Salimesophilobacter vulgaris gen. nov., sp. nov., an anaerobic bacterium isolated from paper-mill wastewater

Yan-Zhou Zhang, Ming-Xu Fang, Wen-Wu Zhang, Tian-Tian Li, Min Wu and Xu-Fen Zhu

College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China

Correspondence
Xu-Fen Zhu
xufenzhu@zju.edu.cn

A novel anaerobic, heterotrophic bacterium, designated strain Zn2^T, was isolated from the wastewater of a paper mill in Zhejiang, China. Cells were Gram-type-positive rods, 0.5–0.8 μm wide and 2–4 μm long, and were motile by a lateral flagellum. The ranges of temperature and pH for growth were 10–50 °C and pH 6.0–9.5. Optimal growth occurred at 35 °C and pH 7.3–7.5. The strain did not require NaCl for growth, but its inclusion in the medium improved growth (optimum concentration 6 %). Substrates utilized as sole carbon sources were peptone, tryptone, Casamino acids, D-xylose, salicin, glycerol, formate, acetate and propionate. The main products of carbohydrate fermentation were acetate, formate, propionate and lactate. Elemental sulfur, thiosulfate and Fe(III) were used as electron acceptors, but sulfate, sulfite, nitrate, nitrite and Mn(IV) were not. Growth was inhibited by the addition of 10 μg ampicillin, penicillin, tetracycline or chloramphenicol ml^{-1}. iso-$C_{15:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ *cis*9 and $C_{18:1}$ *cis*9 were the major fatty acids. Strain Zn2^T did not contain any detectable menaquinones or ubiquinones. The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, two unknown phospholipids and four unknown glycolipids. The genomic DNA G+C content was 37 mol%, as determined by HPLC. 16S rRNA gene sequence analysis revealed that strain Zn2^T was a member of family *Clostridiaceae*, and was most closely related to the type strains of *Geosporobacter subterraneus*, *Thermotalea metallivorans* and *Caminicella sporogenes*, showing 91.2, 90.3 and 91.1 % sequence similarity, respectively. On the basis of its phenotypic and genotypic properties, strain Zn2^T is suggested to represent a novel species of a new genus, for which the name *Salimesophilobacter vulgaris* gen. nov., sp. nov. is proposed. The type strain of *Salimesophilobacter vulgaris* is Zn2^T (=DSM 24770^T =JCM 17796^T).

Industrial wastewater is a specialized ecosystem for bacteria, as it is often characterized by high salinity, low pH and/or low O_2. Microbiological degradation has been shown to be an effective technology to treat industrial wastewater (Fang *et al.*, 2012). While developing this technology, the microbiological composition of the wastewater has been investigated, and several strains with low 16S rRNA gene sequence similarity to known species have been isolated, such as *Citricoccus zhacaiensis* (Meng *et al.*, 2010), *Pectinatus brassicae* (Zhang *et al.*, 2012) and *Brassicibacter mesophilus* (Fang *et al.*, 2012). Here, we describe a novel species of a new genus belonging to the family *Clostridiaceae*, represented by an anaerobic, mesophilic, non-spore-forming bacterium isolated from wastewater of a paper mill.

Strain Zn2^T was isolated in 2010 from a paper mill in the town of Lingqiao (30° 15′ N 120° 10′ E), located in Zhejiang Province, China. For the initial enrichment, we used PYG medium, containing (per l distilled water) 10 g peptone, 10 g yeast extract, 5 g glucose, 0.5 g cysteine, 1 mg resazurin and 40 ml salt solution (see DSMZ medium 104), at pH 6.4. Serum bottles (50 ml) containing a liquid phase of PYG medium under a gas phase of O_2-free N_2 were sealed and autoclaved for 20 min at 121 °C. Bottles were inoculated anaerobically with approximately 10 % (v/v) wastewater sample and incubated at 32 °C for 7 days. The Hungate roll-tube technique (Hungate, 1969; Bryant, 1972) was used to isolate strains from the turbid enrichment cultures, using medium A (pH 6.4), which contained (per l distilled water) 1.0 g Na_2SO_4, 30 g NaCl, 5 g $MgCl_2.6H_2O$, 0.7 g KCl, 1.0 g $CaCl_2.2H_2O$, 0.5 g NH_4Cl, 0.4 g K_2HPO_4, 0.4 g KH_2PO_4, 2 g $NaHCO_3$, 2 mg $FeSO_4$, 5 g yeast extract (Difco), 3 g peptone, 5 g potassium acetate, 0.4 g L-cysteine, 0.5 g $Na_2S.9H_2O$, 0.001 g resazurin and 1 ml trace element solution SL-10

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Zn2^T is HQ880422.

