Paenibacillus chungangensis sp. nov., isolated from a tidal-flat sediment

Mi-Hak Park,^{1,2} Jitsopin Traiwan,^{1,2} Min Young Jung,^{1,2} Yun Sung Nam,³ Ji Hoon Jeong³ and Wonyong Kim^{1,2}

Correspondence
Wonyong Kim
kimwy@cau.ac.kr

¹Department of Microbiology, Chung-Ang University College of Medicine, 221 Heukseok-dong, Dongjak-gu, Seoul 156-756, Republic of Korea

²Research Institute for Translational System Biomixis, Chung-Ang University College of Medicine, 221 Heukseok-dong, Dongjak-gu, Seoul 156-756, Republic of Korea

³Department of Pharmacology, Chung-Ang University College of Medicine, 221 Heukseok-dong, Dongjak-gu, Seoul 156-756, Republic of Korea

A Gram-stain-positive, rod-shaped, endospore-forming bacterium, strain CAU 9038^T, was isolated from a tidal-flat sediment of DaeYiJac Island, Republic of Korea, and its taxonomic position was investigated using a polyphasic approach. The cell-wall peptidoglycan contained meso-diaminopimelic acid. The major polar lipids were diphosphatidylglycerol and phosphatidylglycerol, the major isoprenoid quinone was MK-7 and the dominant cellular fatty acid was anteiso-C_{15:0}. The DNA G+C content was 51.6 mol%. 16S rRNA gene sequence analysis showed that the strain belonged to the genus *Paenibacillus*, with <96.1 % sequence similarity to type strains of *Paenibacillus* species with validly published names. The most closely related type strains to CAU 9038^T were *Paenibacillus thailandensis* S3-4A^T (96.1 % similarity) and *Paenibacillus agaridevorans* DSM 1355^T (95.3 %). The phenotypic, chemotaxonomic and genotypic data clearly indicated that strain CAU 9038^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus chungangensis* sp. nov. is proposed. The type strain is CAU 9038^T (=KCTC 13717^T =CCUG 59129^T).

The genus *Paenibacillus* was originally proposed by Ash *et al.* (1993, 1994) for rRNA group 3 bacilli, mainly on the basis of 16S rRNA gene sequence data. Members of the genus *Paenibacillus* have been isolated from diverse environments such as soils, food, Antarctic environments, warm springs, milk, poultry litter compost, human blood, cerebrospinal fluid and urine (Shida *et al.*, 1997; Berge *et al.*, 2002; Montes *et al.*, 2004; Roux & Raoult, 2004; Scheldeman *et al.*, 2004; Chou *et al.*, 2007; Vaz-Moreira *et al.*, 2007; Roux *et al.*, 2008). At the time of writing, the genus *Paenibacillus* comprised 107 species with validly published names (<http://www.bacterio.cict.fr/p/paenibacillus.html>; last full update 25 January 2010), including the recently described species *Paenibacillus cellulositrophicus* (Akaracharanya *et al.*, 2009), *P. contaminans* (Chou *et al.*, 2009), *P. tundrae* and *P. xylanexedens* (Nelson *et al.*, 2009). During the course of screening micro-organisms for industrial purposes, an aerobic, Gram-stain-positive, endospore-forming bacterium designated CAU 9038^T was

isolated from a sample of tidal-flat sediment of DaeYiJac Island, Republic of Korea. The purpose of the present study was to establish the taxonomic position of this bacterial strain.

The procedure for isolation of strain CAU 9038^T followed that of Gordon & Mihm (1962) using glucose-yeast extract (GYE) agar (10 g yeast extract, 10 g glucose and 15 g agar l⁻¹) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). The sediment sample was diluted with sterilized distilled water and appropriate dilutions were spread onto GYE agar medium and incubated aerobically for 3 days at 30 °C.

