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1 *Methanobacterium alkalithermotolerans* sp. nov., a novel alkaliphilic and hydrogen-utilizing
2 methanogen isolated from an alkaline geothermal spring (La Crouen, New Caledonia)

3

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17

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19

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27 **Abstract**

28

29 An anaerobic, hydrogenotrophic methane-producing archaeon was isolated from an alkaline
30 thermal spring (42°C, pH 9.0) in New Caledonia. This methanogen, designated strain CAN^T, is
31 alkaliphilic, thermotolerant, with Gram-positive staining non-motile cells. Strain CAN^T grows
32 autotrophically using hydrogen exclusively as an energy source and carbon dioxide as the sole
33 carbon source (without the requirement of yeast extract or other organic compounds). It grows
34 at 20–45°C (optimum 45°C) and pH 7.3–9.7 (optimum 9.0). NaCl is not required for growth
35 (optimum 0%) but is tolerated up to 1.5%. It resists to novobiocin, streptomycin, and
36 vancomycin but is inhibited by ampicillin and penicillin, among other antibiotics. The genome
37 consists of a circular chromosome (2.2 Mb) containing 2,126 predicted protein-encoding genes
38 with a G+C content of 36.4 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence
39 indicated that strain CAN^T is a member of the genus *Methanobacterium*, most closely related
40 to the alkaliphilic *Methanobacterium alcaliphilum* WeN4^T with 98.5% 16S rRNA gene
41 sequence identity. The genomes of strain CAN^T and *M. alcaliphilum* DSM 3459, sequenced in
42 this study, share 71.6% average nucleotide identity (ANI) and 14.0% digital DNA–DNA
43 hybridization (dDDH). Therefore, phylogenetic analysis and physiological features determined
44 strain CAN^T as a novel species, for which the name *Methanobacterium alkalithermotolerans*
45 sp. nov. is proposed, and strain CAN^T (= DSM 102889^T = JCM 31304^T) is assigned as the type
46 strain.

47

48

49 Methanogens are a functional group of *Archaea* that produce methane as a by-product
50 of their energy metabolism (1, 2). *Methanobacterium* belongs to the *Methanobacteriaceae*
51 family of the “*Methanobacteriota*” phylum (3, 4). *Methanobacterium* species are mesophilic
52 or thermophilic and occur in various anoxic environments, such as anaerobic digesters (5) and
53 marine sediments (6). More than 41 species of the genus *Methanobacterium* have been
54 described to date. Some of them can use organic compounds, such as formate, to produce
55 methane (5, 7), while other *Methanobacterium* species grow exclusively through the reduction
56 of carbon dioxide (CO₂) by hydrogen (H₂); examples of such include *Methanobacterium*
57 *aarhusense* H2-LR^T (6), *Methanobacterium alcaliphilum* WeN4^T (8), and *Methanobacterium*
58 *movens* TS-2^T (7).

59 In this study, we describe the isolation and characterization of an autotrophic,
60 hydrogenotrophic, alkaliphilic methanogen, which has phenotypic, phylogenetic, and
61 phylogenomic characteristics that allow its assignment to a novel species of the genus
62 *Methanobacterium*. This novel archaeal species was isolated from ‘La Crouen’ geothermal
63 spring, located north of the southern peridotite nappe of New Caledonia (9). This spring
64 discharges alkaline (pH 9.0), anoxic (O₂(aq)<1 mg/L) and warm (42°C) waters (10, 11),
65 enriched in dinitrogen (N₂) with traces of methane (CH₄<3%) (12). This archaeon is the first
66 *Methanobacterium* member isolated and characterized from terrestrial thermal sources (or hot
67 springs) and the first example of methanogens isolated and characterized from the alkaline
68 springs of New Caledonia.

