Methanobacterium Paludis Sp. Nov. And A Novel Strain Of Methanobacterium Lacus Isolated From Northern Peatlands

By: **Suzanna L. Brauer**, Hinsby Cadillo-Quiroz, Noah Goodson, Joseph B. Yavitt & Stephen H. Zinder

Abstract

Two mesophilic, hydrogenotrophic methanogens, designated strains SWAN1T and AL-21, were isolated from two contrasting peatlands: a near circumneutral temperate minerotrophic fen in New York State, USA, and an acidic boreal poor fen site in Alaska, USA, respectively. Cells of the two strains were rod-shaped, non-motile, stained Gram-negative and resisted lysis with 0.1 % SDS. Cell size was 0.6 1.5–2.8 mm for strain SWAN1T and 0.45–0.85 1.5–35 mm for strain AL-21. The strains used H2/CO2 but not formate or other substrates for methanogenesis, grew optimally around 32–37 6C, and their growth spanned through a slightly low to neutral pH range (4.7–7.1). Strain AL-21 grew optimally closer to neutrality at pH 6.2, whereas strain SWAN1T showed a lower optimal pH at 5.4–5.7. The two strains were sensitive to NaCl with a maximal tolerance at 160 mM for strain SWAN1T and 50 mM for strain AL-21. Na2S was toxic at very low concentrations (0.01–0.8 mM), resulting in growth inhibition above these values. The DNA G+C content of the genomes was 35.7 mol% for strain SWAN1T and 35.8 mol% for strain AL-21. Phylogenetic analysis of the 16S rRNA gene sequences showed that the strains are members of the genus Methanobacterium. Strain SWAN1T shared 94–97 % similarity with the type strains of recognized species of the genus Methanobacterium, whereas strain AL-21 shared 99 % similarity with Methanobacterium lacus 17A1T. On the basis of phenotypic, genomic and phylogenetic characteristics, strain SWAN1T (5DSM 25820T5JCM 18151T) is proposed as the type strain of a novel species, Methanobacterium paludis sp. nov., while strain AL-21 is proposed as a second strain of Methanobacterium lacus.

Suzanna L. Brauer, Hinsby Cadillo-Quiroz, Noah Goodson, Joseph B. Yavitt & Stephen H. Zinder (2014) "Methanobacterium Paludis Sp. Nov. And A Novel Strain Of Methanobacterium Lacus Isolated From Northern Peatlands" *International Journal of Systematic and Evolutionary Microbiology* volume 64 pp. 1473-1480 [10.1099/ijs.0.059964-0] Version of Record Available From www.webofknowledge.com

Peatlands cover about 5×10^6 km^2, are concentrated in northern latitudes (temperate to arctic regions), contain nearly one-third of all soil carbon (Gorham, 1991) and contribute about 20 % of the total annual CH_4 emissions into the atmosphere (Cicerone & Oremland, 1988). Thus, methanogenic *Archaea* that inhabit peatlands are important in global carbon and atmospheric CH_4 cycles. Peatlands include numerous habitats, such as acidic moss-dominated bogs and grass-dominated fens with near neutral pH (Gorham, 1991). Studies assessing the methanogenic *Archaea* from northern sites have shown that in addition to new uncultured archaeal lineages, phylotypes associated with the orders *Methanomicrobiales*, *Methanosarcinales*, *Methanobacteriales* and *Methanocellales* are the common inhabitants of these ecosystems (Basiliko *et al.*, 2003; Bridgham *et al.*, 2013; Galand *et al.*, 2003). The importance of each group varies depending on the peatland type, as observed in comparisons of the archaeal community of minerotrophic fens and bogs (Cadillo-Quiroz *et al.*, 2006, 2008; Juottonen *et al.*, 2005; Yavitt *et al.*, 2012). For example, the order *Methanosarcinales* seems to have the greatest diversity in fens, whereas *Methanomicrobiales* dominate in bogs. In contrast, the net contribution of members of the order *Methanobacteriales* to peatland methanogenesis is poorly understood.

Most of the *Methanobacteriales* sequences recovered from peatlands are phylogenetically related to the genus *Methanobacterium*, and have been found in boreal (Kotsyurbenko *et al.*, 2004) and temperate (Basiliko *et al.*,

2003; Cadillo-Quiroz *et al.*, 2006; Yavitt *et al.*, 2012) sites. Although the contribution of *Methanobacterium* species to peatland methanogenesis is not clear, *in vitro* peat soil manipulations showed that lower pH conditions increased the presence of *Methanobacterium* as well as the contribution of hydrogenotrophic methanogenesis (Kotsyurbenko *et al.*, 2007), suggesting a potential role of this group in some low pH peatlands.

