Methanosphaerula palustris gen. nov., sp. nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland

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Peatlands are important sources of CH₄ emissions to the atmosphere and molecular surveys have identified a diverse, but mainly uncultured, euryarchaeal community in them. Characterization of a strain, E1-9cᵀ, associated with uncultured group E1, from a minerotrophic fen is reported. Cells were regular cocci, usually found in pairs, that stained Gram-positive and were resistant to lysis by 0.1 % SDS. Multiple flagella were observed, but motility was not observed in wet mounts. Optimal growth was obtained at moderate temperatures (28–30 °C) and slightly acidic pH (5.5). Total Na⁺ and NaCl were only tolerated at concentrations less than 100 mM and 0.5 %, respectively, and Na₂S concentrations above 0.1 mM were inhibitory. H₂/CO₂ and formate were the only methanogenic substrates used by E1-9cᵀ; formate concentrations above 50 mM were inhibitory for growth. Vitamins, coenzyme M and acetate (4 mM) were required for growth and the doubling time was about 19 h. Phylogenetic analysis of the 16S rRNA gene and inferred McrA amino acid sequences showed that E1-9cᵀ represented an independent lineage within the order Methanomicrobiales. Physiological and phylogenetic comparisons with different members of the order supported classification of E1-9cᵀ in a new genus in the Methanomicrobiales. The name Methanosphaerula palustris gen. nov., sp. nov. is proposed; strain E1-9cᵀ (=ATCC BAA-1565ᵀ =DSM 19958ᵀ) is the type strain of Methanosphaerula palustris.

Abbreviations: TEM, transmission electron microscopy; T-RFLP, terminal restriction fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and mcrA gene sequences of strain E1-9cᵀ are EU156000 and EU296536, respectively.

Several phylogenetic analyses have shown that E1- and E2-related sequences form independent branches within the Methanomicrobiales (Basiliko et al., 2003; Cadillo-Quiroz et al., 2006, 2008; Galand et al., 2003; Hales et al., 1996). The order Methanomicrobiales is diverse and its members exhibit great morphological and physiological variability (Dubach & Bachofen, 1985). This order comprises the families Methanomicrobiaceae, Methanocorpusculaceae and Methanospirillaceae (Boone et al., 2001; Garcia et al., 2000).

Methanomicrobiaceae (Cadillo-Quiroz et al., 2006), have been shown to be highly abundant in clone libraries and by terminal restriction fragment length polymorphism (T-RFLP) analysis of samples from acidic bogs and near-neutral pH fen peatlands (Basiliko et al., 2003; Cadillo-Quiroz et al., 2006, 2008).

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It also includes three genera that are not placed within any family: the genus Methanocalculus, which is listed as a genus incertae sedis (Boone et al., 2001), with four reported species (Lai et al., 2002, 2004; Mori et al., 2000; Ollivier et al., 1998), the proposed genus ‘Candidatus Methanoregula’, with one species, ‘Candidatus Methanoregula boonei’, isolated from an acidic bog (Bräuer et al., 2006a, b), and the recently described genus Methanolinea, with one species, Methanolinea tarda, isolated from sewage sludge (Imachi et al., 2008).
‘Candidatus Methanoaegula boonei’ is a member of the E2 group and was isolated using low-ionic-strength media and reducing agents other than Na₂S to avoid inhibition of growth (Bräuer et al., 2006b). Recently, the isolation of a novel methanogen, designated strain E1-9c¹, using the same culture medium coupled to RFLP analysis as a screening tool (Cadillo-Quiroz et al., 2008) has been reported. Strain E1-9c¹ was isolated from a minerotrophic fen in upstate New York (42° 21’ N 76° 28’ W) where sequences belonging to the E1 group were diverse and abundant (Cadillo-Quiroz et al., 2008). In our previous paper, conditions that enriched and allowed for isolation of a member of the E1 group, using T-RFLP analysis as a selection and tracking tool, were reported. Culture purity was evaluated by culture techniques, phase-contrast microscopy, fluorescent in situ hybridization, T-RFLP analysis and clone library analysis and strain E1-9c¹ was proposed as the first member of a novel genus within the Methanomicrobiales. In this report, a more detailed evaluation of the morphological, physiological and phylogenetic characteristics of strain E1-9c¹ is presented and the novel species Methanosphaerula palustris gen. nov., sp. nov., represented by strain E1-9c¹, is described.