69 For enrichment experiments, a sediment slurry sample was collected in November 2014
70 using both sterilized spatula and glass bottle from the geothermal spring of La Crouen (165°
71 53' 20.6" E 21° 32' 06.6" S), located next to the city of Canala (New Caledonia) (13). For
72 preserving anoxic conditions, the bottle was immediately hermetically sealed and stored at 4°C.
73 In the laboratory, anaerobic enrichments were carried out using a culture medium containing

74 (per liter of distilled water): 0.1 g KH_2PO_4 , 0.1 g K_2HPO_4 , 0.5 g NH_4Cl , 0.5 g NaCl , 0.002 g
75 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 g sodium formate, 1g sodium acetate,
76 0.1 g cysteine hydrochloride, and 1 mL trace element solution SL-10 (14), supplemented with
77 25 mM Tris-Base. The initial pH was adjusted to 9.0 with NaOH. This medium was boiled for
78 5 min and then cooled to room temperature under a flow of O_2 -free N_2 gas. The medium (5 mL)
79 was dispensed into Hungate tubes, degassed under a flow of N_2 , and subsequently autoclaved
80 (20 min, 120°C). The following sterile solutions were injected in each tube: 0.1 mL of 2%
81 $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.1 mL of 8% Na_2CO_3 , 0.1 mL of 10 % tryptone and 0.1 mL of 10% yeast extract.
82 Inoculation at 10% vol. was done with 0.5 mL of the sediment slurry sample. The suspensions
83 were serially diluted in decimal steps using the same medium (up to 10^{-4}), and each tube was
84 supplemented by H_2/CO_2 gas mixture (80/20 v/v at 2 bars). The suspensions were incubated for
85 one month at 37°C under shaking (100 rpm). The Hungate technique for anaerobic cultivation
86 was used throughout this study (15).

87 Isolation and growth experiments were performed using the medium described above
88 (without sodium acetate, sodium formate, yeast extract, and tryptone) with the addition of Balch
89 vitamin solution (16), and H_2/CO_2 (80/20 v/v, 2 bars) as the only sources of energy and carbon,
90 and supplemented with 25 mM Tris-Base (except for testing the optimal pH for growth). For
91 the optimum pH tests, the initial pH of the culture media (ranging between 6.9 and 10.1) was
92 adjusted with an anaerobic sterile stock solution of 1M NaOH. The pH was always checked
93 after H_2/CO_2 injection (following inoculation) and at the end of incubation. Water baths were
94 used for incubation at temperatures ranging from 10 to 60°C . For studies of NaCl requirements
95 (tested between 0 and 3%), NaCl was weighted and added directly into the tubes before medium
96 addition. Each test culture was sub-cultured at least once under the same experimental
97 conditions before determining growth rates. The following compounds were tested as potential
98 substrates: (i) acetate, ethanol, formate, iso-butanol, methanol, trimethylamine, or 2-propanol

99 (at a final concentration of 20 mM) with N₂/CO₂ (80/20, v/v; 1 bar), or (ii) only H₂/CO₂ (80/20,
100 v/v) replacing N₂/CO₂ as the gas phase, or (iii) H₂/CO₂ (80/20, v/v) in the presence of acetate
101 (2 mM) as carbon source. Sensitivity to antibiotics (ampicillin, bacitracin, chloramphenicol,
102 cycloserine, kanamycin, metronidazole, novobiocin, penicillin, rifampicin, streptomycin,
103 tetracycline, vancomycin) was tested by adding each antibiotic separately to the basal medium
104 at a final concentration of 25 µg/mL or 50 µg/mL. The effect of sulfide concentration (0–10
105 g/L) on the growth rate of the isolate was also tested by adding an increasing concentration of
106 a Na₂S solution. Each growth test and control, carried out in parallel, was run in duplicate
107 Hungate tubes. Controls were (i) inoculated tubes without tested compounds and (ii)
108 uninoculated tubes with tested compounds. Growth was monitored by measuring the turbidity
109 increase at 600 nm by inserting Hungate tubes into the tube holder of a spectrophotometer (Cary
110 50, Varian). Gaseous and liquid end-products of metabolism were periodically measured by gas
111 chromatography (Shimadzu GC 8A) and high-performance liquid chromatography (Thermo
112 Separation Products), as described by Mei *et al.* (17).

113 The presence of putative methanogens from CH₄-producing enrichment cultures was
114 checked by observing the autofluorescence of the cofactor F420 characteristic of methanogens.
115 For this purpose, cell observations were performed under UV light using a Nikon Eclipse E600
116 equipped for epifluorescence and an F420 emission filter. Cellular morphology and purity
117 check of the strain were examined using a phase-contrast microscope (Optiphot; Nikon). For
118 transmission electron microscopy (using a MET FEI Tecnai G2), exponentially grown cells
119 were fixed with glutaraldehyde and osmium tetroxide, epon-embedded, and stained with
120 uranyl acetate and lead citrate (to watch the cell wall), as described by Quéméneur *et al.* (18).