The genus *Methanobacterium* contained eight formally accepted species in the most recent edition of *Bergey's Manual of Systematic Bacteriology* (Boone *et al.*, 2001). Since then, 12 other species have been formally described within the genus: *Methanobacterium lacus* (Borrel *et al.*, 2012), *Methanobacterium congolense* (Cuzin *et al.*, 2001), *Methanobacterium oryzae* (Joulian *et al.*, 2000), *Methanobacterium kanagiense* (Kitamura *et al.*, 2011), *Methanobacterium arcticum* (Shcherbakova *et al.*, 2011), *Methanobacterium veterum* (Krivushin *et al.*, 2010), *Methanobacterium aarhusense* (Shlimon *et al.*, 2004), *Methanobacterium petrolearium* and *Methanobacterium ferruginis* (Mori & Harayama, 2011), *Methanobacterium beijingense* (Ma *et al.*, 2005), *Methanobacterium movens* and *Methanobacterium flexile* (Zhu *et al.*, 2011). Members of the genus have been isolated from various sources, including marine and terrestrial environments (Garcia *et al.*, 2000; König, 1984). All species are capable of growth by reduction of CO_2 with H_2, but they present a broad range and variation in phenotypic and genomic characteristics, including catabolism of substrates and DNA G+C content (30–57 %) (Boone *et al.*, 2001).

Only *Methanobacterium palustre* (Zellner *et al.*, 1988) and *Methanobacterium* sp. strains MB2–MB4 (Kotsyurbenko *et al.*, 2007) have been isolated from peatlands. It is unlikely that these strains contain all the potential genetic and physiological diversity of the class *Methanobacteria* in peatlands. Thus, more isolates are necessary to assess their properties and their potential role in peatlands. The present study describes the isolation and characterization of two novel *Methanobacterium* strains from two contrasting sites: strain SWAN1^T, isolated from a minerotrophic fen in a cool temperate climate, is proposed as the type strain of a novel species of the genus *Methanobacterium*, and strain AL-21, isolated from a poor fen under a boreal climate, is proposed as a strain of *Methanobacterium lacus* (Borrel *et al.*, 2012).

Anaerobically sampled peat soil was collected in 2005 and 2006 from the shallow anaerobic layers (approx. 20 cm below the surface) of two peatlands with the following locations and characteristics. Michigan Hollow is a minerotrophic temperate fen in New York State located near Danby (42° 19′ N 76° 29′ W), a sedge-dominated site with near neutral pH (~6.5) (Cadillo-Quiroz *et al.*, 2008). Baxter fen is located near Anchorage, Alaska (61° 32′ N 150° 27′ W), and is an acidic poor fen with pH near 5.4 and is dominated by *Sphagnum* mosses and ericaceous shrubs. All samples were incubated as peat slurries supplemented with H_2/CO_2 and rifampicin as previously described (Bräuer *et al.*, 2006; Cadillo-Quiroz *et al.*, 2008). Tubes that showed high methane accumulation in their headspace were selected for subsequent transfer into anaerobic peat medium 2 (PM2).

PM2 composition and preparation were as previously described (Cadillo-Quiroz *et al.*, 2008) using the modified anaerobic technique of Hungate (Sowers & Noll, 1995). Several hours before inoculation, the following additions were made to the basal medium under sterile and anaerobic conditions: 1.0 mM titanium (III) nitrilotriacetate (7.2 ml 1 M Tris/HCl adjusted to pH 8, 4.8 ml 0.5 M sodium nitrilotriacetate and 0.55 ml 15 % titanium III chloride from Riedel-de-Haen), 10 mM MES (pK_a=6.2 at 28 °C, filter-sterilized 1 M stock solution adjusted to pH 7.8), 0.5 mM coenzyme M (2-mercapthoethanesulfonic acid), 0.4 mM sodium acetate, 1 % (v/v) vitamin solution (Balch *et al.*, 1979) and 0.4 mM Na_2S. The final liquid volumes in the tubes were approximately 5 ml, and 70.7 kPa H_2/CO_2 (80 %/20 %) was added to the headspaces unless otherwise specified.

Isolates were obtained after several dilutions to extinction (10^{-8} or 10^{-9}) in PM2 adjusted to pH 5.6. Purity was evaluated by microscopy, and growth with organic substrates such as yeast extract (0.2 g l^{-1}), glucose or lactate (20 mM), each in the absence of rifampicin, to test for heterotrophic contaminants.

Cell morphology and motility were examined under phase-contrast microscopy with a Nikon Eclipse E600 microscope equipped with a Hamamatsu CCD digital camera. Gram staining and susceptibility to SDS lysis were determined as described by Boone & Whitman (1988) with SDS final concentrations ranging from 0.1 to 2 % (w/v), and cell lysis determined by microscopic observations. Negative staining transmission electron microscopy was performed as described by 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. Micrographs were taken at 100 kV.

