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Methanocalculus

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1 *Genus* **Methanocalculus**

2

3 **Defining publication:** Ollivier, Fardeau, Cayol, Magot, Patel, Prensier and Garcia 1998,

4 821^{VP}

5

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11

12 **Etymology:** Me.tha.no.cal'cu.lus. N.L. n. *methanum* methane; N.L. n. *calculus* pebble;

13 gravel; N.L. n. *Methanocalculus* a methane-producing pebble-shaped bacterium.

14

15

16 **Abstract**

17 The genus *Methanocalculus* comprises six validly published species including

18 *Methanocalculus halotolerans*, *Methanocalculus pumilus*, *Methanocalculus taiwanensis*,

19 *Methanocalculus chunghsingensis*, *Methanocalculus natronophilus*, and *Methanocalculus*

20 *alkaliphilus*.

21 Members of the genus *Methanocalculus* belong to the *Methanocalculaceae* family. They

22 have, with less than 90% 16S ribosomal RNA similarity, *Methanoculleus* and

23 *Methanocorpusculum* species as their closest phylogenetic relatives. *Methanocalculus* spp.

24 use only hydrogen and formate as substrates and require acetate as carbon source for

25 producing methane. They are mesophilic and have a G+C content in the DNA ranging from

26 50.2 to 55.0. NaCl tolerance is observed at concentration up to 125 g/l (e.g. *M. halotolerans*).
27 They inhabit a wide range of environments including extreme ones (e.g. saline oil reservoir,
28 soda lakes) as confirmed by culture dependent and independent techniques. While most
29 *Methanocalculus* spp. are neutrophilic, two species, *Methanocalculus natronophilus* and *M.*
30 *alkaliphilus* are considered as strictly alkaliphilic.

31

32 **Keywords:** *Archaea*, anaerobic, methanogen, hydrogenotrophic

33

34 **Description:**

35 Cells are irregular cocci staining Gram-negative, possibly motile by means of one flagellum
36 or multiple flagella. They possess a proteinaceous cell wall with an S layer. **Mesophilic,**
37 **halotolerant, hydrogenotrophic and anaerobic.** Acetate is required as carbon source for
38 growth. The substrates used for growth are H₂- and formate with CO₂ being used as terminal
39 electron acceptor to produce methane.

40 *DNA G+C content (mol %):* 50-55

41 *Type species:* *Methanocalculus halotolerans*

42 Number of species with validated names: 6.

43

44 **Family classification:** Methanocalculaceae

45

46 Further descriptive information

47 The genus *Methanocalculus* comprises six validly published species: *Methanocalculus*
48 *halotolerans* was isolated from the well-head of the Scheibhardt NDL 103 oilfield in
49 Alsace (France) (Ollivier et al., 1998), *Methanocalculus pumilus* was obtained from the
50 leachate of a sea-based site for solid waste disposal (port of Osaka, Japan) (Mori et al., 2000),

51 *Methanocalculus taiwanensis* was isolated from an estuary in Eriln Shi (Taiwan) (Lai et al.,
52 2002); *Methanocalculus chunghsingensis* was isolated from a marine water aquaculture
53 fishpond near Wong-Kong, Taiwan (Lai et al., 2004), *Methanocalculus natronophilus*
54 originated from the bottom sediments of a collector in the vicinity of a soda lake Tanatar II
55 (Altai, Russian) (Zhilina et al., 2013), and finally, *Methanocalculus alkaliphilus*, which was
56 found to dominate in enrichments from hypersaline soda lake sediments at pH 10 (Sorokin et
57 al., 2015).