Three supplementary figures are available with the online version of this paper.

(Widdel *et al.*, 1983). Strains were purified at least twice before being preserved in 25 % glycerol at −80 °C. One of the isolates obtained, strain Zn2^T, was analysed further.

Genomic DNA was extracted and purified using the method described by Marmur (1961). The 16S rRNA gene was amplified by PCR using universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). An almost-complete 16S rRNA gene sequence (1471 nt) was obtained from strain Zn2^T. Sequence similarity analysis and multiple sequence alignment were performed with the EzTaxon service (Chun *et al.*, 2007) and CLUSTAL W version 1.8 (Thompson *et al.*, 1994), respectively. Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood methods with the MEGA4 program package (Tamura *et al.*, 2007). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Zn2^T was a member of family *Clostridiaceae*, and was most closely related to the type strains of *Geosporobacter subterraneus*, *Thermotalea metallivorans* and *Caminicella sporogenes*, showing 91.2, 90.3 and 91.1 % sequence similarity, respectively (Fig. 1 and Fig. S1, available in IJSEM Online).

The DNA G+C content was determined by HPLC, as described by Mesbah *et al.* (1989), using *Escherichia coli* K-12 DNA as the calibration standard. The genomic DNA G+C content of strain Zn2^T was 37 mol%.

Hungate technique was used throughout the study for physiological and metabolic characterization. The basal medium was medium A, except that the medium was adjust to pH 7.5 before sterilization for 20 min at 121 °C. Cultures were grown under a gas phase of O_2-free N_2. Cell morphology and motility were examined using optical (Olympus BX40) and transmission electron (JEM-1230; JEOL) microscopy during the late-exponential or stationary growth phase (Tan *et al.*, 2012). Cells of strain Zn2^T were rods (0.5–0.8 × 2.0–4.0 μm) with a lateral flagellum. Endospores were not observed. Ultrathin-section electron micrographs revealed a typical Gram-positive cell-wall structure (Fig. S2).

Specific sporulation genes *ssp* and *spo0A* were detected by a PCR method as described by Brill & Wiegel (1997). Strains of *E. coli* and *Bacillus subtilis* were used as negative and positive controls, respectively. The result indicated that strain Zn2^T was potentially a spore-forming bacterium, but endospores were not observed. We speculate that we did not find the right conditions for strain Zn2^T to form endospores. *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T were also tested. The result was in accordance with the observation of endospores of *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T, as mentioned by Klouche *et al.* (2007) and Ogg & Patel (2009).

Determination of optimal growth conditions for strain Zn2^T was conducted in medium A. All tests were performed twice. The strain was cultured at 4, 10, 15, 20, 25, 30, 33, 35, 37, 39, 42, 50 and 55 °C, pH 5–10 (at intervals of 0.5 pH units) and 0, 2–7 (in increments of 0.5 %), 8, 10, 12 and 14 % NaCl. The medium was adjusted to the desired pH using sterile solutions (10 %) of HCl or NaOH, and MES (pH 5.5–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5), CAPSO (pH 9.0–9.5) or CAPS (pH 10.0) was added at a concentration of 25 mM. The optimal temperature for growth of strain Zn2^T was 35 °C (range 10–50 °C); no growth was observed above 50 °C or below 10 °C after incubation for 10 days. The optimal pH range for growth was pH 7.3–7.5 (range pH 6.0–9.5). Optimal growth was observed at 6 % (w/v) NaCl. Growth was observed in the absence of NaCl, but no growth occurred at 12 % NaCl. Strain Zn2^T did not grow in aerobic medium.

