NOTE

Geobacillus toebii sp. nov., a novel thermophilic bacterium isolated from hay compost

Microbial Genomics Lab., Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejon 305-600, Korea

M.-H. Sung, H. Kim, J.-W. Bae, S.-K. Rhee, C. O. Jeon, K. Kim, J.-J. Kim, S.-P. Hong, S.-G. Lee, J.-H. Yoon, Y.-H. Park and D.-H. Baek

Author for correspondence: M.-H. Sung. Tel: +82 42 860 4372. Fax: +82 42 860 4595. e-mail: smoonhee@mail.kribb.re.kr

A thermophilic, spore-forming rod isolated from hay compost in Korea was subjected to a taxonomic study. The micro-organism, designated strain SK-1^T, was identified as being aerobic, Gram-positive, motile and rod-shaped. Growth of the isolate was observed at 45–70 °C (optimum 60 °C) and pH 6·0–9·0 (optimum pH 7·5). The G+C content of the genomic DNA was 43·9 mol%. Chemotaxonomic characteristics of the isolate included the presence of *meso*-diaminopimelic acid in the cell wall and iso-$C_{15:0}$ and iso-$C_{17:0}$ as the major cellular fatty acids. The predominant isoprenoid quinone was MK-7. The chemotaxonomic characteristics of strain SK-1^T were the same as those of the genus *Geobacillus*. Phylogenetic analysis based on 16S rDNA sequences showed that strain SK-1^T is most closely related to *Geobacillus thermoglucosidasius*. However, the phenotypic properties of strain SK-1^T were clearly different from those of *G. thermoglucosidasius*. The level of DNA–DNA relatedness between strain SK-1^T and the type strain of *G. thermoglucosidasius* was 27%. On the basis of the phenotypic traits and molecular systematic data, strain SK-1^T represents a novel species within the genus *Geobacillus*, for which the name *Geobacillus toebii* sp. nov. is proposed. The type strain is strain SK-1^T (= KCTC 0306BP^T = DSM 14590^T).

Keywords: *Geobacillus toebii* sp. nov., thermophile, polyphasic taxonomy, hay compost

The large diversity of the genus *Bacillus* led to the reclassification of seven phylogenetic groups as the new genera *Alicyclobacillus* (Wisotzkey *et al*., 1992), *Paenibacillus* (Ash *et al*., 1993; Heyndrickx *et al*., 1996), *Brevibacillus* (Shida *et al*., 1996), *Aneurinibacillus* (Shida *et al*., 1996; Heyndrickx *et al*., 1997), *Virgibacillus* (Heyndrickx *et al*., 1998), *Salibacillus* and *Gracilibacillus* (Wainø *et al*., 1999). Thermophilic *Bacillus* species previously assigned to rRNA group 5 have recently been transferred to a new genus *Geobacillus*, which was created with two additional novel species, *Geobacillus subterraneus* and *Geobacillus uzenensis* (Ash *et al*., 1991; Rainey *et al*., 1994; Nazina *et al*., 2001). At the time of writing, there are eight validly described *Geobacillus* species. The *Geobacillus* species form a phenotypically and phylogenetically coherent group of thermophilic bacilli with high levels of 16S rRNA sequence similarity (98·5–99·2%). This group comprises established species of thermophilic bacilli (*Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans*). Most of these thermophilic species or strains have been found to grow at temperatures above 55 °C (Nazina *et al*., 2001). Hot compost is considered to offer a favourable habitat for thermophilic bacilli (Fugio & Kume, 1991; Strom, 1985a, b). Strom (1985a, b) isolated more than 750 heterotrophic spore-forming strains from compost; very few of them grew above 60 °C, and only *Bacillus coagulans* and *Geobacillus stearothermophilus* were isolated at 65 °C.

During a routine screening programme of thermophilic

Published online ahead of print on 7 June 2002 as DOI 10.1099/ijs.0.02181-0.

The GenBank accession number for the 16S rDNA sequence of strain SK-1^T is AF326278.

