Pelolinea submarina gen. nov., sp. nov., an anaerobic, filamentous bacterium of the phylum Chloroflexi isolated from subseafloor sediment

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A novel, anaerobic filamentous bacterium, strain MO-CFX1T, was isolated from a methanogenic community, which was originally established from subseafloor sediments collected from off the Shimokita Peninsula, Japan. Cells were non-spore-forming, non-motile, Gram-stain-negative and filamentous. The filaments were longer than 10 μm and 130–150 nm in width. Growth of the strain was observed at 10–37 °C (optimum 25–30 °C), at pH 5.5–8.5 (optimum pH 7.0) and in 0–50 g NaCl l⁻¹ (optimum 15 g NaCl l⁻¹). The strain was able to grow with a number of carbohydrates in the presence of yeast extract. The major cellular fatty acids were monounsaturated C₁₈ : ₁v₉, C₁₆ : ₁v₇ and saturated C₁₈ : ₀ and C₁₆ : ₀. The intact polar lipids of the strain were dominated by diacylglyceride and sphingolipid core lipid structures with monoglycosidic, mixed phosphomonoglycosidic and fatty-acid-modified monoglycosidic polar head groups. The G+C content of the genomic DNA was 52.4 mol%. Based on the comparative 16S rRNA gene sequence analysis, strain MO-CFX1T was affiliated with the class Anaerolineae within the phylum Chloroflexi and was most closely related to Leptolinea tardivitalis YMTK-2T (sequence identity of 91.0%). Based on phenotypic and genetic properties of the novel isolate, we propose a novel species representing a new genus Pelolinea submarina gen. nov., sp. nov., for strain MO-CFX1T (＝JCM 17238T＝KCTC 5975T). This is the first formal description, to our knowledge, of an isolate of the phylum Chloroflexi from the deep-sea sedimentary environment.

Culture-independent molecular studies based on a survey of PCR-amplified 16S rRNA genes have revealed members of the bacterial phylum Chloroflexi to be one of the dominant bacterial components in subseafloor sedimentary environments (Blazejak & Schippers, 2010; Fry et al., 2008; Inagaki et al., 2006; Inagaki & Nakagawa, 2008; Teske, 2013; Yamada & Sekiguchi, 2009). Most of the 16S rRNA gene sequences of members of the phylum Chloroflexi belong to members of the classes Anaerolineae, Caldilineae and Dehalococcoidia. These findings strongly indicate that members of the phylum Chloroflexi play an important role in biogeochemical cycles in the marine subsurface environment. However, until now, cultured representatives from these environments have not been available and their detailed physiological, genetic and ecological properties are currently not well understood.

Recently, we have successfully isolated micro-organisms of the class Anaerolineae, designated strains MO-CFX1T and MO-CFX2, from marine subsurface sediments collected from off the Shimokita Peninsula of Japan in the northwestern Pacific Ocean (Site C9001, 1180 m water depth) (Imachi et al., 2011). Enrichment and isolation of these strains were achieved using a continuous-flow bioreactor...
approach. In the previous publication, we described isolation of these micro-organisms, but we did not characterize the strains fully. We have successfully maintained both isolates, but stable growth and sufficient cell yield were only obtained from the cultures of strain MO-CFX1\textsuperscript{T}. Therefore, in this report, we describe the detailed isolation procedure, morphological, physiological and genetic properties of strain MO-CFX1\textsuperscript{T}. In addition, we also propose this strain as a member of a novel species of a new genus. This is the first formal description, to our knowledge, of an isolate of the phylum Chloroflexi from the deep-sea sedimentary environment.

