Bellilinea caldifistulae gen. nov., sp. nov. and *Longilinea arvoryzae* gen. nov., sp. nov., strictly anaerobic, filamentous bacteria of the phylum *Chloroflexi* isolated from methanogenic propionate-degrading consortia

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Thermophilic (strain GOMI-1^T) and mesophilic (strain KOME-1^T) strains were isolated from two different cultures of propionate-degrading consortia obtained from thermophilic digester sludge and rice paddy soil, respectively. The two strains were non-spore-forming, non-motile and Gram-negative. Both strains were obligately anaerobic micro-organisms, showing multicellular filamentous morphotypes more than 100 µm in length. The cell width for strain GOMI-1^T was 0.2–0.4 µm and that of strain KOME-1^T was 0.4–0.6 µm. Strain GOMI-1^T could grow at 45–65 °C with a pH range of 6.0–7.5 (optimum growth at 55 °C, pH 7.0). The temperature range for growth of strain KOME-1^T was 30–40 °C and the pH range was pH 5.0–8.5 (optimum growth around 37 °C, pH 7.0). Yeast extract was required for growth of both strains. Strain GOMI-1^T was able to grow with a number of carbohydrates in the presence of yeast extract. In yeast extract-containing medium, strain KOME-1^T could utilize proteins and a limited range of sugars for growth. The G+C contents of the DNA of strains GOMI-1^T and KOME-1^T were respectively 54.7 and 57.6 mol%. Major fatty acids of strain GOMI-1^T were C_{16:0}, C_{14:0} and iso-C_{15:0}, whereas those of strain KOME-1^T were iso-C_{15:0}, anteiso-C_{15:0} and C_{14:0}. Based on comparative analysis of 16S rRNA gene sequences of strains GOMI-1^T and KOME-1^T, the strains were placed in different phylogenetic positions in the class *Anaerolineae* of the bacterial phylum *Chloroflexi*. Their phenotypic and genetic traits strongly supported the conclusion that the strains should be described as two independent taxa in the class *Anaerolineae*. Hence, we propose the names *Bellilinea caldifistulae* gen. nov., sp. nov., and *Longilinea arvoryzae* gen. nov., sp. nov., for strains GOMI-1^T and KOME-1^T. The type strains of *Bellilinea caldifistulae* and *Longilinea arvoryzae* are respectively GOMI-1^T (=JCM 13669^T =DSM 17877^T) and KOME-1^T (=JCM 13670^T =KTCC 5380^T).

Abbreviations: NTA, nitrilotriacetate; UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GOMI-1^T and KOME-1^T are respectively AB243672 and AB243673.

The class *Anaerolineae* has recently been proposed as a subclade in the phylum *Chloroflexi* with the related new class *Caldilineae* (Yamada *et al.*, 2006). Analyses based on sequences of 16S rRNA and the 16S rRNA gene have revealed that members of this class are distributed widely in various types of ecosystems, such as the subsurface,

sediments, hot springs and aerobic and anaerobic sludge (Björnsson *et al.*, 2002; Hugenholtz *et al.*, 1998a; Kindaichi *et al.*, 2004; Sekiguchi *et al.*, 1998; Teske *et al.*, 2002), suggesting their ubiquity and functional significance in such environments (Hugenholtz *et al.*, 1998b). However, the *Anaerolineae* have long been considered a group of the yet-to-be-cultured microbes that are recalcitrant to cultivation and isolation (Hugenholtz *et al.*, 1998b; Sekiguchi *et al.*, 2001). Recently, the first tangible micro-organism of the class was isolated and named *Anaerolinea thermophila* (Sekiguchi *et al.*, 2001, 2003). In addition, three filamentous strains belonging to the class (representing *Anaerolinea thermolimos*, *Levilinea saccharolytica* and *Leptolinea tardivitalis*) were further isolated and characterized (Yamada *et al.*, 2005, 2006). So far, these four strains are the only known organisms able to be cultivated and thoroughly described in the class *Anaerolineae*. They are strictly anaerobic, multicellular filamentous microbes and can utilize a number of carbohydrates fermentatively in the presence of yeast extract (Sekiguchi *et al.*, 2001, 2003; Yamada *et al.*, 2005, 2006). Some of the strains require syntrophic association with a hydrogenotrophic methanogen for efficient growth (Sekiguchi *et al.*, 2001, 2003; Yamada *et al.*, 2005, 2006).