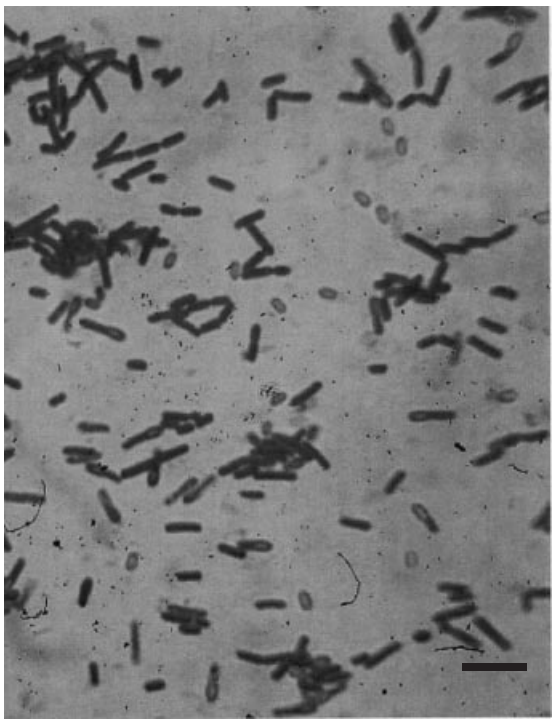

Fig. 1. Phase-contrast photomicrograph of cells of strain SK-1^T. Bar, 5 μm.

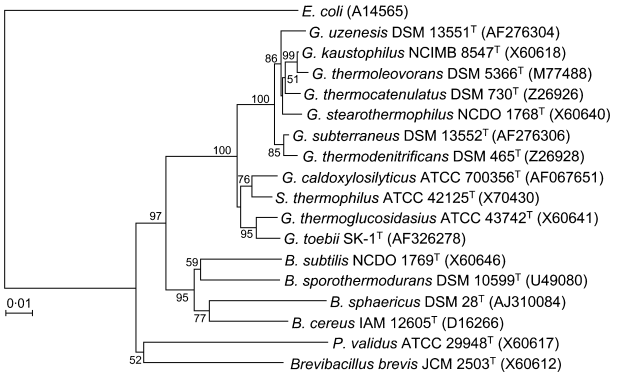

Fig. 2. Phylogenetic tree showing the position of strain SK-1^T within the radiation of the genus *Geobacillus* and related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branching points. Bar, 1 substitution per 100 nt. *B.*, *Bacillus*; *P.*, *Paenibacillus*; *S.*, *Salibacillus*.

bacteria, a thermophilic strain, designated strain SK-1^T, was isolated from hay compost. The isolate exhibited a commensal interaction with the previously described '*Symbiobacterium toebii*' SC-1 (Rhee *et al.*, 2000, 2002). It was clear from a cursory examination that strain SK-1^T has taxonomic properties that allow its assignment to the genus *Geobacillus*. In the present study, strain SK-1^T was subjected to a polyphasic characterization to investigate whether or not it represents a novel species within the genus *Geobacillus*.

A compost sample was taken from a farm-yard in Kongju, Korea. Strain SK-1^T was isolated on a solid modified basal medium (MBM), containing 5 g polypeptone, 1 g yeast extract, 6 g K_2HPO_4, 2 g KH_2PO_4, 0·5 g $MgSO_4$. $7H_2O$ and 0·5 g L-tyrosine l^{-1} deionized water, which was seeded with a compost suspension and incubated at 60 °C for 3 days. Biomass for chemical and molecular systematic studies was obtained by cultivating the organism in shake flasks containing liquid MBM at 60 °C for 3 days. The shape and size of living and stained cells were determined by light microscopy. The Gram reaction was determined using a Gram stain kit (Difco) according to the manufacturer's recommended protocol. Flagellum type was examined using a model H7000 transmission electron microscope (Hitachi) after the preparations were stained with 0·5 to 2% (w/v) phosphotungstic acid. A thin section was stained with uranyl acetate and lead citrate. Scanning electron microscopy of the novel isolate was carried out as described by Padilla *et al.* (1997). Most of the physiological tests were carried out using API 20E and 50CH kits (bioMérieux). Growth in the presence of 0·02% (w/v) sodium azide and 5% (w/v) NaCl was examined according to the method of Gordon *et al.* (1973). MBM was also used for determination of optimal pH and temperature for growth. The effect of pH on growth was determined on solid MBM using four different buffers at a final concentration of 50 mM: citrate/Na_2HPO_4 buffer, pH range 5·0–7·0; phosphate buffer, pH range 6·0–8·0; Tris buffer, pH range 7·0–9·0; glycine/NaOH buffer, pH range 8·5–10·5. The isomer type of diaminopimelic acid in the peptidoglycan was determined by the method of Komagata & Suzuki (1987). Menaquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Fatty acids were extracted and analysed following the instructions of the Microbial Identification System (MIDI).

The chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996). G+C content was determined using the method of Tamaoka & Komagata (1984). The DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. *G. thermoglucosidasius* ATCC 43742^T was used as the reference strain for DNA–DNA hybridization, which was carried out according to the method of Ezaki *et al.* (1989).

16S rDNA was amplified by PCR using two universal primers, as described previously (Yoon *et al.*, 1998). The PCR product was purified using a QIAquick PCR purification kit (Qiagen). The purified 16S rDNA was then sequenced using an ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) as recommended by the manufacturer. The purified sequencing reaction mixtures were electrophoresed automatically using an Applied Biosystems model 310 automatic DNA sequencer. The 16S rDNA sequence of strain SK-1^T was aligned with representative 16S rDNA sequences of related taxa using CLUSTAL W software (Thompson *et al.*, 1994). Any

Table 1. Characteristics that differentiate *Geobacillus toebii* sp. nov. strain SK-1^T from its phylogenetic neighbours

Taxa are indicated as: 1, *G. toebii* strain SK-1^T; 2, *G. thermoglucosidasius*; 3, *G. uzenensis*; 4, *G. subterraneus*; 5, *G. stearothermophilus*; 6, *G. thermocatenulatus*; 7, *G. thermoleovorans*; 8, *G. kaustophilus*; 9, *G. thermodenitrificans*. Characteristics are scored as: +, ⩾ 90 % of strains positive; d, 11–89 % of strains positive; −, ⩾ 90 % of strains negative; ND, not determined. Data were obtained from Nazina *et al.* (2001) (*G. uzenensis*, *G. subterraneus*), Suzuki *et al.* (1983), White *et al.* (1993) and Priest *et al.* (1988) (*G. thermoglucosidasius*), Claus & Berkeley (1986) (*G. stearothermophilus*), Golovacheva *et al.* (1975) (*G. thermocatenulatus*), Zarilla & Perry (1986) (*G. thermoleovorans*), White *et al.* (1993) (*G. kaustophilus*) and Manachini *et al.* (2000) (G. *thermodenitrificans*).

Characteristic	1	2	3	4	5	6	7	8	9
Cell width (μm)	0·5–0·9	< 3	0·9–1·3	0·8–1·5	0·6–1	0·5–1·2	0·9	1·5	0·5–1·0
Cell length (μm)	2·0–3·5	< 0·9	4·7–8·0	4·7–8·0	2–3·5	3–7	6–8	3·5	1·5–2·5
Motility	+	ND	+	+	+	+	+	−	ND
Production of acid from:									
Adonitol	−	+	−	−	ND	−	ND	ND	ND
L-Arabinose	−	−	+	−	d	−	−	d	+
Cellobiose	−	+	+	+	−	+	+	+	+
Galactose	−	d	+	+	−	−	+	+	+
Ribose	−	−	+	+	ND	ND	ND	+	+
Glycerol	−	−	+	+	+	+	+	d	+
Inositol	+	+	−	−	−	−	−	−	ND
Lactose	−	−	−	−	−	−	−	−	+
Rhamnose	−	−	−	−	−	+	−	−	−
Sorbitol	−	−	−	−	−	+	ND	−	ND
D-Xylose	−	+	−	−	d	+	−	d	+
Hydrolysis of:									
Gelatin	−	+	+	−	d	−	−	ND	ND
Casein	+	+	−	−	d	+	ND	+	−
Starch	−	+	+	+	+	+	−	d	+
Aesculin	−	−	+	+	ND	+	ND	ND	ND
Utilization of:									
n-Alkanes	+	ND	+	+	+	ND	+	+	ND
Formate	−	d	−	+	−	ND	ND	ND	ND
Acetate	−	−	+	+	+	ND	ND	+	ND
Lactate	−	−	+	+	−	ND	ND	ND	ND
Citrate (Simmons)	−	+	−	−	d	d	+	ND	ND
Fermentation of glucose	−	−	−	−	d	−	+	−	ND
Denitrification	+	ND	−	+	−	−	+	ND	+
Methyl red test	−	−	−	+	d	d	ND	ND	ND
NaCl range (%, w/v)	0–< 5	0–< 5	0–4	0–5	0–5	0–1·5	0–4	ND	0–3
pH range	6·0–9·0	6·0–8·0	6·2–7·8	6·0–7·8	6·0–8·0	6·5–8·5	6·2–7·8	6·2–7·5	6–8
Temperature range (°C)	45–70	37–68	45–65	45–70	37–65	42–69	35–78	40–75	45–70
G+C content (mol %)	44	53·9	50·4–51·5	49·7–52·3	51·9	45–46	55·2	52–58	48·2–52·3

