Bacillus oceanisediminis sp. nov., isolated from marine sediment

Jianli Zhang,¹ Jiewei Wang,¹ Caiyuan Fang,¹ Fei Song,¹ Yuhua Xin,² Lei Qu¹ and Kai Ding¹

Correspondence

Jianli Zhang
zhangjianli@bit.edu.cn

¹School of Life Science and Technology, Beijing Institute of Technology, Beijing 100081, PR China

²China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

A Gram-stain-positive, spore-forming, rod-shaped and aerobic bacterium was isolated from a sediment sample from the South Sea in China. The isolate, designated H2^T, grew at 4–45 °C (optimum 37 °C) and pH 6–10 (optimum pH 7.0). The cell-wall peptidoglycan contained meso-diaminopimelic acid. The major isoprenoid quinone was MK-7 and the polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown aminophospholipid. The major fatty acid was iso-C_{15:0}. The genomic DNA G + C content of strain H2^T was 44.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate formed a monophyletic clade with *Bacillus firmus* IAM 12464^T. DNA–DNA relatedness between the isolate and *B. firmus* ATCC 14575^T was low (27.5%). Strain H2^T also had a phenotypic profile that readily distinguished it from its closest phylogenetic neighbours. It is evident from the combination of genotypic and phenotypic data that the organism should be classified in a novel species of the genus *Bacillus*, for which the name *Bacillus oceanisediminis* sp. nov. is proposed. The type strain is H2^T (=CGMCC 1.10115^T =JCM 16506^T).

The application of chemotaxonomic, numerical phenetic and molecular systematic methods has led to an improved description and a division of the genus *Bacillus*. Some species have been reclassified in novel genera or transferred to other genera (Ash *et al.*, 1993; Stackebrandt & Liesack, 1993; Shida *et al.*, 1996; Wainø *et al.*, 1999; Nazina *et al.*, 2001; Yoon *et al.*, 2001; Albert *et al.*, 2007; Zhou *et al.*, 2009). Many of these reclassifications are well supported by relatively conserved phenotypic traits such as the quinone system, peptidoglycan composition and polar lipid and cellular fatty acid profiles (Albert *et al.*, 2007). *Falsibacillus* is a newly described genus, which currently comprises a single species, *Falsibacillus pallidus* comb. nov., and is represented by the type strain, CW 7^T. The taxon *F. pallidus* comb. nov. is linked to the illegitimately named taxon *Bacillus pallidus* Zhou *et al.* 2008 and the creation of the genus *Falsibacillus* was primarily based on significant chemotaxonomic differences in comparison with the type species of the genus *Bacillus*, *Bacillus subtilis* (Zhou *et al.*, 2008, 2009). The genus *Bacillus* currently encompasses more than 150 species with validly published names and contains at least six phylogenetically distinct groups on the basis of molecular analyses of 16S rRNA gene sequences

(Ash *et al.*, 1991; Nielsen *et al.*, 1994; Schlesner *et al.*, 2001; Yoon *et al.*, 2004; Carrasco *et al.*, 2007). Members of the genus *Bacillus* have been isolated from a wide variety of aquatic and terrestrial environments. A Gram-stain-positive bacterium, strain H2^T, was isolated during an ecological survey of marine organisms in China and found to have morphological properties consistent with the genus *Bacillus*.

Strain H2^T was isolated from a marine sediment collected at a depth of 823 m in the South Sea, China. A standard dilution-plating technique was used to isolate the strain on marine agar 2216 (MA; Difco) at 37 °C for 3 days. Strain H2^T was maintained on LB agar (10 g tryptone, 5 g yeast extract, 10 g NaCl, 1 l distilled water; pH 7.0) at 4 °C and stored in 20 % (v/v) glycerol suspensions at –20 °C.