PM2 medium, a modified version of PM1 medium (Bräuer et al., 2006b), was used. It contains the following components (mg 1⁻¹): 1.5 KCl, 13.6 KH₂PO₄, 26.8 NH₄Cl, 0.024 CoCl₂, 6H₂O, 0.075 ZnCl₂, 0.019 H₂BO₃, 0.024 NiCl₂, 6H₂O, 0.024 Na₂MoO₄.2H₂O, 1.3 FeCl₃.4H₂O, 0.026 MnSO₄.4H₂O, 1.6 MgSO₄, 2.4 CaCl₂.2H₂O, 0.009 CuSO₄.5H₂O, 3.5 AlK(SO₄)₂.12H₂O and 3.7 disodium EDTA. Medium was prepared under a 70 % N₂/30 % CO₂ atmosphere as described previously (Bräuer et al., 2006b) using the modified anaerobic technique of Hungate (Sowers & Noll, 1995). Several hours before inoculation, sterile anaerobic additions were made to the basal medium with the following final concentrations: 1.0 mM titanium (III) nitritolactate [7.2 ml 1 M Tris/HCl adjusted to pH 8, 4.8 ml 0.5 M sodium nitritolactate and 0.55 ml 15 % titanium (III) chloride (Fluka)]; 10 mM MES (filter-sterilized 1 M stock solution adjusted to pH 7.5); 0.5 mM coenzyme M (2-mercaptopethanesulfonic acid); 0.4 mM sodium acetate; 1% (v/v) vitamin solution (Balch et al., 1979); and 0.04 mM Na₂S.9H₂O (filter-sterilized 20 mM stock solution). The final liquid volumes in the tubes were ~5 ml and H₂/CO₂ (80 %/20 %; 70.7 kPa) was added to the headspace unless otherwise specified. Cultures were incubated on a gyratory shaker at 30 °C and 200 r.p.m. and all evaluations were done for over 29 days.

Cell morphology and motility were examined by phase-contrast microscopy with a Nikon Eclipse E600 microscope. Gram staining and determination of susceptibility to lysis in the presence of SDS were done as described previously (Boone & Whitman, 1988). SDS tests were performed at final concentrations of 0.1–5.0 % (w/v) and lysis was determined by microscopic observations. Negative-staining, ultrathin-sectioning transmission electron microscopy (TEM) was performed as described elsewhere (Firtel et al., 1995) using 2 % uranyl acetate (pH 6.5) and a Phillips Technai 12 Biotwin electron microscope equipped with a Gatan Multiscan model 791 camera and Digital Micrograph software. Photos were taken at 100 kV. Flagella observations were done with low uranyl acetate staining (0.2 %) and images were enhanced digitally using the Photoshop software by a differential adjustment of the brightness and contrast of cell body and flagella versus background surface.

When determining the effect of total sodium on growth of strain E1-9c¹, the background sodium content from the different components of the medium (~15 mM) was added to NaCl values. Substrate utilization tests were performed in duplicate under both N₂/CO₂ and H₂/CO₂ atmospheres. The latter condition was used to verify that the substrate was not inhibitory. The following substrates were tested at a final concentration of 10 mM: sodium acetate, trimethylamine, 2-propanol, methanol, ethanol, 2-butanol, sodium propionate, sodium butyrate and sodium formate. Formate utilization tests also included treatments with or without the addition of sodium selenite (5 μM) and tests at pH 6.4. Antibiotic susceptibility tests were performed by adding anaerobic filter-sterilized stocks of penicillin, ampicillin, vancomycin, tetracycline, bacitracin, kanamycin, spectinomycin, rifampicin and chloramphenicol (each at 100 μg ml⁻¹ final concentration). Both sodium and antibiotic sensitivity tests were done under a H₂/CO₂ atmosphere for over a month. Treatments were monitored for CH₄ production with a flame ionization detector as described previously (Cadillo-Quiroz et al., 2006). Growth rates were calculated from methane production from the exponential portion of the methane accumulation curve (Ni & Boone, 1991).