For experiments on the effect of pH, the pH was adjusted by addition of 30 mM MES adjusted to pH 4.5–7.6. The pH of the cultures was assessed at the end of incubations at 10 days of growth. For the Na_2S sensitivity test, filter-sterilized anaerobic solutions of $Na_2S \cdot 9H_2O$ were prepared with different concentrations so that the same volume of reagent (0.05 ml) was added to replicate 5 ml cultures. To determine the effect of sodium on the growth of strains SWAN1^T and AL-21, additions from sterile anaerobic NaCl stock solutions of different concentrations were made to achieve values of up to 250 mM. The background sodium content with no addition, from the different components of the medium, was approximately 15 mM. The following methanogenic substrates were individually tested: sodium formate and sodium acetate (2 mM), and methanol, 2-propanol, ethanol, 1-butanol, 2-butanol and trimethylamine

(all at 5 mM). Cultures were tested under (i) an N_2/CO_2 and (ii) an N_2 atmosphere with 10 p.s.i. (~69 kPa) H_2 overpressure to test for substrate utilization. In addition, parallel tubes were simultaneously grown (iii) under an H_2/CO_2 atmosphere to verify that amounts of substrates were not inhibitory. Treatments were monitored for CH_4 production using a gas chromatograph with a flame ionization gas detector as previously described (Cadillo-Quiroz *et al.*, 2006). Experiments were conducted in triplicate tubes and incubated for at least 29 days using a gyratory shaker at 28–30 °C and 200 r.p.m., except that temperature tests were performed at static conditions at the corresponding temperatures (4, 10, 16, 22, 28, 32, 37, 40 or 45 °C).

Full 16S rRNA and *mcrA* (alpha subunit of methyl-coenzyme M reductase) gene sequences were obtained from genome sequencing of strains SWAN1^T and AL-21 by the Joint Genome Institute under accession numbers NC_015574 and NC_015216, respectively. Phylogenetic analyses of 16S rRNA gene sequences were completed using ARB software (Ludwig *et al.*, 2004) and the 'Silva 111' database release (July 2012) (Quast *et al.*, 2013). The 16S rRNA gene sequence alignment was exported from ARB and phylogenetic trees were reconstructed by Bayesian analyses. The approximation of posterior probabilities was accomplished with MrBayes version 3.2.1 (Ronquist & Huelsenbeck, 2003) using four-chain Metropolis-coupled Markov chain Monte Carlo analysis. Bayesian consensus trees were built with a burnout of 500 and posterior probabilities were calculated. Tree topology was confirmed using the maximum-likelihood and neighbour joining methods (implemented in ARB) with Olsen evolutionary distance correction. Complementary analysis of the *mcrA* gene was done using nearly complete fragments, available at NCBI, translated to their predicted amino acid sequences (378 aa positions), aligned with CLUSTAL X and followed by tree reconstruction with the neighbour-joining method and 1000 bootstrap trees. DNA G+C measurements were obtained from the genome sequences.

Cells from the two isolates had a rod-shaped morphology, with somewhat variable dimensions (Fig. 1). Cell sizes were 0.6×1.5–2.8 and 0.45–0.85×1.5–35 μm for strains SWAN1^T and AL-21, respectively. Strain AL-21 showed significant variability in cell length, with early growing cultures commonly dominated by 'small' (up to 3 μm long) cells (Fig. 1d) while 'long' cells increased in frequency at late stages of growth (Fig. 1e and f). Cells from the two strains stained Gram-negative and resisted lysis with 0.1 or 2 % SDS. Cells of both strains were non-motile, which corresponded to the absence of flagella. However, fimbriae were observed by electron microscopy. In fact, strain SWAN1^T showed a high abundance of fimbriae and its cell surface had a rugose or coarse configuration (Fig. 1a–c).

Both strains were slightly acidiphilic, with differences in pH optima and range (Table 1 and Fig. S1 available in the online Supplementary Material). Strain SWAN1^T had a narrower pH range (4.8–6.6) with an optimum near pH 5.4–5.7, whereas strain AL-21 had broader pH range from 4.7 to 7.1 with an optimum near pH 6.2. Most members of the genus *Methanobacterium* have been found to grow well near neutral pH with a few alkaliphilic or