gaps at the 5′ and 3′ ends of the alignment were omitted from further analyses. Evolutionary distance matrices were calculated with the algorithm developed by Jukes & Cantor (1969) using the DNADIST program within the PHYLIP package (Felsenstein, 1993). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) implemented within the NEIGHBOR program of the same package. The stability of the relationships was assessed by a bootstrap analysis of 1000 datasets using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package. GenBank accession numbers for reference 16S rRNA gene sequence used in this analysis are shown in Fig. 2.

A thermophilic bacterium, '*Symbiobacterium toebii*' SC-1, exists in an obligate commensal relationship with another thermophilic bacterium, strain SK-1^T, in composts and the commensal partner releases two growth factors during growth (Rhee *et al.*, 2000, 2002). Strain SK-1^T was found to provide higher stimulating activity for the growth of '*Symbiobacterium toebii*' SC-1 than did any other *Bacillus* or *Geobacillus* species tested. Strain SK-1^T exhibited morphological and chemical characteristics that are consistent with those found in the genus *Geobacillus*. Cells of strain SK-1^T were aerobic and Gram-positive rods, 2·0–3·5 μm long and 0·5–0·9 μm wide (Fig. 1). Spores first appeared on MBM agar after 24 h and were ellipsoidal, located

subterminally to terminally in swollen sporangia. Cells were motile. Growth of strain SK-1^T occurred at 45–70 °C, with an optimum of 60 °C. No growth was observed at 80 °C. Growth at 60 °C occurred between pH 6·0 and 9·0, with optimum growth at about pH 7·5. No growth was detected at pH values below 6·0 or above 9·0. Growth was not observed in the presence of 0·02 % sodium azide or 5 % NaCl. Strain SK-1^T was positive for catalase. Whereas acid was produced from D-glucose, no acid was produced from xylose or mannitol. A Voges–Proskauer test proved positive. Strain SK-1^T contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan, MK-7 as its predominant menaquinone and major amounts of iso-$C_{15:0}$ (34·03 %), iso-$C_{16:0}$ (17·46 %) and iso-$C_{17:0}$ (34·86 %) fatty acids. The G+C content of the DNA of strain SK-1^T was 43·9 mol %.

A nearly complete 16S rDNA sequence of 1521 nt (> 96 % of the *Escherichia coli* 16 rRNA sequence) was determined for strain SK-1^T. Comparison of this sequence with sequences of some other bacteria revealed that strain SK-1^T falls within the radiation of the cluster comprising *Geobacillus* species and forms, in particular, a coherent cluster with *G. thermoglucosidasius* ATCC 43742^T (Fig. 2). In the phylogenetic tree based on the neighbour-joining algorithm, the relationship between strain SK-1^T and *G. thermoglucosidasius* ATCC 43742^T was supported by a bootstrap resampling value of 95·2 %. The level of 16S rDNA sequence similarity between strain SK-1^T and *G. thermoglucosidasius* ATCC 43742^T was 98·1 %. 16S rDNA similarity values between strain SK-1^T and the type strains of other validly described *Geobacillus* species were in the range 95·0–95·8 %.