The mcrA gene was amplified using the ME1 and ME2 primer set as described previously (Hales et al., 1996). Phylogenetic analyses of 16S rRNA gene sequences was carried out using ARB software (Ludwig et al., 2004) and the latest release (January 2007) of the ‘greengenes’ database (DeSantis et al., 2006). The 16S rRNA gene sequence alignment was exported from ARB using an ‘Archaeal’ nucleotide base frequency filter that included positions with more than 50 % invariance (1140 valid columns) to avoid possible treeing artefacts. Phylogenetic trees were constructed by Bayesian analyses. The approximation of posterior probabilities was done with MRBAYES version 3.0 (Ronquist & Huelsenbeck, 2003) using four-chain Metropolis-coupled Markov Chain Monte Carlo (MCMC/MC) analysis. Trees and model parameter values were sampled from a target distribution generated when chains converged. Bayesian consensus trees were built with a burnout of 300 and posterior probabilities were calculated. Tree topology was confirmed using maximum-likelihood and neighbour-joining methods with Olsen evolutionary distance correction as implemented in the ARB software (Ludwig et al., 2004). The presented tree represents the most frequently observed topology across
various phylogenetic analyses. For mcrA deduced amino acid sequences, sequences were aligned using CLUSTAL_X and analysed using MRBAYES under similar conditions to those described above. For this analysis, 236 aa residues were compared for the McrA tree.

The genomic DNA G+C content of E1-9cT was calculated by a thermal denaturation fluorimetric method using the Bio-Rad iCycler iQ real-time PCR system as described previously (Gonzalez & Saiz-Jimenez, 2002). The G-nome kit (Qiagen) was used to isolate genomic DNA from strain E1-9cT and the following cultures: ‘Candidatus Methanoregula boonei’ 6A8 (G+C content 54.5 mol%; S. Bräuer, personal communication), Escherichia coli K-12 (50 mol%) and Pseudomonas aeruginosa PAO1 (66 mol%). Additionally, DNA from Micrococcus luteus (G+C content 72 mol%) and Clostridium perfringens (27 mol%) was obtained from commercial sources (Sigma-Aldrich). Thermal denaturation was performed with approximately 2.5 μg DNA from the isolate and standards. Thermal conditions consisted of a ramp from 55 to 90 °C at 0.5 °C min⁻¹. Fluorescent DNA melting curves were generated in triplicate. The DNA G+C content for strain E1-9cT was calculated using a linear regression analysis of melting temperatures against the DNA G+C content of the standards.

Cells of E1-9cT were regular cocci, 0.5–0.8 μm in size and stained Gram-positive. The cells were mostly associated in pairs and planes of division were commonly visible (Fig. 1a). Other cocci in the Methanomicrobiales, such as those of strains of the genus Methanogenium, tend to be irregular and do not form pairs (Table 1). Methanofollis aquaeamiris N2F9704T forms regular cocci when in the exponential phase that change to irregular cocci in the stationary phase (Lai & Chen, 2001). Cultures of E1-9cT did not show such a change in morphology. Under phase-contrast microscopy, E1-9cT cells had a dark centre surrounded by a transparent outer layer; cells were autofluorescent when exposed to near-UV light, indicating the presence of F₄₂₀, a fluorescent coenzyme often present in high concentrations in methanogens (Garcia, 1990). The cell envelope was resistant to lysis with 0.1 % SDS although, at concentrations above 2 %, some evidence of envelope damage was observed.

Fig. 1. TEM of cells of strain E1-9cT. (a) Negative staining showing the regular coccus morphology. Bar, 500 nm. (b) Negative staining with low uranyl acetate addition and digital enhancement (see text) to allow contrast for the observation of multiple flagella (F). Bar, 500 nm. (c–f) Ultrathin sectioning microscopy showing several intracellular details such as S-layer (SL), cytoplasmic membrane (CM), division plane of the cell (DP), tubular-like S-layer-connecting structures (T) and unknown intracellular structures (US). Bars, 500 nm (c, e), 200 nm (d) and 100 nm (f).
**Table 1.** Physiological characteristics of strain E1-9c<sup>T</sup> and other representative cocoid and closely related methanogens belonging to the order *Methanomicrobiales*

Strains: 1, E1-9c<sup>T</sup> (data from this study); 2, *Candidatus Methanoregula boonei* 6A8 (Bräuer et al., 2006a); 3, *Methanolinea tarda* NOBI-1<sup>T</sup> (Imachi et al., 2008); 4, *Methanospirillum hungatii* JF-1<sup>T</sup> (Balch et al., 1979; Ferry et al., 1974); 5, *Methanoculleus palmoi* INSUZ<sup>T</sup> (Zellner et al., 1998); 6, *Methanofollis aquamari* N2<sup>F</sup>9704<sup>T</sup> (Lai & Chen, 2001); 7, *Methanocorpusculum aggregans* MS<sup>T</sup> (Ollivier et al., 1985); 8, *Methanoplanus petrolearius* SEBR 4847<sup>T</sup> (Ollivier et al., 1997); 9, *Methanocorpusculum parvum* XII<sup>T</sup> (Zellner et al., 1987); 10, *Methanocalculus halotolerans* SEBR 4845<sup>T</sup> (Ollivier et al., 1998). All strains used H<sub>2</sub>/CO<sub>2</sub> as methanogenic substrate. ND, No data available.

