Brassicibacter mesophilus gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater

Ming-Xu Fang, Wen-Wu Zhang, Yan-Zhou Zhang, Hai-Qin Tan, Xin-Qi Zhang, Min Wu and Xu-Fen Zhu

College of Life Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, PR China

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

A novel mesophilic, strictly anaerobic bacterium, strain BM^T, was isolated from food industry wastewater. The cells were motile, non-spore-forming rods and stained Gram-negative. Growth of strain BM^T was observed at 16–44 °C (optimum 37 °C) and pH 6.0–9.0 (optimum pH 7.5). The NaCl concentration range for growth was 0–8 % (optimum 1.5 %, w/v). Strain BM^T was chemo-organotrophic, using a few sugars and amino acids as sole carbon and energy sources. The fermentation products from peptone-yeast extract broth were propionate, formate, acetate, ethanol and isovalerate. Indole, NH_3 and H_2S were produced from peptone. No respiratory quinones could be detected. The major fatty acids were iso-$C_{15:0}$ (39.3 %), iso-$C_{15:0}$ dimethyl acetal (10.1 %), anteiso-$C_{15:0}$ (7.6 %), $C_{14:0}$ (6.1 %) and $C_{16:0}$ (5.6 %). The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol and a number of unidentified aminoglycolipids, glycolipids and phospholipids. The DNA G+C content was 28.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain BM^T was related to various genera of the family *Clostridiaceae*, and its closest relatives were *Sporosalibacterium faouarense* SOL3f37^T (94.3 % 16S rRNA gene sequence similarity), *Proteiniborus ethanoligenes* GW^T (92.1 %) and *Clostridiisalibacter paucivorans* 37HS60^T (92.0 %). In recognition of its distinct phenotypic and genotypic characteristics, isolate BM^T is proposed to represent a novel species of a new genus, *Brassicibacter mesophilus* gen. nov., sp. nov. The type strain of *Brassicibacter mesophilus* is BM^T (=JCM 16868^T =DSM 24659^T).

Zhacai is a variety of preserved vegetable that has been favoured in China since it was originally salted during the 1890s. The tumid stem of *Brassica juncea* var. *tumida* is generally used to make the juicy, salty and slightly sour pickle. During the salting process, a microbial community that consists of bacteria, yeasts and fungi plays important roles in the degradation of protein, cellulose and starch from the vegetable to form the distinctive flavour.

Industrial manufacture of zhacai was introduced in China during the 1980s, resulting in an industrial wastewater with high salinity (2–8 %) and organic load. Microbiological degradation has proved to be an effective technology to treat the industrial wastewater. When developing the technology, the microbiological composition of the wastewater was investigated and several strains with low 16S rRNA gene sequence similarity to known species were isolated, such as *Citricoccus zhacaiensis* FS24^T (Meng *et al.*, 2010). Here, we report a strain representing a novel species of a new genus belonging to the family *Clostridiaceae* within the order *Clostridiales* (Wiegel, 2009) that is a strictly anaerobic, mesophilic, non-spore-forming bacterium, isolated from zhacai wastewater.

DSMZ medium 104b was used for isolation and routine cultivation. The wastewater was serially diluted and inoculated into Hungate roll-tubes (Hungate, 1969) under O_2-free N_2. The tubes were incubated at 37 °C until colonies were formed. Hungate roll-tube technique was performed several times to acquire a pure culture of strain BM^T.

The cell morphology was examined under optical (Olympus BX 40) and electron (JEOL JEM-1230) microscopy. Gram staining was performed in all growth phases. Silver-plating staining was performed to observe the flagella, which were also observed under transmission electron microscopy. For transmission electron microscopy, cells were prepared on plates incubated at 37 °C in an anaerobic chamber (Bugbox; Ruskinn) for 48 h. Ultrathin sectioning was performed to examine the cell ultrastructure. Uranyl acetate was used to stain the cells.

Abbreviations: DMA, dimethyl acetal; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BM^T is GU645013.

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

Cells of strain BM^T were long, thin rods (0.3–0.6 × 2.0–6.8 μm) with monotrichous flagella (Fig. S1a, available in IJSEM Online). Cells appeared singly or in pairs. The cells stained Gram-negative in all growth phases and the ultrastructure also revealed a Gram-negative-type cell wall (Fig. S1b). Spores were not observed during cultivation. The heat resistance of the cells was determined as reported by Cayol *et al.* (2000); the cells could not survive heating to 80 °C for 20 min.