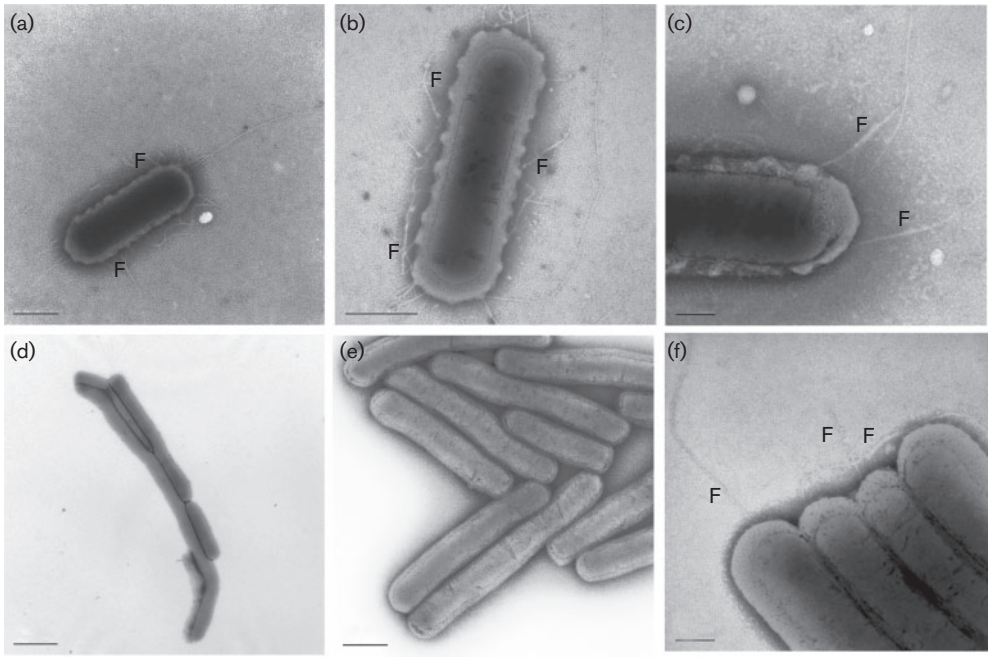

Fig. 1. Negative stain electron microscopy of cells of strains SWAN1^T (a–c) and AL-21 (d–f). F, fimbriae. Bars: (a, b, e), 0.5 μm; (c, f), 0.2 μm; (d), 2 μm.

Table 1. Physiological characteristics of the novel strains as compared with other species of the genus *Methanobacterium*

Strains: 1, SWAN1^T; 2, AL-21; 3, *Methanobacterium lacus* 17A1^T (data from Borrel *et al.*, 2012); 4, *M. congolense* C^T (Cuzin *et al.*, 2001); 5, *Methanobacterium palustre* DSM 3108^T (Zellner *et al.*, 1988); 6, *Methanobacterium beijingense* 8-2^T (Ma *et al.*, 2005); 7, *Methanobacterium veterum* MK4^T (Krivushin *et al.*, 2010); 8, *Methanobacterium uliginosum* DSM 2956^T (Konig, 1984); 9, *Methanobacterium ivanovii* DSM 2611^T (Belyaev *et al.*, 1986); 10, *Methanobacterium bryantii* DSM 863^T (Boone, 1987); 11, *Methanobacterium oryzae* DSM 11106^T (Joulian *et al.*, 2000); 12, *Methanobacterium subterraneum* DSM 11074^T (Kotelnikova *et al.*, 1998); 13, *Methanobacterium movens* TS-2^T (Zhu *et al.*, 2011); 14, *Methanobacterium alcaliphilum* DSM 3387^T (Worakit *et al.*, 1986). NG, Alcohols are oxidized but results in no growth; ND, not determined; G_s, genome sequencing; L_c, liquid chromatography; T_m, melting point; B_d, buoyant density; F, formate; $M+H_2$, methanol plus H_2; iP, 2-propanol; iB, isobutanol.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Source	Minerotrophic fen	Poor fen	Deep lake sediment	Anaerobic digester	Peat bog	Anaerobic digester	Permafrost	Marshy soil	Rock core	Anaerobic digester	Rice field	Granitic groundwater	Lake	Alkaline lake
Cell size (width × length, μm)	0.6 × 1.5–2.8	0.45–0.85 × 1.5–35	0.2–0.4 × 2–15	0.4–0.5 × 2–10	0.5 × 2.5–5	0.4–0.5 × 3–5	0.4–0.45 × 2–8	0.2–0.6 × 1.9–3.8	0.5–0.8 × 1–15	0.5–1 × 1.5–10	0.3–0.4 × 3–10	0.1 × 0.6–1.2	0.4–0.5 × 2–5	0.5–0.6 × 2–25
Substrates:														
F	−	−	−	−	+	+	−	−	−	−	+	+	−	−
$M+H_2$	−	−	+	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND
iP, iB	−	−	−	NG	+	−	−	−	−	NG	−	−	−	ND
Growth temperature (°C):														
Range	16–40	10–37	14–41	25–50	20–45	25–50	10–46	15–45	15–55	ND	20–42	3.6–45	10–50	ND
Optimum	32–37	32–37	30	37–42	33–37	37	28	40	45	37–39	40	20–40	35–38	37
pH for growth														
Range	4.8–6.6	4.7–7.1	5–8.5	5.9–8.2	ND	6.5–8	5.2–9.4	6–8.5	6.5–8.5	ND	6.0–8.5	6.5–9.2	6–9	7.0–9.9
Optimum	5.4–5.7	6.2	6.5	7.2	7	7.2	7.2–7.4	ND	7–7.4	6.9–7.2	7	7.8–8.8	7.2–7.5	8.1–9.1
NaCl range (M)	0–0.25	0–0.06	0–0.4	ND	0–0.3	0–0.5	0–0.3	ND	ND	ND	0–0.4	0.2–1.2	0–1.7	ND
DNA G+C content (mol%)	35.7 (G_s)	35.8 (G_s)	37 (L_c)	39.5 (L_c), 44.8 (T_m)*	34 (T_m)	38.9 (T_m)	22.8 (T_m)	29 (T_m)	36.6 (T_m)	33–38 (B_d)	31 (L_c)	54.5 (T_m)	39.1 (T_m)	57 (B_d)
Na_2S range (mM)	0–0.4	0–0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

*Measured by T_m (Ma *et al.*, 2005).