The basal medium used in this study was prepared with the following components (1\textsuperscript{−1}): 0.53 g NH\textsubscript{4}Cl, 0.1 g KH\textsubscript{2}PO\textsubscript{4}, 4 g MgCl\textsubscript{2}.6H\textsubscript{2}O, 1 g CaCl\textsubscript{2}.2H\textsubscript{2}O, 25 g NaCl, 2 g NaHCO\textsubscript{3}, 0.3 g Na\textsubscript{2}S, 9H\textsubscript{2}O, 0.3 g cysteine. HCl, 1 ml trace element solution (Imachi et al., 2008), 1 ml vitamin solution (Imachi et al., 2009) and resazurin solution (1 mg ml\textsuperscript{−1}). The primary enrichment culture was cultivated anaerobically at 10 °C. For the roll-tube isolation method, solid medium was prepared by adding purified agar (Agar Noble; Difco) to the basal medium at a final concentration of 20 g l\textsuperscript{−1}. After isolation of the strain, all cultivations were performed at 30 °C in 50 ml serum vials containing 20 ml medium (pH at 25 °C, 7.2) under an atmosphere of N\textsubscript{2}/CO\textsubscript{2} (80:20, by volume) without shaking, unless otherwise stated. The serum vials were sealed with butyl rubber stoppers and aluminium crimp seals. Neutralized substrates were added to vials containing basal medium from stock solutions prior to inoculation.

Growth and substrate utilization were determined from OD\textsubscript{400}. All incubations for the substrate utilization test were performed using exponentially growing cultures (10 % inoculum, by volume) at 30 °C for over 3 months. Effects of pH, temperature and NaCl concentration on the growth of strain MO-CFX1\textsuperscript{T} were determined in basal medium containing 20 mM glucose plus 0.1 % (w/v) yeast extract (Difco) medium. To determine the pH range for growth, the medium was adjusted at room temperature to pH 5.0–9.0 with HCl or NaOH solutions under a 100 % N\textsubscript{2} atmosphere prior to inoculation. The pH of the medium was monitored every 4 days during growth using a portable pH meter (Twin pH B-212; Horiba), and the pH was readjusted by using HCl or NaOH if the initial pH had changed significantly. To evaluate the temperature range for growth, cultures were incubated at 0, 2, 4, 10, 15, 20, 25, 30, 37, 45, 50, 55 and 60 °C (pH 7.0). NaCl requirements were determined with varying concentrations of NaCl from 0 to 120 g l\textsuperscript{−1} in the basal medium. In co-culture test, Methanobacterium sp. MO-MB1, a hydrogenotrophic methanogen isolated from the enriched methanogenic community in the same bioreactor (Imachi et al., 2011), was added to the medium at 5 % inoculum (v/v). Effects of antibiotics on growth were evaluated by supplementing the glucose–yeast extract medium with each antibiotic at a final concentration of 50 \mu g ml\textsuperscript{−1}. All incubations for these tests were performed in triplicate culture vessels.

Cell morphology was examined under a fluorescence microscope (BX51F; Olympus) with a colour CCD camera system (DP72; Olympus). The Gram-staining reaction was performed by the Hucker’s method (Doetsch, 1981). Transmission electron microscopy of negatively stained cells was carried out as described by Zillig et al. (1990). Cells grown at 30 °C in the exponential growth phase were negatively stained with 2 % (w/v) phosphotungstic acid and observed under a JEM-1210 electron microscope (JEOL). For ultrathin sectioning, cells were prefixed with 2.5 % glutaraldehyde in the glucose–yeast extract medium at 30 °C for 2 h and then post-fixed in 2 % osmium tetroxide in 0.1 M PBS (Wako) at 4 °C for 2 h. The fixed cells were stained en bloc with 1 % aqueous uranyl acetate for 90 min at room temperature, dehydrated in a graded ethanol series and embedded in EPON812 (TAAB). Cells were sectioned with an ultramicrotome (Ultracut S; Leica Microsystems) and were observed using a JEM-1210 electron microscope (JEOL).