To characterize strain H2^T phenotypically, standard phenotypic tests were performed according to procedures described elsewhere (Gordon *et al.*, 1973, 1977; Smibert & Krieg, 1994; Zhang *et al.*, 2008, 2009). Cellular morphology was examined by light microscopy (BH-2; Olympus). Gram staining was performed as described by Gerhardt *et al.* (1981). The morphology, size and pigmentation of colonies were observed on LB agar, MA and nutrient agar after 1 day at 37 °C. Catalase activity was determined on freshly growing colonies using 3 % (v/v) hydrogen peroxide solution. The oxidase reaction was performed on filter paper moistened with a 1 % (w/v) aqueous solution of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine. Growth under

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H2^T is GQ292772.

A comparison of the fatty acid profiles of strain H2^T and its closest phylogenetic neighbours is available with the online version of this paper.

various pH, temperature and sodium chloride regimes was determined on LB agar after incubation for up to 7 days. Acid production from carbohydrates was determined using methods described by Gordon *et al.* (1974) and utilization of sole carbon sources was determined according to Gordon & Mihm (1957). Resistance to antibiotics was examined using impregnated filter-paper discs (Goodfellow & Orchard, 1974) on LB agar and incubation at 37 °C for up to 7 days. Resistance to lysozyme was determined by the method of Gordon *et al.* (1973). Strain H2^T was Gram-stain-positive and aerobic. The cells were spore-forming rods (0.6–0.8 by 2.0–3.0 µm). After 1 day at 37 °C on LB agar, colonies were circular, creamy white and 1–3 mm in diameter. Strain H2^T grew at 4–45 °C (optimum 37 °C) and pH 6–10 (optimum pH 7.0). A range of phenotypic properties separated strain H2^T from its closest phylogenetic neighbours (Table 1). In particular, the isolate could be differentiated from *Bacillus firmus* ATCC 14575^T in that it was positive for utilization of D-galactose, inulin, *myo*-inositol, lactose, D-sorbitol and xylitol. Additional phenotypic properties are presented in the species description and Table 1.

Biomass for most of the chemotaxonomic and molecular systematic studies was prepared as described by Zhang *et al.* (2002), with the modification of using shake flasks of LB broth. Preparation of the cell wall and determination of the peptidoglycan composition were performed using the methods described by Schleifer (1985), with the modification that cellulose sheets instead of paper were used for TLC. Isoprenoid quinones were extracted and purified according to Collins *et al.* (1987) and purified menaquinones were determined by reversed-phase HPLC (Wu *et al.*, 1989). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin *et al.* (1984). The cellular fatty acid profile was determined for cells cultured on tryptic soy broth agar at 28 °C for 24 h. Cells were saponified and the cellular fatty acids were extracted, purified, methylated and quantified by GC using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G+C content of the DNA was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as a reference.

Table 1. Comparison of properties of strain H2^T with the three most closely related type strains of species in the genus *Bacillus* and the type strain of *Falsibacillus pallidus*

Strains: 1, *Bacillus oceanisediminis* sp. nov. H2^T; 2, *B. firmus* ATCC 14575^T; 3, *B. infantis* SMC 4352-1^T; 4, *B. nealsonii* ATCC BAA-519^T; 5, *F. pallidus* CW 7^T. Data were taken from this study and Gordon *et al.* (1977), Venkateswaran *et al.* (2003), Ko *et al.* (2006) and Zhou *et al.* (2009). +, Positive; w, weakly positive; v, variable; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Isolation source	Marine sediment	Soil	Patient with neonatal sepsis	Spacecraft-assembly facility	Forest soil
Reduction of nitrate	+	+	ND	–	–
Oxidase	+	–	–	–	–
Arginine dihydrolase	+	–	ND	–	–
Growth at/with:					
Temperature range (°C)	4–45	10–40	ND	25–60	15–42
Temperature optimum (°C)	37	37	37	30–35	30–37
NaCl range (% w/v)	0–13	0–10	ND	0–8	0–2
Hydrolysis of:					
Gelatin	+	–	ND	–	+
Starch	+	+	ND	–	–
Sole carbon sources					
D-Galactose	+	–	+	+	–
Glycerol	w	–	–	+	–
Inulin	+	–	+	–	–
<i>myo</i> -Inositol	+	–	–	–	–
Lactose	+	–	+	ND	–
Maltose	w	–	+	+	–
D-Mannitol	+	v	+	–	–
D-Mannose	–	+	–	+	–
D-Sorbitol	+	–	+	–	–
Trehalose	w	–	+	+	–
Xylitol	+	–	–	–	–
Acid production from D-mannitol	–	+	ND	+	–
DNA G+C content (mol%)	44.8	43.7	40.8	ND	42.3