acidiphilic members (Boone *et al.*, 2001). *Methanobacterium espanolae* is the only formally described acidiphilic member in the genus, with the lowest pH range (pH 4.6–7) and optimum (pH 5.6–6.2) (Patel *et al.*, 1990) and the two strains described here showed a similar (AL-21) or slightly lower (SWAN1^T) pH range. Note that a recent report indicated that one *Methanobacterium* strain, MB-4, was able to grow down to pH 3.5 and had optimum growth at pH 5 (Kotsyurbenko *et al.*, 2007); however, no formal description of strain MB-4 has been published at the time of this report. Outside the order *Methanobacteriales*, there are only two other acidiphilic methanogens described: *Methanoregula boonei* and *Methanosphaerula palustris*, both members of the order *Methanomicrobiales* isolated from an acidic (Bräuer *et al.*, 2011) or circumneutral (Cadillo-Quiroz *et al.*, 2009) peatland.

Low amounts of NaCl (10–60 mM) added to the approximately 15 mM Na^+ already present in the medium slightly improved methanogenesis. Concentrations above 160 and 50 mM inhibited the growth of strains SWAN1^T and AL-21, respectively (Fig. S2). Also, strains AL-21 and SWAN1^T were susceptible to inhibition by Na_2S concentrations above 0.4 mM, in agreement with results of our previous studies, which have shown a wide variability of optimal and inhibitory Na_2S additions among methanogenic cultures (Bräuer *et al.*, 2011, 2004; Cadillo-Quiroz *et al.*, 2009). However, many cultured strains are tolerant to Na_2S concentrations around or above 1 mM, as is typically used for reducing anaerobic media (Jarrell & Kalmokoff, 1988). Na_2S sensitivity has been observed in other isolates from peatlands (Bräuer *et al.*, 2011; Cadillo-Quiroz *et al.*, 2009). Thus, for some environments, inhibitory effects may limit the culturability of methanogens in sulfide-containing media.

The two strains were mesophiles with optimal growth between 32 and 37 °C, and a range of growth of 16–40 and 10–37 °C for strains SWAN1^T and AL-21, respectively. In terms of methanogenic substrates, both strains demonstrated growth exclusively with H_2/CO_2 and no significant growth or methanogenesis was observed with the other tested substrates under an N_2/CO_2 or N_2/H_2 atmosphere, and none of the substrates inhibited growth under H_2/CO_2. Coenzyme M and acetate (4 mM) were found to stimulate growth. Acetate is used by hydrogenotrophic methanogens as a carbon source stimulating growth (Borrel *et al.*, 2012; Bräuer *et al.*, 2011; Krivushin *et al.*, 2010; Ma *et al.*, 2005). Under optimal conditions the estimated doubling time for strains SWAN1^T and AL-21 was approximately 35 h, as calculated from the methane production rate.

In terms of 16S rRNA gene sequence phylogeny, the two strains were found to be in the order *Methanobacteriales* within the genus *Methanobacterium* (Fig. 2). Strain SWAN-1^T was related closely to *Methanobacterium* sp. strain MB-4, *Methanobacterium congolense* C^T and '*Methanobacterium curvum*' at 99, 97 and 96 % sequence similarity. Levels of 16S rRNA gene sequence similarity with the type strains of other species of the genus *Methanobacterium* were between 95 and 96 %. Strain MB-4 was isolated from an acidic Siberian bog (Kotsyurbenko *et al.*, 2007), and no formal taxonomic description has been published up to the time of completion of this report. Strain AL-21 was closely related (99 % 16S rRNA gene sequence similarity) to

