

Methanoregula boonei gen. nov., sp. nov., an acidiphilic methanogen isolated from an acidic peat bog

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A novel acidiphilic, hydrogenotrophic methanogen, designated strain 6A8^T, was isolated from an acidic (pH 4.0–4.5) and ombrotrophic (rain-fed) bog located near Ithaca, NY, USA. Cultures were dimorphic, containing thin rods (0.2–0.3 µm in diameter and 0.8–3.0 µm long) and irregular cocci (0.2–0.8 µm in diameter). The culture utilized H₂/CO₂ to produce methane but did not utilize formate, acetate, methanol, ethanol, 2-propanol, butanol or trimethylamine. Optimal growth conditions were near pH 5.1 and 35 °C. The culture grew in basal medium containing as little as 0.43 mM Na⁺ and growth was inhibited completely by 50 mM NaCl. To our knowledge, strain 6A8^T is one of the most acidiphilic (lowest pH optimum) and salt-sensitive methanogens in pure culture. Acetate, coenzyme M, vitamins and yeast extract were required for growth. It is proposed that a new genus and species be established for this organism, *Methanoregula boonei* gen. nov., sp. nov. The type strain of *Methanoregula boonei* is 6A8^T (=DSM 21154^T =JCM 14090^T).

H₂/CO₂-utilizing methanogens of the order *Methanomicrobiales* are found in diverse environments and have been isolated from oil wells (Ollivier *et al.*, 1997), estuaries and marine sediments (Blotevogel *et al.*, 1986; Chong *et al.*, 2002; Mikucki *et al.*, 2003; Rivard *et al.*, 1983), anaerobic digesters (Ollivier *et al.*, 1985; Paynter & Hungate, 1968; Zellner *et al.*, 1998), rumen fluid (Paynter & Hungate, 1968), lakes and other freshwater systems (Franzmann *et al.*, 1997; Harris *et al.*, 1984) and as endosymbionts of ciliate protists (Van Bruggen *et al.*, 1986). In the past 10 years, many novel members of the *Methanomicrobiales* have been described, including three new genera, *Methanocalculus* (Ollivier *et al.*, 1998), *Methanolinea* (Imachi *et al.*, 2008) and *Methanosphaerula* (Cadillo-Quiroz *et al.*, 2009).

Culture-independent environmental surveys of acidic (pH 3.5–5.0) peat-forming wetlands revealed an abundance

of *mcrA* and small-subunit (SSU) or 16S rRNA gene sequences that cluster in the *Methanomicrobiales*, sometimes considered related to *Methanospirillum*, but divergent from all methanogens in pure culture (Basiliko *et al.*, 2003; Galand *et al.*, 2002; Hales *et al.*, 1996). These results have been supported using terminal restriction fragment length polymorphism (T-RFLP) profiles (Cadillo-Quiroz *et al.*, 2006) or studies of mixed methanogenic cultures (Bräuer *et al.*, 2006b; Horn *et al.*, 2003; Sizova *et al.*, 2003). Recently, an acidiphilic isolate, given the tentative name '*Candidatus Methanoregula boonei*' 6A8 (Bräuer *et al.*, 2006a), was obtained from one of the enrichment cultures. This organism had the unusual features of being dimorphic, with both rods and cocci, and acidiphilic, with a pH optimum near 5. Recently, the complete genome sequence of this organism was determined. In this communication, we present a complete taxonomic description of strain 6A8^T.

Enrichment of an acidiphilic methanogenic culture from McLean bog, an acidic (pH 4.0–4.5) and ombrotrophic (rain-fed) site near Ithaca, NY, USA (42° 30' N 76° 30' W) (Bräuer *et al.*, 2004), followed by the isolation of the methanogenic strain 6A8^T (Bräuer *et al.*, 2006a) were described previously. The 6A8^T culture was maintained on H₂/CO₂ in a modified PM1 low-ionic-strength basal medium (Bräuer *et al.*, 2006a) amended with titanium(III) nitrilotriacetate [Ti(III) NTA], HOMOPIPES buffer [homopiperazine-*N,N'*-bis-2-(ethanesulfonic acid), 5 mM, pH 5.4], coenzyme M (2-mercaptoethanesulfonic acid), sodium acetate, yeast extract and a vitamin solution. Cultures were