Lipids were extracted in a Teflon tube after addition of combusted sea sand with a modified Bligh and Dyer approach with phosphate buffer and an ultrasonic probe (HD2200; Bandelin Electronic) following the protocol described by Sturt et al. (2004). After phase separation, the organic phase was dried under a nitrogen stream and stored as total lipid extract (TLE) at −20 °C. An aliquot of the TLE was saponified by reacting with 500 μl KOH (6 % in methanol) for 2.5 h at 70 °C, quenching of the reaction with 1 ml water and 200 μl of 2 M hydrochloric acid and extraction with n-hexane in several steps before evaporation. Fatty acids were converted to methyl esters by reaction with 400 μl boron trifluoride in methanol (Elvert et al., 2003). In order to properly detect hydroxylated fatty acids, the fatty acid methyl esters (FAMEs) were further derivatized to trimethylsilyl-ethers by treatment with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine at 70 °C for 1 h. Analysis was performed on 6890N-GC (Agilent) coupled to a 5973 inert mass selective detector (Agilent). Identification of fatty acid derivatives was based on comparison of retention time and mass spectra with standards and commercial spectral libraries. Quantification of relative concentrations of fatty acids was based on integration of total ion current chromatograms. Intact polar lipids (IPLs) were directly analysed from the TLE using a Ultimate 3000RS UHPLC system (Dionex) coupled to a maXis ultrahigh resolution quadrupole time of flight mass spectrometer (Bruker) and using an Acquity BEH Amide column (Waters) in hydrophilic interaction chromatography mode with electrospray ionization (Wo¨rmer et al., 2013). Identification of IPLs was based on sum formulas calculated from exact mass (mass accuracy better than 2 p.p.m.) and comparison of fragment spectra with typical fragmentation pathways in positive and negative ionization. The G+C content of the DNA was determined by HPLC with a DNA-GC kit (Yama sh Shoyu) after digestion of the DNA with nuclease P1.

Procedures for DNA extraction, PCR amplification, cloning and sequencing were performed as described...
previously (Imachi et al., 2011). The nearly full-length 16S rRNA gene sequence of the isolate was amplified with a bacterial universal primer pair 8f/UN1490R. The comparative 16S rRNA sequence phylogenetic analysis was performed as described elsewhere (Imachi et al., 2006). To estimate the confidence of the tree topologies, bootstrap-resampling analysis with 1000 replicates was performed for neighbour-joining, maximum-parsimony and maximum-likelihood methods using the MEGA 5.1 program package (Tamura et al., 2011). 16S rRNA gene sequence identity values were calculated using the Calculate Matrix function of the ARB program with Jukes and Cantor correction (Jukes & Cantor, 1969; Ludwig et al., 2004).

Carbohydrates, short-chain fatty acids and alcohols were determined by HPLC using a Rezex ROA-Organic Acid Aminex HPX-87H column (Phenomenex; eluent, 0.0025 M H2SO4; column temperature, 50 °C), a UV-VIS detector (SPD-10Avp; Shimadzu) and a refractive index detector (RID-10A; Shimadzu). Hydrogen, methane and carbon dioxide were measured by GC (model GC-3200; GL Science; detector type, thermal conductivity detector; packing material, SHINCARBON; column temperature, 100 °C).

To isolate a variety of anaerobic micro-organisms in the bioreactor, the enriched methanogenic community was inoculated into basal media containing various substrates in 50 ml serum vials. Of these, we found that filamentous micro-organisms morphologically resembling bacteria of the class Anaerolineae dominated in a yeast extract [0.01 % (w/v)] medium supplemented with rifampicin, which was incubated at 10 °C. The enrichment culture also contained rod-shaped and coccoid microbes as minor populations. The cell density of microbes in the medium was very low: turbidity of the culture was not clearly visible. Therefore, the following cultivation conditions were tested to obtain better cell yields of the filamentous microbe in the subsequent cultures: yeast extract concentration was increased from 10 to 20 %, 10 mM glucose was added as an additional energy source and incubation temperature was increased from 10 to 20 °C. As a result, cell density of the filamentous microbe drastically increased and the filamentous cells became the dominant population in the culture after two successive transfers (>98 % of the population based on distinct cell morphology). To identify the microbes present in the enrichment culture, we performed 16S rRNA gene-based clone analysis. The analysis indicated that all eight of the bacterial rRNA gene clones were identical and affiliated with the class Anaerolineae within the phylum Chloroflexi. To isolate the microbe of the class Anaerolineae, the roll-tube method was employed twice. However, no colony formation was observed for over 6 months. Purification of the microbe of the class Anaerolineae was performed by serial dilution in liquid media containing 10 mM glucose, 0.1 % yeast extract and 50 μg rifampicin ml−1. After two successive transfers, we obtained a pure culture of the member of the class Anaerolineae, strain MO-CFX1T. The purity of strain MO-CFX1T was demonstrated by the failure to grow any strain other than strain MO-CFX1T in a thioglycollate medium (Difco) containing approximately 2 mM sucrose, 2 mM glucose, 2 mM fructose and 2 mM xylose and an AC medium (Difco) at 10, 25 or 55 °C. In addition, we evaluated purity by the failure to recover archaeal 16S rRNA gene amplification products by PCR with archaeal universal primer pairs Arc9F/Ar912r and Arc9F/UN1490R. This PCR survey also indicated that the MO-CFX1T culture was axenic.