The pure culture of CAU 9038^T was preserved at –70 °C in GYE broth supplemented with 25 % (v/v) glycerol. Cell morphology was examined by light microscopy (Leica DM 1000). Flagellum type was examined using cells from exponentially growing cultures. The cells were negatively stained with 1 % (w/v) phosphotungstic acid and, after air drying, the grids were examined using a transmission electron microscope (Philips CM-20). Gram-staining was determined by microscopic examination following described procedures (Smibert & Krieg, 1981). Growth at various temperatures was determined on tryptic soy agar

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 9038^T is GU187432.

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

(TSA; Difco) at 20–45 °C in an aerobic incubator (model MIR-253; Sanyo) or anaerobic chamber (model Bactron; Sheldon). Growth at 0–15 % NaCl at 30 °C was investigated in tryptic soy broth (TSB; Difco). The pH range for growth was examined in TSB buffered with 12 mM MES (Sigma) at pH 3.0, 4.5 and 6.0 or with 15 mM CAPS (Sigma) at pH 8.0, 9.0 and 10.0. A nutrient sporulation medium (NSM) was used to produce spores (Schaeffer *et al.*, 1965; Nicholson & Setlow, 1990). After 3 days of growth at 32 °C, endospores were examined using malachite green staining (Smibert & Krieg, 1994).

Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was tested by means of the oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine (Merck). Hydrolysis of casein, starch and urea was determined on TSA according to methods described previously (Lányi, 1987; Smibert & Krieg, 1994). Tests for acid production from carbohydrates and enzyme activities and other physiological and biochemical tests were carried out using API 50CHB, API 20E and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Cellular fatty acid methyl esters were prepared from cells grown on TSA at 30 °C for 3 days by acid methanolysis (Minnikin *et al.*, 1980) and analysed by gas chromatography (Agilent Technologies 56890N) equipped with a flame-ionization detector using a cross-linked 5 % phenyl methyl silicon fused silica capillary column (Hewlett Packard 19091B-102). Preparation of cell walls and analysis of peptidoglycan structures were carried out using the methods described by Schleifer (1985), with the modification that TLC on cellulose sheets was performed instead of paper chromatography. Polar lipids were extracted and analysed by two-dimensional TLC according to Minnikin *et al.* (1984). Menaquinones were analysed as described previously (Komagata & Suzuki, 1987) using reversed-phase HPLC.

Genomic DNA of strain CAU 9038^T was isolated and purified by the method of Marmur (1961). The G+C content of the genomic DNA was determined using HPLC by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. PCR amplification and DNA sequencing of the 16S rRNA gene of the strain were carried out following established procedures (Nam *et al.*, 2004). The amplified 16S rRNA gene was sequenced directly using a BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (Applied Biosystems 3730). Multiple alignments with sequences of a broad selection of *Paenibacillus* strains and calculations of sequence similarity were carried out by using the EzTaxon server (Chun *et al.*, 2007) and CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distance matrices were generated by the neighbour-joining method described by Jukes & Cantor (1969). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch &

Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) methods from the PHYLIP suite of programs (Felsenstein, 1989) and tree topology was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein, 1985) of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package.

Colonies of strain CAU 9038^T were strictly aerobic, yellow, convex and circular with a diameter of 0.3–0.4 mm after 3 days of cultivation at 30 °C on TSA. Cells were rods, approximately 1.1–1.3 µm wide and 0.3 µm long. Spherical terminal endospores were produced within swollen sporangia (Supplementary Fig. S1, available in IJSEM Online) and were motile by means of peritrichous flagella (Supplementary Fig. S2). Growth was observed at 20–45 °C (optimum 30 °C) and at pH 5.0–11.0 (optimum pH 7.0) on GYE agar. Strain CAU 9038^T grew in the presence of 0–15 % (w/v) NaCl (optimum 3 % NaCl). Cells of strain CAU 9038^T were catalase- and oxidase-positive, but were negative for nitrate reduction and hydrolysis of casein, gelatin and starch.