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
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<td>S</td>
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<td>5.1</td>
<td>7</td>
<td>6.6–7.4</td>
<td>6.9–7.5</td>
<td>6.5</td>
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<td>6.3–8.0</td>
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<td>30–37</td>
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<td><strong>NaCl concentration for growth (%)</strong></td>
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<td>ND</td>
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<td>8</td>
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<td>54.5</td>
<td>56.3</td>
<td>45</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
<td>ND</td>
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<td>−</td>
<td>ND</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>+</td>
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*F, Filaments; IC, irregular cocci; RC, regular cocci; S, spirilla; TR, thin rods.

TEM of negatively stained cells (Fig. 1b) showed that cells of E1-9c<sup>T</sup> possessed multiple flagella. Flagella were around 14 nm thick and could reach lengths of 8–12 μm. Flagella were readily lost under stress conditions such as high-speed centrifugation and oxic environments, perhaps explaining the absence of motility in light microscopy observations of wet mounts. An electron-dense cell wall about 30–70 nm thick was observed in thin sections (Fig. 1c, d, f), and negative staining of the cell surface did not show a pattern that was characteristic of outer S-layers (not shown). Most strains of the *Methanomicrobiales* are sensitive to lysis with dilute detergents because they possess a single S-layer cell wall, and strains with this type of cell wall commonly stain Gram-negative (Boone et al., 1993). Electron microscopy, resistance to SDS lysis and Gram-positive staining observations suggest that the cell wall of E1-9c<sup>T</sup> is different from a single S-layer and that this thick layer may play a role in adaptation to low-ionic-strength environments, analogous to the methanochondriotin layer in *Methanosarcina* species (Sprott & Beveridge, 1994). In thin sections, the cytoplasm of some cells apparently shrank away from the outer layer as a result of the osmotic strength of the 2% glutaraldehyde fixative, and tubular structures were visible in the intervening space (T in Fig. 1c, e, f). It has been suggested that these tubular structures connect the S-layer to the cytoplasmic membrane (Sprott & Beveridge, 1994) and they are identical in appearance to those observed in *Methanosarcina mazei* (Robinson, 1986). Additionally, in many cells, an unknown structure was observed in the cytoplasm (US in Fig. 1c, d, f) resembling arrays of protein complexes (~10 nm in diameter), that were less electron-dense than ribosomes. These structures do not resemble the ‘methanochondrión’ internal membrane systems, now believed to be preparation artefacts (Sprott & Beveridge, 1994). The potential protein complexes are similar in size to purified methylcoenzyme M methylreductase complexes (~10 nm) and smaller than F<sub>420</sub>-reducing hydrogenase complexes (~15 nm) (Wackett et al., 1987).

Our previous report (Cadillo-Quiroz et al., 2008) showed that E1-9c<sup>T</sup> is a mesophile with optimal growth at 28–30 °C and that it is mildly acidiphilic, with an optimum pH of about 5.5 and a narrow pH range of 4.8–6.4. Only a few
Other methanogens have been described with a pH optimum <5.5. ‘Candidatus Methanoregula booneii’ 6A8, in the order Methanomicrobiales, isolated from an acidic (pH 4.5) bog peatland, is the most acidiphilic methanogen described to date, with optimal pH around 5.1 (Brauer et al., 2006a). Methanobacterium espanolae, isolated from pulp mill waste sludge, represents the next most acidiphilic reported methanogen, with optimum pH around 5.6 (Patel et al., 1990). Strain E1-9cT has a lower optimum pH than that of the fen from which it was isolated (pH 6.5).