58 Based on molecular approaches, the presence of *Methanocalculus* spp. has been confirmed in
59 soda lakes (e.g. the East African Rift Valley in Kenya or the Lonar Lake in India) (Surakasi et
60 al., 2007; Mwirichia et al., 2010), in oilfield ecosystems including oil-reservoir of the Bokor
61 Oil Field in Malaysia (Li et al., 2012), the oil-containing produced water from five production
62 wells of a Canadian oil field (Kryachko et al., 2017), and in produced water from separators
63 to storage tanks of shale gas wells in Sichuan Basin, China (Zhang et al., 2017). They have
64 been retrieved in deep subsurface gas-associated water at the Minami-Kanto gas field in Japan
65 (Mochimaru et al., 2007), but also in marine, estuarine, terrestrial natural environments. Their
66 presence has been highlighted in anaerobic bioreactors treating wastewaters (Shin et al., 2010;
67 Calderon et al., 2011) and the design of *Methanocalculus*-specific primer/probe has
68 demonstrated the relative abundance of these archaeons in anaerobic digestion of dairy waste
69 (Chen, 2014; Chen and He, 2015). Altogether these results thus provide evidence of the
70 ecological significance of these hydrogenotrophic archaeons regarding organic matter
71 degradation in anaerobic processes. It is noteworthy that a *Methanocalculus* sp. was recently
72 identified as a potential candidate to microbiologically influence corrosion (Tidwell et al.,
73 2017).

74 Properties of the species of the genus are described in Table 1. Cells are irregular cocci (0.2-
75 1.5 μm) occurring singly, in pairs or in small aggregates. Possibly motile by means of one

76 flagellum or multiple flagella. Cells stain Gram-negative, and electron microscopy reveals a
77 cytoplasmic membrane surrounded by an S-layer-like surface layer.

78 **Growth conditions.** The temperature range for growth is 15–45°C, with an optimum between
79 35 and 38°C. The pH range for growth is between 5.8 and 10 with four species being
80 neutrophilic and two species being alkaliphilic. The salinity range for growth is from 0–
81 12.5% NaCl (w/v) with an optimum depending upon the species. *Methanocalculus* spp. are
82 considered as halotolerant archaeons.

83 The six characterized species of *Methanocalculus* require acetate as carbon source for growth.
84 Methane is produced only from H₂ + CO₂ or formate (Table 1).

85

86 Enrichment and isolation procedures

87

88 Anaerobically prepared liquid bicarbonate-buffered medium is used to enrich for
89 *Methanocalculus* spp. (for details see Ollivier et al., 1998). Pure cultures are obtained by
90 using roll tubes following the procedures of Hungate (1969), and colonies developed after 2
91 weeks incubation at 37°C.

92

93 Maintenances procedures

94

95 Stock cultures can be maintained in culture medium described by Ollivier et al. (1998). Liquid
96 cultures retain viability for several months after storage at -80°C in the basal medium
97 containing 20% glycerol (v/v).

98

99 Phylogenetic and taxonomic comments

100

101

102 Members of the genus *Methanocalculus* belong to the family *Methanocalculaceae* (Oren,
103 2014, Zhilina et al, 2013), order *Methanomicrobiales* within the phylum *Euryarchaeota*
104 (Figure 1). They differ markedly phylogenetically (less than 90% similarity of the 16S rRNA
105 gene) from its closest relatives including *Methanoculleus* and *Methanocorpusculum* spp.
106 which in contrast to *Methanocalculus* spp. may require, beside acetate, a complex organic
107 compound (e.g. yeast extract, rumen fluid) for growth and possibly use alcohols (e.g. 2-
108 propanol/CO₂, 2-butanol/CO₂) for methanogenesis.

109

110 ***List of species of the genus Methanocalculus***

111

112 1. ***Methanocalculus halotolerans*** Ollivier, Fardeau, Cayol, Magot, Patel, Prensier and Garcia
113 1998, 826^{VP}

114

115 ha.lo.to.le'rans. Gr. n. hals, halos salt; tolerans L. pres. part. of tolero tolerate; M.L.
116 masc. adj. halotolerans salt-tolerating

117

118 Mesophilic, halotolerant, anaerobic archaeon. Cells are 0.8-1 µm in diameter. They occur
119 singly or in pairs and possess two to three peritrichous flagella. The temperature range for
120 growth is 24-50°C, with optimum growth at 38°C. The optimum pH for growth is 7.6. The
121 salinity range for growth is 0-125 g/l (optimum 50 g/l). Yeast extract is stimulatory for
122 growth. Produces methane from H₂ + CO₂ and formate. Requires acetate for growth. The
123 cellular fatty acids are not reported. Isolated from the well-head of the Scheibhardt ND
124 103 oilfield in Alsace (France) (Ollivier et al., 1998).