121 The purity of the strain was further checked after DNA extraction (19) by the amplification
122 of partial 16S rRNA gene using the bacteria-specific primers 341F/518R (20) to verify the
123 absence of any bacterial contaminant. Near-complete 16S rRNA genes were amplified using

124 the primer pairs A109F/1492R for *Archaea* domain (21, 22), then sequenced by GATC-Biotech
125 (Konstanz, Germany). The 16S rRNA gene sequence was deposited in Genbank and aligned
126 with available sequences from the NCBI database using the MUSCLE program (23),
127 implemented in MEGA7 software (24). Evolutionary analyses were conducted from 16
128 nucleotide sequences (including the isolate, fourteen *Methanobacterium* type species, and
129 *Methanopyrus kandleri*) using three different methods: maximum-likelihood (ML) (25),
130 maximum-parsimony (MP) (26), and neighbor-joining (NJ) (27).

131 High molecular weight DNA of the isolate and *Methanobacterium alcaliphilum* DSM 3459
132 was extracted using a phenol-chloroform method (28) modified by adding a step including
133 several cycles of grinding the frozen cell pellet in liquid nitrogen to optimize cell wall lysis.
134 Whole genomes were then sequenced at the GeT-PlaGe platform (INRAe Toulouse, France)
135 by combining long reads (Oxford Nanopore) and short reads (Illumina MiSeq) technologies, as
136 described by Quéméneur *et al.* (13). The genomes of the isolate and *Methanobacterium*
137 *alcaliphilum* DSM 3459 were assembled with Unicycler v0.4.6 (29) and SPAdes v3.7.1 (30).
138 Their sequences were deposited in the NCBI database and annotated using the MaGe system
139 (Microscope Genome Annotation) (31). The average nucleotide identity value (ANI) was
140 calculated using OrthoANI with Usearch (<http://www.ezbiocloud.net/tools/ani>). Digital (or *in*
141 *silico*) DNA-DNA hybridization (dDDH) was calculated using the Genome-to-Genome
142 Distance Calculator (GGDC) 2.1 (<http://ggdc.dsmz.de/>) (32). Whole-genome- and proteome-
143 based taxonomic analyses were performed using the bioinformatics platform Type (Strain)
144 Genome Server (TYGS) (<https://tygs.dsmz.de>) (33).

145 Methane production and a simultaneous decrease in both H₂ and CO₂ were detected in all
146 enrichment cultures of the 'La Crouen' slurry sample after two weeks of incubation at 37°C.
147 The highest dilution culture (10⁻⁴) was transferred into a minimum liquid medium with H₂/CO₂
148 as sole energy and carbon sources. The Roll-tube method described originally by Hungate (15)

149 was used for strain isolation in a solid medium. The culture was ten-fold serially diluted in roll
150 tubes containing a molten 1.6% agar medium. Single white and circular colonies (1 mm in
151 diameter after 2-week incubation) were resuspended in basal medium with the addition of Balch
152 vitamins, which serves to inoculate a second round of ten-fold serially diluted in roll tubes.
153 After the third round of roll tube isolation, one single colony was finally grown in liquid culture,
154 resulting in the strain designated CAN^T, further characterized physiologically and
155 metabolically.

156 Cells of strain CAN^T are straight rods 1.3–4.0 μm long and 0.5–0.7 μm wide, occurring
157 singly, in pairs, or chains (Fig. 1A and 1B). They are non-motile and Gram-positive staining
158 (Fig. 1B).

159 Strain CAN^T grows only under strictly anaerobic conditions. It is mesophilic but
160 thermotolerant and grows at temperatures ranging from 20°C to 45°C (Table 1). The optimal
161 temperature for growth is 45°C. The pH range for growth is 7.3–9.7, with an optimum growth
162 at pH 9.0 (checked 15 min after H₂/CO₂ addition and at the end of the experiments). NaCl is
163 not required for growth, and the NaCl range for growth is 0–1.5% (optimum at 0%), indicating
164 that strain CAN^T is adapted to the low-salinity, warm, and high-pH fluid emitted by the alkaline
165 spring of La Crouen (New Caledonia).