Substrate utilization was tested under a gas phase of O_2-free N_2, using the salt solution of medium A minus potassium acetate. The medium was adjusted to pH 7.3. All substrates were tested in the presence of 0.1 g yeast extract l^{-1} (BD). Complex proteinaceous substrates (10 g l^{-1}) such as Casamino acids (BD) and peptone (BD), and organic acids (20 mM), methanol (20 mM), ethanol (20 mM), glycerol

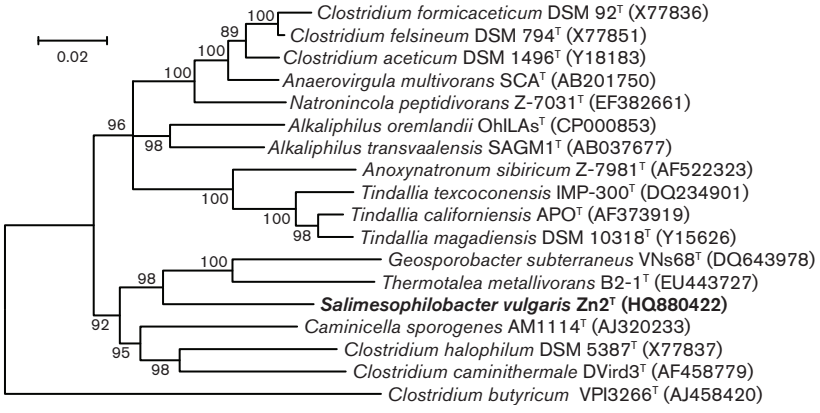

Fig. 1. Neighbour-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequences of strain Zn2^T and its closest relatives. Bar, 2 changes per 100 nucleotide positions. Maximum-parsimony and maximum-likelihood trees are shown in Fig. S1.

(25 mM), sorbitol (25 mM) and glycine were autoclaved for 20 min at 121 °C. Sugars (20 mM) and other amino acids (20 mM) were sterilized with UV light overnight as described by Tan *et al.* (2012) and added to the culture medium directly before cultivation. All tests were performed in triplicate. Growth could be observed in the presence of D-xylose, salicin, glycerol, acetate, formate, propionate, peptone and Casamino acids, but not in the presence of D-glucose, D-fructose, D-galactose, maltose, D-ribose, L-arabinose, lactose, mannose, cellobiose, raffinose, sucrose, sorbose, xylan, inositol, mannitol, D-sorbitol, methanol, ethanol, citrate, fumarate, lactate, pyruvate, succinate, L-alanine, L-arginine, aspartate, asparagine, L-cysteine, L-glutamine, glutamate, glycine, L-histidine, L-leucine, L-lysine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, tryptophan or L-valine. The main products of carbohydrate fermentation were acetate, lactate, formate and propionate. We supposed that strain Zn2^T degrades carbohydrate first to simple organic acids and then to CO_2 and H_2.

To analyse the reduction of electron acceptors, sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM) and sodium nitrate (20 mM) were added from filter-sterilized solutions to the basal medium (lacking the reductant Na_2S, L-cysteine and Casamino acids). Elemental sulfur (1 %), amorphous Fe(III) oxyhydroxide (0.2 %) and manganese dioxide (0.2 %) were added to the basal medium directly before autoclaving. Reduction of elemental sulfur, thiosulfate, sulfite and sulfate was tested using the method of Ramamoorthy *et al.* (2006). $Fe(NH_4)_2(SO_4)_2$ (0.5 %) was added to the culture tubes. Tubes were scored as positive if a black FeS precipitate was formed. The reduction of nitrate to nitrite was determined by using Griess reagent; tubes were scored as positive if the Griess reagent turned red. Reduction of Fe(III) was inferred when a transformation of the reddish-brown colour of the Fe(III) oxide to a dark precipitate [Fe(II)] and a clearing of the medium was observed (Ogg & Patel, 2009). Mn(IV) reduction was inferred by a clearing of the medium (Ogg & Patel, 2009). Elemental sulfur, thiosulfate and Fe(III) were used as electron acceptors, while sulfite, sulfate, nitrate, nitrite and Mn(IV) were not.

Sensitivity to antibiotics was tested under increasing concentrations (10, 100, 200 and 300 μg ml^{-1}) of ampicillin, chloramphenicol, penicillin, streptomycin and tetracycline. Growth of strain Zn2^T was inhibited completely by the addition of ampicillin, penicillin, tetracycline or chloramphenicol at 10 μg ml^{-1}.