125 *DNA G+C content (mol %): 55 (HPLC).*

126 *Type strain:* SEBR 4845, (DSM 14092, OCM 470).

127 *EMBL/GenBank accession number (16S rRNA gene):* AF033672.

128

129 2. *Methanocalculus pumilus* Mori, Yamamoto, Kamagata, Hatsu, and Takamizawa 2000,

130 1728^{VP}

131 pu'mi.lus. L. adj. pumilus little

132

133 Mesophilic anaerobic archaeon. Cells are irregular cocci 0.8-1 µm in diameter. They are non-
134 motile. Gram-staining is negative. The temperature range for growth is 25-45°C, with
135 optimum growth at 35°C. The optimum pH for growth is around 7.0. Grows in the presence
136 of 1% NaCl (optimum growth). Produces methane from H₂ + CO₂ and formate. Requires
137 acetate for growth. The strain is resistant to high concentrations of several heavy metals such
138 as CdCl₂ and CuSO₄. It was isolated from leachate at a waste-disposal site in Osaka, Japan.

139 *DNA G+C content (mol %): 51.9 (HPLC).*

140 *Type strain:* MHT-1 (ATCC BAA 129, JCM 10627, DSM 12632).

141 *EMBL/GenBank accession numbers (16S rRNA gene):* AB008353.

142

143 3. *Methanocalculus taiwanensis* Lai, Chen, Shu, Chiou, Wang, Chuang, Hong, Liu, Lai and

144 Hua 2002, 1805^{VP}

145 tai.wa.nensis. N.L. adj. taiwanensis of Taiwan, indicating the source of the type strain.

146

147 Mesophilic anaerobic archaeon. Cells are irregular cocci 0.9-1.4 µm in diameter. They are
148 non-motile. Gram-staining is negative. The temperature range for growth is 25-42°C, with
149 optimum growth at 37°C. The optimum pH for growth is 6-7, with a range of 6.3-8.3. Grows

150 in the presence of 0.5% NaCl (optimum growth), in a range of 0-4%. Produces methane from
151 H₂ + CO₂ and formate. Requires acetate for growth. Tungsten is not required but stimulates
152 growth. Was isolated from the water of an estuary in Eirln Shi near Wong-Kong, Taiwan.

153

154 *Type strain:* P2F9704a (CCRC 16182, DSM 14663, OCM 671).

155 *EMBL/GenBank accession numbers (16S rRNA gene):* AF172443.

156

157 **4. *Methanocalculus chunghsingensis*** Lai, Lin, Yu, Huang and Chen 2004 189^{VP}

158

159 chung.hsing.ensis. N.L. masc. adj. *chunghsingensis* from Chung Hsing University, to

160 honour the university where this research was performed.

161 Mesophilic halotolerant anaerobic archaeon. Cells are irregular cocci 1.1-1.6 µm in diameter.

162 They are non-motile or weakly motile with flagella. Gram-staining is negative. The

163 temperature range for growth is 20-45°C, with optimum growth at 37°C. The optimum pH for

164 growth is 7, with a range of 6.0-8.0. Grows in the presence of 1.0% NaCl (optimum growth),

165 in a range of 0-12%. Produces methane from H₂ + CO₂ and formate. Requires acetate for

166 growth. Tungsten is highly stimulatory for some strains. Was isolated from a marine water

167 aquaculture fishpond near Wong-Kong, Taiwan.

168 *DNA G+C content (mol %):* 50.8 (Tm). *Type strain:* K1F9705b (DSM 14539, OCM 772).

169 *EMBL/GenBank accession number (16S rRNA gene):* AF347025.

170

171 **5. *Methanocalculus natronophilus*** Zhilina, Zavarzina, Kevbrin and Kolganov 2013, 703^{VP}

172 na.tro.no.phi'lus. L. adj. natronophilus liking soda

173

174 Mesophilic, alkaliphilic, natronophilic, and halotolerant anaerobic archaeon. Cells are
175 irregular angular cocci 0.2-1.2 μm in diameter. They are motile by means of peritrichous
176 flagella. Gram-staining is negative. The temperature range for growth is 15-45°C, with
177 optimum growth at 35°C. The optimum pH for growth is around 9.0-9.5 with a pH growth
178 range of 8.0-10.2. Grows within the range of 0-10% NaCl. Produces methane from $\text{H}_2 + \text{CO}_2$
179 and formate. Requires acetate for growth. Was isolated from the bottom sediments of a
180 collector in the vicinity of a soda lake Tanatar II (Altai, Russia).

