Clostridium arbusti sp. nov., an anaerobic bacterium isolated from pear orchard soil

Min Young Jung,^{1,2} In-Soon Park,¹ Wonyong Kim,² Hong Lim Kim,³ Woon Kee Paek⁴ and Young-Hyo Chang¹

Correspondence

Young-Hyo Chang
yhchang@kribb.re.kr

¹Korean Collection for Type Cultures, Biological Resource Center, KRIIBB, 52 Oeundong, Daejeon 305-806, Republic of Korea

²Department of Microbiology, College of Medicine, Chung-Ang University, 221 Heukseok-dong, Dongjak-ku, Seoul 156-756, Republic of Korea

³Namhae Sub-Station, National Horticultural Research Institute, RDA, Namhae, 668-812, Republic of Korea

⁴National Science Museum, 32-2 Guseong-dong, Yuseong-gu, Daejeon 305-705, Republic of Korea

An obligately anaerobic, Gram-positive, spore-forming bacterial strain, designated SL206^T, was isolated from pear orchard soils. Strain SL206^T cells were straight or slightly curved rods, with motility by peritrichate flagella. Cell walls contained *meso*-diaminopimelic acid; wall sugars were glucose, rhamnose and mannose. The major fatty acids were C_{16:0}, C_{18:1 ω 9c} and summed feature 10 (containing C_{18:1 ω 11c/9t/6t}). API 20A reactions were negative for oxidase, catalase and acid production from L-rhamnose, sucrose, trehalose, D-xylose, melezitose, salicin and D-sorbitol, and positive for acid production from D-glucose, sucrose, maltose, D-mannose and raffinose. Glucose was fermented to acetate, butyrate, CO₂, H₂ and ethanol in culture. The G + C content of the genomic DNA was 31.1 mol%. Based on comparative 16S rRNA gene sequence analysis, the isolate belonged to the genus *Clostridium* and formed a clade with *Clostridium pasteurianum*. The species most closely related to strain SL206^T were *C. pasteurianum* (98.6 % similarity) and *Clostridium acidisoli* (97.8 % similarity). In DNA–DNA relatedness studies, the isolate had 59.5 % relatedness with *C. pasteurianum* and thus represented a unique species. On the basis of these studies, strain SL206^T (=KCTC 5449^T =JCM 14858^T) is proposed to represent the type strain of a novel species, *Clostridium arbusti* sp. nov.

The genus *Clostridium* is a large and phenotypically heterogeneous taxon, currently comprising more than 190 species (Wiegel *et al.*, 2006). The DNA of these anaerobic bacteria are classified as belonging to three major groups (Wiegel *et al.*, 2006): the low-G + C Gram-positive bacteria, the *Clostridium coccoides*–*Eubacterium rectale* group (cluster XIVa), and the *Clostridium leptum* group (cluster IV). The species within this genus are divided into several cluster groups based on 16S rRNA gene sequences (Collins *et al.*, 1994). Cluster I, which includes the type species of the genus, *Clostridium butyricum*, is the largest of the clostridial groups, and members of this group exhibit similarity values above 90 % despite exhibiting a range of saccharolytic, proteolytic, psychrophilic, mesophilic and thermophilic phenotypes. Some of the strains within cluster I have been isolated and described as novel micro-organisms obtained from soil or sediments (Jeong *et al.*,

2004; Kim *et al.*, 2006, 2007; Lee *et al.*, 2007). In this study, we report the taxonomic description of a strictly anaerobic bacterium within cluster I, designated strain SL206^T.

Strain SL206^T was isolated from soil samples (30 cm depth) collected from a pear orchard in Daejeon, Republic of Korea. The samples were serially diluted in 0.85 % (w/v) saline solution, spread onto Reinforced Clostridial Medium (RCM; Difco) that was heated at 80 °C for 10 min, and incubated at 30 °C under anaerobic conditions (Forma Anaerobic System; Thermo Fisher Scientific) using a gas phase of N₂/H₂/CO₂ (88:7:5 %, v/v). Strain SL206^T was subcultured several times to obtain a pure culture on agar plates. The reference strains used in the study were *Clostridium pasteurianum* KCTC 1674^T and *Clostridium acidisoli* KCTC 5384^T. Strains were cultured routinely on RCM in Hungate tubes (Bellco) under anaerobic conditions and stored at –80 °C as skim milk (Difco) suspensions (10 %, w/v).