Abbreviation: SSU, small subunit.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and complete genome sequence of strain 6A8^T are DQ282124 and CP000780. The NCBI Protein accession number for the deduced McrA sequence is ABS55100.

incubated on a gyratory shaker at 28 °C and 200 r.p.m. As described elsewhere (Bräuer *et al.*, 2006a), culture purity was verified by several tests including lack of heterotrophic growth, fluorescence *in situ* hybridization with a species-specific probe, lack of a PCR amplicon using bacterial 16S rRNA gene primers and homogeneity of clone libraries of PCR amplicons based on two different archaeal primer sets.

DNA G+C content was determined from the complete genome sequence. A phylogenetic analysis of the SSU rRNA gene sequence relative to other sequences from methanogens across all five methanogen orders, including closely related environmental sequences, has been presented elsewhere (Bräuer *et al.*, 2006a). Here, we constructed a phylogenetic tree that includes primarily sequences from cultured and named members of the order *Methanomicrobiales*, allowing a more in-depth look at the phylogenetic placement of 6A8^T among other members of the *Methanomicrobiales*. Additionally, we included several isolates and clone sequences from mixed cultures, as well as some environmental sequences from peatlands in the E1/E2 group (Cadillo-Quiroz *et al.*, 2006). Sequences of representative members of the *Methanomicrobiales* were aligned using the Silva database, release 94 (09.04.08), of the ARB package (Ludwig *et al.*, 2004). Phylogenetic trees were constructed from 1212 bp well-aligned sequences using neighbour-joining and maximum-likelihood methods in the PHYLIP software package (Felsenstein, 2004). The topologies of the two phylograms were identical for nodes with bootstrapping values >70 %, and the neighbour-joining output is presented here. Methyl-coenzyme M reductase alpha subunit amino acid sequences, predicted from *mcrA* gene sequences, were aligned using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were constructed from 236 aligned amino acid positions using neighbour-joining and maximum-likelihood methods in the PHYLIP software package (Felsenstein, 2004). The branching pattern in the maximum-likelihood analysis was identical to that in the neighbour-joining analysis.

The following substrates for methanogenesis were added individually to cultures of strain 6A8^T: sodium acetate, sodium formate (pH 5) (both at 2 mM), methanol, ethanol, 2-propanol, butanol and trimethylamine (all at 5 mM). Cells were grown under a N₂/CO₂ atmosphere to test for substrate utilization and parallel tubes were grown under a H₂/CO₂ atmosphere to verify that the amount of substrate added was not inhibitory. Additionally, cultures were grown under a H₂/CO₂ atmosphere or under a N₂/CO₂ atmosphere with no added substrate to serve as positive and negative controls, respectively. Growth rates, as determined by methanogenesis (Boone & Whitman, 1988), were examined with respect to Na⁺ concentration. For experiments on the effect of Na⁺, the medium was modified to limit the total Na⁺ concentration. The pH of the HOMOPIES buffer and Ti(III) NTA solutions was adjusted using KOH rather than NaOH, and potassium acetate rather than sodium acetate was used, so that coenzyme M (which can only be purchased as the sodium

salt) and yeast extract were the only known sources of Na⁺ in the medium. The final Na⁺ concentration of the medium was determined to be 0.432 ± 0.007 mM by the Cornell Nutrient Analysis Laboratory using inductively coupled plasma (ICP) spectroscopy. Methane production by cultures was quantified using a flame ionization gas detector as described previously (Bräuer *et al.*, 2004). Growth yield was determined in triplicate by drying 120 ml culture on pre-weighed 0.2 µm polycarbonate filters (Nucleopore) first on the bench top and then in a desiccator overnight. Uninoculated medium was filtered onto membranes (in triplicate) to calculate the blank. Specific growth rates (μ) were determined using the equation $\mu = (\ln X - \ln X_0)/t$ (Madigan *et al.*, 2000). Physiological tests were performed in triplicate, and either standard deviation or standard error of the mean (where noted) was calculated.