Cells of strain MO-CFX1T were filaments longer than 10 μm (Fig. S1, available in IJSEM Online). Cell width of the strain was 130–150 nm. Electron microscopy showed that the strain did not possess a clearly visible sheath-like structure (Fig. S1). The cells were Gram-staining-reaction negative. Spore formation and motility were not observed under any of the conditions we tested. The strain was strictly anaerobic because its growth was completely inhibited in the presence of trace quantities of oxygen (0.1 and 0.2 % O2, by volume). Strain MO-CFX1T required yeast extract for growth and could grow with yeast extract as sole energy source (clear cell growth could be observed at ≥0.1 % yeast extract). In the presence of 0.01 % yeast extract, strain MO-CFX1T could utilize the following substrates (20 mM final concentration unless otherwise indicated): glucose, ribose, arabinose, fructose, sucrose, galactose, raffinose, pectin (5 g l−1), xylose (5 g l−1), xylan (5 g l−1) and starch (5 g l−1). In medium containing 20 mM glucose plus 0.1 % yeast extract, major fermentative end products were acetate, lactate, ethanol and hydrogen. Small amounts of pyruvate and propionate were also produced. The following substrates did not support growth with 0.01 % yeast extract: Casamino acids (0.1 %), tryptone (0.1 %), peptone (0.1 %), crotonate, H2/CO2 (80:20, v/v, approximately 100 kPa in head space) plus acetate (1 mM), betaine, mannose, lactate, glycerol (5 mM), formate (10 mM) plus acetate (1 mM), acetate, propionate, butyrate, malate, fumarate, succinate, pyruvate, ethanol (10 mM) and 1-propanol (10 mM). In addition, strain MO-CFX1T did not reduce the following inorganic substances in glucose–yeast extract medium: sulphate, sulphite (2 mM), thiosulphate, elemental sulphur, nitrate (10 mM) and Fe(III)-nitritolriacetate (5 mM).

Strain MO-CFX1T grew at temperatures between 10 and 37 °C with optimum growth at 25–30 °C. We performed the experiment in triplicate culture vessels and confirmed the growth of the strain in all three culture vessels at every temperature tested except 10 °C. At 10 °C, only one of the cultures was visually observed to be turbid, but the other two cultures did not display cell proliferation. We confirmed that 16S rRNA gene sequence of the cells grown at 10 °C was identical to that of strain MO-CFX1T. These observations indicate that 10 °C is indeed around the lower temperature threshold for the growth of strain MO-CFX1T. The pH range for growth of the strain was measured to be 5.5–8.5 with an optimum of 7.0. The strain had been cultured on seawater-based medium, but it could grow at low NaCl concentrations down to 0 g l−1. The NaCl
concentration range of growth was between 0 and 50 g l\(^{-1}\) with optimum growth at 15 g l\(^{-1}\). Under the optimum conditions (pH 7.0, 30 °C, 15 g NaCl l\(^{-1}\)), the doubling time was about 37 h, calculated based on measurement of OD\(_{400}\). Co-culture with a hydrogenotrophic methanogen *Methanobacterium* sp. strain MO-MB1 did not promote the growth of the strain, although hydrogenotrophic methanogens accelerated the growth for some species of the genus *Anaerolineae* to prevent hydrogen accumulation (Sekiguchi et al., 2001; Yamada & Sekiguchi, 2009). Strain MO-CFX\(^{+}\) tolerated rifampicin. Ampicillin, chloramphenicol, kanamycin, neomycin, penicillin G and vancomycin completely inhibited growth.