To date, all authentic species of the *Anaerolineae* have been initially found in and isolated from a special wastewater treatment system operating under anaerobic conditions called the upflow anaerobic sludge blanket (UASB) system. However, besides the UASB system, there are many anaerobic ecosystems in natural and artificial environments, and it is quite likely that an enormous number of unknown species of the *Anaerolineae* are hidden in such environments. We therefore searched anaerobic environments other than UASB sludges for novel species of the *Anaerolineae*. As a result, we successfully isolated two *Anaerolineae*-type filamentous anaerobes, designated strains GOMI-1^T and KOME-1^T, from a thermophilic digester sludge and rice paddy soil, respectively. In this report, the isolation and detailed morphological, physiological and chemotaxonomic characteristics and genetic features of the strains are described, assigning taxonomic placements to the strains in the class *Anaerolineae*.

The two filamentous strains were isolated from thermophilic (55 °C) and mesophilic (37 °C) methanogenic propionate-degrading consortia enriched from thermophilic digester sludge (55 °C) and rice paddy soil, respectively. Both consortia contained propionate-degrading *Pelotomaculum* spp. and hydrogenotrophic methanogens (Imachi *et al.*, 2006). Besides these organisms, various morphotypes such as ovals, rods or filaments were observed in the enrichment cultures. Fluorescence *in situ* hybridization using the *Chloroflexi*-specific probe GNSB941* (Yamada *et al.*, 2005) was performed according to previous reports (Sekiguchi *et al.*, 1999; Yamada *et al.*, 2005). The analysis showed that GNSB941* probe-reactive filamentous cells were present as a significant constituent in the two enrichments. To isolate GNSB941*-positive filamentous cells that possibly belonged to the *Anaerolineae*

from the cultures, we first transferred part of the enrichments to a fresh medium supplemented with sucrose (20 mM) and yeast extract (0.1%), because species of the *Anaerolineae* are commonly known as saccharolytic anaerobes that require yeast extract. The medium used for isolation and cultivation of the filamentous bacteria was prepared as described previously (Sekiguchi *et al.*, 2000; Yamada *et al.*, 2005). Primary enrichment cultures inoculated with thermophilic digester sludge and rice paddy soil were cultivated anaerobically at 55 °C and 37 °C, respectively. A number of successive transfers to fresh sucrose-yeast extract medium were conducted, and stable cultures that consisted mainly of the filamentous cells were finally established. Two strains, GOMI-1^T and KOME-1^T, were isolated from these enriched cultures by the roll-tube isolation method (Hungate, 1969) with sucrose-yeast extract medium supplemented with purified agar (20 g agar noble l⁻¹; Difco). Isolation of strains GOMI-1^T and KOME-1^T was performed at 55 °C and 37 °C, respectively. Tiny, colourless, lens-shaped colonies, 0.1–0.2 mm in diameter, were formed within 1 month of cultivation in both cases.

For microscope observations, agar-coated slides were prepared and microbial cells were immobilized on the slides (Pfennig & Wagener, 1986). Both strains (strains GOMI-1^T and KOME-1^T) showed flexible filaments of indefinite length (generally longer than 100 µm) under the microscope (Olympus AX80T) (Fig. 1). Cell widths of strains GOMI-1^T and KOME-1^T were respectively 0.2–0.4 µm and 0.4–0.6 µm (Fig. 1). Gram-staining done according to Hucker's method (Doetsch, 1981) was negative for both strains. Spore formation was not observed in either strain under any culture conditions. Gliding motility was not observed (based on the observation of colony forms on solid agar medium).