DNA–DNA hybridization was performed to determine the genomic relatedness between strain SK-1^T and the type strain of *G. thermoglucosidasius*. Since the DNA–DNA relatedness was only 27 %, they appeared to be members of distinct genomic species (Wayne *et al.*, 1987). Some phenotypic properties also showed that strain SK-1^T could be clearly distinguished from *G. thermoglucosidasius* ATCC 43742^T (Table 1). Moreover, strain SK-1^T has higher activity for stimulation of growth of '*Symbiobacterium toebii*' SC-1 than does *G. thermoglucosidasius* ATCC 43742^T. It is apparent from the genotypic and phenotypic data that strain SK-1^T merits recognition as a member of a novel species. Therefore, we propose the creation of a novel species, *Geobacillus toebii* sp. nov., for strain SK-1^T.

Description of *Geobacillus toebii* sp. nov.

Geobacillus toebii (to.e′bi.i. N.L. neut. gen. n. *toebii* derived from toebi, a special farmland compost in Korea, from which the organism was isolated).

Cells are Gram-positive, aerobic rods, 2·0–3·5 μm long and 0·5–0·9 μm wide. Motile. Spores are ellipsoidal, located subterminally to terminally in swollen sporangia. Growth occurs at 45–70 °C with an optimum of 60 °C. No growth is observed at 80 °C. Growth at 60 °C occurs between pH 6·0 and 9·0, with an optimum pH of about 7·5. No growth is found at pH values below 6·0 or above 9·0. Growth is not observed in the presence of 0·02 % azide or 5 % NaCl. Catalase-positive. Acid is produced from D-glucose but not from xylose or mannitol. The Voges–Proskauer test is positive. The major cellular fatty acids are iso-$C_{15:0}$ and iso-$C_{17:0}$. The G+C content is 43·9 mol % (as determined by HPLC). Isolated from farmland compost in Kongju, Korea. The type strain is strain SK-1^T (= KCTC 0306BP^T = DSM 14590^T).

Acknowledgements

This work was supported by National Research Laboratory (NRL) program grant NLM0020224 from the Ministry of Science and Technology of Korea. We thank Professor Tairo Oshima (Tokyo University of Pharmacy and Life Science) for very helpful comments and discussion.

References

Andersson, M., Laukkanen, M., Nurmiaho-Lassila, E.-L., Rainey, F. A., Niemelä, S. I. & Salkinoja-Salonen, M. (1995). *Bacillus thermosphaericus* sp. nov. a new thermophilic ureolytic bacillus isolated from air. *Syst Appl Microbiol* **18**, 203–220.

Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.

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.

Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1993). PHYLIP: Phylogenetic Inference Package, version 3.5. Department of Genetics, University of Washington, Seattle, USA.

Fugio, Y. & Kume, S. J. (1991). Isolation and identification of thermophilic bacteria from sewage sludge compost. *J Ferment Bioeng* **72**, 334–337.

Golovacheva, R. S., Loginova, L. G., Salikhov, T. A., Kolesnikov, A. A. & Zaitzeva, G. N. (1975). A new thermophilic species *Bacillus thermocatenulatus* nov. sp. *Mikrobiologiya* **44**, 265–268 (in Russian).

Gordon, R. E. (1981). One hundred and seven years of the genus *Bacillus*. In *The Aerobic Endospore-forming Bacteria: Classification and Identification*, pp. 1–15. Edited by R. C. W. Berkeley & M. Goodfellow. London, New York: Academic Press.

Gordon, R. E., Haynes, W. C. & Pang, C. H. (1973). *The Genus Bacillus*. Agricultural Handbook 427. Washington, DC: US Department of Agriculture.