For lipid analysis, cells were harvested in late exponential phase, centrifuged immediately and lyophilized before use. Extraction of total lipids and fractionation followed a procedure modified from Zhang *et al.* (2006). Analysis of the lipid components were conducted on an LC-MS as described previously (Sturt *et al.*, 2004).

Microscopic analyses of cell motility and morphology were conducted under phase-contrast with a Nikon Eclipse E600 microscope. The cell envelope was tested using Gram staining and by evaluating susceptibility of the cells to SDS lysis, as described previously (Boone & Whitman, 1988). Concentrations of 0, 0.05, 0.1, 0.5, 1, 2 and 3 % SDS were used, and cell integrity was evaluated using microscopy. Negative-staining and thin-sectioning transmission electron microscopy was performed at the Cornell Integrated Microscopy Center. Cells were concentrated by centrifugation, washed and resuspended in deionized water. A drop (~5 µl) of the concentrated cells was mixed with a drop of bacitracin (100 µg ml⁻¹) and allowed to settle onto Formvar-coated copper grids (100 mesh) for 5 min before staining with 2 % uranyl acetate (pH 6.5) for a few seconds. The electron microscope used for low-resolution micrographs was a Phillips EM-201 equipped with a Gotan model 780 camera and that used for high-resolution micrographs was a Phillips Technai 12 Biotwin, equipped with a Gatan Multiscan model 791 camera and Digital Micrograph software. Photomicrographs were taken at 100 kV.

Cultures of strain 6A8^T were dimorphic, consisting of thin, straight rods, 0.2–0.3 µm in diameter and 0.8–3.0 µm long, and irregular cocci, 0.3–0.8 µm in diameter (Fig. 1; Bräuer *et al.*, 2006a). Asymmetrical cell division was sometimes observed in rods, but cell division was not observed in cocci greater than 0.3 µm in diameter (Bräuer *et al.*, 2006a). In addition, two other mixed cultures containing organisms closely related to 6A8^T (98–99 % identity in the SSU rRNA gene), clone KB1 (S. H. Zinder, personal observation) and clone NTA (Bräuer *et al.*, 2006b), also appear to be dimorphic. Using fluorescence microscopy,

cofactor F_{420} autofluorescence was not observed in cells during any growth phase, indicating that the cells contained low concentrations of this cofactor, or possibly none. Transmission electron micrographs of negatively stained cell cultures of strain 6A8^T (Fig. 1) showed that the two morphotypes had similar cell-wall structures that were visually identical to the S-layer cell envelopes found in most archaea (Kandler & König, 1998). The two morphotypes had similar 'notches', which are attributed to fracturing of the S-layer by flattening of the cells during preparation. Filamentous appendages, 9–11 nm in diameter, were also observed, and some preparations contained darkly stained inclusions, which may have been storage granules. The diameters of the filaments are at the lower boundary of those for archaeal flagella (10–14 nm; Thomas *et al.*, 2001), and, while archaeal flagella genes are present in the genome, motility of cells of 6A8^T has not been observed. Thus, the function of the filaments is unknown. Cells were also sensitive to lysis by low

concentrations of detergent (0.1% SDS) and stained Gram-negative. These results suggest that strain 6A8^T has a more typical archaeal proteinaceous S-layer that is more similar to that of *Methanolinea tarda* (Imachi *et al.*, 2008) than to the thicker, SDS-resistant layer of *Methanosphaerula palustris* (Cadillo-Quiroz *et al.*, 2009).