166 H₂ and CO₂ were the only substrates used for growth and methanogenesis (Table 1, Fig. 2).
167 Acetate, ethanol, formate, isobutanol, methanol, trimethylamine, and 2-propanol were not used
168 as substrates for growth or methane production. Yeast extract and tryptone were not required
169 for growth. The maximal growth rate was found to be 0.02 h⁻¹ using H₂/CO₂ as sole energy and
170 carbon sources for growth in optimal conditions (45°C, pH 9.0, and 0% NaCl).

171 Strain CAN^T was resistant to antibiotics such as novobiocin, streptomycin, and
172 vancomycin added at 25 μg/mL or 50 μg/mL in the medium. It also grew in the presence of 25
173 μg/mL of tetracycline, but it was sensitive to this antibiotic at 50 μg/mL. Ampicillin, bacitracin,

174 chloramphenicol, cycloserine, kanamycin, metronidazole, penicillin, and rifampicin at 25
175 $\mu\text{g/mL}$ inhibited cell growth. Sulfide concentration greater than 1.5 g/L also inhibited cell
176 growth of strain CAN^T.

177 Phylogenetic analysis based on the almost full-length 16S rRNA gene sequence (1227 nt)
178 indicated that strain CAN^T is a member of the genus *Methanobacterium*, in the order
179 *Methanobacteriales*, class *Methanobacteria*, phylum “*Methanobacteriota*” (Fig. 3). The
180 phylogenetic position of strain CAN^T was confirmed by using three different methods:
181 maximum-likelihood (ML, Fig 3), maximum-parsimony (MP, Fig. S1A), and neighbor-joining
182 (NJ, Fig. S1B). The closest relatives of strain CAN^T are type strains of the
183 species: *Methanobacterium alcaliphilum* WeN4^T (98.5% identity) isolated from sediments of a
184 low-salt alkaline lake of the Wadi el Natrun (Egypt) (8), *Methanobacterium movens* TS-2^T
185 (98.4% identity) isolated from sediments of the alkaline saline Tuosu Lake (Qaidam basin,
186 China) (7), and *Methanobacterium flexile* GH^T (97.5% identity) isolated from sediments of an
187 alkaline saline lake, the Gahai Lake (Qaidam basin, China) (7). It is more distantly related to
188 the other alkaliphilic *Methanobacterium* type strains of the species: *Methanobacterium*
189 *subterraneum* A8p^T (95.1% identity).

190 The 16S rRNA gene sequence of strain CAN^T was closely related (>99% identity) to
191 a few environmental sequences retrieved in March 2022 by NCBI web BLAST search (34) and
192 originated from deep subsurface gas-associated water (DQ841242) (35) as well as H₂-fed
193 bioreactor operated at halo-alkaline conditions (KJ491002) (36). Sequences of the 16S rRNA
194 gene affiliated to the genus *Methanobacterium* were also detected in the alkaline spring of La
195 Crouen (representing 4.5% of prokaryotes in the sample studied) (11) and in the hyperalkaline
196 springs of the Ligurian Alps ophiolite massif and in subsurface fluids of the Sultanate of Oman
197 ophiolite massif (19, 37-39), where the methanogen could potentially consume H₂ produced by
198 serpentinization reactions.

199 The complete genome sequence of strain CAN^T is a unique circular chromosome 2,2
200 Mb long with a 36.4 mol% G+C content (Table 2). Its G+C content is identical to that of *M.*
201 *flexile* GH^T (36.4 mol%), higher than that of *M. alcaliphilum* WeN4^T (33.5 mol%), and lower
202 than *M. movens* TS-2^T (39.1 mol%). Its size is much smaller than that of its closest phylogenetic
203 neighbor *M. alcaliphilum* WeN3 (2.4 Mb). The ANI and dDDH values between CAN^T and *M.*
204 *alcaliphilum* WeN3 were 71.55% and 14.0%, i.e., far below the species boundaries of 95~96%
205 and 70%, respectively, recommended by Chun *et al.* (40). The CAN^T strain genome comprises
206 a single chromosome and 2,189 genes, including 2,126 protein-encoding genes and 49 total
207 RNA encoding genes. A complete pathway for hydrogenotrophic methanogenesis was found
208 using MaGe with both genes encoding formylmethanofuran dehydrogenase and methyl
209 coenzyme-M reductase that catalyze the initial and final step of methanogenesis on H₂/CO₂,
210 respectively. The genome of *M. alcaliphilum* WeN3 comprises 2,375 genes, including 2,325
211 protein-encoding genes and 46 total RNA encoding genes. The distribution of protein-encoding
212 genes of both strain CAN^T and *M. alcaliphilum* WeN3 into COGs functional categories is very
213 similar (Supplementary Table S1). In a whole-proteome-based phylogenetic analysis (Fig. S2),
214 strain CAN^T is also closely related to *M. alcaliphilum* (consistently with the 16S rRNA gene-
215 based phylogenetic analysis) and clustered with other members of the family
216 *Methanobacteriaceae*, including the genera *Methanobacterium*, *Methanobrevibacter*, and
217 *Methanothermobacter* (previously placed in the genus *Methanobacterium* (41)).