API test strips provided adequate phenotypic data to describe the new isolate and a detailed description of the key phenotypic characteristics of the isolate is given in Table 1 and in the species description. Acids were produced from cellobiose, glycogen and 5-ketogluconate but not from glycerol, L-arabinose, ribose, methyl β-D-xylopyranoside, galactose, glucose, fructose, rhamnose, mannitol, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, maltose, lactose, sucrose, trehalose, melezitose, raffinose, gentiobiose, turanose or gluconate. Citrate utilization and reduction of nitrates to nitrites were positive. Activities of esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-fucosidase were detected, whereas activities of alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucosidase (naphthol), α-glucosidase, β-glucosidase (6-Br), N-acetyl-β-glucosaminidase and α-mannosidase were not detected.

The predominant isoprenoid quinone was unsaturated menaquinone MK-7. TLC analysis of whole-cell hydrolysates revealed mainly ribose. The diagnostic diamino acid in the cell-wall hydrolysate was *meso*-diaminopimelic acid. The polar lipids were made up of diphosphatidylglycerol, phosphatidylglycerol, an unidentified glycolipid and a minor amount of unknown phospholipids. The cellular fatty acid profile of the strain grown on TSA contained saturated, branched and unsaturated fatty acids as follows: C_{10:0} (2.5 %), iso-C_{14:0} (3.7 %), C_{14:0} (1.1 %), iso-C_{15:0} (8.7 %), anteiso-C_{15:0} (39.0 %), C_{15:0} (23.5 %), iso-C_{16:0} (7.9 %), C_{16:1ω11c} (1.3 %), C_{16:0} (3.7 %), anteiso-C_{17:0} (1.7 %), iso-C_{17:0} (2.0 %) and C_{17:0} (3.0 %) (Table 2).

The DNA G+C content of strain CAU 9038^T was 51.6 mol%. The nearly complete 16S rRNA gene sequence of strain CAU 9038^T (1469 bp) was determined and compared with the corresponding sequences of other

Table 1. Differential characteristics between strain CAU 9038^T and the type strains of recognized *Paenibacillus* species

Strains: 1, CAU 9038^T; 2, *P. thailandensis* KCTC 13043^T; 3, *P. agaridevorans* KCTC 3849^T; 4, *P. dendritiformis* KCTC 3765^T; 5, *P. montaniterrae* KCTC 13036^T. +, Positive; –, negative; w, weakly positive. Data were obtained in this study unless indicated.

Characteristic	1	2	3	4	5
Growth at 50 °C	–	+	–	–	–
Acid from:					
Glycerol	–	–	–	+	+
L-Arabinose	–	+	w	–	+
Ribose	–	+	–	+	+
D-Xylose	–	+	–	–	+
Methyl β-D-xylopyranoside	–	–	+	–	+
Galactose	–	–	+	–	+
Glucose	–	+	+	+	+
Fructose	–	+	–	+	+
Mannose	w	+	–	+	+
Rhamnose	–	+	–	–	–
Mannitol	–	–	–	–	+
Methyl α-D-glucopyranoside	–	–	+	–	+
N-Acetylglucosamine	–	–	–	–	+
Amygdalin	–	–	–	–	+
Arbutin	–	–	–	+	+
Cellobiose	+	–	+	–	+
Maltose	–	–	+	+	+
Lactose	–	+	+	–	+
Melibiose	w	+	+	–	+
Sucrose	–	+	+	–	+
Trehalose	–	–	+	+	+
Melezitose	–	–	+	–	–
Raffinose	w	+	+	–	+
Glycogen	+	+	–	+	+
Gentiobiose	–	–	+	–	+
Turanose	–	–	w	–	+
D-Tagatose	w	–	–	–	–
Gluconate	–	–	–	–	+
5-Ketogluconate	+	–	–	–	–
Hydrolysis of:					
Casein	–	–	–	–	+
Gelatin	–	–	+	–	–
Starch	–	–	w	+	+
Enzyme activity					
Alkaline phosphatase	–	–	–	+	–
Esterase (C4)	+	–	–	+	+
Esterase lipase (C8)	–	–	–	+	–
α-Chymotrypsin	–	–	–	+	+
Acid phosphatase	–	–	–	+	–
Naphthol-AS-BI-phosphohydrolase	+	–	–	–	–
α-Galactosidase	–	+	–	–	–
β-Galactosidase	+	+	+	–	+
α-Glucosidase	–	–	–	–	+
β-Glucosidase (6-Br)	–	+	–	–	+
N-Acetyl-β-glucosaminidase	–	+	–	–	–
DNA G + C content (mol%)*	51.6	52.7 ^a	51 ^b	55 ^c	48.8 ^d