Analysis of the cell-wall peptidoglycan showed that strain H2^T possessed the A1 γ type, with *meso*-diaminopimelic acid as the diagnostic diamino acid, which is in common with a large majority of members of the genus *Bacillus* (Priest *et al.*, 1988). Strain H2^T contained MK-7 as the major menaquinone. The polar lipids detected were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown aminophospholipid. The cellular fatty acid profile of strain H2^T contained iso-C_{15:0} (35.65 %), C_{16:1} ω 7c alcohol (9.97 %), iso-C_{14:0} (9.71 %), anteiso-C_{15:0} (9.61 %), iso-C_{16:0} (9.21 %) and summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B; 6.73 %) as the major fatty acids (>5 %) (Supplementary Table S1, available in IJSEM Online). These iso- and anteiso-branched fatty acids of the 14–17-carbon series are typical of those found in the cell membranes of members of the genus *Bacillus* (Kämpfer, 1994; Albert *et al.*, 2005). Thus, these chemotaxonomic features of strain H2^T were typical of those for the genus *Bacillus* (Priest *et al.*, 1988; Heyrman *et al.*, 2004; Albert *et al.*, 2005, 2007; Wieser *et al.*, 2005; Lim *et al.*, 2006a, b; Xue *et al.*, 2008). In addition, the DNA G+C content of strain H2^T was 44.8 mol%, which is within the range for the genus *Bacillus* (Nielsen *et al.*, 1995; Priest *et al.*, 1988).

Chromosomal DNA preparation from strain H2^T, PCR-mediated amplification of the 16S rRNA gene and purification of the amplification product were carried out according to Rainey *et al.* (1996) and the amplification product was sequenced directly using the method of Lu *et al.* (2001). Nucleotide sequences were sequenced automatically using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. The resultant 16S rRNA gene sequence was compared with those available from the GenBank database using the BLAST program to determine an approximate phylogenetic affiliation for strain H2^T. Multiple alignment with closely related sequences was performed using CLUSTAL X version 1.8 (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution and maximum-parsimony algorithms in MEGA version 3.1 (Kumar *et al.*, 2004). Evolutionary distance matrices were generated according to Kimura (1980). Topologies of the resultant unrooted trees were evaluated by bootstrap analysis (Felsenstein, 1985) using 1000 resamplings of the neighbour-joining dataset.

An almost-complete 16S rRNA gene sequence (1393 nt) was obtained for strain H2^T. Phylogenetic analysis showed that the isolate belonged in the phyletic group classically defined as the genus *Bacillus*. The type strains of species of the genus *Bacillus* with the greatest pairwise similarity to strain H2^T were *B. firmus* IAM 12464^T (98.5 % 16S rRNA gene sequence similarity), *B. infantis* SMC 4352-1^T (98.3 %) and *B. nealsonii* FO-92^T (97.7 %). The sequence similarities between strain H2^T and other members of the genus *Bacillus* were below 97.0 %. The initial alignment included 16S rRNA gene sequences from members of

nearly all of the species of the genus *Bacillus* with validly published names. Of these, 25 sequences were found to have a close relationship with strain H2^T and were selected for further analysis. It is apparent from the neighbour-joining phylogenetic tree (Fig. 1) that strain H2^T forms a coherent cluster with *B. firmus* IAM 12464^T and *B. infantis* SMC 4352-1^T within the genus *Bacillus* and is distantly related to other members of the genus. The same results were obtained with the other methods of phylogenetic reconstruction used in the study, minimum evolution and maximum parsimony (data not shown).