218 Table 1 summarizes the physiological and biochemical differences between strain
219 CAN^T and several type species of the genus *Methanobacterium* (including the four closest
220 phylogenetic neighbor type species and other alkaliphilic members). Strain CAN^T shares
221 similar properties with several *Methanobacterium* members, such as the exclusive utilization
222 of H₂/CO₂ for methanogenesis. However, strain CAN^T differs from its closest phylogenetic
223 neighbors, *M. alcaliphilum* WeN4^T, *M. movens* TS-2^T, and *M. flexile* GH^T, by its ability: (i) to

224 grow at 45°C (its optimum temperature), similarly to *M. aarhusense* H2-LR^T (6), and (ii) to
225 grow autotrophically in mineral medium without any organic additions (e.g., yeast extract),
226 similarly to *Methanobacterium subterraneum* A8p^T (5). To our knowledge, strain CAN^T is also
227 singular by its ability to grow optimally at higher pH (i.e., pH 9.0) than other type species of
228 the genus *Methanobacterium*. Indeed, the two other alkaliphilic *Methanobacterium* members,
229 i.e., *M. alcaliphilum* WeN4^T (8) and *M. subterraneum* A8p^T (5), display optimal growth at pH
230 8.4 and pH 7.8–8.8, respectively. Based on morphological, phenotypic, phylogenetic, and
231 genomic analyses, strain CAN^T is proposed to represent a novel species of the genus
232 *Methanobacterium*, *M. alkalithermotolerans* sp. nov.

233

234 Description of *Methanobacterium alkalithermotolerans*

235

236 *Methanobacterium alkalithermotolerans* (al.ka.li.ther.mo.to'le.rans. N.L. n. *alkali*, alkali (from
237 Arabic article *al*, the; Arabic n. *qaliy*, ashes of saltwort); Gr. fem. adj. *therme*, heat; L. pres.
238 part. *tolerans*, tolerating, enduring; N.L. part. adj. *alkalithermotolerans*, thermophilic alkali-
239 tolerating).

240

241 Displays similar traits as described for the genus. Cells are non-motile, straight rods 1.3–4.0
242 µm long and 0.5–0.7 µm wide, occurring singly, in pairs, or in chains. Colonies are white and
243 circular, 1 mm in diameter after two weeks of incubation. Alkaliphilic, mesophilic but
244 thermotolerant. Growth occurs at 20–45°C (optimum 45°C), at pH 7.3–9.7 (optimum pH 9.0)
245 with 0–1.5% NaCl (optimum 0%). Added NaCl and yeast extract were not required for growth.
246 Hydrogen and carbon dioxide were used as substrates for growth and methanogenesis. Acetate,
247 ethanol, formate, isobutanol, methanol, trimethylamine, and 2-propanol were not used for
248 growth. Resistant to novobiocin, streptomycin, and vancomycin (at 50 µg/mL) but sensitive to

249 ampicillin, bacitracin, chloramphenicol, cycloserine, kanamycin, metronidazole, penicillin,
250 rifampicin (at 25 µg/mL), and tetracycline (at 50 µg/mL). Most closely related to
251 *Methanobacterium alcaliphilum* WeN4^T (98.5% 16S rRNA gene sequence identity). The G+C
252 content of the genomic DNA is 36.4%. The GenBank accession number for the 16S rRNA gene
253 sequence of strain CAN^T is KR349725, and that for the whole genome sequence is CP058560.
254 The type strain CAN^T (= DSM 102889^T = JCM 31304^T) was isolated from a sediment slurry of
255 La Crouen geothermal spring near Canala, New Caledonia.