*Data taken from: a, Khianngam *et al.* (2009b); b, Uetanabaro *et al.* (2003); c, Tcherpakov *et al.* (1999); d, Khianngam *et al.* (2009a).

Table 2. Cellular fatty acid compositions of strain CAU 9038^T and closely related type strains of the genus *Paenibacillus*

Strains: 1, CAU 9038^T; 2, *P. thailandensis* KCTC 13043^T; 3, *P. agaridevorans* KCTC 3849^T; 4, *P. dendritiformis* KCTC 3765^T; 5, *P. montaniterrae* KCTC 13036^T. Values are percentages of total fatty acids, and were obtained in this study. –, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain saturated					
C _{10:0}	2.5	–	–	–	–
C _{14:0}	1.1	–	1.2	4.8	2.1
C _{15:0}	23.5	–	6.2	–	8.5
C _{16:0}	3.7	4.1	6.7	3.3	10.9
C _{17:0}	3.0	–	–	–	1.3
Straight-chain unsaturated					
C _{16:1ω11c}	1.3	–	1.2	–	6.0
Branched-chain					
anteiso-C _{13:0}	–	–	–	1.8	–
anteiso-C _{15:0}	39.0	54.1	46.2	5.5	45.8
anteiso-C _{17:0}	1.7	12.6	3.7	–	4.5
iso-C _{13:0}	–	–	–	15.0	–
iso-C _{14:0}	3.7	1.7	5.0	4.6	3.2
iso-C _{15:0}	8.7	7.8	8.6	38.5	4.6
iso-C _{16:0}	7.9	15.4	18.0	3.7	8.0
iso-C _{17:0}	2.0	3.2	3.5	5.4	2.6
iso-C _{17:1ω5c}	–	–	–	2.8	–
iso-C _{17:1ω10c}	–	–	–	1.5	–
Summed features*					
2	–	–	–	1.6	–
3	–	–	–	8.0	–

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI System. Summed feature 2 contains C_{14:0} 3-OH and/or iso-C_{16:1} 1; summed feature 3 contains C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

bacterial strains in the GenBank database. A phylogenetic analysis based on 16S rRNA gene sequence data revealed that strain CAU 9038^T belonged to the genus *Paenibacillus*. The tree topology, supported by high bootstrap values, clearly separated strain CAU 9038^T within the lineage of the genus *Paenibacillus*, distinct from *Bacillus simplex* NBRC 15720^T, forming an outlying, single-member cluster. It is evident from our 16S rRNA gene sequence study that strain CAU 9038^T forms a distinct subclade with closely related type strains such as *Paenibacillus thailandensis* S3-4A^T (96.1 % similarity), *P. agaridevorans* DSM 1355^T (95.3 %), *P. dendritiformis* CIP 105967^T (95.1 %), *P. montaniterrae* MXC2-2^T (95.1 %), *P. granivorans* A30^T (95.1 %), *P. nanensis* MX2-3^T (95.1 %), *P. agarexedens* DSM 1327^T (95.1 %), *P. harenae* B519^T (95.1 %), *Fontibacillus aquaticus* GPTSA19^T (95.0 %) and *P. alkaliterrae* KSL-134^T (95.0 %) (Fig. 1). These data, together with the phenotypic and chemotaxonomic data for strain CAU 9038^T, provide sufficient evidence to support the proposal to recognize this strain as a member of a novel species, as