Chemotaxonomic analyses were performed on strain Zn2^T, *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T. The cells were harvested in late exponential growth. Fatty acid methyl esters were obtained from freeze-dried cells of all three strains. Identification and quantification of fatty acid methyl esters as well as numerical analysis of the fatty acid profiles was carried out by using the Sherlock Microbial Identification System with the standard MIS library generation software (Microbial ID Inc.) as described by Fang *et al.* (2012), matching the results with the Anaerobe Moore 3.90 library. The results are presented in Table 1. The major fatty acids of strain Zn2^T were iso-$C_{15:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ *cis*9 and $C_{18:1}$ *cis*9. Significant qualitative and quantitative differences existed in the fatty acid profiles of the three strains (Table 1).

Isoprenoid quinones was extracted using the method described by Minnikin *et al.* (1984), and analysed by

Table 1. Fatty acid contents of strain Zn2^T and related type strains

Strains: 1, Zn2^T; 2, *G. subterraneus* DSM 17957^T; 3, *T. metallivorans* JCM 15105^T. Values are percentages of total fatty acids and were determined in this study. Major components (>5 %) are in bold. Fatty acids present at less than 0.5 % in all strains are not shown. −, Not detected; tr, trace amount (<0.5 %); ALDE, aldehyde; DMA, dimethylacetal; ECL, equivalent chain length; FAME, fatty acid methyl ester.

Fatty acid	1	2	3
$C_{10:0}$ FAME	−	2.3	0.9
$C_{11:0}$ FAME	−	0.6	−
iso-$C_{11:0}$ FAME	−	tr	0.9
$C_{11:0}$ DMA	−	1.3	tr
$C_{12:0}$ FAME	0.8	3.7	1.1
$C_{12:0}$ 3-OH FAME	−	2.1	−
$C_{13:0}$ FAME	−	**8.0**	tr
iso-$C_{13:0}$ FAME	1.9	0.8	**7.5**
$C_{13:1}$ *cis*12 FAME	−	1.9	1.0
$C_{14:0}$ FAME	**19.8**	**24.5**	**13.6**
iso-$C_{14:0}$ FAME	0.6	1.1	tr
$C_{14:1}$ *cis*9 FAME	1.2	−	tr
$C_{14:0}$ DMA	−	**5.7**	2.6
$C_{14:1}$ *cis*7 DMA	−	1.5	tr
$C_{15:0}$ FAME	1.0	**7.9**	tr
iso-$C_{15:0}$ FAME	**18.6**	1.1	**45.4**
anteiso-$C_{15:0}$ FAME	1.2	1.0	3.1
iso-$C_{15:0}$ 3-OH FAME	−	−	0.6
anteiso-$C_{15:0}$ 3-OH FAME	−	0.7	−
$C_{15:0}$ DMA	−	4.3	−
iso-$C_{15:0}$ DMA	tr	−	**11.5**
anteiso-$C_{15:0}$ DMA	−	−	1.0
iso-$C_{15:0}$ ALDE	−	−	3.4
$C_{15:1}$ *cis*9/*trans*8 FAME	−	1.9	−
Unknown ECL 14.762 $C_{15:2}$ FAME	tr	**7.8**	tr
$C_{16:0}$ FAME	**16.5**	2.9	1.7
$C_{16:1}$ *cis*7 FAME	−	1.1	tr
$C_{16:1}$ *cis*9 FAME	**24.8**	4.8	tr
$C_{16:1}$ *cis*11 FAME	1.2	−	−
$C_{16:0}$ DMA	−	2.4	1.0
$C_{16:1}$ *cis*9 DMA	−	3.8	tr
$C_{16:0}$ ALDE	−	0.5	tr
$C_{18:1}$ *cis*9 FAME	**7.6**	0.7	−
$C_{18:0}$ FAME	2.8	tr	tr
$C_{18:1}$ *cis*11/*trans*9/*trans*6 FAME	1.8	−	−

HPLC as described by Tindall (1990). No quinone could be detected in any of the three strains.

Polar lipids were extracted and separated on silica-gel plates (10 × 10 cm; Merck 5554) and analysed further as described by Minnikin *et al.* (1984) and Xu *et al.* (2011). We used sulfuric acid to reveal total lipids, ninhydrin for aminolipids, α-naphthol for glycolipids and Zinzadze's reagent for phospholipids. The results were analysed as described by Fang *et al.* (2012) and are shown in Fig. S3. The main polar lipids of strain Zn2^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, two unknown phospholipids and four unknown glycolipids. Although the main polar lipids of the three trains were similar, differences existed in their content and in the composition of minor lipids.