256

257 **Conflicts of interest**

259 The authors declare that there are no conflicts of interest.

260

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270

271

272 **Figures legends**

273

274 **Fig 1:** Phase-contrast micrographs of cells of strain CAN^T (A; bar 10 μm) and transmission
275 electron micrograph showing cells of strain CAN^T (B; bar 1 μm), with Gram-positive type cell
276 wall (C; bar 0.5 μm).

277

278 **Fig. 2:** Kinetics of hydrogen (H₂) consumption, methane (CH₄) production, and growth (optical
279 density OD 600 nm × 10) of strain CAN. This monitoring was determined at pH 9.0 and 37°C
280 in the minimal medium with the addition of H₂/CO₂ (80/20 v/v, 2 bars) as the only source of
281 energy and carbon. Values are means ± standard deviations of triplicated cultures.

282

283 **Fig. 3:** Maximum-likelihood (ML) phylogenetic tree based on 1227 aligned bp of 16S rRNA
284 gene sequences showing the position of strain CAN^T among its closest neighbors belonging to
285 the genus *Methanobacterium*. *Methanopyrus kandleri* AV19^T (NR_074539) was used as an
286 outgroup (not shown). Bootstrap values (based on 1000 replicates) are shown at branch nodes.
287 Accession numbers are indicated in brackets. Bar: 0.02 substitutions per 100 nucleotides.

288

289

290 **Table 1.** Differential characteristics between strain CAN^T (obtained in this study) and data obtained from previous studies for several type species
 291 of the genus *Methanobacterium*. Strains: 1, CAN^T; 2, *M. alcaliphilum* WeN4^T (8); 3, *M. movens* TS-2^T (7); 4, *M. flexile* GH^T (7); 5, *M. aarhusense*
 292 H2-LR^T (6); 6, *M. subterraneum* A8p^T (5).

Characteristics	1	2	3	4	5	6
Origin	Sediment of thermal spring, New Caledonia	Alkaline lake sediment, Egypt	Sediment of Tuoso Lake, China	Sediment of Gahai Lak, China	Marine sediment, Denmark	Deep groundwater, Sweden
Cell size (µm)	0.5–0.7 x 1.3–4.0	0.5–0.6 x 2–25	0.4–0.5 x 2–5	0.3–0.5 x 2–5	0.7 x 5–18	0.1–0.15 x 0.6–1.2
Gram staining	-	-	+	+		+
Motility	-	-	+	-	-	-
Growth substrates						
H ₂ + CO ₂	+	+	+	+	+	+
Formate	-	-	-	+	-	+
Alcohols	-	-	-		-	-
Acetate requirement	-	-	-		-	-
Yeast extract requirement	-	+	+	+	-	-
Optimum temperature (°C)	45	37	35–38	35–38	45	30–40
Optimum pH for growth	9.0	8.4	7.2–7.5	7.0–.5	7.5–8.0	7.8–8.8
Optimum NaCl (%)	0	0–0.6	0–0.3	0–0.1	0.05–.9	0.1–0.2
DNA G+C content (mol%) ¹	36.4 [*]	57 ²	39.5 ²	37.3 ²	34.9 ²	39.5 ¹ (54.5 ²)

+, Positive; -, negative.

¹ G+C content determined from genomic data.

² G+C content determined by using HPLC.

293
294
295

296 **Table 2.** Genome statistics of strain CAN^T, and *Methanobacterium alcaliphilum* DSM3459
 297 (both genomes obtained in this study).

298

Genome / Sample Name	Strain CAN ^T	<i>Methanobacterium alcaliphilum</i> DSM 3459
GenBank accession number	CP058560	JALKIF000000000
MaGe Genome ID	MAGE_000013558.1	MAGE_000016954.1
Genome Size (bp)	2,187,077	2,377,238
Contig count	NA	65
Scaffold count	1	65
GC (%)	36.4	33.4
Gene Count	2,189	2,375
Protein count	2,126	2,325
rRNA Count	7	4
tRNA Count	40	40
Other RNA	2	2

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302 **References**

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304 1. Lyu Z, Shao N, Akinyemi T, Whitman WB. Methanogenesis. *Current Biology*.
305 2018;28(13):R727-R32.