There are widely accepted criteria for delineating species in current bacteriology, stating that strains with <70 % DNA–DNA relatedness or with <97 % 16S rRNA gene sequence similarity are considered to represent different species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). Many members of the genus *Bacillus* with >98.5 % 16S rRNA gene sequence similarity are considered to belong to different species. For example, the 16S rRNA gene sequences of the type strains of *Bacillus bataviensis*, *B. soli*, *B. drementensis*, *B. novalis* and *B. vireti* show 98.7–99.6 % pairwise similarity (Ko *et al.*, 2006). DNA–DNA relatedness provides a reliable way of distinguishing between representatives of species that share high 16S rRNA gene sequence similarity (Goodfellow *et al.*, 1998). In the present study, DNA–DNA relatedness studies were carried out between the isolate and its closest phylogenetic neighbours, *B. firmus* ATCC 14575^T, *B. infantis* SMC 4352-1^T and *B. nealsonii* ATCC BAA-519^T, using the liquid renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) using a model Lambda 35 UV/VIS spectrometer equipped with a temperature program controller (Perkin–Elmer) and the results were expressed as the mean of three determinations. Strain H2^T showed low levels of DNA–DNA relatedness with *B. firmus* ATCC 14575^T (27.5 %), *B. infantis* SMC 4352-1^T (23.1 %) and *B. nealsonii* ATCC BAA-519^T (20.7 %); all of these values are well below the 70 % cut-off point.

Although strain H2^T showed a higher 16S rRNA gene sequence similarity with *F. pallidus* CW 7^T (97 %) than with the type strain of the type species of the genus *Bacillus*, *B. subtilis* NCDO 1769^T (96 %), the isolate did not belong to the genus *Falsibacillus*. Chemotaxonomic data showed some significant differences in comparison with *F. pallidus* CW 7^T (Supplementary Table S1). Polar lipid profiles have proved to be a most useful tool for the classification and determination of members of the family *Bacillaceae* in recent years (Zhou *et al.*, 2009). The polar lipid profile of *B. subtilis* has been shown to be characteristic of the genus *Bacillus* and should form part of the genus description (Kämpfer *et al.*, 2006). The polar lipid profile of *B. subtilis* comprises diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown aminophospholipid and β -gentiobiosyldiacylglycerol (Kämpfer *et al.*, 2006; Albert *et al.*, 2007), while the polar lipids of *F. pallidus* comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two aminophosphoglycolipids, five unknown

Fig. 1. Neighbour-joining tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain H2^T and related type strains in the genus *Bacillus*. Bootstrap values (>50%) based on 1000 resampled datasets are shown at branch nodes. Bar, 0.005 substitutions per nucleotide position.

phospholipids, two phosphoglycerolipids and one glycolipid (Zhou *et al.*, 2009). The polar lipid profile of strain H2^T was similar to those for the genus *Bacillus*. The significant differences in the cellular fatty acid profile and phenotypic properties (Table 1 and Supplementary Table S1) can be used to distinguish strain H2^T from *F. pallidus* CW 7^T and, therefore, it is concluded that the isolate should be placed in the genus *Bacillus*.

On the basis of the taxonomic data described above, strain H2^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus oceanisediminis* sp. nov. is proposed.

Description of *Bacillus oceanisediminis* sp. nov.

Bacillus oceanisediminis (o.ce.a.ni.se.di'mi.nis. L. n. *oceanus* ocean; L. n. *sedimen* -inis sediment; N.L. gen. n. *oceanisediminis* of/from sediment of the ocean).