Based on the phylogenetic analysis described above, strain Zn2^T is likely to represent a novel species of the family *Clostridiaceae*. Moreover, its phylogenetic distance from members of the genera *Geosporobacter*, *Thermotalea* and *Caminicella* (91.2, 90.3 and 91.1 % 16S rRNA gene sequence similarity to the type strains of the respective type species) suggests that strain Zn2^T represents a new genus. This is strongly supported by significant differences in phenotypic and genotypic characteristics, as detailed in Tables 1 and 2. The DNA G + C content of strain Zn2^T was much lower (37 mol%) than the values reported for *G. subterraneus* DSM 17957^T (42 mol%) and *T. metallivorans* JCM 15105^T (48 mol%), but was much higher than the value for *C. sporogenes* DSM 14501^T (24 mol%). Moreover, strain Zn2^T differs from *G. subterraneus* DSM 17957^T, *T. metallivorans* JCM 15105^T and *C. sporogenes* DSM 14501^T in major phenotypic traits, such as the use of carbon sources and electron acceptors, products of carbohydrate fermentation, optimal growth temperature and NaCl tolerance. Strain Zn2^T did not use glucose, while the other three strains did. Additionally, strain Zn2^T differs from members of the closest genera in sporulation and the structure of its cell envelope. Strain Zn2^T has the potential to form spores, whereas but *T. metallivorans* JCM 15105^T does not. Strain Zn2^T has a typical Gram-positive cell wall, while *T. metallivorans* JCM 15105^T and *C. sporogenes* DSM 14501^T have a Gram-negative-type cell wall.

Finally, the evidence from chemotaxonomy showed distant relatedness among strain Zn2^T, *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T. Strain Zn2^T contained no dimethylacetal (DMA) or aldehyde (ALDE) fatty acids, whereas *G. subterraneus* DSM 17957^T ($C_{14:0}$ DMA and $C_{16:0}$ DMA) and *T. metallivorans* JCM 15105^T (iso-$C_{15:0}$ ALDE) contained significant proportions (Table 1). Strain Zn2^T possessed the fatty acid methyl esters (FAMEs) $C_{16:0}$ FAME, $C_{16:1}$ *cis*9 FAME and $C_{18:1}$ *cis*9 FAME as main components, while they were minor constituents in *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T. Additionally, $C_{14:1}$ *cis*9 FAME, $C_{16:1}$ *cis*11 FAME, $C_{18:0}$ FAME and $C_{18:1}$ *cis*11/*trans*9/*trans*6 FAME were detected in extracts of strain Zn2^T, but not in *G. subterraneus* DSM 17957^T or *T. metallivorans* JCM 15105^T. Moreover, strain Zn2^T contained no fatty acids with fewer than 12 carbons, in contrast to *G. subterraneus* DSM 17957^T ($C_{10:0}$ FAME and $C_{11:0}$ FAME) and *T. metallivorans* JCM 15105^T ($C_{10:0}$ FAME and iso-$C_{11:0}$ FAME). Strain Zn2^T possessed diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as main phospholipids; *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T contained smaller amounts of phosphatidylglycerol and larger amounts of phosphatidylethanolamine (Fig. S3). Strain Zn2^T possessed phosphatidylserine, while *G. subterraneus* DSM 17957^T and *T. metallivorans* JCM 15105^T did not. The compositions of unknown glycolipids and phospholipids were also different in the three strains.

On the basis of the phenotypic differences and the large phylogenetic distance that separates strain Zn2^T from other members of the family *Clostridiaceae*, it is suggested that strain Zn2^T represents a novel species of a new genus, for which the name *Salimesophilobacter vulgaris* gen. nov., sp. nov. is proposed.

Description of *Salimesophilobacter* gen. nov.

Salimesophilobacter [Sa.li.me.so.phi.lo.bac′ter. L. n. *sal*, *salis* salt; Gr. adj. *mesos* middle; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend to, loving; N.L. masc. n. *bacter* rod, staff; N.L. masc. n. *Salimesophilobacter* halotolerant, mesophilic rod].

