Paenibacillus motobuensis sp. nov., isolated from a composting machine utilizing soil from Motobu-town, Okinawa, Japan

Ken-ichiro Iida,¹ Yasuichi Ueda,² Yoshiaki Kawamura,³ Takayuki Ezaki,³ Akemi Takade,¹ Shin-ichi Yoshida¹ and Kazunobu Amako¹

Correspondence

Ken-ichiro Iida

iida@bact.med.kyushu-u.ac.jp

¹Department of Bacteriology, Faculty of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka-city, Fukuoka, 812-8582, Japan

²Motobu Noge Hospital, 880-1 Ohama, Motobu-cho, Kunigami-gun, Okinawa, 905-0212, Japan

³Department of Microbiology, Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu-city, Gifu, 501-1194, Japan

A novel bacterial strain, MC10^T, was isolated from a compost sample produced in a composting machine utilizing soil from Motobu-town, Okinawa, Japan. The isolate was Gram-negative, but produced endospores. These conflicting characters prompted a taxonomic study of the isolate. The isolate was examined using a combination of phenotypic characterization, cellular fatty acid analysis, DNA base composition determination and 16S rRNA gene sequence analysis. Phylogenetic analysis, based on 16S rRNA gene sequence comparisons, placed strain MC10^T within the genus *Paenibacillus*. As in other *Paenibacillus* species, the isolate contained anteiso-C_{15:0} as the major fatty acid and the DNA G + C content was 47.0 mol%. However, 16S rRNA gene sequence similarity values of less than 95.6% were found between this isolate and all members of the genus *Paenibacillus*. Based upon these results, strain MC10^T (= GTC 1835^T = JCM 12774^T = CCUG 50090^T) should be designated as the type strain of a novel species within the genus *Paenibacillus*, *Paenibacillus motobuensis* sp. nov.

Until the early 1990s, all aerobic, endospore-forming bacteria were classified as the genus *Bacillus*. In recent years, the genus *Bacillus* has been separated into several distinct genera, such as *Amphibacillus* (Niimura *et al.*, 1990), *Alicyclobacillus* (Wisotzkey *et al.*, 1992), *Paenibacillus* (Ash *et al.*, 1993), *Aneurinibacillus* and *Brevibacillus* (Shida *et al.*, 1996), *Halobacillus* (Spring *et al.*, 1996), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Gracilibacillus* and *Salibacillus* (Wainø *et al.*, 1999), *Anoxybacillus* (Pikuta *et al.*, 2000), *Coprobacillus* (Kageyama & Benno, 2000), *Filobacillus* (Schlesner *et al.*, 2001), *Geobacillus* (Nazina *et al.*, 2001), *Ureibacillus* (Fortina *et al.*, 2001), *Jeotgalibacillus* and *Marinibacillus* (Yoon *et al.*, 2001). The genus *Paenibacillus* was created by the separation of the so-called group 3 bacilli (Ash *et al.*, 1993); the new genus included 11 species. Since

this reclassification, additional transfers to the genus *Paenibacillus* and proposals for novel strains to be designated as *Paenibacillus* species have increased. Members of the genus *Paenibacillus* are aerobic, or facultatively anaerobic, organisms that produce ellipsoidal endospores in swollen sporangia and the cell wall shows structures typical of Gram-positive bacteria, but sometimes stains negatively. The DNA G + C content ranges from 40 to 54 mol% and anteiso-C_{15:0} is the major cellular fatty acid.

Recently, the problems of garbage produced from everyday living have become serious issues, especially in urban areas. Incineration has been used in the past as a common method of waste disposal. However, the production of toxic substances, especially dioxin, during incineration has become a major problem. Alternative methods for waste disposal are needed in order to avoid environmental pollution.

A heat-producing reaction was observed in a mixture of garbage and soil at Motobu-town, Okinawa, Japan. Using this phenomenon, a composting machine was developed by one of us (Y. U.). This machine comprises an airtight container that can withstand three times atmospheric pressure and a motor used for mixing the garbage inside the vessel. After an hour of operation, the temperature in the

Published online ahead of print on 29 April 2005 as DOI 10.1099/ijs.0.63636-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MC10^T is AY741810.