Strain 6A8^T grows using H_2/CO_2 exclusively for methanogenesis. No growth or methane production was observed in the absence of H_2/CO_2 or upon addition of acetate, formate (pH 5), methanol, ethanol (Bräuer *et al.*, 2006a), 2-propanol, butanol or trimethylamine (this study). Acetate and formate at 2 mM partially inhibited growth on H_2/CO_2 and were completely inhibitory at 5 mM. The other substrates did not inhibit methanogenesis significantly at 5 mM. Growth inhibition by 5 mM acetate and formate may be partially explained by the toxicity of their protonated forms at low pH (Bräuer *et al.*, 2004). Strain 6A8^T also required 0.2 mM acetate, coenzyme M, vitamins

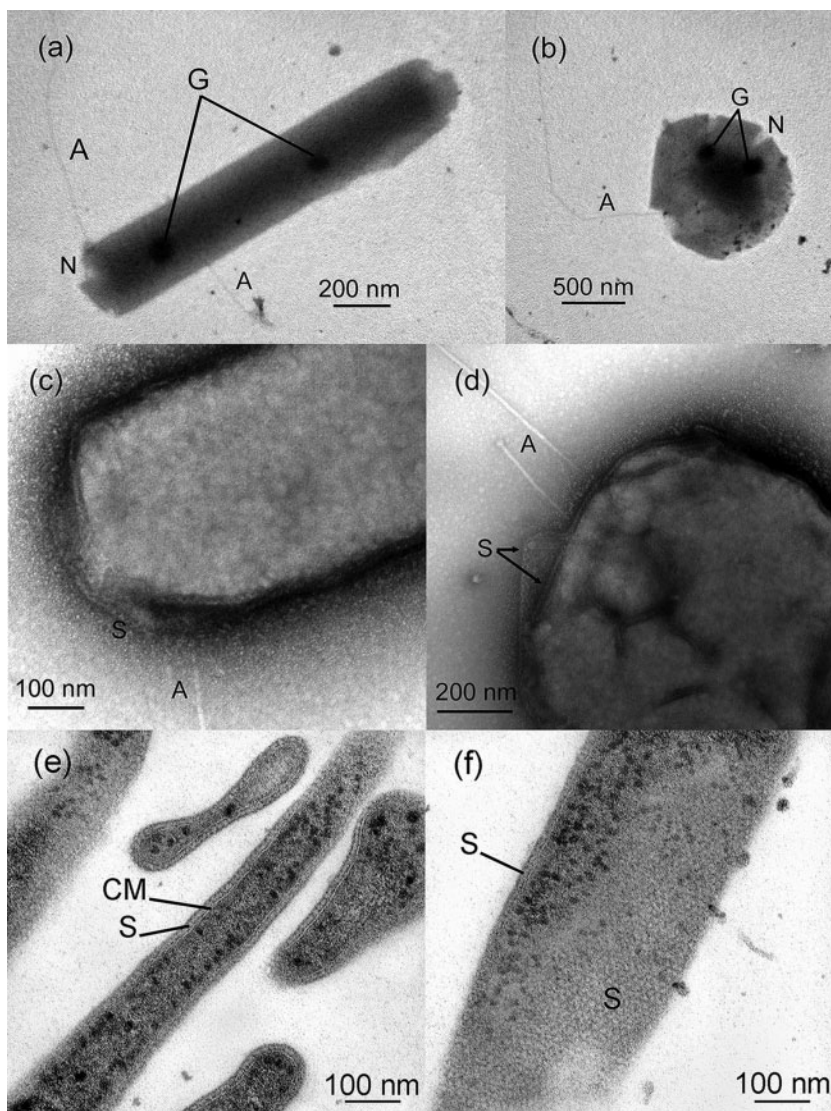


Fig. 1. Electron micrographs of strain 6A8^T including uranyl acetate-stained rod-shaped (a, c) and coccus-shaped (b, d) cells, as well as ultrathin-sectioned cells (e, f). (a, b) Low-resolution micrographs showing notches (N) in the cell wall, putative storage granules (G) and pilus-like appendages (A). (c, d) Higher-resolution micrographs showing subunit structure at the periphery of the outer S-layer (S) and pilus-like appendages (A). (e, f) Ultrathin-section micrographs that highlight intercellular structures such as the outer S-layer (S) and cytoplasmic membrane (CM). Note the subunit structure of the S-layer that is apparent in (f).