Strains GOMI-1^T and KOME-1^T were obligately anaerobic, filamentous micro-organisms. Neither aerobic nor phototrophic growth in PE medium was observed in either strain (Hanada *et al.*, 1995). The following physiological tests, nutritional properties, usage of electron acceptors, fermentative end products and acid formation, were done according to methods described previously (Imachi *et al.*, 2000, 2002; Roden & Lovley, 1993; Sekiguchi *et al.*, 2000; Yamada *et al.*, 2005). A small amount of yeast extract was required for growth in both strains. In the presence of yeast extract (0.01%), strain GOMI-1^T could utilize the following substrates (20 mM each unless specified otherwise): ribose, arabinose, fructose, glucose, galactose, mannose, sucrose, raffinose and pectin (5 g l⁻¹). The following substrates supported weak growth of strain GOMI-1^T and caused acid formation in medium supplemented with yeast extract (0.01%) (substrates added at a final concentration of 20 mM unless mentioned otherwise): Casamino acids (0.1%), tryptone (0.1%), peptone (0.1%), xylose and xylan (5 g l⁻¹). The following substrates tested did not support growth of strain GOMI-1^T and did not lead to acid formation even in the

Fig. 1. Phase-contrast photomicrographs of strains GOMI-1^T (a) and KOME-1^T (b) grown on sucrose-yeast extract medium. Bars, 10 μ m.

presence of yeast extract (0.01 %) (20 mM each unless specified): crotonate, H_2/CO_2 (1 atm) plus acetate (10 mM), betaine, lactate, glycerol, fumarate, acetate, propionate, butyrate, malate, succinate, ethanol, methanol, 1-propanol, starch, ethylene glycol, hydroquinone (1 mM), phenol (1 mM), benzoate (5 mM) and formate plus acetate (10 mM).

Under parallel conditions, strain KOME-1^T could utilize the following substrates (20 mM each unless specified): tryptone (0.1 %), xylose, raffinose, sucrose, xylan (5 g l⁻¹), pectin (5 g l⁻¹) and peptone (0.1 %). Casamino acids (0.1 %) and fructose (20 mM) led to weak growth and acid formation. None of the following substrates tested supported growth (20 mM each unless specified): pyruvate, glucose, ribose, arabinose, galactose, mannose, crotonate, H_2/CO_2 (1 atm) plus acetate (10 mM), lactate, glycerol, fumarate, acetate, propionate, butyrate, malate, succinate, ethanol, methanol, 1-propanol, starch, ethylene glycol, hydroquinone (1 mM), phenol (1 mM), benzoate (5 mM) and formate plus acetate (10 mM), betaine and starch (5 g l⁻¹).

In medium containing sucrose (20 mM) and yeast extract (0.01 %), major fermentative products of strain GOMI-1^T were acetate, lactate, formate and hydrogen. Small amounts of propionate and pyruvate were also produced. On the other hand, strain KOME-1^T produced only acetate, lactate and hydrogen as end products. Strains GOMI-1^T and KOME-1^T did not use any of the following electron acceptors within 4 weeks of incubation on sucrose-yeast extract medium: 20 mM sulphate, 1 mM sulphite, 20 mM thiosulphate, 20 mM elemental sulphur, 20 mM nitrate, 20 mM fumarate and 5 mM Fe(III) nitrilotriacetate (NTA).

Effects of pH, temperature and NaCl concentration on growth of strains GOMI-1^T and KOME-1^T were determined by using the basal medium containing 20 mM sucrose plus 0.1 % yeast extract (2 % inoculum). All tests were performed in triplicate and growth was evaluated by measuring optical density (OD_{400}) and formation of acid and hydrogen. Growth of strain GOMI-1^T occurred in a pH range of 6.0–7.5 and growth of strain KOME-1^T was observed between pH 5.0 and 8.5; both strains showed optimum growth at around pH 7.0. The optimum growth temperature of strain GOMI-1^T was 55 °C (growth occurred at 45–65 °C). Strain KOME-1^T could grow between 30 and 40 °C with an optimum temperature of around 37 °C. The two strains were basically freshwater organisms, but they could tolerate significant amounts of salt. Strain GOMI-1^T could tolerate NaCl up to 30 g l⁻¹, while KOME-1^T could not grow in 15 g NaCl l⁻¹.