A table of 16S rRNA gene sequence similarities between MC10^T and several *Paenibacillus* species, a transmission electron micrograph showing flagella and an expanded phylogenetic tree are available as supplementary material in IJSEM Online.

machine increases to over 130 °C. By using this machine, garbage was broken down and turned into a soil-like substance. The mechanisms by which such high temperatures are produced are not clear and studies to analyse this phenomenon with biological and biochemical techniques have been performed. During a study to identify bacteria in the samples from this machine, a Gram-negative, but spore-forming, strain was isolated. The strain was named MC10^T and was considered to represent a *Paenibacillus* species based on the phylogenetic analysis of the 16S rRNA gene sequence. Based on the 16S rRNA gene sequence, phenotypic characteristics, DNA base composition and cellular fatty acid composition analysis, we propose that strain MC10^T is classified as the type strain of *Paenibacillus motobuensis* sp. nov.

Strain MC10^T was isolated from a compost sample collected from a composting machine. Serially-diluted compost suspensions were inoculated onto Luria–Bertani agar (pH 6) (Sambrook *et al.*, 1989). After colony isolation, the strain was cultivated at 37 °C for 48 h on trypticase soy agar (TSA; Difco) for chromosomal DNA extraction and on BUGM medium (Biolog) for the Biolog substrate utilization test. Tests for the utilization of substrates as the sole carbon source were performed with Biolog GP microplates (Biolog) containing 95 different carbon compounds, according to the manufacturer's instructions. Catalase and oxidase activity assays, the nitrate reduction test and the Voges–Proskauer test were carried out as described previously (Smibert & Krieg, 1994). Cell morphology was observed using light microscopy and transmission electron microscopy (TEM). Flagellum type was determined by TEM. Bacteria grown statically in trypticase soy broth (TSB; Difco) for 24 h were shadowed with chromium and examined with a JEM2000EX (JEOL) electron microscope. An ultrastructural study was carried out by using colonies and cells grown on TSA at 37 °C for 48 h. The freeze-substitution fixation technique was used for thin sectioning of samples for TEM (Amako *et al.*, 1983; Amako & Takade, 1985).

Cells of strain MC10^T were rods and approximately 0.6–1.0 × 1.0–3.0 µm in size. The strain was facultatively anaerobic and motile by means of peritrichous flagella (Supplementary Fig. S1 in IJSEM Online). The isolate produced ellipsoidal endospores in swollen sporangia in the terminal region of the cell (Fig. 1a). Colonies of the isolate grown on TSA were flat, smooth and opaque white. Growth was found to be optimal at 37 °C, with a range from 25 to 55 °C. Although the optimal growth temperature for the majority of the members of the genus *Paenibacillus* has been reported to be 28–30 °C (Shida *et al.*, 1997a), several novel *Paenibacillus* species have been described with temperature optima at around 37–40 °C (Shida *et al.*, 1997b; Yoon *et al.*, 1998; Tcherpakov *et al.*, 1999; Meehan *et al.*, 2001; Velázquez *et al.*, 2004). Strain MC10^T was found to be Gram-negative, but no apparent outer membrane structure was observed in the cell wall (Fig. 1b) and the cell wall

Fig. 1. Ultrastructure of strain MC10^T. (a) TEM of an ultrathin section showing an endospore in a swollen sporangium. Cross-sectionally cut spores are shown in box. (b) Magnified part of (a) (in rectangle). Bar, 1 µm.

structure was more like that of the Gram-positive bacteria. Some members of the genus *Paenibacillus*, such as *Paenibacillus glucanolyticus* and *Paenibacillus macquariensis*, have been known to give a Gram-negative reaction (Marshall & Ohye, 1966; Dasman *et al.*, 2002). The endospores had nine projections from the outer spore coat (Fig. 1a). Strain MC10^T showed catalase and oxidase activity and reduced nitrate to nitrite. The utilization of 95 substrates as the sole carbon source was tested using the Biolog GP plate and the results are noted in the description. Physiological characteristics, including selected data from the carbon utilization test for strain MC10^T, were compared with those of its closest phylogenetic relatives, *Paenibacillus azoreducens*, *P. cineris*, *P. cookii*, *P. favisporus* and *P. chibensis* (Table 1).