Table 1. Characteristics of strain 6A8^T and other representatives of the order *Methanomicrobiales*

Strains: 1, strain 6A8^T (data from this study); 2, *Methanosphaerula palustris* E1-9C^T (Cadillo-Quiroz *et al.*, 2009); 3, *Methanolinea tarda* NOBI-1^T (Imachi *et al.*, 2008); 4, *Methanospirillum hungatei* JF1^T (Balch *et al.*, 1979; Ferry *et al.*, 1974); 5, *Methanofollis aquaemaris* N2F9704^T (Lai & Chen, 2001); 6, *Methanogenium aggregans* MSt^T (Ollivier *et al.*, 1985); 7, *Methanogenium marinum* AK-1^T (Chong *et al.*, 2002); 8, *Methanoplanus limicola* M3^T (Wildgruber *et al.*, 1982); 9, *Methanoculleus palmolei* INSLUZ^T (Zellner *et al.*, 1998); 10, *Methanocorpusculum parvum* XII^T (Zellner *et al.*, 1987); 11, *Methanocalculus halotolerans* SEBR 4845^T (Ollivier *et al.*, 1998). All strains used H₂/CO₂ as a methanogenic substrate. ND, No data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Source	Acidic bog	Rich fen	Sewage sludge	Sewage sludge	Fish pond	Anaerobic digester	Marine sediment	Drilling waste	Anaerobic digester	Anaerobic digester	Oil well
Cell shape*	TR, IC	RC	TR, F	S	RC, IC	IC	IC	P	IC	IC	IC
pH for growth											
Optimum	5.1	5.7	7	6.6–7.4	6.5	6.5–7	6.0–6.6	6.5–7.5	6.9–7.5	6.8–7.5	7.6
Range	4.5–5.5	4.8–6.4	6.7–8	ND	6.3–8	6–8	5.5–7.5	ND	6.5–8	ND	7–8.4
Temperature for growth (°C)											
Optimum	35–37	30	50	30–37	37	37	25	40	40	37	38
Range	10–40	14–35	35–55	ND	20–43	25–40	5–25	17–41	22–50	25–44	25–45
NaCl concentration for growth (% w/v)											
Optimum	<0.1	<0.2	0	ND	0.5	ND	ND	1	ND	0–4.7	5
Range	0–0.3	0–0.6	0–1.5	ND	0–6	0–2.5	1.4–7.3	0.4–5.4	ND	ND	0–12.5
Minimum <i>t</i> _{gen} (h)	44	19	144	17	63	8	60	6	13.5	8	12
DNA G + C content (mol%)	54.5	58.9	56.3	45	ND	52	ND	47.5	59	49	55
Substrates used											
Formate	–	+	+	+	+	+	+	+	+	+	+
2-Propanol	–	–	–	ND	–	ND	–	ND	+	+	–
2-Butanol	–	–	–	ND	–	ND	–	ND	+	+	–
Acetate required for growth	+	+	+	+	–	+	+	+	ND	+	+

*F, Filaments; IC, irregular cocci; P, plate-shaped; RC, regular cocci; S, spirilla; TR, thin rods.

and yeast extract for growth. Acetate is commonly used by methanogens as a carbon source, and is required by strains of many members of the *Methanomicrobiales* (Table 1), including *Methanosphaerula palustris* (Cadillo-Quiroz *et al.*, 2009) and *Methanospirillum hungatei* (Sprott & Jarrell, 1981). The growth yield on H_2/CO_2 was 0.68 ± 0.13 g dry weight per mol methane and the maximum specific growth rate (μ) was 0.017 h^{-1} at pH 5.1 and 28°C (i.e. doubling time was 44 ± 10 h). Generation time (t_{gen}) was estimated to be 1.5–2 days during early exponential growth and 2–2.5 days during late exponential growth.