306 2. Garcia J-L, Patel BK, Ollivier B. Taxonomic, phylogenetic, and ecological diversity of
307 methanogenic Archaea. *Anaerobe*. 2000;6(4):205-26.

308 3. Boone DR. *Methanobacterium*. *Bergey's manual of systematics of archaea and bacteria*.
309 2015:1-8.

310 4. Rinke C, Chuvochina M, Mussig AJ, Chaumeil P-A, Davin AA, Waite DW, et al. A
311 standardized archaeal taxonomy for the Genome Taxonomy Database. *Nature Microbiology*.
312 2021;6(7):946-59.

313 5. Kotelnikova S, Macario AJ, Pedersen K. *Methanobacterium subterraneum* sp. nov., a
314 new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic
315 groundwater. *International journal of systematic and evolutionary microbiology*.
316 1998;48(2):357-67.

317 6. Shlimon AG, Friedrich MW, Niemann H, Ramsing NB, Finster K. *Methanobacterium*
318 *aarhusense* sp. nov., a novel methanogen isolated from a marine sediment (Aarhus Bay,
319 Denmark). *International journal of systematic and evolutionary microbiology*. 2004;54(3):759-
320 63.

321 7. Zhu J, Liu X, Dong X. *Methanobacterium movens* sp. nov. and *Methanobacterium*
322 *flexile* sp. nov., isolated from lake sediment. *International journal of systematic and*
323 *evolutionary microbiology*. 2011;61(12):2974-8.

324 8. Worakit S, Boone DR, Mah RA, Abdel-Samie M-E, El-Halwagi M. *Methanobacterium*
325 *alcaliphilum* sp. nov., an H₂-utilizing methanogen that grows at high pH values. *International*
326 *Journal of Systematic and Evolutionary Microbiology*. 1986;36(3):380-2.

327 9. Maurizot P, Sevin B, Lesimple S, Collot J, Jeanpert J, Bailly L, et al. Mineral resources
328 and prospectivity of non-ultramafic rocks of New Caledonia. *Geological Society, London,*
329 *Memoirs*. 2020;51(1):215-45.

330 10. Cox ME, Launay J, Paris J, editors. *Geochemistry of low temperature geothermal*
331 *systems in New Caledonia*. Pacific Geothermal conference and 4th NZ Geothermal Workshop;
332 1982.

333 11. Quéméneur M, Mei N, Monnin C, Postec A, Wils L, Bartoli M, et al. Prokaryotic
334 Diversity and Hydrogenotrophic Methanogenesis in an Alkaline Spring (La Crouen, New
335 Caledonia). *Microorganisms*. 2021;9(7):1360.

336 12. Deville E, Prinzhofer A. The origin of N₂-H₂-CH₄-rich natural gas seepages in
337 ophiolitic context: A major and noble gases study of fluid seepages in New Caledonia. *Chemical*
338 *Geology*. 2016;440:139-47.

339 13. Quéméneur M, Erauso G, Bartoli M, Vandecasteele C, Wils L, Gil L, et al. *Alkalicella*
340 *caledoniensis* gen. nov., sp. nov., a novel alkaliphilic anaerobic bacterium isolated from 'La
341 Crouen' alkaline thermal spring, New Caledonia. *International Journal of Systematic and*
342 *Evolutionary Microbiology*. 2021;71(5):004810.

343 14. Widdel F, Kohring G-W, Mayer F. Studies on dissimilatory sulfate-reducing bacteria
344 that decompose fatty acids. *Archives of microbiology*. 1983;134(4):286-94.

345 15. Hungate R. *Methods in microbiology. A Roll Tube Method for Cultivation of Strict*
346 *Anaerobes*, Academic Press, New York. 1969:117-32.

347 16. Balch W, Fox GE, Magrum LJ, Woese CR, Wolfe R. Methanogens: reevaluation of a
348 unique biological group. *Microbiological reviews*. 1979;43(2):260-96.

349 17. Mei N, Zergane N, Postec A, Erauso G, Ollier A, Payri C, et al. Fermentative hydrogen
350 production by a new alkaliphilic *Clostridium* sp.(strain PROH2) isolated from a shallow
351 submarine hydrothermal chimney in Prony Bay, New Caledonia. *international journal of*

hydrogen energy. 2014;39(34):19465-73.