The temperature range for growth was determined using a water bath maintained at 10–50 °C at 1 °C intervals. To study the salinity requirement, DSMZ medium 104b was prepared without NaCl and NaCl was added at 0–10 % (w/v) at 0.5 % intervals. To examine the pH range for growth, 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 10.0–11.5) was added at 25 mM and the pH was adjusted using sterile solutions (10 %) of HCl or NaOH. All experiments were performed in triplicate.

Strain BM^T was a strict anaerobe. The optimal temperature for growth was 37 °C (range 16–44 °C). NaCl was not necessary for growth; the strain grew in the presence of 0–8 % NaCl, with optimum growth at 1.5 %. The pH range for growth was 6.0–9.0 with an optimum at pH 7.5; no growth was observed at or below pH 5.5 or at or above pH 9.5.

Substrate utilization was tested in a basal medium containing (l^{-1}) 0.3 g KH_2PO_4, 0.3 g K_2HPO_4, 1.0 g NH_4Cl, 15 g NaCl, 0.1 g KCl, 0.1 g $CaCl_2.2H_2O$, 0.1 g $MgCl_2.6H_2O$ and 1 ml 0.1 % (w/v) resazurin. The medium was adjusted to pH 7.5. Sugars (20 mM), alcohols (0.1 %), organic acids and amino acids (20 mM) were added individually to the basal medium in the presence of 0.1 g yeast extract l^{-1}. Growth on Casamino acids, peptone, tryptone and yeast extract (all from BD) was also examined. Utilization of substrates was confirmed by analysing fermentation products using HPLC with an ion exclusion column (Aminex hpx-87h; Bio-Rad). To determine potential electron acceptors, elemental sulfur (0.1 %, w/v), sulfate (20 mM), thiosulfate (20 mM), nitrate (10 mM) and nitrite (5 mM) were added to the basal medium lacking the reductant Na_2S, and the results were analysed as described by Ogg & Patel (2009). The methyl red and Voges–Proskauer reactions, H_2S and indole production and catalase and oxidase activities were determined as described by Wu *et al.* (2010a).

Strain BM^T grew heterotrophically on yeast extract, peptone, tryptone and Casamino acids. The fermentation products in peptone-yeast extract broth were propionate, formate, acetate, ethanol and isovalerate. Strain BM^T could use four amino acids (glutamic acid, glycine, methionine and valine) as sole carbon and energy sources, and formed formate, acetate and ethanol. Fructose, glucose, ribose, sucrose and pyruvate were also used, with acetate and ethanol produced. The following substrates were not used: alanine, arginine, asparagine, aspartate, cysteine, glutamine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, arabinose, cellobiose, galactose, lactose, maltose, mannose, melibiose, raffinose, rhamnose, salicin, sorbose, starch, trehalose, xylose, inositol, mannitol, sorbitol, methanol, ethanol, propanol, citrate, fumarate, malate, succinate, malonate, formate, acetate, propionate, lactate, cellulose and xylan. Although tryptophan was not used as a sole carbon source, indole was produced from peptone. Elemental sulfur and sulfate could enhance growth, but not thiosulfate, nitrate or nitrite; however, neither sulfur nor sulfate was reduced to sulfide.

Chemotaxonomic analyses were performed on strain BM^T and *Sporosalibacterium faouarense* JCM 15487^T. Cells used in analyses of the two strains were cultivated in DSMZ medium 104b until the OD_{600} reached 0.2, which was considered to be an indication of late-exponential phase. Fatty acid methyl esters (FAMEs) were obtained from freeze-dried cells as described by Kuykendall *et al.* (1988). Identification and quantification of the FAMEs were performed automatically by using the Sherlock Microbial Identification System with the standard MIS Library Generation Software (Microbial ID Inc.). The main FAMEs of strain BM^T were iso-$C_{15:0}$ (39.3 %), iso-$C_{15:0}$ dimethyl acetal (DMA) (10.1 %), ante-iso-$C_{15:0}$ (7.6 %), $C_{14:0}$ (6.1 %) and $C_{16:0}$ (5.6 %). The fatty acid profiles of the two strains are shown in Table 1. Although the main fatty acids of the two strains were similar, differences were observed in their proportions and also in the minor components observed. Isoprenoid quinones were extracted according to Minnikin *et al.* (1984) and analysed by HPLC as described by Tindall (1990). No quinone was detected from either strain. The 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.* (2007). Molybdatophosphoric acid was used to reveal total lipids, Zinzadze's reagent for phospholipids, ninhydrin for aminolipids and α-naphthol for glycolipids. The polar lipid composition of strain BM^T was distinguishable from that of *S. faouarense* JCM 15487^T (Fig. S2).