Cells are 0.6–0.8 µm in width and 2–3 µm in length, Gram-stain-positive, aerobic, round-ended rods that occur singly or in pairs and occasionally in short chains. Ellipsoidal endospores are formed subterminally, paracentrally or centrally. After 1 day at 37 °C on LB agar, colonies are creamy white, slightly convex in the centre, smooth, round and 1–3 mm in diameter; edges are usually irregular with pointed projections that may spread and become rhizoid in old cultures. Diffusible pigments are not formed. Grows at 4–45 °C (optimum 37 °C), at pH 6–10 (optimum pH 7.0) and with 0–13% (w/v) NaCl. Positive for catalase, oxidase, β-galactosidase and arginine dihydrolase, but negative for lipase, phenylalanine deaminase and urease. Nitrate is reduced. Indole and methyl red tests are positive, but

Voges–Proskauer and egg-yolk reactions are negative. Hydrogen sulphide is not produced. Adenine, casein, gelatin, starch and tyrosine (weak) are hydrolysed; aesculin and arbutin are not hydrolysed. Acid is formed from D-glucose, inulin, glycogen, raffinose and sucrose but not from L-arabinose, cellobiose, D-fructose, D-galactose, glycerol, myo-inositol, lactose, maltose, D-mannitol, melezitose, melibiose, α-L-rhamnose, D-sorbitol, trehalose, D-xylitol or D-xylose. As sole carbon and energy sources, utilizes cellobiose, meso-erythritol, D-glucose, glycogen, melezitose, raffinose, α-L-rhamnose, sucrose, acetate, fumarate, lactic acid, malate, pyruvic acid, sorbic acid, succinate and tartrate (weak), but not L-arabinose, D-fructose, melibiose, D-ribose, L-sorbose, D-xylose, benzoate, citrate, malonate, propionate or oxalate. Resistant to (µg per disc unless otherwise stated) aztreonam (30), but susceptible to lysozyme (0.005%, w/v), amikacin (30), amoxicillin plus clavulanic acid (10), ampicillin (10), cefotaxime (30), chloramphenicol (30), ciprofloxacin (5), clindamycin hydrochloride (2), erythromycin (15), gentamicin sulphate (10), kanamycin sulphate (30), mezlocillin (75), ofloxacin (5), penicillin G (10 U), rifampicin (5), streptomycin sulphate (10), tetracycline hydrochloride (30) and tobramycin sulphate (10). Additional phenotypic properties are shown in Table 1. Chemotaxonomic properties are typical of the genus *Bacillus*: the cell-wall peptidoglycan contains meso-diaminopimelic acid, the major isoprenoid quinone type is MK-7 and the polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown aminophospholipid. The fatty acid profile (>2%) is composed of iso-C_{15:0}, C_{16:1}ω7c alcohol, iso-C_{14:0}, ante-iso-C_{15:0}, iso-C_{16:0}, summed feature 4 (iso-C_{17:1} I and/or ante-iso-C_{17:1} B), iso-C_{16:1} H, iso-C_{17:1}ω10c, ante-iso-C_{17:0}, iso-C_{17:0} and C_{16:1}ω11c. The genomic DNA G + C content of the type strain is 44.8 mol%.

The type strain, H2^T (=CGMCC 1.10115^T =JCM 16506^T), was isolated from a sediment sample from the South Sea, China.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NSFC, grant number 30970009), and by the University Basic Fund of Beijing Institute of Technology of China (grant number 20081642008).