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU 9038^T and type strains of closely related taxa of the genus *Paenibacillus*. Branches of the tree that were also found using the least-squares and/or maximum-likelihood treeing algorithms are indicated by asterisks (both algorithms) or by M (maximum-likelihood only). Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >50% are given. Bar, 0.01 substitutions per nucleotide position.

recommended by Stackebrandt *et al.* (2002) and Logan *et al.* (2009). Although a species description is given for a single isolate, this taxonomic status is warranted because this strain was isolated during the course of routine screening of bacteria for industrial purposes and is associated with a special industrial application. The name *Paenibacillus chungangensis* sp. nov. is proposed for this new taxon.

Description of *Paenibacillus chungangensis* sp. nov.

Paenibacillus chungangensis (chung.an.gen'sis. N.L. masc. adj. *chungangensis* pertaining to Chung-Ang University, where taxonomic studies on the type strain were performed).

Cells are strictly aerobic, Gram-stain-positive, motile, endospore-forming, short rods, 1.1–1.3 µm wide and 0.3 µm long. Colonies are yellow, convex and circular on TSA after 72 h of incubation at 30 °C. Grows at 20–45 °C (optimum 30 °C), pH 5.0–11.0 (optimum pH 7.0) and 0–15% (w/v) NaCl (optimum 3%). Catalase- and oxidase-positive. Acid production occurs from cellobiose, glycogen and 5-ketogluconate. Citrate utilization and reduction of nitrates to nitrites are positive. Activities of esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-fucosidase are detected. Whole-cell hydrolysates contain mainly ribose. The diagnostic diamino acid in the cell-wall hydrolysate is *meso*-diaminopimelic

acid. The major polar lipids are diphosphatidylglycerol and phosphatidylglycerol. The major isoprenoid quinone is MK-7. The predominant cellular fatty acids (>5%) are anteiso-C_{15:0}, C_{15:0}, iso-C_{15:0} and iso-C_{16:0}, with smaller amounts of iso-C_{14:0}, C_{16:0}, C_{17:0}, C_{10:0}, iso-C_{17:0}, anteiso-C_{17:0}, C_{16:1ω11c} and C_{14:0}. The DNA G+C content of the type strain is 51.6 mol%.

The type strain, CAU 9038^T (=KCTC 13717^T =CCUG 59129^T), was isolated from a tidal-flat sediment sample taken from DaeYijac Island, Republic of Korea.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Applications Centre Program, Ministry of Education, Science & Technology, Republic of Korea (grant no. 11-2008-03-002-00).

References

- Akaracharanya, A., Lorliam, W., Tanasupawat, S., Lee, K. C. & Lee, J. C. (2009). *Paenibacillus cellulositrophicus* sp. nov., a cellulolytic bacterium from Thai soil. *Int J Syst Evol Microbiol* **59**, 2680–2684.
- 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.
- Ash, C., Priest, F. G. & Collins, M. D. (1994). *Paenibacillus* gen. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 51. *Int J Syst Bacteriol* **44**, 852.
- Berge, O., Guinebreteire, M. H., Achouak, W., Normand, P. & Heulin, T. (2002). *Paenibacillus graminis* sp. nov. and *Paenibacillus odorifer* sp. nov., isolated from plant roots, soil and food. *Int J Syst Evol Microbiol* **52**, 607–616.
- Chou, J. H., Chou, Y. J., Lin, K. Y., Sheu, S. Y., Sheu, D. S., Arun, A. B., Young, C. C. & Chen, W. M. (2007). *Paenibacillus fonticola* sp. nov., isolated from a warm spring. *Int J Syst Evol Microbiol* **57**, 1346–1350.
- Chou, J. H., Lee, J. H., Lin, M. C., Chang, P. S., Arun, A. B., Young, C. C. & Chen, W.-M. (2009). *Paenibacillus contaminans* sp. nov., isolated from a contaminated laboratory plate. *Int J Syst Evol Microbiol* **59**, 125–129.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.
- Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. H. Munro. New York: Academic Press.