Genomic DNA was extracted and the 16S rRNA gene was amplified as described by Wu *et al.* (2010b). The almost-complete 16S rRNA sequence (1490 nt) was compared with sequences from closely related species with the EzTaxon service (Chun *et al.*, 2007). Sequence data were aligned with CLUSTAL W version 1.8 (Thompson *et al.*, 1994). Neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods in MEGA 5 (Tamura *et al.*, 2011) were used to reconstruct the phylogenetic tree. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining tree (Fig. 1). Its topology was also supported by the maximum-parsimony and maximum-likelihood trees (see Figs S3 and S4). The DNA G+C content of strain BM^T, determined by reverse-phase HPLC according to Mesbah & Whitman (1989), was 28.2 mol%.

On the basis of 16S rRNA gene sequence similarity, the three closest relatives of strain BM^T were *S. faouarense*

Table 1. Cellular fatty acid contents of strain BM^T and *S. faouarense* JCM 15487^T

Data were obtained in this study under identical conditions. Values are percentages of total fatty acids; fatty acids representing >5 % of the total are in bold. ALDE, Aldehyde; DMA, dimethyl acetal; FAME, fatty acid methyl ester; ND, not detected.

Fatty acid	Strain BM^T	*S. faouarense* JCM 15487^T
iso-$C_{11:0}$ FAME	ND	0.3
iso-$C_{13:0}$ FAME	1.5	1.4
anteiso-$C_{13:0}$ FAME	0.3	0.5
Summed feature 1*	0.5	0.6
Summed feature 3*	2.1	**5.5**
iso-$C_{14:0}$ FAME	1.8	0.5
$C_{14:0}$ FAME	**6.1**	1.6
$C_{14:0}$ DMA	2.0	1.9
iso-$C_{15:0}$ FAME	**39.3**	**42.8**
anteiso-$C_{15:0}$ FAME	**7.6**	**8.7**
$C_{16:0}$ ALDE	0.5	0.3
$C_{15:0}$ FAME	0.3	ND
iso-$C_{15:0}$ DMA	**10.1**	**22.5**
anteiso-$C_{15:0}$ DMA	1.6	2.5
iso-$C_{16:0}$ FAME	0.6	ND
$C_{16:1}$ *cis*7 FAME	0.2	ND
$C_{16:1}$ *cis*9 FAME	1.5	0.2
$C_{16:0}$ FAME	**5.6**	1.8
Summed feature 6*	0.2	ND
$C_{16:1}$ *cis*9 DMA	0.7	0.3
$C_{16:0}$ DMA	2.9	1.9
iso-$C_{17:0}$ FAME	1.0	0.5
anteiso-$C_{17:0}$ FAME	0.9	ND
$C_{17:0}$ cyclo FAME	0.9	1.8
anteiso-$C_{17:0}$ DMA	ND	0.4
$C_{18:2}$ *cis*9,12 FAME	0.6	ND
$C_{18:1}$ *cis*9 FAME	1.1	0.6
Summed feature 10*	0.8	0.6
$C_{18:0}$ FAME	3.7	1.1
Summed feature 11*	0.2	ND
$C_{18:0}$ DMA	0.3	ND
anteiso-$C_{19:0}$ FAME	0.3	ND
$C_{19:0}$ FAME	0.6	ND
$C_{20:1}$ *cis*11 FAME	0.6	ND
$C_{20:1}$ *cis*13/*trans*11 FAME	0.5	ND
$C_{20:0}$ FAME	1.9	ND