The fatty acid derivative analysis showed that the strain contained C\(_{18:1}\)o9\(\alpha\) (52.2 %), C\(_{16:1}\)o7\(\alpha\) (15.6 %), C\(_{18:0}\) (11.3 %) and C\(_{16:0}\) (8.0 %) as the major fatty acids; minor fatty acids were C\(_{14:0}\) (2.7 %), 10-methyl-C\(_{16:0}\) (2.2 %), C\(_{18:1}\) 3-OH (1.9 %), 10-oxo-C\(_{18:0}\) (1.5 %) and 10-oxo-C\(_{19:0}\) (1.3 %) (Table S1). Fatty acids hydroxylated at position 3 have been described as membrane constituents of some cultures of pseudomonas (Moss & Dees, 1976) or gliding bacteria (Fautz et al., 1979), whereas the more unusual oxo-fatty acids are thought to be formed in response to environmental stress (e.g. Taranto et al., 2003) and as lipid oxidation products (e.g. el-Sharkawy et al., 1992; Marchand & Rontani, 2003). The IPIs were dominated by diacylglyceride (DAG) and sphingolipid (ceramide) core lipid structures with monoglycosidic, mixed phosphomonoglycosidic and fatty-acid-modified monoglycosidic polar head groups (one additional fatty acid attached to the sugar) (Fig. S2). The G+C content of the total DNA of MO-CFX\(^{+}\) was 52.4 mol%. The nearly complete 16S rRNA gene sequence (1432 bp) of strain MO-CFX\(^{+}\) was determined. Comparative 16S rRNA gene sequence analysis showed that strain MO-CFX\(^{+}\) was affiliated with the class *Anaerolineae* within the phylum *Chloroflexi* (Fig. 1). The most closely related cultured relative of strain MO-CFX\(^{+}\) was *Leptolinea tarditvalis* YMTK-2\(^{+}\) (sequence identity, 91.0 %) (Yamada et al., 2006).

Phenotypic and genetic analyses in this study indicated that strain MO-CFX\(^{+}\) is a member of the class *Anaerolineae* within the phylum *Chloroflexi*. To date, nine species of the class *Anaerolineae* have been characterized taxonomically (Table 1). Our isolate MO-CFX\(^{+}\) has several phenotypic traits in common with these organisms of the class *Anaerolineae* with validly published names, e.g. filamentous morphology, no motility, no spore formation, strictly anaerobic growth and chemo-organotrophic metabolism with sugars and polysaccharides. However, there are also major phenotypic differences between strain MO-CFX\(^{+}\) and other species of class *Anaerolineae* (Table 1): (i) strain MO-CFX\(^{+}\) could grow at relatively low temperature such as 10 °C, while the other species could not; (ii) all species of the class *Anaerolineae* , except for very recent isolate *Thermominilinea lacunofontana* SW7\(^{+}\) (Nunoura et al., 2006).