Heyndrickx, M., Vandemeulebroecke, K., Scheldeman, P., Kersters, K., De Vos, P., Logan, N. A., Aziz, A. M., Ali, N. & Berkeley, R. C. W. (1996). A polyphasic reassessment of the genus *Paenibacillus*, reclassification of *Bacillus lautus* (Nakamura 1984) as *Paenibacillus lautus* comb. nov. and of *Bacillus peoriae* (Montefusco *et al.* 1993) as *Paenibacillus peoriae* comb. nov., and emended descriptions of *P. lautus* and of *P. peoriae*. *Int J Syst Bacteriol* **46**, 988–1003.

Heyndrickx, M., Lebbe, L., Vancanneyt, M. & 7 other authors (1997). A polyphasic reassessment of the genus *Aneurinibacillus*, reclassification of *Bacillus thermoaerophilus* (Meier-Stauffer *et al*. 1996) as *Aneurinibacillus thermoaerophilus* comb. nov., and emended descriptions of *A. aneurinilyticus* corrig., *A. migulanus*, and *A. thermoaerophilus*. *Int J Syst Bacteriol* **47**, 808–817.

Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, G. & Logan, N. A. (1998). *Virgibacillus*: a new genus to accommodate *Bacillus pantothenticus* (Proom and Knight 1950). Emended description of *Virgibacillus pantothenticus*. *Int J Syst Bacteriol* **48**, 99–106.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–232. Edited by H. N. Munro. New York: Academic Press.

Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.

Manachini, P. L., Mora, D., Nicastro, G., Parini, C., Stackebrandt, E., Pukall, R. & Fortina, M. G. (2000). *Bacillus thermodenitrificans* sp. nov., nom. rev. *Int J Syst Evol Microbiol* **50**, 1331–1337.

Nazina, T. N., Tourova, T. P., Poltaraus, A. B. & 8 other authors (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* 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.

Padilla, J. A., Uno, F., Yamada, M., Namba, H. & Nii, S. (1997). High-resolution immuno-scanning electron microscopy using a non-coating method: study of herpes simplex virus glycoproteins on the surface of virus particles and infected cells. *J Electron Microsc* **46**, 171–180.

Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* **134**, 1847–1882.

Rainey, F. A., Fritze, D. & Stackebrandt, E. (1994). The phylogenetic diversity of thermophilic members of the genus *Bacillus* as revealed by 16S rDNA analysis. *FEMS Microbiol Lett* **115**, 205–211.

Rhee, S.-K., Lee, S.-G., Hong, S.-P., Choi, Y.-H., Park, J.-H., Kim, C.-J. & Sung, M.-H. (2000). A novel microbial interaction: obligate commensalism between a new gram-negative thermophile and a thermophilic *Bacillus* strain. *Extremophiles* **4**, 131–136.

Rhee, S.-K., Jeon, C. O., Bae, J.-W. & 9 other authors (2002). Characterization of *Symbiobacterium toebii*, an obligate commensal thermophile isolated from compost. *Extremophiles* **6**, 57–64.

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

Shida, O., Takagi, H., Kadowaki, K. & Komagata, K. (1996). Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol* **46**, 939–946.

Strom, P. F. (1985a). Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. *Appl Environ Microbiol* **50**, 899–905.

Strom, P. F. (1985b). Identification of thermophilic bacteria in solid-waste composting. *Appl Environ Microbiol* **50**, 906–913.

Suzuki, Y., Kishigami, T., Inoue, K., Mizoguchi, Y., Eto, N., Takagi, M. & Abe, S. (1983). *Bacillus thermoglucosidasius* sp. nov., a new species of obligately thermophilic bacilli. *Syst Appl Microbiol* **4**, 487–495.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Thomson, 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.

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.

White, D., Sharp, R. J. & Priest, F. G. (1993). A polyphasic taxonomic study of thermophilic bacilli from a wide geographical area. *Antonie van Leeuwenhoek* **64**, 357–386.

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., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.

Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.

Zarilla, K. & Perry, J. J. (1987). *Bacillus thermoleovorans*, sp. nov., a species of obligately thermophilic hydrocarbon utilizing endospore-forming bacteria. *Syst Appl Microbiol* **9**, 258–264.