References

- Albert, R. A., Archambault, J., Rosselló-Mora, R., Tindall, B. J. & Matheny, M. (2005). *Bacillus acidicola* sp. nov., a novel mesophilic, acidophilic species isolated from acidic *Sphagnum* peat bogs in Wisconsin. *Int J Syst Evol Microbiol* 55, 2125–2130.
- Albert, R. A., Archambault, J., Lempa, M., Hurst, B., Richardson, C., Gruenloh, S., Duran, M., Worliczek, H. L., Huber, B. E. & other authors (2007). Proposal of *Viridibacillus* gen. nov. and reclassification of *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei* as *Viridibacillus arvi* gen. nov., comb. nov., *Viridibacillus arenosi* comb. nov. and *Viridibacillus neidei* comb. nov. *Int J Syst Evol Microbiol* 57, 2729–2737.
- 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.
- Carrasco, I. J., Márquez, M. C., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2007). *Bacillus chagannorensis* sp. nov., a moderate halophile from a soda lake in Inner Mongolia, China. *Int J Syst Evol Microbiol* 57, 2084–2088.
- Collins, M. D., Howarth, O. W., Grund, E. & Kroppenstedt, R. M. (1987). Isolation and structural determination of new members of the vitamin K₂ series in *Nocardia brasiliensis*. *FEMS Microbiol Lett* 41, 35–39.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurements of DNA hybridization from renaturation rates. *Eur J Biochem* 12, 133–142.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (editors) (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- Goodfellow, M. & Orchard, V. A. (1974). Antibiotic sensitivity of some nocardioform bacteria and its value as a criterion for taxonomy. *J Gen Microbiol* 83, 375–387.
- Goodfellow, M., Stainsby, F. M., Davenport, R., Chun, J. & Curtis, T. (1998). Activated sludge foaming: the true extent of actinomycete diversity. *Water Sci Technol* 37, 511–519.
- Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J Bacteriol* 73, 15–27.
- Gordon, R. E., Haynes, W. C. & Pang, C. H.-N. (1973). *The Genus Bacillus*. US Department of Agriculture Handbook no. 427. Washington, DC: Agricultural Research Service.
- Gordon, R. E., Barnett, D. A., Handerman, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* 24, 54–63.
- Gordon, R. E., Hyde, J. L. & Moore, J. A., Jr (1977). *Bacillus firmus*-*Bacillus lentus*: a series or one species? *Int J Syst Bacteriol* 27, 256–262.
- Heyrman, J., Vanparys, B., Logan, N. A., Balcaen, A., Rodriguez-Diaz, M., Felske, A. & De Vos, P. (2004). *Bacillus novalis* sp. nov., *Bacillus vireti* sp. nov., *Bacillus soli* sp. nov., *Bacillus botaviensis* sp. nov. and *Bacillus drentensis* sp. nov., from the Drentse A grasslands. *Int J Syst Evol Microbiol* 54, 47–57.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* 4, 184–192.
- Jahnke, K. D. (1992). BASIC computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrophotometer on a PC/XT/AT type personal computer. *J Microbiol Methods* 15, 61–73.
- Kämpfer, P. (1994). Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* 17, 86–98.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* 42, 989–1005.
- Kämpfer, P., Rosselló-Mora, R., Falsen, E., Busse, H.-J. & Tindall, B. J. (2006). *Cohnella thermotolerans* gen. nov., sp. nov. and classification of '*Paenibacillus hongkongensis*' as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* 56, 781–786.
- 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.
- Ko, K. S., Oh, W. S., Lee, M. Y., Lee, J. H., Lee, H., Peck, K. R., Lee, N. Y. & Song, J.-H. (2006). *Bacillus infantis* sp. nov. and *Bacillus idriensis* sp. nov., isolated from a patient with neonatal sepsis. *Int J Syst Evol Microbiol* 56, 2541–2544.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5, 150–163.
- Lim, J.-M., Jeon, C. O. & Kim, C.-J. (2006a). *Bacillus taeanensis* sp. nov., a halophilic Gram-positive bacterium from a solar saltern in Korea. *Int J Syst Evol Microbiol* 56, 2903–2908.
- Lim, J.-M., Jeon, C. O., Lee, S.-M., Lee, J.-C., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2006b). *Bacillus salarius* sp. nov., a halophilic spore-forming bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* 56, 373–377.