*Summed features are groups of two or three fatty acids that could not be separated under the conditions used. They contain the following components: summed feature 1, $C_{13:1}$ *cis*12 FAME and/or $C_{11:1}$ 2-OH FAME; summed feature 3, iso-$C_{15:0}$ ALDE and/or unknown fatty acid ECL 13.570; summed feature 6, anteiso-$C_{15:0}$ 3-OH FAME and/or $C_{16:1}$*cis*7 DMA; summed feature 10, one or more of $C_{18:1}$ *cis*11/*trans*9/*trans*6; summed feature 11, iso-$C_{17:0}$ 3-OH FAME and/or $C_{18:2}$ DMA.

SOL3f37^T (94.3 % pairwise similarity), *Proteiniborus ethanoligenes* GW^T (92.1 %) and *Clostridiisalibacter paucivorans* 37HS60^T (92.0 %). Strain BM^T was also related

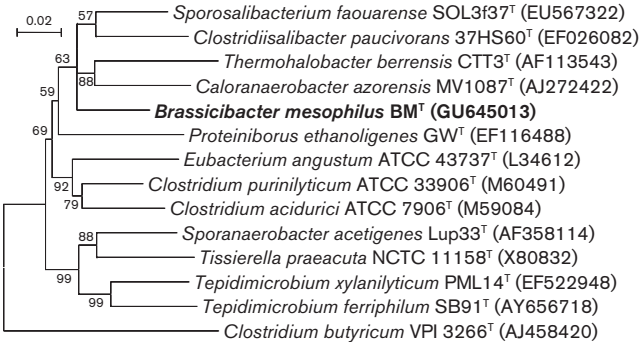

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strain BM^T and related species. Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.02 substitutions per nucleotide position.

to two thermophilic strains, *Thermohalobacter berrensis* CTT3^T (91.3 % pairwise similarity) and *Caloranaerobacter azorensis* MV1087^T (91.1 %). Comparisons of 16S rRNA gene sequences revealed that strain BM^T belonged to cluster XII of the *Clostridiales* (Collins *et al.*, 1994). According to the latest edition of *Bergey's Manual of Systematic Bacteriology* (Wiegel, 2009), strain BM^T should be classified into the family *Clostridiaceae* within the order *Clostridiales*, and probably represents a novel species of a new genus.

Genotypic and phenotypic traits suggest that strain BM^T is distinct from its phylogenetic relatives (Table 2). Strain BM^T differed from *T. berrensis* CTT3^T (Cayol *et al.*, 2000) and *Caloranaerobacter azorensis* MV1087^T (Wery *et al.*, 2001) in that the latter strains are thermophilic (and hence were not included in Table 2), with optimal growth temperatures of 65 °C, whereas the upper temperature for growth of strain BM^T was 44 °C. Strain BM^T fermented amino acids and sugars, which were not metabolized by *P. ethanoligenes* GW^T (Niu *et al.*, 2008), and the G+C content of *P. ethanoligenes* GW^T (38.0 mol%) was significantly higher than that of strain BM^T. In contrast to *Clostridiisalibacter paucivorans* 37HS60^T (Liebgott *et al.*, 2008), strain BM^T could use four sugars as sole carbon and energy sources, and was able to use glutamic acid, glycine, methionine and valine, while *Clostridiisalibacter paucivorans* 37HS60^T fermented cysteine, lysine, serine and valine.

Finally, chemotaxonomic evidence showed the distant relatedness between strain BM^T and *S. faouarense* JCM 15487^T. Strain BM^T had a lower content (10.1 %) of iso-$C_{15:0}$ DMA than did *S. faouarense* JCM 15487^T (22.5 %), and also possessed $C_{14:0}$ and $C_{16:0}$ as major components, while these two fatty acids were minor components in *S. faouarense* JCM 15487^T (Table 1). Moreover, nonadecanoic acids ($C_{19:0}$, anteiso-$C_{19:0}$) and eicosaenoic acids ($C_{20:0}$, $C_{20:1}$ *cis*11, $C_{20:1}$ *cis*13/*trans*11) were present in extracts of

Table 2. Characteristics that distinguish strain BM^T from the type strains of phylogenetically related species

Strains: 1, BM^T; 2, *S. faouarense* SOL3f37^T (data from Rezgui *et al.*, 2011); 3, *P. ethanoligenes* GW^T (Niu *et al.*, 2008); 4, *Clostridiisalibacter paucivorans* 37HS60^T (Liebgott *et al.*, 2008). +, Positive; −, negative; ND, no data available; UASB, upflow anaerobic sludge blanket.