Under optimum growth conditions for strains GOMI-1^T (55 °C, pH 7.0) and KOME-1^T (37 °C, pH 7.0), doubling times on sucrose-yeast extract medium were respectively 45 and 92 h (evaluated by the method described previously; Sekiguchi *et al.*, 2003; Yamada *et al.*, 2006). Growth of each strain was found to stagnate after a certain amount of hydrogen accumulated in the vial. Co-culture with a hydrogen scavenger such as a methanogen prevented the hydrogen accumulation and obviously promoted growth. In the sucrose-yeast extract medium, syntrophic growth properties of the two strains with hydrogenotrophic methanogens were evaluated in co-cultivation with *Methanospirillum hungatei* DSM 864^T for strain KOME-1^T and *Methanothermobacter thermautotrophicus* DSM 1053^T for strain GOMI-1^T. A significant increase in the growth rate was observed in co-culture of strain KOME-1^T with *Methanospirillum hungatei* DSM 864^T and the doubling time decreased to 29 h (evaluated as described above). A similar result was obtained in co-culture of strain GOMI-1^T with *Methanothermobacter thermautotrophicus* DSM 1053^T, and the doubling time was 38 h.

For determination of genomic DNA G + C contents, DNA of each isolate was extracted and purified as described previously (Kamagata & Mikami, 1991) and analysed by HPLC (Shintani *et al.*, 2000). The DNA G + C contents of strains GOMI-1^T and KOME-1^T were respectively 54.7 and 57.6 mol%. For cellular fatty acid methyl ester analysis

(Hanada *et al.*, 2002), cells of strains GOMI-1^T and KOME-1^T were harvested from cultures grown under optimum growth conditions with medium containing sucrose (20 mM) plus yeast extract (0.1 %). The analyses showed that strain GOMI-1^T contained C_{16:0} (28 %), C_{14:0} (24 %) and iso-C_{15:0} (13 %) as the major cellular fatty acids, with C_{18:0} (7 %), C_{16:1}Δ9 *cis* (8 %), branched C_{17:0} (5 %), anteiso-C_{15:0} (3 %), C_{18:1}Δ9 *cis* (3 %), C_{12:0} (2 %), anteiso-C_{17:0} (2 %), iso-C_{17:0} (1 %), branched C_{13:0} (1 %), branched C_{14:0} (1 %), iso-C_{16:0} (1 %) and branched C_{18:0} (1 %) as minor fatty acids. Strain KOME-1^T contained iso-C_{15:0} (27 %), anteiso-C_{15:0} (24 %) and C_{14:0} (20 %) as the major cellular fatty acids, with C_{16:0} (8 %), branched C_{13:0} (6 %), branched C_{14:0} (5 %), C_{12:0} (2 %), anteiso-C_{17:0} (2 %), C_{15:0} (1 %), C_{13:0} (1 %), C_{15:0} (1 %), iso-C_{16:0} (1 %), branched C_{17:0} (1 %) and C_{18:1}Δ9 *cis* (1 %) as minor fatty acids. Quinone analysis by HPLC (Zhang *et al.*, 2003) revealed that neither isolate contained any type of quinone.

To determine 16S rRNA gene sequences for both isolates, we extracted DNA from the strains with the methods of Hiraishi (1992). 16S rRNA genes were amplified with the bacteria-specific 16S rRNA gene-targeted primer set 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTACCTTGTTACGACTT-3') (Weisburg *et al.*, 1991), and sequenced with a CEQ DTC kit-Quick start kit (Beckman Coulter) and a Beckman Coulter CEQ-2000 automated sequence analyser (Imachi *et al.*, 2006; Yamada *et al.*, 2005). Totals of 1425 nt (strain GOMI-1^T) and 1422 nt (strain KOME-1^T) were determined. Phylogenetic analysis based on the neighbour-joining method (Saitou & Nei, 1987) with the ARB program (Ludwig *et al.*, 2004) and the maximum-likelihood method with the TREEFINDER program (Jobb, 2007) indicated that the strains were affiliated with the class *Anaerolineae* (Fig. 2). However, they were clearly distant from any known species of the *Anaerolineae*. The most closely related organism to strain GOMI-1^T was *Levilinea saccharolytica* KIBI-1^T (1424 nt), with 93 % 16S rRNA gene similarity. The closest strain to strain KOME-1^T was *Leptolinea tardivitalis* YMTK-2^T (1426 nt), with 92 % sequence similarity. The sequence similarity between strains KOME-1^T and GOMI-1^T was 92 %. These low sequence similarities strongly suggest that new genera and species should be created for each isolate.