181 *DNA G+C content (mol %):* 50.2 (Tm).

182 *Type strain:* Z-7105 (VKM B-2765, DSM 25006).

183 *EMBL/GenBank accession numbers (16S rRNA gene):* JX966306.

184 .

185 6. ***Methanocalculus alkaliphilus*** Sorokin, Ben Abbas, Merkel, Rijpstra, Damsté, Sukhacheva
186 and Loosdrecht 2015, 3744^{VP}

187 al.ka.li.phi'lus. N. L. n. alkali (from Arabic article al-, the, and Arabic n. qaliy) ashes of
188 saltwort; N.L. adj. philus (from Gr. adj. philos) loving; N.L. adj. *alkaliphilus* loving alkaline
189 conditions

190 Mesophilic, halotolerant and alkaliphilic anaerobic archaeon. Cells are angled flattened
191 coccoids, 1.5-2.5 μm , motile by multiple peritrichous archaella. The cell wall consists of a
192 thin proteinaceous layer. Core membrane lipids included archaeol and GDGT-0. The
193 temperature range for growth is 20-41°C, with optimum growth at 35°C. The optimum pH for
194 growth is 9.5, with a range of 8.0-10.2. Grows in the presence of NaCl, in a range of 0.5-
195 3.5%. Produces methane from $\text{H}_2 + \text{CO}_2$ and formate. Requires acetate for growth. The
196 species encompasses 11 closely related isolates from south Siberian soda lakes and several

197 isolates from alkaline Lonar Crater Lake in India. Was isolated from mixed sediments of soda
198 lakes in Kulunda Steppe (Altai, Russia).

199 *DNA G+C content (mol %):* 51.1 (Tm).

200 *Type strain:* AMF2, (DSM 24457, UNIQEM U859).

201 *EMBL/GenBank accession number (16S rRNA gene):* HM053969.

202

203

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267

268 **FIGURE CAPTIONS**

269 **Figure 1**

270 Phase-contrast micrograph of *Methanocalculus halophilus* showing irregular coccoid cells
271 (bar, 5 μ m).

272

273 **Figure 2**

274 Phylogenetic tree based on 16S rRNA genes showing the position of the species of genus
275 *Methanocalculus* relative to the type species of the genera of the order *Methanomicrobiales*.
276 *Methanocella paludicola* SANA E DSM 17711 (AB 196288) was used as an outgroup (not
277 shown). Phylogenetic relationships were determined with the maximum likelihood algorithm
278 embedded in the MEGA6 software package. Bootstrap analysis was used to evaluate the tree
279 topology by means of 1,000 resamplings. Only bootstrap values above 70% are shown. Bar,
280 0.02 substitutions per nucleotide position.

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Table 1. Differential characteristics between, *Methanocalculus halotolerans* (1), *Methanocalculus pumilus* (2), *Methanocalculus taiwanensis* (3), *Methanocalculus chunghsingensis* (4), *Methanocalculus natronophilus* (5), and *Methanocalculus alkaliphilus* (6) ND, not determined.

Characteristics	1	2	3	4	5	6
Motility/flagella	+	-	-	-	+	+
Temperature range (optimum) (°C)	25-45 (38)	24-45 (35)	28-37 (37)	20-45 (37)	15-45 (35)	ND-41 (35)
pH range (optimum)	7.0-8.4 (7.6)	6.5-7.5 (ND)	6.3-8.3 (6.8)	5.8-7.7 (7.2)	8-10.2 (9.0-9.5)	8-10.2 (9.5)
NaCl range (optimum) (%)	0-12 (5)	0-7 (1.0)	0-3 (0.5)	0-12 (1.0)	0-10 (ND)	
G + C content (mol %)	55 (HPLC)	51.9 (HPLC)	ND	50.8 (Tm)	50.2 (Tm)	51.1 (Tm)

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 296
 297
 298

[VOIR TABLEAU](#)