Characteristic	1	2	3	4
Isolation source	Food industry wastewater	Hydrocarbon-polluted soil	UASB sludge	Olive-mill wastewater
Cell width × length (μm)	0.3–0.6 × 2.0–6.8	0.5 × 5.0–10.0	0.5–0.6 × 1.4–3.8	0.5 × 3.0–8.0
Motility	+	+	−	+
Gram stain	−	+	−	+
Cell-wall type	−	+	+	+
Spore formation	−	+	−	+
Yeast extract dependence	−	+	−	−
NaCl dependence	−	+	−	+
Type of flagella	Monotrichous	Monotrichous	None	Peritrichous
Temperature for growth (°C)				
Range	16–44	20–48	20–48	20–50
Optimum	37	40	37	42
pH for growth				
Range	6.0–9.0	6.2–8.1	6.4–10.0	5.5–8.5
Optimum	7.5	6.9	8.5–8.8	6.8
NaCl concentration for growth (%, w/v)				
Range	0–8	0.5–15	0–2	1–10
Optimum	1.5	4	ND	5
DNA G+C content (mol%)	28.2	30.7	38.0	33.0
Growth on Casamino acids	+	−	+	+
Substrates used				
Cysteine	−	−	−	+
Glutamic acid	+	−	ND	−
Glycine	+	−	ND	−
Lysine	−	−	−	+
Methionine	+	−	−	−
Serine	−	−	−	+
Valine	+	−	−	+
Fructose	+	+	−	−
Glucose	+	+	−	−
Ribose	+	−	−	−
Sucrose	+	−	−	−
Fumarate	−	+	−	+
Succinate	−	−	−	+
Pyruvate	+	+	−	+

strain BM^T but not in *S. faouarense* JCM 15487^T. Strain BM^T possessed diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as major phospholipids; however, in *S. faouarense* JCM 15487^T, there was little phosphatidylethanolamine, and the glycolipids were also different in the two strains (Fig. S2). Other differences between strain BM^T and *S. faouarense* JCM 15487^T (Rezgui *et al.*, 2011) included Gram-staining and cell-wall type, spore formation, dependence on yeast extract and NaCl, tolerance of NaCl and substrates used.

On the basis of the genotypic and phenotypic characteristics described above, we propose that strain BM^T represents a novel species of a new genus, with the name *Brassicibacter mesophilus* gen. nov., sp. nov.

Description of *Brassicibacter* gen. nov.

Brassicibacter (Bras′si.ci.bac′ter. L. n. *brassica* a cabbage, and also a scientific genus name; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Brassicibacter* rod-shaped bacterium from *Brassica*, referring to the isolation of the first strain from wastewater of the preserved vegetable *Brassica juncea*).

Motile, rod-shaped, non-spore-forming bacteria. Stain Gram-negative. Mesophilic and strictly anaerobic. Chemoorganotrophs able to grow on Casamino acids, peptone, tryptone and yeast extract. Ferment a few sugars and amino acids. Propionate is formed from peptone-yeast extract broth. No respiratory quinones can be detected. The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:0}$ DMA, anteiso-$C_{15:0}$,

$C_{14:0}$ and $C_{16:0}$. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as well as various unidentified aminoglycolipids, glycolipids and phospholipids. The DNA G+C content of the only known strain is 28.2 mol%. 16S rRNA gene sequence comparisons locate the genus in the lineage of low-G+C-content Gram-positive bacteria, in the *Clostridiaceae*, the type family of the order *Clostridiales*. The type species is *Brassicibacter mesophilus*.

Description of *Brassicibacter mesophilus* sp. nov.

Brassicibacter mesophilus [me.so.phi′lus. Gr. adj. *mesos* middle; N.L. adj. *philus -a -um* from Gr. adj. *philos -ê -on* friend to, loving; N.L. masc. adj. *mesophilus* middle (temperature)-loving, mesophilic].