Chromosomal DNA was extracted and purified as described by Ezaki *et al.* (1994). G + C content was determined by the HPLC method (Ezaki *et al.*, 1990) using a packed column (Wakosil 5C18; Wako). The G + C content was calculated using *Escherichia coli* K-12 strain DNA as a standard (51.12 mol% G + C). The 16S rRNA gene sequence corresponding to positions 8–1485 in the *E. coli* numbering system (Brosius *et al.*, 1978) was amplified by PCR and sequenced according to Ezaki *et al.* (1994). The PCR product was purified with a Microcon Spincolumn (Millipore). Sequencing of the purified PCR product was performed using an ABI PRISM BigDye Terminator cycle sequencing

Table 1. Characteristics of *P. motobuensis* sp. nov. and closely related *Paenibacillus* species

Strains: 1, *Paenibacillus motobuensis* sp. nov. MC10^T; 2, *P. azoreducens* DSM 13822^T (data from Meehan *et al.*, 2001); 3, *P. cineris* LMG 18439^T (Logan *et al.*, 2004); 4, *P. cookii* LMG 18419^T (Logan *et al.*, 2004); 5, *P. favisporus* GMP01^T (Velázquez *et al.*, 2004); 6, *P. chibensis* NRRL B-142^T (Shida *et al.*, 1997b). +, Positive; –, negative; w, weak; v, variable; ND, not determined.

Characteristic	1	2	3	4	5	6
Growth at 50 °C	+	+	+	+	–	+
Optimum growth temp. (°C)	37	37	30	30	37	37
Oxidase	+	–	+	+	+	–
Nitrate reduction	+	–	ND	ND	+	+
Voges–Proskauer test	–	–	v	+	+	–
Utilization of:						
N-Acetylglucosamine	–	+	+	–	ND	ND
D-Arabinose	–	+	v	–	ND	–
L-Fucose	–	–	–	+	ND	ND
Glycerol	+	+	–	+	ND	–
Glycogen	–	+	v	+	ND	ND
myo-Inositol	–	–	+	–	ND	ND
Mannitol	–	+	+	–	+	+
D-Melezitose	–	–	+	+	ND	ND
Sorbitol	–	–	v	–	ND	–
Growth in NaCl at 5 %	w	–	–	+	w	–
G + C content (mol%)	47	47	48	52	53	53

ready reaction kit (Applied Biosystems) as recommended by the manufacturer. The purified sequencing reaction mixtures were electrophoresed automatically (model 3100; Applied Biosystems). The 16S rRNA gene sequence of strain MC10^T was aligned with those of all established *Paenibacillus* species and some other taxa using CLUSTAL W version 1.5 (Thompson *et al.*, 1994). Other reference sequences were obtained from the DDBJ database. 16S rRNA gene sequence similarity values were calculated using the CLUSTAL W package. A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). A bootstrap analysis (Felsenstein, 1985) with 1000 replications was performed with the CLUSTAL W package to evaluate the topology of the phylogenetic tree. The *Paenibacillus*-specific primer PAEN515F and universal primer 1377R were applied to isolate MC10^T for *Paenibacillus*-specific PCR according to the method of Shida *et al.* (1996, 1997a).

The DNA G + C content of strain MC10^T was 47.0 mol%. The phylogenetic position of the strain, based on the almost-complete 16S rRNA gene sequence of *Paenibacillus* species, including strain MC10^T, shown in Fig. 2, was constructed from evolutionary distances (Kimura, 1980) calculated by the neighbour-joining method (Saitou & Nei, 1987). The 15 strains with the highest binary similarity values of 16S rRNA sequences are presented in Supplementary Table S1 in IJSEM Online. The levels of 16S rRNA gene sequence similarity between strain MC10^T and *Paenibacillus* species

ranged from 95.6 % (*Paenibacillus azoreducens*, DSM 13822^T, GenBank accession no. AJ272249) to 90.2 % (*Paenibacillus alginolyticus*, DSM 5050^T, accession no. D78465). The phylogenetic tree (Fig. 2) constructed from the 16S rRNA gene sequence data shows that strain MC10^T appeared within the genus *Paenibacillus* and occupied a distinct phylogenetic position within the genus (an expanded phylogenetic tree is available as Supplementary Fig. S2 in IJSEM Online). Species found to be closely related to strain MC10^T were *P. azoreducens*, *P. chibensis*, *P. cineris*, *P. cookii* and *P. favisporus*. The definition of a bacterial species suggests that strains with approximately 70 % or greater DNA–DNA relatedness are members of the same species (Wayne *et al.*, 1987). According to the available data (Stackebrandt & Goebel, 1994), if the 16S rRNA gene sequence similarities are less than 97.0 %, the total chromosomal DNA extracted from two bacterial strains will not reassociate by more than 60 %, regardless of the hybridization method used. Keswani & Whitman (2001) suggested a threshold value of 98.6 % similarity for taxa where the rRNA gene sequences possess ultrameric properties and Drancourt *et al.* (2000) used 99 % for species identification. Our isolate, MC10^T, showed less than 95.6 % sequence similarity values among all members of the genus *Paenibacillus*. A PCR fragment (0.8 kb) could be amplified from the DNA of MC10^T using the primer combination of the genus-specific primer PAEN515F and universal primer 1377R (Shida *et al.*, 1997a).