The optimum total Na+ concentration for strain E1-9cT was between 15 (background Na+ concentration in medium) and 35 mM; concentrations above 100 mM strongly inhibited growth (Supplementary Fig. S1, available in IJSEM Online). Strain E1-9cT tolerated 0.5 % NaCl, a value lower than those found for many strains isolated from other non-marine sources (Table 1). The inhibitory concentration of total Na+ for E1-9cT was nearly twice that of ‘Candidatus Methanoregula booneii’ (S. Brauer, personal communication). The difference in Na+ tolerance between E1-9cT and ‘Candidatus Methanoregula booneii’ could hypothetically reflect an adaptation to the difference in total Na+ concentration observed in the pore water of Michigan Hollow (~30 μM Na+) versus McLean Bog (~2 μM Na+) (Dettling et al., 2007), the respective sources of each bacterium. E1-9cT was also highly sensitive to Na2S concentrations. E1-9cT required Na2S as a sulfur source (Rajagopal & Daniels, 1986), but concentrations above 0.1 mM were inhibitory (Cadillo QUIROZ et al., 2008). Na2S is commonly used as a reducing agent at concentrations between 1 and 2 mM (Sowers & Noll, 1995).

In terms of methanogenic substrates, E1-9cT cultures used H2/CO2 and formate, but not acetate, trimethylamine, 2-propanol, methanol, ethanol, 2-butanol, propionate or sodium butyrate (all at 10 mM final concentration) as substrates for methanogenesis. With the exception of formate, none of these substrates were inhibitory during growth with H2/CO2. Formate additions (10 or 20 mM) caused a 3- to 4-week lag in growth in tubes also supplemented with H2/CO2 (Supplementary Fig. S2). Formate is commonly used by members of the order Methanomicrobiales (Boone et al., 2001); however, formate can be toxic at concentrations <100 mM at low pH (<6) and, in some strains, formate dehydrogenase activity is dependent on the presence of selenium (Belay et al., 1986; Jarrell & Kalmokoff, 1988; Sparling & Daniels, 1990). Therefore, additional experiments were performed to address the potential formate toxicity at low pH and the selenium requirement. At pH 5.7, strain E1-9cT was able to use formate at low concentrations, as shown by the stoichiometric increase of methane following multiple additions of 10–20 mM formate (Supplementary Fig. S2).
Formate additions above 50 mM inhibited growth of strain E1-9cT. Higher pH (6.4) or the addition of selenite (1 and 10 μM) did not improve the use of formate (Supplementary Fig. S2). The addition of 1 μM selenite accentuated the inhibitory effects of formate in tubes with H₂/CO₂ (Supplementary Fig. S2).

In addition to H₂/CO₂ as methanogenic substrate and mineral nutrients, E1-9cT required vitamins, coenzyme M and acetate for growth. The use of acetate as a carbon source has been observed in many H₂/CO₂-utilizing methanogens (Balch et al., 1979; Belay et al., 1986; Bott et al., 1985; Jarrell & Kalmokoff, 1988). For instance, Methanospirillum hungatei requires acetate at levels around 18 mM (Sprott & Jarrell, 1981) and acetate can contribute up to 65% of total cellular carbon in Methanothermobacter thermoautotrophicus (Fuchs et al., 1978). It was found that acetate concentrations around 4 mM were optimal for growth of E1-9cT under H₂/CO₂ and that concentrations above 20 mM did not further stimulate growth (data not presented). E1-9cT doubling times were ~30 h with 0.4 mM acetate (concentration used in the isolation of the strain) and ~19 h with 4 mM acetate under standard growth conditions of pH 5.7 and 30 °C.

Strain E1-9cT was resistant to penicillin, ampicillin, vancomycin, tetracycline, bacitracin, kanamycin, spectinomycin and rifampicin (all at 100 μg ml⁻¹), but chloramphenicol inhibited growth.