Cells are anaerobic, long, thin rods, motile by a lateral flagellum. Mesophilic and halotolerant. Gram-type-positive and show genetic potential for sporulation. No respiratory quinones can be detected. The major cellular fatty acids are iso-$C_{15:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ *cis*9 and $C_{18:1}$ *cis*9. The main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and unknown phospholipids and glycolipids. The genomic DNA G + C content of the type strain of the type species is 37 mol%. 16S rRNA gene analysis indicates that the genus is a member of the family *Clostridiaceae* and is most closely related to the genera *Geosporobacter*, *Thermotalea* and *Caminicella*. The type species is *Salimesophilobacter vulgaris*.

Description of *Salimesophilobacter vulgaris* sp. nov.

Salimesophilobacter vulgaris (vul.ga′ris. L. fem. adj. *vulgaris* common, referring to the lack of specific characteristics).

Cells are 0.5–0.8 × 2.0–4.0 μm. Growth occurs at 10–50 °C (optimum 35 °C) and pH 6.0–9.5 (optimum pH 7.3–7.5). The range of salt concentration for growth is 0–10 % (w/v) NaCl (optimum at 6 %). Grows on peptone as a sole carbon source. Growth can be observed in the presence of D-xylose, salicin, glycerol, acetate, formate, propionate and Casamino acids; but not in the presence of D-glucose, D-fructose, D-galactose, maltose, D-ribose, L-arabinose, lactose, mannose, cellobiose, raffinose, sucrose, sorbose, xylan,

Table 2. Differential phenotypic, physiological and genotypic characteristics of strain Zn2^T and its closest relatives

Strains: 1, Zn2^T; 2, *G. subterraneus* DSM 17957^T (data from Klouche *et al.*, 2007); 3, *T. metallivorans* JCM 15105^T (Ogg & Patel, 2009); 4, *C. sporogenes* DSM 14501^T (Alain *et al.*, 2002). +, Positive; −, negative; ND, no data available.

Characteristic	1	2	3	4
Cell width (μm)	0.5–0.8	0.5	0.6–0.7	0.5–0.7
Cell length (μm)	2.0–4.0	3.0–5.0	3.0–3.5	3.0–10.0
Gram stain	+	+	−	−
Cell-wall Gram type	+	+	−	−
Sporulation genes	+	+	−	+
Motility	+	−	+	+
Temperature for growth (°C)				
Optimum	35	42	50	60
Range	10–50	25–55	30–55	45–65
pH for growth				
Optimum	7.3–7.5	7.5	8.0	7.5
Range	6.5–9.0	4.5–8.0	6.5–9.0	4.5–8.0
NaCl concentration for growth (%, w/v)				
Optimum	6	4	<4	3
Range	0–10	0–5	<4	2–6
DNA G+C content (mol%)*	36.9 (HPLC)	42.2 (HPLC)	48 (T_m)	24.2 (T_m)
Carbohydrate fermentation:				
Glucose	−	+	+	+
Fructose	−	+	+	−
Cellobiose	−	+	+	−
Salicin	+	ND	ND	ND
Mannose	−	−	+	ND
Mannitol	−	ND	+	ND
Ribose	−	+	−	−
Xylose	+	+	−	−
Alcohol fermentation				
Glycerol	+	−	−	ND
Inositol	−	ND	+	ND
Carboxylic acid fermentation				
Formate	+	−	−	−
Acetate	+	−	−	−
Propionate	+	−	−	ND
Amino acid fermentation				
Alanine	−	+	−	ND
Arginine	−	+	−	ND
Glutamate	−	+	−	ND
Glutamine	−	+	−	ND
Isoleucine	−	+	−	−
Serine	−	+	+	−
Reduction of electron acceptors				
Fe(III)	+	ND	+	−
Elemental sulfur	+	−	+	+
Thiosulfate	+	−	−	+
16S rRNA gene sequence similarity to strain Zn2^T (%)	(100)	91.2	90.3	91.1
Products from carbohydrate fermentation†	A, F, P, L	A, F, H_2, CO_2, (E)‡	E, A‡	B, A, E, H_2, CO_2

*Determined by HPLC or thermal denaturation (T_m).

†A, Acetate; B, butyrate; E, ethanol; F, formate; L, lactate; P, propionate. Minor products are indicated in parentheses.

