2 M NaCl, respectively (Paterek and Smith, 1988; Zhilina and Zavarzin, 1987). *Methanococcoides* is difficult to distinguished

from *Methanlobus* (Chen et al., 2018) and differentiation is achieved by 16S rRNA phylogeny or genome sequencing. The genus *Methanococcoides* is distinguished to the genus *Methanosalsum* by the pH range requirement for growth (pH 8-10) (Abbas et al., 2015). The genus *Methanomicrococcus* catabolyzed the methylated compounds (trimethylamine, dimethylamine, monomethylamine) and the methanol only in the presence of hydrogen as electron donor (Sprenger et al., 2000), members of the genus *Methanococcoides* have no requirement for hydrogen.

Taxonomic comments.

Phylogenetic analysis using either the 16S rRNA gene (**Figure 2**) or the *mcrA* gene (L'Haridon et al., 2014), places all known *Methanococcoides* spp. as a monophyletic clade within the family *Methanosarcinaceae*.

*List of species of the genus **Methanococcoides***

1. *Methanococcoides alaskense* Singh et al., 2005

alaskense (a.las'ken.se. N.L. neut. adj. *alaskense* referring to Skan Bay, Alaska, from where the type strain was isolated).

Irregular cocci of 1.5–2.0 µm in diameter, occurring singly. Non-motile. Pili are present. TMA serves as catabolic substrate with methane as the product. Methanol, acetate, dimethyl sulfide, formate and CO₂ are not used as catabolite substrates. TMA serves as sole organic substrate. Fastest growth occurs at 23.6 °C, with salinity between 0.3 and 0.4 M and a pH of 6.3–7.5.

The type strain, AK-5^T (=OCM 775^T=DSM 17273^T), was isolated from permanently cold, anoxic marine sediments at Skan Bay, Alaska.

222

223 *The mol% G+C content of the DNA: 39.5(Tm).*

224 *Type strain: AK-5^T, OCM 775, DSM 17273*

225 *EMBL/GenBank accession (16S rRNA gene): AY941801*

226

227 Other strain

228 Strain: AK-9, OCM793

229 *EMBL/GenBank accession (16S rRNA gene): AY941802*

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231 **2. *Methanococcoides burtonii*** Franzmann, Springer, Ludwig, Conway de Macario and
232 Rhode 1993, 398^{VP} (Effective publication: Franzmann, Springer, Ludwig, Conway de Macario
233 and Rhode 1992, 579).

234 *bur.ton'* i.i. M.L. gen. n. *burtonii* of Burton; named after Harry R. Burton, a limnologist
235 who discovered methane in Ace Lake.

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237 Irregular cocci of 0.8 to 1.8 µm; occur singly or in pairs. Motile with a single flagellum.
238 Colonies (<1mm) are yellow, circular and convex. Cells exhibit a blue-green autofluorescence
239 under UV light to blue light illumination. Growth occurs between 1.7 and 35 °C with an optimum
240 around 23°C. NaCl and Mg are required for growth, NaCl range for growth is 0.2-0.5 M NaCL
241 with an optimum at 0.2 M; 10 mM of Mg²⁺ are required for growth. Neutrophilic with an optimal
242 pH at 7.7. Methanol, methylamine, dimethylamine, trimethylamine are catabolic substrates for
243 growth. Acetate, CO₂, formate, ethanol, 2-butanol, 1-propanol, 2-propanol, acetone, dimethyl
244 sulfide are not. Yeast extract and trypticase improved growth.

245 The type strain ACE-M was isolated Ace lake Antartica.

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247 *The mol% G+C content of the DNA:* 40.8(genome analysis).

248 *Type strain:* ACE-M, DSM 6242

249 *EMBL/GenBank accession (16S rRNA gene):* X65537

250 *EMBL/GenBank accession (genome):* NC_007955

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252 **3. *Methanococcoides methylutens*** Sowers and Ferry 1985b, 223^{VP} (Effective publication:
253 Sowers and Ferry 1983, 688).

254 *me.thyl.u' tens*. M.L. n. *methylum* methyl; L. part. adj. *utens* using; *methylutens* using
255 methyl.

256 Cells are highly irregular cocci of 1 to 3 μm in diameter which stain Gram negative and
257 occur singly or in pairs. Non-motile. Surface colonies are yellow, circular, and convex with entire
258 edges and fluoresce blue-green under longwave UV light. Cells are lysed by sodium dodecyl
259 sulfate and possess a thin protein cell wall (10 nm thickness). Growth and methanogenesis occur
260 with the methylotrophic substrates. TMA, methylamine, and methanol serve as substrates for
261 growth and methanogenesis; $\text{H}_2\text{-CO}_2$, formate, and acetate do not. Yeast extract, Trypticase,
262 rumen fluid, or B vitamins stimulate growth. Sodium and magnesium are required for growth.
263 Optimal growth temperatures at 30-35°C and pH 7.0 to 7.5.

264 The type strain, TMA-10 has been isolated from marine sediments from the Sumner Branch
265 of Scripps Canyon located near La Jolla, California.

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267 *The mol% G+C content of the DNA:* 42.5 (genome analysis).

268 *Type strain:* TMA-10, ATCC 33938, DSMZ 2657, OCM 158.

269 *EMBL/GenBank accession (16S rRNA gene):* FR733669

270 *EMBL/GenBank accession (genome):* NZ_JRHO000000

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Other strain

Strain: MM1, DSM 16625

EMBL/GenBank accession (16S rRNA gene): FJ477324

4. *Methanococcoides vulcani* L’Haridon et al., 2014.

vulcani (vul.ca ' ni. L. gen. n. vulcani, of Vulcanus, the Roman god of fire; to the place of isolation Napoli mud volcano).