Analysis of cellular fatty acids was performed at the National Collections of Industrial and Marine Bacteria Japan Ltd. Briefly, biomass for cellular fatty acid analysis was prepared from a 24 h culture grown on a TSA plate at 30 °C. Fatty acid methyl esters were prepared according to the manufacturer's instructions for analysis using the MIDI Microbial Identification System (Hewlett Packard). The resultant esters were separated using a gas chromatograph (model 6890; Hewlett Packard).

The major fatty acids found in isolate MC10^T are shown in Table 2 and are compared with values available for phylogenetically related *Paenibacillus* strains. Anteiso-branched C_{15:0}, the major fatty acid in the genus *Paenibacillus* (Shida *et al.*, 1997a), was also the major fatty acid component of strain MC10^T, comprising 39.8 % of the total. Other major (>10 %) fatty acids were straight-chain C_{16:0} and iso-branched C_{15:0}, C_{16:0} and C_{17:0}. The fatty acid profile of strain MC10^T was similar to those of selected members of the genus *Paenibacillus*, but there were differences in the proportions of some fatty acids.

In conclusion, according to the combined morphological, physiological, chemotaxonomic and phylogenetic data discussed here, it is evident that strain MC10^T belongs to the genus *Paenibacillus*. The genetic distinctiveness inferred from the phylogenetic study validates the proposal of this strain as representing a novel species, *Paenibacillus motobuensis* sp. nov.

Fig. 2. Phylogenetic tree showing the position of *P. motobuensis* MC10^T in relation to several *Paenibacillus* species and some rod-shaped, endospore-forming bacteria based on 16S rRNA gene sequences. *Gemella haemolysans* ATCC 10379^T (L14326) was used as the outgroup. Numbers at branching points refer to bootstrap values (1000 resampling). Bar, 1.0% sequence divergence.

Table 2. Cellular fatty acid profiles of some *Paenibacillus* species and strain MC10^T

Species: 1, *Paenibacillus motobuensis* sp. nov. MC10^T; 2, *P. azoreducens* DSM 13822^T (data from Meehan *et al.*, 2001); 3, *P. cineris* LMG 18439^T (Logan *et al.*, 2004); 4, *P. cookii* LMG 18419^T (Logan *et al.*, 2004); 5, *P. favisporus* GMP01^T (Velázquez *et al.*, 2004); 6, *P. chibensis* NRRL B-142^T (Shida *et al.*, 1997b). ND, Not determined. Values are percentages of total fatty acids.

Fatty acid	1	2	3	4	5	6
Straight-chain:						
C _{14:0}	1.1	3.5	ND	ND	ND	0.3
C _{15:0}	1.7	0.1	ND	ND	3.6	0.6
C _{16:0}	10.3	22.1	18.0	11.0	8.7	5.3
Iso-branched:						
C _{14:0}	1.5	0.8	ND	ND	0.9	0.8
C _{15:0}	14.7	5.9	6.0	6.5	6.5	4.0
C _{16:0}	10.5	8.6	9.0	13.0	11.1	12.2
C _{17:0}	10.1	5.6	ND	ND	ND	3.2
Anteiso-branched:						
C _{13:0}	0.2	5.6	ND	ND	3.3	3.2
C _{15:0}	39.8	33.9	46.0	36.0	48.8	57.8
C _{17:0}	9.3	19.8	10.0	20.0	15.0	14.3

Description of *Paenibacillus motobuensis* sp. nov.

Paenibacillus motobuensis (mo.to.bu.en'sis. N.L. masc. adj. *motobuensis* pertaining to Motobu in Okinawa, Japan, where the type strain was isolated).