Strain 6A8^T is a slow-growing methanogen with a maximum specific growth rate (μ) comparable to those of the moderate acidiphile *Methanobacterium espanolae* (approx. 0.022 h^{-1} at pH 5.6 and 0.016 h^{-1} at pH 5.0; Patel *et al.*, 1990) and the psychrophile *Methanogenium frigidum* (0.014 h^{-1} ; Franzmann *et al.*, 1997). Another related strain of the *Methanomicrobiales*, *Methanolinea tarda* NOBI-1^T, was reported to have an even lower μ of 0.007 h^{-1} (Imachi *et al.*, 2008). Strain 6A8^T had a temperature optimum for growth near 35°C , with no growth at 4 or 45°C (Bräuer *et al.*, 2006a). The pH optimum was near 5.1, and cultures did not grow below pH 4 or above pH 6 (Bräuer *et al.*, 2006a), making it a moderate acidiphile. Recently, Kotsyurbenko *et al.* (2007) isolated an acidiphilic *Methanobacterium* strain with a pH optimum between 5.0 and 5.5 and a growth range of pH 3.8–6.0, similar to that of 6A8^T. It will be interesting to determine factors that regulate the distribution of these two acidiphilic methanogen groups. Overall, members of the E1/E2 group of the *Methanomicrobiaceae*, such as 6A8^T, are the predominant group in many temperate peatlands of various pH ranges throughout the world (Basiliako *et al.*, 2003; Cadillo-Quiroz *et al.*, 2006, 2008; Galand *et al.*, 2003; Hales *et al.*, 1996; Jouttonen *et al.*, 2005).

Peat bogs have low ion concentrations, and the Na^+ concentration in McLean bog was near $2 \mu\text{M}$ (Bräuer *et al.*, 2004). A salient property of strain 6A8^T is that it grew well at the lowest Na^+ concentration we could achieve in our growth medium, approx. 0.4 mM , and growth was inhibited almost completely by 50 mM NaCl (Fig. 2). Other hydrogenotrophic methanogens, even ones considered freshwater strains, tolerate much higher NaCl concentrations (Jarrell & Kalmokoff, 1988), up to 250 mM . In addition, a requirement of about 1 mM Na^+ for growth (Perski *et al.*, 1982) has been attributed to a bioenergetic circuit involving Na^+ pumping by a membrane-bound methyltetrahydromethanopterin-coenzyme M methyltransferase (Deppenmeier *et al.*, 1999). The low Na^+ requirement of 6A8^T and the low concentrations of Na^+ in the habitat from which it was isolated suggest a mechanism for energy conservation with a much higher affinity for Na^+ than is found in other methanogens, or one that does not involve Na^+ . In terms of NaCl tolerance, the recently described *Methanosphaerula palustris* was somewhat less sensitive, and did not tolerate 100 mM NaCl (Cadillo-Quiroz *et al.*, 2009). This is not surprising, as *Methanosphaerula*

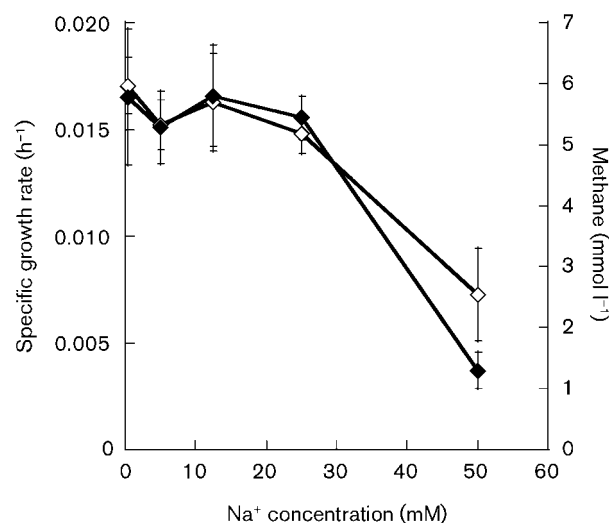


Fig. 2. Specific growth (\diamond) and total CH_4 production after 1 week (\blacklozenge) for cultures of strain 6A8^T in response to a range of NaCl concentrations. Each point represents a mean of triplicate tubes and error bars represent SEM.

palustris was isolated from a neutral pH peatland with higher mineral nutrient concentrations. Growth of *Methanolinea tarda*, isolated from sewage sludge, was inhibited by 250 – 350 mM NaCl (Imachi *et al.*, 2008), more typical of freshwater methanogens. Also, similar to *Methanosphaerula palustris* (Cadillo-Quiroz *et al.*, 2009), strain 6A8^T did not tolerate the millimolar concentrations of sulfide (Bräuer *et al.*, 2006b) typically added as a reducing agent to growth media for methanogens.