To determine the phenotypic characteristics of strain SL206^T, cells were grown on RCM agar at 37 °C for 48 h.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SL206^T is EU816420.

Gram staining (Gram stain kit; Difco) and the KOH test were carried out as described previously (Chang *et al.*, 2008). Cell morphology and flagellation were examined by phase-contrast microscopy (Nikon 80i) and transmission electron microscopy (H-7600, Hitachi). Catalase activity was detected by placing drops of 3 % (v/v) H₂O₂ onto plate-grown cultures and observing the production of oxygen bubbles. The presence of oxidase activity was determined by using an Oxy-swab (bioMérieux). Various biochemical properties were determined by using the API 20A, 32A and ZYM strips (bioMérieux) according to the manufacturer's instructions. The pH range for growth was determined by growing cells in 500 ml bottles (Corning) containing 250 ml RCM broth at pH 4–9 in 0.5-unit increments. Broths were buffered with Na₂HPO₄/NaH₂PO₄, succinic acid/NaOH or 2-amino-2-methyl-1,3-propanediol/HCl. The pH and NaCl (0–5 %) ranges for growth were determined after 5 days of incubation at 32 °C in RCM. The temperature range for growth (5–50 °C) was also determined. Growth was checked by monitoring optical density at 595 nm (Bio-Rad). Motility was observed in a Hungate tube containing 0.4 % semi-solid agar. The cell-wall sugar and diaminopimelic acid in cell-wall hydrolysates were analysed by using solvent systems as described by Komagata & Suzuki (1987).

Colonies of strain SL206^T cultivated on RCM agar were circular (approx. 1.0–1.5 mm in diameter), entire, convex and white or ivory. Cells were Gram-positive, strictly anaerobic and straight or slightly curved rods (1.0–1.3 × 3.5–4.5 µm). Cells were motile with flagella that were primarily peritrichately arranged. Oval endospores were observed in a terminal position (Fig. 1a). Electron micrographs of thin sections of cells showed that the cytoplasmic membrane of the strain was surrounded by a peptidoglycan layer and an outer layer (Fig. 1b). Cells grew at 20–40 °C and at pH 5.5–9.0. Optimal growth was observed at 37 °C

and pH 7.0. No growth occurred with greater than 3.0 % (w/v) NaCl.

The cell wall of strain SL206^T contained *meso*-diaminopimelic acid; wall sugars were glucose, rhamnose and mannose. In contrast, *C. pasteurianum* KCTC 1674^T contained glucose, rhamnose, mannose and galactose, and *C. acidisoli* KCTC 5384^T contained glucose, rhamnose and galactose. Cell wall sugars of strain SL206^T were significantly different from those of closely related species. Strain SL206^T was similar to the most closely related species with respect to fatty acid profile, utilization of glucose, sucrose, maltose and mannose (Heyndrickx *et al.*, 1991; Holdeman *et al.*, 1977), and nitrogen-fixing activity in nitrogen-free media (Kasap & Chen, 2005; Kuhner *et al.*, 2000). However, it differed from the related species in certain phenotypic characteristics such as enzyme reaction and cell wall sugar composition. Detailed phenotypic characteristics of strain SL206^T are reported in Table 1 and the species description.

The nearly complete 16S rRNA gene sequence (1347 bp) for strain SL206^T was determined according to Chang *et al.* (2002). The resulting sequence was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the model of Jukes & Cantor (1969). The PHYLIP package (Felsenstein, 1993) was used for all analyses. The tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining methods based on 1000 resamplings.