To date, four cultured representatives have been described within the class *Anaerolineae*. Differential phenotypic characteristics of the four species of the *Anaerolineae* and our isolates are shown in Table 1. These organisms share several common phenotypic traits, e.g. filamentous morphology, strictly anaerobic metabolism and fermentative growth with a number of sugars in the presence of yeast extract. The thermophilic strain GOMI-1^T was very similar to the two thermophilic species within the genus *Anaerolinea*, *A. thermophila* and *A. thermolimos*, in many physiological respects, but there were obvious phenotypic differences between them: (i) strain GOMI-1^T was able to grow at 65 °C and pH 8.5, whereas the *Anaerolinea* species

could not grow under such conditions, (ii) the isolate did not hydrolyse starch, while the *Anaerolinea* species showed significant starch hydrolysis, and (iii) strain GOMI-1^T has a cellular fatty acid composition that is easily distinguished from those of the *Anaerolinea* species. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain GOMI-1^T was distant from the genus *Anaerolinea* (the sequence similarities between strain GOMI-1^T and *Anaerolinea* species were lower than 90 %), and strongly suggested the creation of a new genus for the isolate (Fig. 2).

While the phylogenetic analysis showed that the other isolate, strain KOME-1^T, was also distant from any known species in the class *Anaerolineae*, physiologically it resembled the mesophilic species within the class *Anaerolineae*, *Levilinea saccharolytica* and *Leptolinea tardivitalis*. However, strain KOME-1^T could be clearly differentiated from the related mesophiles by the following phenotypic differences (Table 1): (i) strain KOME-1^T had a narrow temperature range for growth and showed growth at 40 °C and (ii) strain KOME-1^T had a narrow nutritional range in substrate utilization and could not grow on glucose. In addition, a clear difference was found in syntrophic growth with a methanogen. Co-culture with a hydrogenotrophic methanogen enhanced growth of KOME-1^T; similar enhancement was also found in the two species of the genus *Anaerolinea* (Yamada *et al.*, 2005, 2006) and strain GOMI-1^T, but was not observed in the mesophilic species belonging to the genera *Levilinea* and *Leptolinea*.

Based on the phenotypic and genetic comparisons mentioned above, we propose the names *Bellilinea caldifistulae* gen. nov., sp. nov., and *Longilinea arvoryzae* gen. nov., sp. nov., for strains GOMI-1^T and KOME-1^T.

Description of *Bellilinea* gen. nov.

Bellilinea (Bel.li.li'ne.a. L. adj. *bellus* beautiful; L. fem. n. *linea* line; N.L. fem. n. *Bellilinea* beautifully line-shaped organism).

Gram-negative. Cells are non-motile. Multicellular and filamentous. Spores are not formed. Grow under strictly anaerobic conditions. No photosynthetic growth is observed. Thermophilic. The main fatty acids are C_{16:0}, C_{14:0} and iso-C_{15:0}. The G + C content of genomic DNA of the type strain of the type species is 54.7 mol%. Phylogenetic position is in the class *Anaerolineae*. The type species is *Bellilinea caldifistulae*.

Description of *Bellilinea caldifistulae* sp. nov.

Bellilinea caldifistulae (cal.di.fis'tu.lae. L. adj. *calidus* hot; L. n. *fistula* a tube, pipe; N.L. gen. n. *caldifistulae* of a hot pipe, referring to a thermophilic reactor vessel for wastewater/waste treatment, from where the type strain was isolated).