- Khiannangam, S., Tanasupawat, S., Lee, J. S., Lee, K. C. & Akaracharanya, A. (2009a). *Paenibacillus siamensis* sp. nov., *Paenibacillus septentrionalis* sp. nov. and *Paenibacillus montaniterrae* sp. nov., xylanase-producing bacteria from Thai soils. *Int J Syst Evol Microbiol* **59**, 130–134.
- Khiannangam, S., Akaracharanya, A., Tanasupawat, S., Lee, K. C. & Lee, J. S. (2009b). *Paenibacillus thailandensis* sp. nov. and *Paenibacillus nanensis* sp. nov., xylanase-producing bacteria isolated from soil. *Int J Syst Evol Microbiol* **59**, 564–568.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Logan, N. A., Berge, O., Bishop, A. H., Busse, H.-J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Rabinovitch, L. & other authors (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* **59**, 2114–2121.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.
- 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.
- Montes, M. J., Mercade, E., Bozal, N. & Guinea, J. (2004). *Paenibacillus antarcticus* sp. nov., a novel psychrotolerant organism from the Antarctic environment. *Int J Syst Evol Microbiol* **54**, 1521–1526.
- Nam, S. W., Kim, W., Chun, J. & Goodfellow, M. (2004). *Tsukamurella pseudospumae* sp. nov., a novel actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **54**, 1209–1212.
- Nelson, D. M., Glawe, A. J., Labeda, D. P., Cann, I. K. O. & Mackie, R. I. (2009). *Paenibacillus tundrae* sp. nov. and *Paenibacillus xylanexedens* sp. nov., psychrotolerant, xylan-degrading bacteria from Alaskan tundra. *Int J Syst Evol Microbiol* **59**, 1708–1714.
- Nicholson, W. L. & Setlow, P. (1990). Sporulation, germination, and outgrowth. In *Molecular Biological Methods for Bacillus*, pp. 391–450. Edited by C. R. Harwood & S. M. Cutting. Chichester: Wiley.
- Roux, V. & Raoult, D. (2004). *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov. and *Paenibacillus timonensis* sp. nov., isolated from blood cultures. *Int J Syst Evol Microbiol* **54**, 1049–1054.
- Roux, V., Fenner, L. & Raoult, D. (2008). *Paenibacillus provencensis* sp. nov., isolated from human cerebrospinal fluid, and *Paenibacillus urinalis* sp. nov., isolated from human urine. *Int J Syst Evol Microbiol* **58**, 682–687.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schaeffer, P., Millet, J. & Aubert, J.-P. (1965). Catabolic repression of bacterial sporulation. *Proc Natl Acad Sci U S A* **54**, 704–711.
- Scheldeman, P., Goossens, K., Rodriguez-Diaz, M., Pil, A., Goris, J., Herman, L., De Vos, P., Logan, N. A. & Heyndrickx, M. (2004). *Paenibacillus lactis* sp. nov., isolated from raw and heat-treated milk. *Int J Syst Evol Microbiol* **54**, 885–891.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). 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. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Microbiology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- 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., Frederiksen, 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.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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., 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.
- Uetanabaro, A. P., Wahrenburg, C., Hunger, W., Pukall, R., Spröer, C., Stackebrandt, E., de Canhos, V. P., Claus, D. & Fritze, D. (2003). *Paenibacillus agarexedens* sp. nov., nom. rev., and *Paenibacillus agaridevorans* sp. nov. *Int J Syst Evol Microbiol* **53**, 1051–1057.
- Vaz-Moreira, I., Faria, C., Nobre, M. F., Schumann, P., Nunes, O. C. & Manaia, C. M. (2007). *Paenibacillus humicus* sp. nov., isolated from poultry litter compost. *Int J Syst Evol Microbiol* **57**, 2267–2271.