Cells exhibit a slight tumbling motility by means of 1 to 4 polar flagella. They are irregular cocci (diameter 0.6 - 1.7 µm) and occurred singly. Colonies are yellow, circular and convex. Cells exhibit a specific F420 blue autofluorescence under UV light illumination. TMA, DMA, MMA betaine, choline, *N,N*-dimethylethanolamine and methanol serve as catabolite substrates with methane as the product. DMS, formate, acetate, hydrogen are not used as catabolic substrates. Hydrogen was not used with methanol. Growth is stimulated by vitamins, yeast extract, peptone, tryptone, and casamino acids.

The fastest growth occurs at 30°C, with salinity of 0.5 M of Na⁺ and a pH of 7.0. Doubling time under optimal conditions was 21 hours.

The type strain, SLH33^T was isolated from sediment in the Napoli mud volcano covered by a dense orange microbial mat at 1,938 m water depth.

The mol% G+C content of the DNA: 43.4 (genome analysis).

Type strain: SLH33^T, DSM 26966^T, JCM 19278^T

EMBL/GenBank accession (16S rRNA gene): KC631821

EMBL/GenBank accession (genome): NZ_FOHQ00000000

296 Other species

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298 *Methanococcoides* sp.

299 strain: AM1, DSM 26764

300 EMBL/GenBank accession (16S rRNA gene): HE862406

301

302 *Methanococcoides* sp.

303 strain: HCM 6, DSM 7059, OCM 152

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305 *Methanococcoides* sp.

306 strain: 28, UBOCC-M-3246

307 EMBL/GenBank accession (16S rRNA gene): MK568477

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309 *Methanococcoides* sp.

310 strain: 32, UBOCC-M-3250

311 EMBL/GenBank accession (16S rRNA gene): MK568478

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313 *Methanococcoides* sp.

314 strain: 35, UBOCC-M-3239

315 EMBL/GenBank accession (16S rRNA gene): MK568479

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317 *Methanococcoides* sp.

318 strain: 38, UBOCC-M-3249

319 EMBL/GenBank accession (16S rRNA gene): MK568480

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321 *Methanococcoides sp.*

322 *strain:* 56, UBOCC-M-3255

323 *EMBL/GenBank accession (16S rRNA gene):* MK568481

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Table 1. Diagnostic and descriptive features of the species of *Methanococcoides*. Species: 1, *Methanococcoides vulcani* SLH33^T (this study); 2, *Methanococcoides methylutens* (Sowers & Ferry, 1983); 3, *Methanococcoides burtonii* (Franzmann et al., 1992); 4, *Methanococcoides alaskense* (Singh et al., 2005).
Legend: +, positive; -, negative.

	<i>M. vulcani</i>	<i>M. methylutens</i>	<i>M. burtonii</i>	<i>M. alaskense</i>
Origin	Napoli Mud Volcano Mediterranean sea	Submarine Canyon sediment California	Hypolimnion Ace Lake Antartica	Anoxic sediment Skan Bay Alaska
Cells diameter (µm)	0.6 – 1.7	1 – 3	0.8 – 1.8	1.5 - 2
Extracellular structure	Flagella		Flagella	Pili
Motility	+	-	+	-
Temperature range for growth(°C)				
min-(opt)-max	nd-(30)-35	15-(30-35)-nd	1.7-(23.4)-29.5	-2.3-(23.6)-30.6
pH range for growth	6- 7.8	6 -8.0	5.5-8.0	6.0-8.0
NaCl concentration for growth (%)	0.5-6.0	1.0-6.0	1.0-6.0	0-4.0
Catabolic substrate*				
Methanol	+	+	+	-
DMS	-	_*	_*	-
<i>N,N</i> -dimethylethanolamine	+	+*	+*	_*
Choline	+	_*	_*	_#
Betaine	+	_*	_*	-

*: (L’Haridon et al., 2014)

Table 2. Available genomic features of *Methanococcoides* isolated strains.

Characteristics	<i>M. burtonii</i>	<i>M. vulcani</i>	<i>M. methylutens</i>	<i>M. methylutens</i>	<i>Methanococcoides</i> sp
Strain	DSM 6242 ^T	DSM 26966 ^T	DSM 2657 ^T	MM1	AM1
Accession number	NC_007955	NZ_FOHQ00000000	NZ_JRHO00000000	NZ_CP009518.	GCA_900774055
Genome size (Mb)	2.58	2.31	2.51	2.39	2.48
Total genes	2571	2355	2288	2341	ND
Protein-coding genes	2406	2179	2192	2245	ND
rRNA genes	7	7	9	12	ND
tRNA genes	12	53	51	52	ND
G + C (%)	40.8	43.4	42.5	44	42.7

ND: Not Determined

Adapted from Allen et al, 2009, Guan et al, 2014, updated from NCBI genome.

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Figure 1: Electron micrograph of *Methanococcoides vulcani* strain SLH33^T.

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Figure 2. Phylogenetic tree of 16S rRNA gene sequences of type strains and related strains of *Methanococcoides* genus in the order *Methanosarcinales*. The tree was constructed by using the maximum-likelihood (RaxML with rate distribution model GTRGAMMA) method in ARB software based on 780 unambiguous bases and 1,000 bootstrap replications. Bootstrap values are indicated at nodes for branch boostrapp values >50%. The sequence of *Halococcus morrhuae* strain L.D.3.1^T (D11106) was used as an outgroup. Bar, 1% estimated difference in nucleotide sequence.

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