A preliminary comparison of the 16S rRNA gene sequence for strain SL206^T was performed by an initial BLAST search

Fig. 1. Transmission electron micrographs of thin sections of strain SL206^T cells showing (a) cell division (bar, 1 µm) and (b) Gram-positive-type cell wall (bar, 200 nm). CM, cell membrane; P, peptidoglycan layer; S, septum; SP, spore; W, outer layer.

Table 1. Differential physiological characteristics of strain SL206^T and closely related species of the genus *Clostridium*

Strains: 1, SL206^T; 2, *C. pasteurianum* KCTC 1674^T; 3, *C. acidisoli* KCTC 5384^T. +, Positive; –, negative; w, weakly positive. Data from this study.

Characteristic	1	2	3
Growth temperature range (optimum) °C	20–40 (37)	25–45 (32)	5–37 (25–30)
Growth pH range (optimum)	5.5–9.0 (7.0)	4.7–7.5 (7.0)	4.0–7.0 (4.0–6.5)
Growth in 3% (w/v) NaCl	–	+	–
Cell wall sugar*	R, M, Gc	R, M, Gc, Gl	R, Gc, Gl
Nitrogen-fixation	+	+	–
DNA G + C content (mol%)	31.1	26–28	30.7
API 20A			
D-Mannitol	–	+	+
Lactose	–	+	+
Salicin	–	+	+
D-Xylose	–	+	+
L-Arabinose	–	+	+
Aesculin	–	–	+
Glycerol	–	+	+
Cellobiose	–	+	+
Melezitose	–	+	+
D-Sorbitol	–	+	+
D-Rhamnose	–	+	+
Trehalose	–	+	–
API 32A or ZYM			
α-Galactosidase	+	–	–
β-Galactosidase	–	–	+
β-Glucosidase	–	–	+
Glutamic acid decarboxylase	+	–	–
Acid phosphatase	–	–	+
Alkaline phosphatase	–	–	w

*R, Rhamnose; M, mannose; Gc, glucose; Gl, galactose.

against the GenBank database. The result clearly indicated that the isolate belonged to cluster I of the genus *Clostridium*; this cluster contains the type species of the genus, *C. butyricum*. Aligned sequences of strain SL206^T and members of cluster I of this genus were analysed on the basis of the secondary structure of the bacterial 16S rRNA gene. Phylogenetically, strain SL206^T was closest to *C. pasteurianum* ATCC 6013^T. The results of comprehensive phylogenetic analyses are summarized in Fig. 2. The strain formed a monophyletic clade with the type strain of *C. pasteurianum* (M23930). The clade was confirmed by other treeing algorithms and was supported by a 97% bootstrap value. In the pairwise comparison of 16S rRNA gene similarities, the closest phylogenetic relatives to strain SL206^T were *C. pasteurianum* (98.6%), *C. acidisoli* (97.8%) and *Clostridium akagii* (96.9%). Strain SL206^T and its closest relatives exhibited more than 97% sequence similarity. Therefore, we examined their genomic relatedness using the spectrophotometric DNA–DNA reassociation method.

DNA–DNA hybridization was performed, as described previously (Chang *et al.*, 2008), with photobiotin-labelled probes at 45 °C using a Fluoroskan Ascent Fluorescent plate reader (Thermo Life Science). Three replicates of each sample were tested. DNA–DNA hybridization values between strain SL206^T and strains *C. pasteurianum* KCTC 1674^T and *C. acidisoli* KCTC 5384^T were 59.5 and 53.2%, respectively. These values were moderate but below the suggested threshold value for species delineation (Wayne *et al.*, 1987). These values indicate that strain SL206^T represents a novel species that is distinct from *C. pasteurianum* and *C. acidisoli*.