Displays the following properties in addition to those given for the genus. Cells are 0.3–0.6 × 2.0–6.8 μm, occurring singly or in pairs. Monotrichously flagellated. Grows at 16–44 °C (optimum 37 °C) and at pH 6.0–9.0 (optimum pH 7.5). The NaCl range for growth is 0–8 % (w/v), with an optimum at 1.5 %. Heterotrophic. Cells are able to ferment the following substrates in the presence of yeast extract (0.1 g l^{-1}): glutamic acid, glycine, methionine, valine, fructose, glucose, ribose, sucrose and pyruvate. Not able to reduce elemental sulfur, sulfate, thiosulfate, nitrate or nitrite. Methyl red and Voges–Proskauer tests are negative. Tests for catalase and oxidase activities are negative. H_2S, NH_3 and indole are produced from peptone.

The type strain, BM^T (=JCM 16868^T =DSM 24659^T), was isolated from food industry wastewater in China. The DNA G+C content of strain BM^T is 28.2 mol%.

Acknowledgements

We thank Dr Jean Euzéby for his help with etymology and nomenclature. This work was supported by grants from the National Natural Science Foundation of China (grant no. 30970002) and from the National Science and Technology Major Projects – Water Pollution Control and Treatment Project (2008ZX07101-006-3).

References

Cayol, J. L., Ducerf, S., Patel, B. K., Garcia, J. L., Thomas, P. & Ollivier, B. (2000). *Thermohalobacter berrensis* gen. nov., sp. nov., a thermophilic, strictly halophilic bacterium from a solar saltern. *Int J Syst Evol Microbiol* **50**, 559–564.

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.

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

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 the 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.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Liebgott, P. P., Joseph, M., Fardeau, M. L., Cayol, J. L., Falsen, E., Chamkh, F., Qatibi, A. I. & Labat, M. (2008). *Clostridiisalibacter paucivorans* gen. nov., sp. nov., a novel moderately halophilic bacterium isolated from olive mill wastewater. *Int J Syst Evol Microbiol* **58**, 61–67.

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. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.

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.

Niu, L., Song, L. & Dong, X. (2008). *Proteiniborus ethanoligenes* gen. nov., sp. nov., an anaerobic protein-utilizing bacterium. *Int J Syst Evol Microbiol* **58**, 12–16.

Ogg, C. D. & Patel, B. K. (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.

Rezgui, R., Ben Ali Gam, Z., Ben Hamed, S., Fardeau, M. L., Cayol, J. L., Maaroufi, A. & Labat, M. (2011). *Sporosalibacterium faouarense* gen. nov., sp. nov., a moderately halophilic bacterium isolated from oil-contaminated soil. *Int J Syst Evol Microbiol* **61**, 99–104.

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

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

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.

Wery, N., Moricet, J. M., Cueff, V., Jean, J., Pignet, P., Lesongeur, F., Cambon-Bonavita, M. A. & Barbier, G. (2001). *Caloranaerobacter azorensis* gen. nov., sp. nov., an anaerobic thermophilic bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **51**, 1789–1796.

Wiegel, J. (2009). Family I. *Clostridiaceae* Pribram 1933, 90^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 3, pp. 736–738. Edited by G. M. Garrity, P. De Vos, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K. H. Schleifer & W. B. Whitman. New York: Springer.

Wu, X.-Y., Zheng, G., Zhang, W.-W., Xu, X.-W., Wu, M. & Zhu, X.-F. (2010a). *Amphibacillus jilinensis* sp. nov., a facultatively anaerobic, alkaliphilic bacillus from a soda lake. *Int J Syst Evol Microbiol* **60**, 2540–2543.

Wu, X.-Y., Shi, K.-L., Xu, X.-W., Wu, M., Oren, A. & Zhu, X.-F. (2010b). *Alkaliphilus halophilus* sp. nov., a strictly anaerobic and halophilic bacterium isolated from a saline lake, and emended description of the genus *Alkaliphilus*. *Int J Syst Evol Microbiol* **60**, 2898–2902.

Xu, X.-W., Wu, Y.-H., Zhou, Z., Wang, C.-S., Zhou, Y.-G., Zhang, H.-B., Wang, Y. & Wu, M. (2007). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619–1624.