18. Quéméneur M, Erauso G, Frouin E, Zeghal E, Vandecasteele C, Ollivier B, et al. Hydrostatic pressure helps to cultivate an original anaerobic bacterium from the Atlantis Massif seafloor (IODP Expedition 357): *Petrocella atlantisensis* gen. nov. sp. nov. *Frontiers in microbiology*. 2019;10:1497.

19. Quéméneur M, Palvadeau A, Postec A, Monnin C, Chavagnac V, Ollivier B, et al. Endolithic microbial communities in carbonate precipitates from serpentinite-hosted hyperalkaline springs of the Voltri Massif (Ligurian Alps, Northern Italy). *Environmental Science and Pollution Research*. 2015;22(18):13613-24.

20. Muyzer G, De Waal EC, Uitterlinden A. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and environmental microbiology*. 1993;59(3):695-700.

21. Großkopf R, Janssen PH, Liesack W. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Applied and environmental microbiology*. 1998;64(3):960-9.

22. Nicol GW, Leininger S, Schleper C, Prosser JI. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental microbiology*. 2008;10(11):2966-78.

23. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research*. 2004;32(5):1792-7.

24. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*. 2016;33(7):1870-4.

25. Felsenstein J. Phylogenies from restriction sites: a maximum - likelihood approach. *Evolution*. 1992;46(1):159-73.

26. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Biology*. 1971;20(4):406-16.

27. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*. 1987;4(4):406-25.

28. Marteinsson V, Birrien J-L, Kristjánsson JK, Prier D. First isolation of thermophilic aerobic non-sporulating heterotrophic bacteria from deep-sea hydrothermal vents. *FEMS microbiology ecology*. 1995;18(3):163-74.

29. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS computational biology*. 2017;13(6):e1005595.

30. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of computational biology*. 2012;19(5):455-77.

31. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, et al. MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic acids research*. 2013;41(D1):D636-D47.

32. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC bioinformatics*. 2013;14(1):1-14.

33. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nature communications*. 2019;10(1):1-10.

34. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *Journal of molecular biology*. 1990;215(3):403-10.

35. Mochimaru H, Uchiyama H, Yoshioka H, Imachi H, Hoaki T, Tamaki H, et al. Methanogen diversity in deep subsurface gas-associated water at the Minami-Kanto gas field in Japan. *Geomicrobiology Journal*. 2007;24(2):93-100.

- 402 36. Sousa JA, Plugge CM, Stams AJ, Bijmans MF. Sulfate reduction in a hydrogen fed
403 bioreactor operated at haloalkaline conditions. *Water research*. 2015;68:67-76.
- 404 37. Rempfert KR, Miller HM, Bompard N, Nothaft D, Matter JM, Kelemen P, et al.
405 Geological and geochemical controls on subsurface microbial life in the Samail Ophiolite,
406 Oman. *Frontiers in Microbiology*. 2017;8:56.
- 407 38. Fones EM, Colman DR, Kraus EA, Stepanauskas R, Templeton AS, Spear JR, et al.
408 Diversification of methanogens into hyperalkaline serpentinizing environments through
409 adaptations to minimize oxidant limitation. *The ISME journal*. 2021;15(4):1121-35.
- 410 39. Brazelton WJ, Thornton CN, Hyer A, Twing KI, Longino AA, Lang SQ, et al.
411 Metagenomic identification of active methanogens and methanotrophs in serpentinite springs
412 of the Voltri Massif, Italy. *PeerJ*. 2017;5:e2945.
- 413 40. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, et al. Proposed
414 minimal standards for the use of genome data for the taxonomy of prokaryotes. *International
415 journal of systematic and evolutionary microbiology*. 2018;68(1):461-6.
- 416 41. Wasserfallen A, Nölling J, Pfister P, Reeve J, De Macario EC. Phylogenetic analysis of
417 18 thermophilic *Methanobacterium* isolates supports the proposals to create a new genus,
418 *Methanothermobacter* gen. nov., and to reclassify several isolates in three species,
419 *Methanothermobacter thermotrophicus* comb. nov., *Methanothermobacter wolfeii* comb.
420 nov., and *Methanothermobacter marburgensis* sp. nov. *International Journal of Systematic and
421 Evolutionary Microbiology*. 2000;50(1):43-53.
- 422