To determine the DNA G + C (mol%) content, DNA was prepared according to Chang *et al.* (2002) and analysed by reversed-phase HPLC as described by Tamaoka & Komagata (1984). The relative values were calculated by using *Escherichia coli* KCTC 2441^T DNA as the standard. The DNA G + C content of strain SL206^T was 31.1 mol%. Although this value was a little higher than those reported for *C. pasteurianum* (26–28 mol%; Holdeman *et al.*, 1977) and *C. acidisoli* (30.7 mol%; Kuhner *et al.*, 2000), its taxonomic position suggests that strain SL206^T represents a member of cluster I and the low-G + C Gram-positive bacteria of the order *Clostridiales* (Wiegel *et al.*, 2006).

Cellular fatty acid patterns of cells grown on PYG medium (DSMZ medium 104) at 37 °C were determined after 24 h by extracting and analysing the fatty acid methyl esters (FAMES) according to a standard protocol (Sherlock Microbial Identification System; MIDI). The FAMES were separated with an automated GC system (model 6890N and 7683 autosampler; Agilent) and identified by using the Microbial Identification software package (Moore library).

Cellular fatty acid patterns for the novel strain were examined and compared with the patterns of *C. pasteurianum* KCTC 1674^T and *C. acidisoli* KCTC 5384^T (Table 2). In all three strains, C_{16:0}, C_{18:1ω9c} and summed feature 10 (containing C_{18:1ω11c/9t/6t}) were the most common fatty acids, comprising approximately 75–86% of total extracted lipids. The major fatty acids for strain SL206^T (C_{16:0}, C_{18:1ω9c}) were similar to those of other species, whereas the strain differed from this group by the presence of C_{18:0} 12-OH and the absence of C_{16:0} DMA (dimethyl acetate), C_{18:1ω9c} DMA and C_{19:0} cyclo 9,10 DMA.

For detection of end-products from glucose metabolism, strain SL206^T was cultivated in PY basal medium (DSMZ medium 104 without glucose and Tween 80, and with only 1 g yeast extract l^{–1}) at 37 °C for 24 h. Prior to cultivation, pre-sterilized glucose solution was added to the medium to a final concentration of 20 mM. All cultivations were carried out in 50 ml serum vials (working volume of 20 ml) under an atmosphere of N₂/CO₂ (85:15, v/v). Fermentation end-products were analysed by GC (Varian 3400 GC; Varian Associates) equipped with a flame-ionization detector and CP wax 52 CB column, according to methods described by Holdeman *et al.* (1977) and Steer *et al.* (2001).

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1347 bp) showing relationships of strain SL206^T within the genus *Clostridium*. Bootstrap values are calculated from 1000 replications and values >50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 changes per nucleotide position.

Nitrogen-fixing cultures were grown on a nitrogen-free medium, as described by Kasap & Chen (2005), consisting of: sucrose, 30 g; K₂HPO₄, 0.69 g; Na₂SO₄·10H₂O, 0.19 g; mineral solution, 1 ml (KCTC medium no. 650); CaCO₃, 5 g and resazurin 1 mg (per litre of distilled water). Cultures were grown at 32 °C under N₂ in rubber-stoppered 160 ml serum bottles containing 50 ml of medium. Acetylene-reduction assay was used to measure nitrogenase activity, as described by Lee *et al.* (2002), by gas chromatography (Shimadzu 17A) with a flame-ionization detector (FID). The head space of cultures was replaced by argon gas and 10 % acetylene gas was added. Tubes were incubated at 32 °C for 1 h with shaking, and then 300 µl sample was used for GC analysis.

Analysis of fermentation end-products from glucose metabolism showed that acetate, butyrate, acids, abundant

CO₂ and H₂ were formed by strain SL206^T, whereas lactate was not detected. Ethanol was produced. Pyruvate was converted principally to acetate, butyrate, CO₂ and H₂; lactate was not utilized (Wiegel *et al.*, 2006).