The lipids of 6A8^T were dominated by the non-cyclic glycerol dialkyl glycerol tetraether (GDGT-0) or caldarchaeol, which accounted for about 96 % of the lipids. The other 4 % of the lipids was composed mainly of diether archaeol. These results are consistent with archaeal lipids of other methanogens from the *Methanomicrobiales* (Koga *et al.*, 1998).

Based upon phylogenetic analysis of SSU rRNA gene sequences, strain 6A8^T (Bräuer *et al.*, 2006a) was related to a clade of previously uncultured members of the *Methanomicrobiales* called the R10 group (Hales *et al.*, 1996) or the E1/E2 group (Cadillo-Quiroz *et al.*, 2006). This clade includes the two recently described species *Methanolinea tarda* (Imachi *et al.*, 2008) and *Methanosphaerula palustris* (Cadillo-Quiroz *et al.*, 2009). The sequence of the *mcrA* gene from the original 6A enrichment culture (Bräuer *et al.*, 2006b) placed it within the 'Fen cluster' in the *Methanomicrobiales*, demonstrating that this group is congruent with the SSU rRNA R10 or E1/E2 clade. The SSU rRNA gene sequence of strain 6A8^T was close (>98 % identity) but not identical to many environmental sequences recovered from McLean bog and other acidic wetlands (Bräuer *et al.*, 2006a, b). Fig. 3 shows the SSU

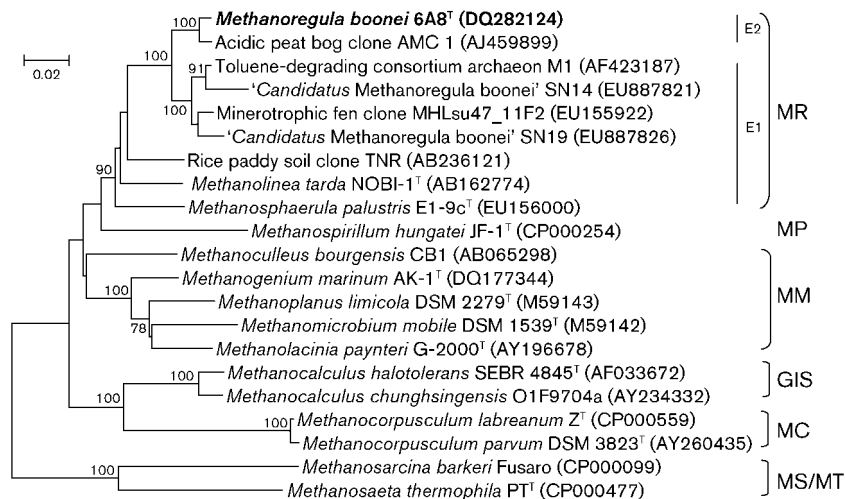


Fig. 3. Dendrogram showing the phylogenetic relationship of 16S rRNA gene sequences from strain 6A8^T and cultured members of families in the order *Methanomicrobiales*, as well as some uncultured members of group E1/E2. Families are abbreviated as follows: MM, *Methanomicrobiaceae*; MP, *Methanospirillaceae*; MC, *Methanocorpusculaceae*; GIS, *genus incertae sedis*; MS/MT, *Methanosarcinaceae*/Methanosaetaceae (Boone *et al.*, 2001). Sequences of *Methanosarcina barkeri* Fusaro and *Methanosaeta thermophila* PT^T were used as the outgroup. Alignments were performed in ARB (Ludwig *et al.*, 2004) and the tree was constructed based on a distance-matrix analysis using the neighbour-joining method in the PHYLIP software package (Felsenstein, 2004). All nodes with bootstrapping values >70% were supported using maximum-likelihood analysis. Bootstrapping values for those nodes are depicted here. Bar, 0.02 substitutions per nucleotide position.