Cells are longer than 100 µm and 0.2–0.4 µm in width. Growth occurs between 45 and 65 °C with optimum

Fig. 2. Evolutionary dendrogram of the bacterial phylum *Chloroflexi* inferred from 16S rRNA gene sequences, showing the phylogenetic positions of strains GOMI-1^T and KOME-1^T (indicated by arrows). The sequences were aligned and the phylogenetic tree was reconstructed by the neighbour-joining method. 16S rRNA gene sequences of *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725), *Bacillus subtilis* 168 (D26185) and *Corynebacterium diphtheriae* NCTC 11397^T (X84248) were used as outgroups (not shown). Bootstrap resampling analysis (Felsenstein, 1985) for 1000 replicates was performed with the PAUP* 4.0 program package (Swofford, 2002) for neighbour-joining and maximum-likelihood methods and with the TREEFINDER program (Jobb, 2007) for the maximum-likelihood method to estimate the confidence of the tree topologies. Branching points with support probabilities above 95 % by all the analyses (estimated using neighbour-joining and maximum-likelihood methods) are indicated by solid circles, whereas nodes with open circles indicate >85 % bootstrap probability support by the two analyses. Nodes with open squares indicate >75 % probability support by both analyses. Nodes without symbols were not highly resolved (<75 %) as specific groups in the analyses. Bar, 10 nucleotide substitutions per 100 nucleotides. Class-level lineages in the phylum *Chloroflexi* are numbered (in brackets) according to the reports of Hugenholtz & Stackebrandt (2004) and Yamada *et al.* (2006).

growth at 55 °C. Growth is observed at pH 6.0–7.5 with optimum growth at pH 7.0. Doubling time for growth is 45 h under optimum growth conditions. Growth is enhanced in co-cultivation with hydrogenotrophic methanogens. Yeast extract is required for growth. In the presence of 0.01 % yeast extract, growth is observed with the following substrates: ribose, arabinose, fructose, glucose, galactose, mannose, sucrose, raffinose and pectin. Weak growth occurs with the following substrates with medium supplemented with 0.01 % yeast extract: Casamino acids, tryptone, peptone, xylose and xylan. The following substrates are not utilized in the presence of yeast extract: crotonate, H₂/CO₂ plus acetate, betaine, lactate, glycerol, fumarate, acetate, propionate, butyrate, malate, succinate, ethanol, methanol, 1-propanol, starch, ethylene glycol, hydroquinone, phenol, benzoate and formate plus acetate.

None of following compounds is utilized as an electron acceptor: sulphate, sulphite, thiosulphate, elemental sulphur, nitrate, fumarate and Fe(III) NTA.

The type strain is GOMI-1^T (=JCM 13669^T =DSM 17877^T), which was isolated from thermophilic sludge in which sewage sludge and domestic garbage had been digested.

Description of *Longilinea* gen. nov.

Longilinea (Lon.gi.li'ne.a. L. adj. *longus* long; L. fem. n. *linea* line; N.L. fem. n. *Longilinea* long line-shaped organism).

Gram-negative. Cells are non-motile. Multicellular and filamentous. Spores are not formed. Grow under strictly anaerobic conditions. No photosynthetic growth is

Table 1. Phenotypic characteristics of species with validly published names affiliated with the class *Anaerolineae* in the phylum *Chloroflexi*

Data for *A. thermolimosa* IMO-1^T, *Levilinea saccharolytica* KIBI-1^T and *Leptolinea tardivitalis* YMTK-2^T were obtained in our previous study (Yamada *et al.*, 2006) and this study. Data for *A. thermophila* UNI-1^T were taken from Sekiguchi *et al.* (2003). –, Negative; ±, variable; +, positive; ND, not determined. Only differences found among the strains are listed. All strains show the following characteristics: they form multicellular filaments; optimum growth at pH 7.0; no respiration of O₂; positive for fermentation; no quinones detected; positive for utilization of yeast extract. All species show the same response for use of substrates in the presence of yeast extract as follows: sucrose, raffinose (positive); Casamino acids (variable); lactate, fumarate, acetate, succinate, ethanol (negative).