![Phylogenetic tree of the phylum Chloroflexi based on comparative analyses of 16S rRNA gene sequences, showing the placement of strain MO-CFX\(^{+}\). The tree was produced based on a subset of 32 nearly complete 16S rRNA gene sequences by neighbour-joining analysis in combination with a filter of ≥50% conserved regions of 16S rRNA gene sequences to exclude the influence of highly variable positions. For sequences taken from complete genomes or rrn operons, their position on the genome/operon is indicated in parentheses. Three 16S rRNA gene sequences, Escherichia coli ATCC 11775\(^{T}\) (GenBank accession no. X80725), Bacillus subtilis IAM 12118\(^{T}\) (AB042061) and Corynebacterium diphtheriae NCTC 11397 (X84248), were used to root the tree (not shown). Bootstrap values obtained with the neighbour-joining/maximum-parsimony/maximum-likelihood methods based on 1000 replicates; −, <50%. Bar, 0.1 changes per nucleotide sequence position.](http://ijs.sgmjournals.org)
| Strains: 1, MO-CFX1T (data from this study); 2, *Leptolinea tardivitalis* YMTK-2T; 3, *Levilinea saccharolytica* KIBI-1T (Yamada et al., 2006); 4, *Bellilinea caldifistulae* GOMI-1T; 5, *Longilinea arvoryzae* KOME-1T (Yamada et al., 2007); 6, *Ornitholinea approxima* P3M-1T (Podosokorskaya et al., 2013); 7, *Anaerolinea thermophila* UNI-1T (Sekiguchi et al., 2003); 8, *Anaerolinea thermolimosa* IMO-1T (Yamada et al., 2006); 9, *Thermaanaerobacter daxensi* GNS-1T (Gre´goire et al., 2011); 10, *Thermomarinilinea lacunofontana* SW7T (Nunoura et al., 2013). ND, No data available; +, positive growth or reaction; −, negative growth or reaction; ±, variable/weakly positive growth. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cell diameter (µm)** | 0.13–0.15 | 0.15–0.2 | 0.4–0.5 | 0.2–0.4 | 0.4–0.6 | 0.3–0.7 | 0.2–0.3 | 0.3–0.4 | 0.2–0.3 | 0.2 |
| **Optimum pH (range)** | 7.0 (5.5–8.5) | 7.0 (6.0–7.2) | 7.0 (6.0–7.2) | 7.0 (5.0–8.5) | 7.5–8.0 (6.5–9.0) | 7.0 (6.0–8.0) | 6.5 (5.8–8.5) | 6.0 (5.5–7.3) | 6.0 (5.5–7.3) | 6.0 |
| **Optimum NaCl concentration (g l⁻¹) (range)** | 15 (0–50) | ND (0–2.5) | ND (0–2.5) | ND (0–30) | ND (0–15) | 1 (0–<20) | ND (0–5) | ND (0–2.5) | 2 (0–10) | 10 (5–35) |
| **Substrates for growth:** | | | | | | | | | | |
| Arabinose | + | ± | − | + | − | ± | + | ± | ND |
| Fructose | + | + | + | + | ± | − | + | + | − | ± |
| Glucose | + | + | + | + | − | + | + | + | − | ± |
| Sucrose | + | + | + | + | − | + | + | + | − | ± |
| Raffinose | + | + | + | + | − | + | + | + | − | ± |
| Pectin | + | + | ± | + | − | ± | + | ± | ND |
| Tryptone | − | + | + | ± | − | ± | + | ± | ND |
| Casamino acids | − | ± | ± | − | ± | − | ± | − | ± |
| Xylose | + | + | + | + | ± | − | + | + | − | ± |
| Xylan | + | + | + | + | ± | − | + | + | − | ± |
| Pyruvate | − | ± | ± | + | ± | − | ± | − | ± |
| Starch | + | + | + | + | − | ± | + | + | − | ± |
| **Growth products (minor products)* | Ace, Lac, Eth, H₂, (Pyr, Pro) H₂ (Succ, For) Ace, For, H₂ (Lac) Ace, Lac, For, H₂ (Pyr, Pro) Ace, Lac, H₂, H₂ (Pyr, For) Ace, Eth, H₂, H₂ (Lac, Pyr) Ace, H₂ (Lac, Suc, For) Ace, Lac, H₂, H₂ (Lac, Pro) | + /− /− | + /+ /+ | + /+ /+ | − / + /− | − /− /− | − /− /− | − /− /− | − /− /− | + /+ /+ |
| **Resistance to rifampicin/ampicillin/kanamycin†** | Marine subsurface anaerobic sediments | Mesophilic anaerobic sludge | Mesophilic anaerobic sludge | Thermophilic anaerobic sludge | Rice paddy soil | Deep terrestrial hot aquifer | Thermophilic anaerobic sludge | Mesophilic anaerobic sludge | Deep hot aquifer | Shallow sea hydrothermal vent |

*For *Bellilinea caldifistulae* GOMI-1T and *Longilinea arvoryzae* KOME-1T, end products were measured from sucrose plus 0.01 % yeast extract. For the other strains, they were detected in glucose plus 0.01 % yeast extract medium. The abbreviations for the end products are as follows: Ace, acetate; Lac, lactate; Succ, succinate; Pyr, pyruvate; For, formate; Eth, ethanol; H₂, hydrogen; Pro, propionate.