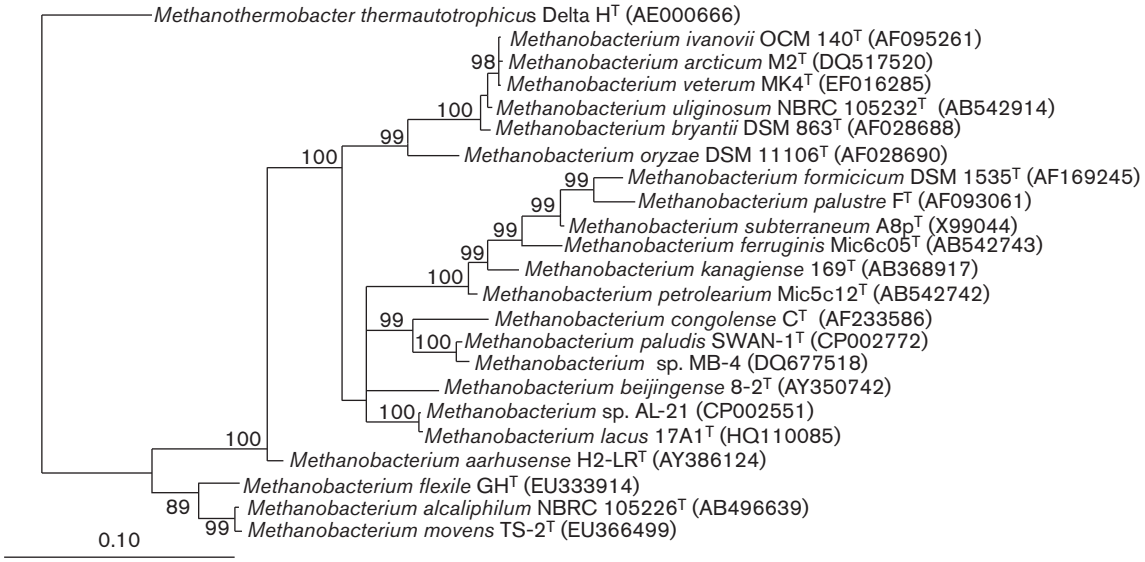

Fig. 2. 16S rRNA gene sequence phylogenetic analysis of strains SWAN1^T and AL-21 and members of the order *Methanobacteriales*. Strain numbers are given following the species name, and accession numbers for 16S rRNA gene sequences are given in parentheses. Posterior probabilities above 89 % are indicated at corresponding nodes in the tree. Bar, 0.1 changes per nucleotide position.

Methanobacterium lacus 17A1^T (Borrel *et al.*, 2012), and to clones from a pH 4.5 H_2/CO_2 enrichment culture derived from a German acidic bog (98 % similarity) (Horn *et al.*, 2003). The *mcrA* gene sequence tree (Fig. S3) showed a similar topology for the close relatives of strain SWAN-1^T with nucleotide sequence similarity of 97, 87 and 79 to strain MB-4, *Methanobacterium congolense* C^T and strain AL-21, respectively. The *mcrA* gene tree supported strain AL-21 as an independent lineage although bootstrap support for this was low and the *mcrA* gene sequence of *Methanobacterium lacus* 17A1^T was not available for comparison. The DNA G+C content from full genome sequencing was 35.7 and 35.8 mol% for strains SWAN1^T and AL-21, respectively.

The differences in sequence similarity between strain SWAN1^T and other species in this group were as low as or lower than among the recognized species, suggesting that strain SWAN1^T represents a novel species in the genus *Methanobacterium*. The high sequence similarity (99 %) between strain AL-21 and *Methanobacterium lacus* 17A1^T (Borrel *et al.*, 2012) suggests the two strains are members of the same species. However, note that recent reports on *Methanobacterium* species taxonomy have found that even strains sharing 99 % 16S rRNA gene sequence similarity can be considered to represent different species in conjunction with other genomic assessments including DNA G+C content and DNA–DNA hybridization (Shcherbakova *et al.*, 2011). Differences in DNA G+C content between strain AL-21 (35.8 mol%) and *Methanobacterium lacus* 17A1^T (37 mol%) are within limits previously found between different species of the genus *Methanobacterium* with 99 % 16S rRNA gene sequence similarity (Shcherbakova *et al.*, 2011). However, DNA–DNA hybridization tests between strain AL-21 and *Methanobacterium lacus* 17A1^T have been precluded by the lack of growth of strain 17A1^T to densities high enough to provide sufficient DNA yields for those tests (H. Cadillo-Quiroz, personal observation; DSMZ, personal communication). A more fine-scale approach with less DNA demand such as high-throughput genome sequencing of *Methanobacterium lacus* 17A1^T for comparison with the already available genome of strain AL-21 could be used to further resolve the taxonomic placement of these strains. The genome of strain 17A1^T was not available at the time of completion of this work.

Based on morphological, physiological (Table 1) and phylogenetic characteristics, these two novel isolates are considered to be members of the genus *Methanobacterium*. Several lines of physiological evidence were found differentiating these strains from their closest formally described relative. Shorter cells, a lower optimal pH and range, lower optimal temperature and range, and variable DNA G+C content (3–9 % difference) differentiated strain SWAN1^T from *Methanobacterium congolense* C^T. Strain AL-21 also showed several physiological characteristics differentiating it from *Methanobacterium lacus* 17A1^T, including a lack of growth on methanol in the presence of H_2, a lower optimal pH and range, a narrower temperature range and higher optimal temperature, and a nearly ten times higher sensitivity to NaCl. However, strain AL-21 and *Methanobacterium lacus* 17A1^T also showed a high genetic similarity (99 %), and sufficient genomic evidence is not available for their separation as independent groups. Therefore, we suggest that strain SWAN1^T represents a novel species of the genus *Methanobacterium*, for which the name *Methanobacterium paludis* sp. nov. is proposed, and provisionally propose that strain AL-21 represents a second strain of *Methanobacterium lacus*.

Description of *Methanobacterium paludis* sp. nov.

Methanobacterium paludis (pa.lu′dis. L. gen. n. *paludis* of a marsh).

Cells are Gram-stain-negative, non-motile rods (1.5–2.8 μm) that are resistant to lysis by 0.1 % (w/v) SDS. The cell surface has a rugose appearance when observed by electron microscopy. Cells are obligately hydrogenotrophic and do not consume formate or other substrates in the absence or presence of H_2. Growth is observed over a pH range of 4.8–6.6 with an optimum near pH 5.4–5.7, and over a temperature range of 16–40 °C with optimal growth around 32–37 °C. Sensitive to sodium ions and the reducing agent Na_2S, with inhibition occurring above 160 and 0.4 mM, respectively.