Acetylene-reduction assay was used to measure whole-cell nitrogenase activity for strain SL206^T. When grown on medium in the presence of molybdenum, the strain reduced acetylene to ethylene (approx. 0.51 mg l⁻¹). *C. pasteurianum* KCTC 1674^T also showed nitrogenase activity (approx. 0.64 mg ethylene l⁻¹), but *C. acidisoli* KCTC 5384^T did not. No nitrogenase activity was evident in the absence of Mo. Molybdenum was essential for the biosynthesis and activity of the nitrogenase involved (Wiegel *et al.*, 2006).

On the basis of its cell wall sugar composition, nitrogenase activity, fatty acid pattern, DNA G + C content (31.1 mol%), DNA–DNA hybridization values (<59.5 %), 16S rRNA gene sequence analyses (<98.6 % similarity), growth temperature and pH range, strain SL206^T can be differentiated from members of the genus *Clostridium* as a novel species, for which the name *Clostridium arbusti* sp. nov. is proposed.

Description of *Clostridium arbusti* sp. nov.

Clostridium arbusti (ar.bu'sti. L. gen. n. *arbusti* of an orchard, isolated from pear orchard soil).

Cells in RCM agar culture are Gram-staining-positive, straight to slightly curved rods, 1.0–1.3 × 3.5–4.5 µm, occurring singly or in pairs. Motile cells are peritrichous. Spores are oval, terminal and do not swell the cell. Cell walls contain *meso*-diaminopimelic acid; wall sugars are glucose, rhamnose and mannose. Surface colonies on RCM agar plates are 1–1.5 mm in diameter, circular to irregular, low convex, translucent to semiopaque, white or ivory and shiny with a smooth or erose margin and an entire internal structure. Cultures in PYG broth are turbid with a smooth sediment and have a pH of 5.0–5.2 after incubation for 5 days. The optimum temperature for growth is 37 °C. Strains grow nearly as well at 35 °C, moderately well at 32 °C and poorly if at all at 40 °C. Growth is stimulated by fermentable carbohydrate but inhibited by 3.0 % NaCl or a

Table 2. Cellular fatty acid compositions of strain SL206^T and closely related species of the genus *Clostridium*

Strains: 1, SL206^T; 2, *C. pasteurianum* KCTC 1674^T; 3, *C. acidisoli* KCTC 5384^T. Values are percentages of total fatty acids detected. –, Not detected; DMA, dimethyl acetate. Data from this study.

Fatty acid	1	2	3
C _{14:0}	2.6	2.5	2.8
C _{16:1ω9c}	4.6	5.7	5.5
C _{16:0}	31.8	29.7	31.6
C _{16:1ω9c} DMA	2.3	–	1.7
C _{16:0} DMA	–	–	1.9
C _{17:0} cyclo DMA	3.0	–	5.7
C _{18:1ω9c}	38.0	44.9	34.2
C _{18:0}	5.8	6.3	3.4
C _{18:1ω9c} DMA	–	–	3.0
C _{18:0} 12-OH	2.9	–	–
C _{19:0} cyclo 9,10 DMA	–	–	1.2
Summed feature 10*	9.0	11.0	8.9

*Fatty acids that could not be separated by GC using the Microbial Identification System (Microbial ID) software were considered summed features. Summed feature 10 contains C_{18:1ω11d/9t/6t}.

pH of 9.0. Growth occurs in synthetic medium. Abundant gas is detected in RCM and PYG deep agar cultures. Negative API 20A reactions for oxidase, catalase and acid production from L-rhamnose, sucrose, trehalose, D-xylose, melezitose, salicin and D-sorbitol; positive reactions for acid production from D-glucose, sucrose, maltose, D-mannose and raffinose. Positive Rapid ID 32A reactions for α -galactosidase, α -glucosidase and glutamic acid decarboxylase. Positive API ZYM reactions for naphthol-AS-BI-phosphohydrolase, α -galactosidase, α -glucosidase; negative reactions for acid phosphatase, alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, β -galactosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Atmospheric N₂ is fixed; molybdenum is essential for biosynthesis and activity of the nitrogenase involved. Cellular fatty acids include mainly the straight chain C_{16:0}, monounsaturated C_{18:0} ω 9c and summed feature 10 (containing C_{18:1} ω 11c/9t/6t). The DNA G+C content of the type strain is 31.1 mol%. Products in PYG broth cultures include butyric and acetic acids; abundant CO₂ and H₂ are detected. Ethanol is produced. Pyruvate is converted principally to acetate, butyrate, CO₂ and H₂; lactate is not utilized.