that strain MO-CFX1T is distinct from other members of the class Anaerolineae with validly published names; (iv) all the previously described members of the class Anaerolineae have saturated fatty acids as major cellular fatty acids, while the strain possesses unsaturated fatty acids; and (v) strain MO-CFX1T produces ethanol from glucose, but all the members of the class Anaerolineae are unique in comparison with other members of the class Anaerolineae. Although antibiotic tolerance has not been determined for Ornatilinea apprima P3M-1T and ‘Thermoanaerotrix daxensis’ GNS-1T (Grégoire et al., 2011), many species of the class Anaerolineae tolerate rifampicin (Table 1). Moreover, many species of the class Anaerolineae can tolerate ampicillin and kanamycin (T. Yamada and Y. Sekiguchi, personal communication), while strain MO-CFX1T cannot. Thus, these antibiotics may function as selective agents for the enrichment/isolation of particular members of the class Anaerolineae. In addition to physiological characteristics, phylogenetic analysis clearly shows that strain MO-CFX1T1 is distinct from other members of the class Anaerolineae (Fig. 1). The closest relative of strain MO-CFX1T is Leptolinea tardivitalis YMTK-2T and their 16S rRNA gene sequence identity is 91.0%. This value is too low to classify strain MO-CFX1T as a member of the genus Leptolinea. Based on these phenotypic and phylogenetic properties, we propose strain MO-CFX1T as the type strain of a novel genus and species with the name of Pelolinea submarina gen. nov., sp. nov.

Description of Pelolinea gen. nov.
Pelolinea (Pe.lo.li’ne.a. Gr. adj. pelos dark-coloured, hence anaerobic mud; L. fem. n. linea, line; N.L. fem. n. Pelolinea line-shaped organism living in anaerobic environments).

A strictly anaerobic organism. Cells are non-motile. Filamentous. Gram staining reaction is negative. Phylogenetic position is in the class Anaerolineae within the phylum Chloroflexi.

The type species is Pelolinea submarina.

Description of Pelolinea submarina sp. nov.
Pelolinea submarina (sub.ma.ri’na. L. prep. sub under; L. adj. marina marine; N.L. fem. adj. submarina from a submarine area).

Strictly anaerobic. Cells are filamentous, >10 μm long and 130–150 nm in width. In the presence of 0.01% yeast extract, growth occurs with glucose, ribose, arabinose, fructose, sucrose, galactose, raffinose, pectin, xylose, xylan and starch. The following organic substrates are not utilized: Casamino acids, tryptone, peptone, mannose, crotonate, betaine, lactate, glycerol, H$_2$CO$_2$ plus acetate, formate plus acetate, acetate, propionate, butyrate, malate, fumarate, succinate, pyruvate, ethanol and 1-propanol. The following inorganic substrates are not utilized: sulphate, sulphite, thiosulphate, elemental sulphur, nitrate and Fe(II)-nitritotriacetate. Temperature range for growth is 10–37 °C (optimum, 25–30 °C). The pH range for growth is 5.5–8.5 (optimum, pH 7.0). Growth is observed in the presence of 0–50 g NaCl l$^{-1}$ and its optimum is 15 g NaCl l$^{-1}$. The major fatty acids are C$_{18:1}$O9, C$_{18:1}$O7, C$_{18:0}$ and C$_{16:0}$; minor fatty acids were C$_{14:0}$, 10-methyl-C$_{16:0}$ C$_{18:1}$ 3-OH, 10-oxo-C$_{18:0}$ and 10-oxo-C$_{19:0}$. The IPLs are dominated by DAG and sphingolipid (ceramide) core lipid structures with monoglycosidic, mixed phosphomonoglycosidic and fatty-acid-modified monoglycosidic polar head groups.

The type strain, MO-CFX1T (=JCM 17238T, =KCTC 5595T), was isolated from marine subsurface sediments collected off the Shimokita Peninsula of Japan, north-western Pacific Ocean (Site C9001, 41° 10.6389’N 142° 12.081’E, 1180 m water depth). The strain is most closely related to Leptolinea tardivitalis YMTK-2T (91.0% 16S rRNA gene sequence identity). The G+C content of the DNA was 52.4 mol% (HPLC).

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References