The type strain, SWAN1^T (=DSM 25820^T=JCM 18151^T), was isolated from Michigan Hollow fen, a minerotrophic circumneutral (~pH 6.5) peatland in New York State, USA. The genomic G+C content of the type strain is 35.7 mol%, as determined by genome sequencing.

Acknowledgements

We thank Dr. K. Duddleston at the University of Alaska Faribanks Anchorage for generous assistance with field sampling at Baxter Fen. We also thank A. Qadir and A. Hammond-Cadillo for their help with media preparation and comments on the manuscript.

References

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Mol Biol Rev* **43**, 260–296.

Basiliko, N., Yavitt, J. B., Dees, P. M. & Merkel, S. (2003). Methane biogeochemistry and methanogen communities in two Northern peatland ecosystems, New York State. *Geomicrobiol J* **20**, 563–577.

Belyaev, S. S., Obraztsova, A. Y., Laurinavichus, K. S. & Bezrukova, L. V. (1986). Characteristics of rod-shaped methane-producing bacteria from an oil pool and description of *Methanobacterium ivanovii* sp. nov. *Microbiology* **55**, 821–826.

Boone, D. R. (1987). Replacement of the type strain of *Methanobacterium formicicum* and reinstatement of *Methanobacterium bryantii* sp. nov. nom. rev. (Ex Balch and Wolfe, 1981) with M.o.H. (DSM 863) as the type strain. *Int J Syst Bacteriol* **37**, 172–173.

Boone, D. R. & Whitman, W. B. (1988). Proposal of minimal standards for describing new taxa of methanogenic bacteria. *Int J Syst Bacteriol* **38**, 212–219.

Boone, D. R., Whitman, W. B. & Koga, Y. (2001). Order I. *Methanobacteriales.* In *Bergey's Manual of Systematic Bacteriology*, pp. 214–245. Edited by D. R. Boone & G. M. Castenholz. New York: Springer.

Borrel, G., Joblin, K., Guedon, A., Colombet, J., Tardy, V., Lehours, A.-C. & Fonty, G. (2012). *Methanobacterium lacus* sp. nov., isolated from the profundal sediment of a freshwater meromictic lake. *Int J Syst Evol Microbiol* **62**, 1625–1629.

Bräuer, S. L., Yavitt, J. B. & Zinder, S. H. (2004). Methanogenesis in McLean Bog, an acidic peat bog in upstate New York: stimulation by H_2/CO_2 in the presence of rifampicin, or by low concentrations of acetate. *Geomicrobiol J* **21**, 433–443.

Bräuer, S. L., Yashiro, E., Ueno, N. G., Yavitt, J. B. & Zinder, S. H. (2006). Characterization of acid-tolerant H/CO-utilizing methanogenic enrichment cultures from an acidic peat bog in New York State. *FEMS Microbiol Ecol* **57**, 206–216.

Bräuer, S. L., Cadillo-Quiroz, H., Ward, R. J., Yavitt, J. B. & Zinder, S. H. (2011). *Methanoregula boonei* gen. nov., sp. nov., an acidiphilic methanogen isolated from an acidic peat bog. *Int J Syst Evol Microbiol* **61**, 45–52.

Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K. & Zhuang, Q. (2013). Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. *Glob Change Biol* **19**, 1325–1346.

Cadillo-Quiroz, H., Bräuer, S., Yashiro, E., Sun, C., Yavitt, J. & Zinder, S. (2006). Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environ Microbiol* **8**, 1428–1440.

Cadillo-Quiroz, H., Yashiro, E., Yavitt, J. B. & Zinder, S. H. (2008). Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen. *Appl Environ Microbiol* **74**, 2059–2068.

Cadillo-Quiroz, H., Yavitt, J. B. & Zinder, S. H. (2009). *Methanosphaerula palustris* gen. nov., sp. nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland. *Int J Syst Evol Microbiol* **59**, 928–935.

Cicerone, R. J. & Oremland, R. S. (1988). Biogeochemical aspects of atmospheric methane. *Global Biogeochem Cycles* **2**, 299–327.

Cuzin, N., Ouattara, A. S., Labat, M. & Garcia, J. L. (2001). *Methanobacterium congolense* sp. nov., from a methanogenic fermentation of cassava peel. *Int J Syst Evol Microbiol* **51**, 489–493.

Firtel, M., Southam, G., Mok, T., Harris, R. & Beveridge, T. J. (1995). Electron microscopy techniques for the Archaea. In *Archaea: a laboratory manual. Methanogens*, pp. 123–140. Edited by K. R. Sowers & H. J. Schreier. Cold Spring Harbor, NY: Cold Spring Harbor.

Galand, P. E., Fritze, H. & Yrjälä, K. (2003). Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. *Environ Microbiol* **5**, 1133–1143.

Garcia, J. L., Patel, B. K. C. & Ollivier, B. (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic *Archaea*. *Anaerobe* **6**, 205–226.

Gorham, E. (1991). Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecol Appl* **1**, 182–195.