The type strain is SL206^T (=KCTC 5449^T =JCM 14858^T), isolated from soil samples from a pear orchard in Daejeon, Republic of Korea.

Acknowledgements

We are grateful to Dr Jean P. Euzéby (Society for Systematic and Veterinary Bacteriology, France) for his advice on nomenclatural queries and Dr Jeong Kug Lee (Dept. Life Science, Sogang University, Korea) for his courtesy of performing the acetylene reduction analysis. This work was supported by the Korea Science and Engineering Foundation and the KRIBB Research Initiative Program funded by the Ministry of Education, Science and Technology (MEST), Republic of Korea (grants NMC0300837 and 2009-0080150).

References

- Chang, Y.-H., Han, J., Chun, J., Lee, K. C., Rhee, M.-S., Kim, Y.-B. & Bae, K. S. (2002). *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea. *Int J Syst Evol Microbiol* **52**, 377–381.
- Chang, Y.-H., Jung, M. Y., Park, I.-S. & Oh, H.-M. (2008). *Sporolactobacillus vineae* sp. nov., a spore-forming lactic acid bacterium isolated from vineyard soil. *Int J Syst Evol Microbiol* **58**, 2316–2320.
- 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. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.
- Heyndrickx, M., De Vos, P. & De Ley, J. (1991). Fermentation characteristics of *Clostridium pasteurianum* LMG 3285 grown on glucose and mannitol. *J Appl Bacteriol* **70**, 52–58.
- Holdeman, L. V. & Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.
- Jeong, H., Yi, H., Sekiguchi, Y., Muramatsu, M., Kamagata, Y. & Chun, J. (2004). *Clostridium jejuense* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **54**, 1465–1468.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kasap, M. & Chen, J.-S. (2005). *Clostridium pasteurianum* W5 synthesizes two NifH-related polypeptides under nitrogen-fixing conditions. *Microbiology* **151**, 2353–2362.
- Kim, S., Jeong, H. & Chun, J. (2006). *Clostridium ganghwense* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **56**, 691–693.
- Kim, S., Jeong, H. & Chun, J. (2007). *Clostridium aestuarii* sp. nov., from tidal flat sediment. *Int J Syst Evol Microbiol* **57**, 1315–1317.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kühner, C. H., Matthies, C., Acker, G., Schmittroth, M., Gößner, A. S. & Drake, H. L. (2000). *Clostridium akagii* sp. nov. and *Clostridium acidisoli* sp. nov.: acid-tolerant, N₂-fixing clostridia isolated from acidic forest soil and litter. *Int J Syst Evol Microbiol* **50**, 873–881.
- Lee, I.-H., Park, J. Y., Kho, D. H., Kim, M.-S. & Lee, J. K. (2002). Reductive effect of H₂ uptake and poly- β -hydroxybutyrate formation on nitrogenase-mediated H₂ accumulation of *Rhodobacter sphaeroides* according to light intensity. *Appl Microbiol Biotechnol* **60**, 147–153.
- Lee, Y.-J., Romanek, C. S. & Wiegel, J. (2007). *Clostridium aciditolerans* sp. nov., an acid-tolerant spore-forming anaerobic bacterium from constructed wetland sediment. *Int J Syst Evol Microbiol* **57**, 311–315.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Steer, T., Collins, M. D., Gibson, G. R., Hippe, H. & Lawson, P. A. (2001). *Clostridium hathewayi* sp. nov., from human faeces. *Syst Appl Microbiol* **24**, 353–357.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wiegel, J., Tanner, R. & Rainey, F. A. (2006). An introduction to the family Clostridiaceae. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 4, pp. 654–678. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.