The full 16S rRNA gene and intergenic transcribed sequence were determined for strain E1-9cT and phylogenetic analysis showed that this strain was closely associated with environmental sequence subcluster ‘c’ of the E1 group (Cadillo-Quiroz et al., 2008). The E1 group is an independent lineage within the order Methanomicrobiales (Cadillo-Quiroz et al., 2006, 2008). Therefore, 16S rRNA gene and mcrA-based phylogenetic trees were constructed (Fig. 2) with predicted sequences of representative strains of the reported genera in this order. Sequences from the recently described methanogens ‘Candidatus Methanoregula boonei’ 6A8 (Bräuer et al., 2006a) and Methanolinea tarda NOBI-1T (Imachi et al., 2008; Sakai et al., 2007) were also included. Phylogenetic analysis based on 16S rRNA gene sequences showed that E1-9cT was divergent from all other described genera within the Methanomicrobiales and possessed no more than 85–92 % sequence identity to strains of any other genus. The phylogenetic branching order in Fig. 2(a) indicates that Methanospirillum, and hence the family Methanospirillaceae, is the most closely related group (90 % identity). However, in terms of sequence identity, the closest relatives of strain E1-9cT were Methanolinea tarda NOBI-1T (94 % identity) followed by ‘Candidatus Methanoregula boonei’ 6A8 and Methanoculleus palmolei INSLUZT (both with 92 % identity). Phylogenetic analysis of amino acid sequences predicted from the mcrA gene supported strain E1-9cT as an independent branch in the Methanomicrobiales although, when compared with the 16S rRNA gene sequence analysis, some differences were observed in the branching order and order of sequence similarity with its closest relatives (Fig. 2). McrA sequence analysis showed the following order in terms of the predicted amino acid sequence identities (%) to E1-9cT: Methanoculleus palmolei INSLUZT (83 %) as the closest, followed by Methanospirillum hungatei JF-1T (82 %) and then ‘Candidatus Methanoregula boonei’ 6A8 and Methanolinea tarda NOBI-1T (both 81 %). In contrast to the 16S rRNA gene analysis, NOBI-1T was more distantly related to E1-9cT; however, the branching positions of strains NOBI-1T, E1-9cT and 6A8 were highly variable in the multiple analyses of predicted amino acid sequences of the mcrA gene, indicating that these sequences did not provide a robust phylogeny for these organisms.

A comparison of phenotypic (Table 1) and phylogenetic (Fig. 2) characteristics of strain E1-9cT indicates its uniqueness among members of the order Methanomicrobiales, allowing us to propose it as the type strain of a novel species in a new genus, Methanosphaerula palustris gen. nov., sp. nov. It is proposed that this genus be a genus incertae sedis, temporarily not affiliated to any of the known families in this order. Phylogenetic analysis based on 16S rRNA gene sequences of members of the order Methanomicrobiales showed that the phylogenetic divergence of E1-9cT from members of the other families (85–92 % identity) was close to the divergence levels observed among the families themselves (82–89 %), which suggests that E1-9cT could represent a new family. It is expected that the more detailed genomic analysis of this strain presently under way and the isolation and description of additional related strains will allow a better evaluation of the taxonomy of these novel organisms within the Methanomicrobiales.

**Description of Methanosphaerula gen. nov.**

*Methanosphaerula* [Me.tha.no.sphae.ru’la. N.Gr. n. methyl-an (from N. Gr. n. methyl-yl and chemical suffix -ane) methane; L. fem. n. sphaerula a small sphere; N.L. fem. n. Methanosphaerula small spherical methane-producer].

Cells are coccis and are often found in pairs. Methanogenic and strictly anaerobic members of the domain *Archaea*. Mesophilic and mildly acidiphilic; produce methane from H₂/CO₂ and formate. The type species is *Methanosphaerula palustris*.

**Description of Methanosphaerula palustris sp. nov.**

*Methanosphaerula palustris* (pa.lus’tris. L. fem. adj. palus-tris marshy, swampy or muddy, living in marshes).

Cells stain Gram-positive and do not lyse with 0.1 % SDS. Cells are 0.5–0.8 μm in diameter and autofluorescent when exposed to near-UV light. The optimum pH is about 5.5; growth occurs at pH 4.8–6.4. The temperature optimum is about 30 °C with growth occurring at 14–35 °C. Optimal
growth is observed in 15–35 mM total Na\(^+\), but no growth is observed in the presence of \(\geq 100\) mM total Na\(^+\) or \(\geq 0.5\) % NaCl. Doubling time is about 19 h under optimal conditions. Methane is produced from H\(_2\)/CO\(_2\) or formate, but not from ethanol, methanol, 2-propanol, 2-butanol, acetate, propionate or butyrate. Formate concentrations \(>50\) mM are inhibitory at pH 5.5. Vitamins, coenzyme M, acetate (4 mM) and low concentrations of Na\(_2\)S (\(<0.08\) mM) are required for growth.

The type strain is E1-9c\(^T\) (=ATCC BAA-1565\(^T\) =DSM 19958\(^T\)), isolated from minerotrophic fen peatland. The DNA G+C content of the type strain is 58.9 \(\pm\) 2 mol%.

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References


Methanosphaerula palustris gen. nov., sp. nov.