- Lu, Z., Liu, Z., Wang, L., Zhang, Y., Qi, W. & Goodfellow, M. (2001). *Saccharopolyspora flava* sp. nov. and *Saccharopolyspora thermophila* sp. nov., novel actinomycetes from soil. *Int J Syst Evol Microbiol* 51, 319–325.
- Marmur, J. & Doty, P. (1962). Determination of base composition of deoxyribonucleic acid from its denaturation temperature. *J Mol Biol* 5, 109–118.
- 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.
- Nazina, T. N., Tourova, T. P., Poltarau, A. B., Novikova, E. V., Grigoryan, A. A., Ivanova, A. E., Lysenko, A. M., Petrunyaka, V. V., Osipov, G. A. & other authors (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzonensis* 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.
- Nielsen, P., Rainey, F. A., Outtrup, H., Priest, F. G. & Fritze, D. (1994). Comparative 16S rDNA sequence analysis of some alkaliphilic bacilli and the establishment of a sixth rRNA group within the genus *Bacillus*. *FEMS Microbiol Lett* **117**, 61–66.
- Nielsen, P., Fritze, D. & Priest, F. G. (1995). Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. *Microbiology* **141**, 1745–1761.
- Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* **134**, 1847–1882.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsiaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- 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 *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol* **46**, 939–946.
- Smibert, R. M. & 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.
- 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.
- Stackebrandt, E. & Liesack, W. (1993). Nucleic acids and classification. In *Handbook of New Bacterial Systematics*, pp. 152–189. Edited by M. Goodfellow & A. G. O'Donnell. London: Academic Press.
- Stackebrandt, E., Fredericksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Venkateswaran, K., Kempf, M., Chen, F., Satomi, M., Nicholson, W. & Kern, R. (2003). *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are γ -radiation resistant. *Int J Syst Evol Microbiol* **53**, 165–172.
- 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., 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.
- Wieser, M., Worliczek, H., Kämpfer, P. & Busse, H.-J. (2005). *Bacillus herbersteinensis* sp. nov. *Int J Syst Evol Microbiol* **55**, 2119–2123.
- Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* [English translation of *Microbiology (Beijing)*] **16**, 176–178.
- Xue, Y., Ventosa, A., Wang, X., Ren, P., Zhou, P. & Ma, Y. (2008). *Bacillus aidingensis* sp. nov., a moderately halophilic bacterium isolated from Ai-Ding salt lake in China. *Int J Syst Evol Microbiol* **58**, 2828–2832.
- 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* Rüger 1983 as *Marinibacillus marinus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 2087–2093.
- Yoon, J.-H., Kim, I.-G., Kang, K.-H., Oh, T.-K. & Park, Y.-H. (2004). *Bacillus hwajinpoensis* sp. nov. and an unnamed *Bacillus* genom-species, novel members of *Bacillus* rRNA group 6 isolated from sea water of the East Sea and the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **54**, 803–808.
- Zhang, J., Zhang, Y., Xiao, C., Liu, Z. & Goodfellow, M. (2002). *Rhodococcus maanshanensis* sp. nov., a novel actinomycete from soil. *Int J Syst Evol Microbiol* **52**, 2121–2126.
- Zhang, J., Wu, D., Zhang, J., Liu, Z. & Song, F. (2008). *Saccharopolyspora shandongensis* sp. nov., isolated from wheat-field soil. *Int J Syst Evol Microbiol* **58**, 1094–1099.
- Zhang, J., Song, F., Xin, Y.-H., Zhang, J. & Fang, C. (2009). *Microvirga guangxiensis* sp. nov., a novel alphaproteobacterium from soil, and emended description of the genus *Microvirga*. *Int J Syst Evol Microbiol* **59**, 1997–2001.
- Zhou, Y., Wei, W., Chen, Q., Xu, Y., Wang, X., Huang, X. & Lai, R. (2008). *Bacillus pallidus* sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* **58**, 2850–2854.
- Zhou, Y., Xu, J., Xu, L. & Tindall, B. J. (2009). *Falsibacillus pallidus* to replace the homonym *Bacillus pallidus* Zhou et al. 2008. *Int J Syst Evol Microbiol* **59**, 3176–3180.