Horn, M. A., Matthies, C., Küsel, K., Schramm, A. & Drake, H. L. (2003). Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl Environ Microbiol* **69**, 74–83.

Jarrell, K. F. & Kalmokoff, M. L. (1988). Nutritional requirements of the methanogenic archaebacteria. *Can J Microbiol* **34**, 557–576.

Joulian, C., Patel, B. K. C., Ollivier, B., Garcia, J. L. & Roger, P. A. (2000). *Methanobacterium oryzae* sp. nov., a novel methanogenic rod isolated from a Philippines ricefield. *Int J Syst Evol Microbiol* **50**, 525–528.

Juottonen, H., Galand, P. E., Tuittila, E.-S., Laine, J., Fritze, H. & Yrjälä, K. (2005). Methanogen communities and *Bacteria* along an ecohydrological gradient in a northern raised bog complex. *Environ Microbiol* **7**, 1547–1557.

Kitamura, K., Fujita, T., Akada, S. & Tonouchi, A. (2011). *Methanobacterium kanagiense* sp. nov., a hydrogenotrophic methanogen, isolated from rice-field soil. *Int J Syst Evol Microbiol* **61**, 1246–1252.

König, H. (1984). Isolation and characterization of *Methanobacterium uliginosum* sp. nov. from a marshy soil. *Can J Microbiol* **30**, 1477–1481.

Kotelnikova, S., Macario, A. J. & Pedersen, K. (1998). *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater. *Int J Syst Bacteriol* **48**, 357–367.

Kotsyurbenko, O. R., Chin, K.-J., Glagolev, M. V., Stubner, S., Simankova, M. V., Nozhevnikova, A. N. & Conrad, R. (2004). Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ Microbiol* **6**, 1159–1173.

Kotsyurbenko, O. R., Friedrich, M. W., Simankova, M. V., Nozhevnikova, A. N., Golyshin, P. N., Timmis, K. N. & Conrad, R. (2007). Shift from acetoclastic to H_2-dependent methanogenesis in a west Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium* strain. *Appl Environ Microbiol* **73**, 2344–2348.

Krivushin, K. V., Shcherbakova, V. A., Petrovskaya, L. E. & Rivkina, E. M. (2010). *Methanobacterium veterum* sp. nov., from ancient Siberian permafrost. *Int J Syst Evol Microbiol* **60**, 455–459.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Ma, K., Liu, X. & Dong, X. (2005). *Methanobacterium beijingense* sp. nov., a novel methanogen isolated from anaerobic digesters. *Int J Syst Evol Microbiol* **55**, 325–329.

Mori, K. & Harayama, S. (2011). *Methanobacterium petrolearium* sp. nov. and *Methanobacterium ferruginis* sp. nov., mesophilic methanogens isolated from salty environments. *Int J Syst Evol Microbiol* **61**, 138–143.

Patel, G. B., Sprott, G. D. & Fein, J. E. (1990). Isolation and characterization of *Methanobacterium espanolae* sp. nov., a mesophilic, moderately acidiphilic methanogen. *Int J Syst Bacteriol* **40**, 12–18.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41** (D1), D590–D596.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Shcherbakova, V. A. N., Rivkina, E. M., Pecheritsyna, S. A., Laurinavichius, K. S., Suzina, N. E. & Gilichinsky, D. A. (2011). *Methanobacterium arcticum* sp. nov., a methanogenic archaeon from Holocene Arctic permafrost. *Int J Syst Evol Microbiol* **61**, 144–147.

Shlimon, A. G., Friedrich, M. W., Niemann, H., Ramsing, N. B. & Finster, K. (2004). *Methanobacterium aarhusense* sp. nov., a novel methanogen isolated from a marine sediment (Aarhus Bay, Denmark). *Int J Syst Evol Microbiol* **54**, 759–763.

Sowers, K. R. & Noll, K. M. (1995). Techniques for anaerobic growth. In *Archaea: a Laboratory Manual Methanogens*, pp. 15–48. Edited by F. T. Robb, A. R. Place, K. R. Sowers, H. J. Schreier, S. DasSarma & E. M. Fleischmann. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Worakit, S., Boone, D. R., Mah, R. A., Abdelsamie, M. E. & Elhalwagi, M. M. (1986). *Methanobacterium alcaliphilum* sp. nov., an H_2 utilizing methanogen that grows at high pH values. *Int J Syst Bacteriol* **36**, 380–382.

Yavitt, J., Yashiro, E., Cadillo-Quiroz, H. & Zinder, S. (2012). Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region, North America. *Biogeochemistry* **109**, 117–131.

Zellner, G., Bleicher, K., Braun, E., Kneifel, H., Tindall, B. J., Macario, E. C. & Winter, J. (1988). Characterization of a new mesophilic, secondary alcohol-utilizing methanogen, *Methanobacterium palustre* spec. nov. from a peat bog. *Arch Microbiol* **151**, 1–9.

Zhu, J., Liu, X. & Dong, X. (2011). *Methanobacterium movens* sp. nov. and *Methanobacterium flexile* sp. nov., isolated from lake sediment. *Int J Syst Evol Microbiol* **61**, 2974–2978.