‡Products of glucose fermentation.

inositol, mannitol, D-sorbitol, methanol, ethanol, citrate, fumarate, lactate, pyruvate, succinate, L-alanine, L-arginine, aspartate, asparagine, L-cysteine, L-glutamine, glutamate, glycine, L-histidine, L-leucine, L-lysine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, tryptophan or L-valine. The main products of carbohydrate fermentation are acetate, formate, propionate and lactate. Elemental sulfur, thiosulfate and Fe(III) are used as electron acceptors. Sulfite, sulfate, nitrate, nitrite and Mn(IV) are not used as electron acceptors. The main polar lipids include two unknown phospholipids and four unknown glycolipids.

The type strain, Zn2^T (=DSM 24770^T =JCM 17796^T), was isolated from wastewater of a paper mill in Zhejiang, China.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant no. 30970002).

References

Alain, K., Pignet, P., Zbinden, M., Quillevere, M., Duchiron, F., Donval, J. P., Lesongeur, F., Raguenes, G., Crassous, P. & other authors (2002). *Caminicella sporogenes* gen. nov., sp. nov., a novel thermophilic spore-forming bacterium isolated from an East-Pacific Rise hydrothermal vent. *Int J Syst Evol Microbiol* **52**, 1621–1628.

Brill, J. A. & Wiegel, J. (1997). Differentiation between spore-forming and asporogenic bacteria using a PCR and Southern hybridization based method. *J Microbiol Methods* **31**, 29–36.

Bryant, M. P. (1972). Commentary on the Hungate technique for culture of anaerobic bacteria. *Am J Clin Nutr* **25**, 1324–1328.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Fang, M.-X., Zhang, W.-W., Zhang, Y.-Z., Tan, H.-Q., Zhang, X.-Q., Wu, M. & Zhu, X.-F. (2012). *Brassicibacter mesophilus* gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. *Int J Syst Evol Microbiol* **62**, 3018–3023.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Hungate, R. E. (1969). A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Klouche, N., Fardeau, M. L., Lascourrèges, J. F., Cayol, J. L., Hacene, H., Thomas, P. & Magot, M. (2007). *Geosporobacter subterraneus* gen. nov., sp. nov., a spore-forming bacterium isolated from a deep subsurface aquifer. *Int J Syst Evol Microbiol* **57**, 1757–1761.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.

Meng, F.-X., Yang, X.-C., Yu, P.-S., Pan, J.-M., Wang, C.-S., Xu, X.-W. & Wu, M. (2010). *Citricoccus zhacaiensis* sp. nov., isolated from a bioreactor for saline wastewater treatment. *Int J Syst Evol Microbiol* **60**, 495–499.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Ogg, C. D. & Patel, B. K. C. (2009). *Thermotalea metallivorans* gen. nov., sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia aquifer. *Int J Syst Evol Microbiol* **59**, 964–971.

Ramamoorthy, S., Sass, H., Langner, H., Schumann, P., Kroppenstedt, R. M., Spring, S., Overmann, J. & Rosenzweig, R. F. (2006). *Desulfosporosinus lacus* sp. nov., a sulfate-reducing bacterium isolated from pristine freshwater lake sediments. *Int J Syst Evol Microbiol* **56**, 2729–2736.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tan, H.-Q., Wu, X.-Y., Zhang, X.-Q., Wu, M. & Zhu, X.-F. (2012). *Tepidibacter mesophilus* sp. nov., a mesophilic fermentative anaerobe isolated from soil polluted by crude oil, and emended description of the genus *Tepidibacter*. *Int J Syst Evol Microbiol* **62**, 66–70.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Widdel, F., Kohring, G. W. & Mayer, F. (1983). Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. *Arch Microbiol* **134**, 286–294.

Xu, X.-W., Huo, Y.-Y., Wang, C.-S., Oren, A., Cui, H.-L., Vedler, E. & Wu, M. (2011). *Pelagibacterium halotolerans* gen. nov., sp. nov. and *Pelagibacterium luteolum* sp. nov., novel members of the family *Hyphomicrobiaceae*. *Int J Syst Evol Microbiol* **61**, 1817–1822.

Zhang, W.-W., Fang, M.-X., Tan, H.-Q., Zhang, X.-Q., Wu, M. & Zhu, X.-F. (2012). *Pectinatus brassicae* sp. nov., a Gram-negative, anaerobic bacterium isolated from salty wastewater. *Int J Syst Evol Microbiol* **62**, 2145–2149.