rRNA gene phylogeny of selected members of the order *Methanomicrobiales*, including clone sequences from mixed cultures, clones AMC 1 and M1, the recently isolated 'Candidatus *Methanoregula boonei*' strains SN14 and SN19 (in pure culture) and two environmental clones, TNR and MHLsu47_11F2. Several representatives from mixed cultures, AMC 1 as well as clone NTA (GenBank accession no. DQ205188) and KB-1 (AY780566), share 98–99% identity with 6A8^T. However, only AMC 1 was chosen for tree analysis because of the presence of a longer sequence in the database. 'Candidatus *Methanoregula boonei*' SN14 and SN19 sequences represent undescribed isolates from sewage sludge and share only 95% SSU rRNA gene sequence identity with strain 6A8^T. Both strains cluster more closely

with members of group E1 and clearly represent different species, perhaps even a different genus from 6A8^T. Overall, phylogenetic analyses as well as physiological and structural characteristics support the placement of strain 6A8^T in a novel genus and species, and perhaps a novel family (Fig. 4; Cadillo-Quiroz *et al.*, 2009).

Description of *Methanoregula* gen. nov.

Methanoregula (Me.tha.no.re'gu.la. N.L. pref. *methano-* pertaining to methane; L. fem. n. *regula* slat or ruler; N.L. fem. n. *Methanoregula* methane-producing thin slat).

Cells are dominated by thin rods and are commonly dimorphic, forming irregular cocci as well. Cultures are

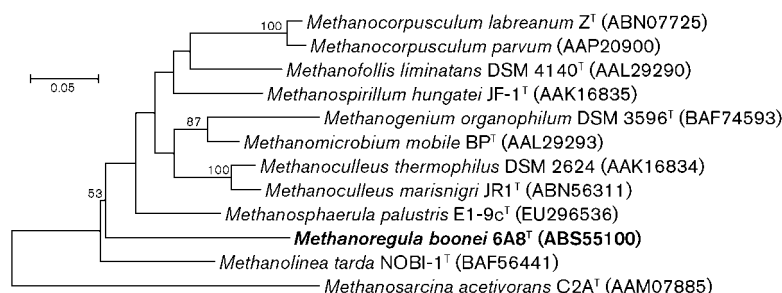


Fig. 4. Neighbour-joining dendrogram showing the phylogenetic relationship of the deduced McrA sequence from strain 6A8^T with sequences from cultured members of the order *Methanomicrobiales*. The sequence of *Methanosarcina acetivorans* C2A^T was used as the outgroup. Predicted amino acid sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were constructed from 236 aligned amino acids using neighbour-joining and maximum-likelihood methods in the PHYLIP software package (Felsenstein, 2004). The branching pattern in the maximum-likelihood analysis was identical to that in the neighbour-joining analysis. Nodes with bootstrapping values >50% are presented here. Bar, 0.05 substitutions per amino acid position.

mesophilic, produce methane from H_2/CO_2 and are strict anaerobes. *Methanoregula boonei* is the type species.

Description of *Methanoregula boonei* sp. nov.

Methanoregula boonei (boo'ne.i. N.L. masc. gen. n. *boonei* of Boone, to honour the pioneering work of David Boone on methanogenic archaea).

Displays the following properties in addition to those described for the genus. Cells occur as thin rods, dividing irregularly and sometimes producing longer rods, shorter rods or irregular cocci. They have a mean diameter of 0.2–0.8 μm and are non-motile, and many cells have pilus-like appendages. The cell envelope is composed of an SDS-sensitive S-layer. Cells are obligately hydrogenotrophic. Grows at pH 4.5–5.5, with optimum growth near pH 5.1. Grows between 10 and 40 $^{\circ}C$, with an optimum near 35–37 $^{\circ}C$. Cultures are also extremely salt sensitive, producing methane at exponential rates within a narrow salt range from 0.4 mM Na^+ to 25 mM NaCl, and they are adapted to oligotrophic and low pH environments. Growth is inhibited by millimolar quantities of sulfide as a reducing agent, while Ti(III) and coenzyme M (2-mercaptoethanesulfonic acid) are tolerated. The genomic DNA G+C content of the type strain is 54.5 mol%, as determined from the genome sequence.

The type strain is 6A8^T (=DSM 21154^T =JCM 14090^T).

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