Rod-shaped cells with a length of approx. 1.0–3.0 µm and a width of 0.6–1.0 µm. Cells are motile by means of peritrichous flagella and produce terminally located ellipsoidal spores. Colonies are circular, flat, smooth and opaque white. Facultatively anaerobic. Cells stain Gram-negative; however, the cell wall structure is that of Gram-positive bacteria. Positive catalase and oxidase reactions. Negative Voges–Proskauer reaction. Nitrate is reduced to nitrite. The temperature range for growth is 20–55 °C, with the optimum being 37 °C. The pH range for growth is 6.0–8.0, the optimum being pH 8.0. Growth occurs in the presence of 5% NaCl but is inhibited by 10% NaCl. Anteiso-branched C_{15:0} (39.8%) represents the major cellular fatty acid. Utilizes α-cyclodextrin, β-cyclodextrin, dextrin, amygdalin, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose, D-gluconic acid, α-D-glucose, maltose, maltotriose, D-mannose, methyl α-D-galactoside, palatinose, D-psicose, salicin, sucrose, D-trehalose, turanose, D-xylose, α-ketovaleric acid, methyl

pyruvate, 2,3-butanediol, glycerol and adenosine (utilization data were obtained with Biolog GP microplates). The DNA G + C content of the type strain is 47.0 mol% (determined by HPLC).

The type strain MC10^T (=GTC 1835^T=JCM 12774^T=CCUG 50090^T), was isolated from a composting machine containing soil from Motobu, Okinawa, Japan. The 16S rRNA gene sequence of this strain is deposited at DDBJ under accession number AY741810.

References

- Amako, K. & Takade, A. (1985). The fine structure of *Bacillus subtilis* revealed by the rapid-freezing and substitution fixation method. *J Electron Microsc* **34**, 13–17.
- Amako, K., Murata, K. & Umeda, A. (1983). Structure of the envelope of *Escherichia coli* observed by the rapid-freezing and substitution fixation method. *Microbiol Immunol* **27**, 95–99.
- Ash, C., Priest, F. G. & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Dasman, Kajiya, S., Kawasaki, H., Yagi, M., Seki, T., Fukasaki, E. & Kobayashi, A. (2002). *Paenibacillus glycanilyticus* sp. nov., a novel species that degrades heteropolysaccharide produced by the cyanobacterium *Nostoc commune*. *Int J Syst Evol Microbiol* **52**, 1669–1674.
- Drancourt, M., Bollet, C., Carlouz, A., Martelin, R., Gayral, J. P. & Raoult, D. (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J Clin Microbiol* **38**, 3623–3630.
- Ezaki, T., Saidi, S. M., Liu, S. L., Hashimoto, Y., Yamamoto, H. & Yabuuchi, E. (1990). Rapid procedure to determine the DNA base composition from small amounts of Gram-positive bacteria. *FEMS Microbiol Lett* **67**, 127–130.
- Ezaki, T., Li, N., Hashimoto, Y., Miura, H. & Yamamoto, H. (1994). 16S ribosomal DNA sequence of anaerobic cocci and proposal of *Ruminococcus hansenii* comb. nov. and *Ruminococcus productus* comb. nov. *Int J Syst Bacteriol* **44**, 130–136.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fortina, M. G., Pukall, R., Schumann, P., Mora, D., Parini, C., Manachini, P. L. & Stackebrandt, E. (2001). *Ureibacillus* gen. nov., a new genus to accommodate *Bacillus thermosphaericus* (Andersson et al. 1995), emendation of *Ureibacillus thermosphaericus* and description of *Ureibacillus terrenus* sp. nov. *Int J Syst Evol Microbiol* **51**, 447–455.
- Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, G. & Logan, N. A. (1998). *Virgibacillus*: a new genus to accommodate *Bacillus pantothenicus* (Proom and Knight 1950). Emended description of *Virgibacillus pantothenicus*. *Int J Syst Bacteriol* **48**, 99–106.
- Kageyama, A. & Benno, Y. (2000). *Coprobacillus catenaformis* gen. nov., sp. nov., a new genus and species isolated from human feces. *Microbiol Immunol* **44**, 23–28.
- Keswani, J. & Whitman, W. B. (2001). Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. *Int J Syst Evol Microbiol* **51**, 667–678.
- 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.
- Logan, N. A., De Clerck, E., Lebbe, L., Verhelst, A., Goris, J., Forsyth, G., Rodriguez-Diaz, M., Heyndrickx, M. & De Vos, P. (2004). *Paenibacillus cineris* sp. nov. and *Paenibacillus cookii* sp. nov., from Antarctic volcanic soils and a gelatin-processing plant. *Int J Syst Evol Microbiol* **54**, 1071–1076.
- Marshall, B. J. & Ohye, D. F. (1966). *Bacillus macquariensis* n.sp., a psychrotrophic bacterium from sub-antarctic soil. *J Gen Microbiol* **44**, 41–46.
- Meehan, C., Bjorson, A. J. & McMullan, G. (2001). *Paenibacillus azoreducens* sp. nov., a synthetic azo dye decolorizing bacterium from industrial wastewater. *Int J Syst Evol Microbiol* **51**, 1681–1685.
- Nazina, T. N., Tourova, T. P., Poltarau, A. B. & 8 other authors (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. *Int J Syst Evol Microbiol* **51**, 433–446.
- Niimura, Y., Koh, E., Yanagida, F., Suzuki, K., Komagata, K. & Kozaki, M. (1990). *Amphibacillus xylanus* gen. nov., sp. nov., a facultatively anaerobic spore-forming xylan-digesting bacterium which lacks cytochrome, quinone, and catalase. *Int J Syst Bacteriol* **40**, 297–301.
- Pikuta, E., Lysenko, A., Chuvilskaya, N., Mendrock, U., Hippe, H., Suzina, N., Nikitin, D., Osipov, G. & Laurinavichius, K. (2000). *Anoxybacillus pushchinensis* gen. nov., sp. nov., a novel anaerobic, alkaliphilic, moderately thermophilic bacterium from manure, and description of *Anoxybacillus flavithermus* comb. nov. *Int J Syst Evol Microbiol* **50**, 2109–2117.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schlesner, H., Lawson, P. A., Collins, M. D., Weiss, N., Wehmeyer, U., Völker, H. & Thomm, M. (2001). *Filobacillus milensis* gen. nov., sp. nov., a new halophilic spore-forming bacterium with Orn-D-Glu-type peptidoglycan. *Int J Syst Evol Microbiol* **51**, 425–431.
- Shida, O., Takagi, H., Kadowaki, K. & Komagata, K. (1996). Proposal for two new genera. *Brevibacillus* gen. nov. and *Aneurini-bacillus* gen. nov. *Int J Syst Bacteriol* **46**, 939–946.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997a). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis* and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997b). Emended description of *Paenibacillus amylolyticus* and description of *Paenibacillus illinoisensis* sp. nov. and *Paenibacillus chibensis* sp. nov. *Int J Syst Bacteriol* **47**, 299–306.
- Smibert, R. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