Characteristic	<i>Bellilinea caldifistulae</i> (strain GOMI-1 ^T)	<i>Longilinea arvoryzae</i> (strain KOME-1 ^T)	<i>Levilinea saccharolytica</i> KIBI-1 ^T	<i>Leptolinea tardivitalis</i> YMTK-2 ^T	<i>Anaerolinea thermolimosa</i> IMO-1 ^T	<i>Anaerolinea thermophila</i> UNI-1 ^T
Cell diameter (µm)	0.2–0.4	0.4–0.6	0.4–0.5	0.15–0.2	0.3–0.4	0.2–0.3
Temperature range (°C)	45–65	30–40	25–50	25–50	42–55	50–60
Optimum growth temperature (°C)	55	37	37–40	37	50	55
pH range	6.0–8.5	5.0–7.5	6.0–7.2	6.0–7.2	6.0–7.5	6.0–8.0
Doubling time (h)*	45 (29)	92 (38)	56 (56)	50 (50)	48 (10)	72 (48)
Major cellular fatty acids	C _{16:0} , C _{14:0} , i-C _{15:0}	i-C _{15:0} , ai-C _{15:0} , C _{14:0}	C _{14:0} , i-C _{15:0} , C _{16:0}	Branched C _{17:0} , C _{16:0} , C _{14:0}	ai-C _{17:0} , i-C _{15:0} , C _{16:0}	C _{16:0} , C _{15:0} , C _{14:0}
DNA G + C content (mol%)	54.7	57.6	59.5	48.2	53.3	54.5
Utilization in the presence of yeast extract of:						
Tryptone	±	+	+	+	+	±
Betaine	–	–	±	+	–	ND
Pyruvate	+	–	+	±	+	±
Glucose	+	–	+	+	+	+
Mannose	+	–	±	+	+	+
Galactose	+	–	±	±	+	+
Fructose	+	±	+	+	+	+
Arabinose	+	–	–	±	+	±
Xylose	±	–	+	+	+	±
Ribose	+	–	+	+	+	±
Pectin	+	+	±	+	±	±
Starch	–	–	–	±	±	+
Isolation source	Thermophilic anaerobic sludge	Rice paddy soil	Mesophilic anaerobic sludge	Mesophilic anaerobic sludge	Thermophilic anaerobic sludge	Thermophilic anaerobic sludge

*Doubling times in parentheses indicate those in syntrophic growth with hydrogenotrophic methanogens.

observed. Mesophilic. The main fatty acids are iso-C_{15:0}, anteiso-C_{15:0} and C_{14:0}. The G + C content of genomic DNA of the type strain of the type species is 57.6 mol%. Phylogenetic position is in the class *Anaerolineae*. The type species is *Longilinea arvoryzae*.

Description of *Longilinea arvoryzae* sp. nov.

Longilinea arvoryzae (L. n. *arvum* an arable field, cultivated land; L. n. *oryza* rice; N.L. gen. n. *arvoryzae* of a rice paddy field).

Cells are longer than 100 µm and 0.4–0.6 µm in width. Growth occurs between 30 and 40 °C with optimum growth around 37 °C. Growth is observed at pH 5.0–8.5,

with optimum growth around pH 7.0. Doubling time for growth is 92 h under optimum growth conditions. Growth is enhanced in co-cultivation with hydrogenotrophic methanogens. In the presence of 0.01 % yeast extract, growth is observed with the following substrates: xylose, raffinose, sucrose, xylan, pectin and peptone. Weak growth occurs with Casamino acids and fructose in medium supplemented with 0.01 % yeast extract. The following substrates are not utilized in the presence of yeast extract: pyruvate, glucose, ribose, arabinose, galactose, mannose, crotonate, H₂/CO₂ plus acetate, lactate, glycerol, fumarate, acetate, propionate, butyrate, malate, succinate, ethanol, methanol, 1-propanol, starch, ethylene glycol, hydroquinone, phenol, benzoate, formate plus acetate, betaine and starch. None of following compounds is utilized as

an electron acceptor: sulphate, sulphite, thiosulphate, elemental sulphur, nitrate, fumarate and Fe(III) NTA.

The type strain is KOM-1^T (=JCM 13670^T =KTCC 5380^T), isolated from rice paddy soil soaked with water.

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