- Spring, S., Ludwig, W., Marquez, M. C., Ventosa, A. & Schleifer, K.-H. (1996). *Halobacillus* gen. nov., with description of *Halobacillus litoralis* sp. nov. and *Halobacillus truperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int J Syst Bacteriol* **46**, 492–496.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tcherpakov, M., Ben-Jacob, E. & Gutnick, D. L. (1999). *Paenibacillus dendritiformis* sp. nov., proposal for a new pattern-forming species and its localization within a phylogenetic cluster. *Int J Syst Bacteriol* **49**, 239–246.
- 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.
- Velázquez, E., De Miguel, T., Poza, M., Rivas, R., Rosselló-Mora, R. & Villa, T. G. (2004). *Paenibacillus favisporus* sp. nov., a xylanolytic bacterium isolated from cow faeces. *Int J Syst Evol Microbiol* **54**, 59–64.
- Wainø, M., Tindall, B. J., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.
- Wisotzkey, J. D., Jurtshuk, P., Jr, Fox, G. E., Deinhard, G. & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* **42**, 263–269.
- Yoon, J.-H., Yim, D. K., Lee, J.-S., Shin, K.-S., Sato, H. H., Lee, S. T., Park, Y. K. & Park, Y.-H. (1998). *Paenibacillus campinasensis* sp. nov., a cyclodextrin-producing bacterium isolated in Brazil. *Int J Syst Bacteriol* **48**, 833–837.
- Yoon, J.-H., Weiss, N., Lee, K.-C., Lee, I.-S., Kang, K. H. & Park, Y.-H. (2001). *Jeotgalibacillus alimentarius* gen. nov., sp. nov., a novel bacterium isolated from jeotgal with L-lysine in the cell wall, and reclassification of *Bacillus marinus* Rger 1983 as *Marinibacillus marinus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 2087–2093.