Paenibacillus sputi sp. nov., isolated from the sputum of a patient with pulmonary disease

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Strain KIT 00200-70066-1^T was isolated from the sputum of a patient with pulmonary disease. Cells of the strain were Gram-variable, facultatively anaerobic, motile, spore-forming rods and formed colourless to white colonies on tryptic soy agar at 30 °C and pH 7. The pathogenicity of the strain is not known. The strain contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, MK-7 as the predominant menaquinone, anteiso-C_{15:0}, iso-C_{16:0} and C_{16:0} as the major fatty acids and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and several unknown lipids in the polar lipid profile. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belongs to the genus *Paenibacillus*, sharing the highest levels of sequence similarity with *Paenibacillus nanensis* MX2-3^T, *Paenibacillus agaridevorans* DSM 1355^T and *Paenibacillus alkaliterrae* KSL-134^T (95.4, 95.2 and 94.8 %, respectively), and that it occupied a distinct position within this genus. Combined phylogenetic and phenotypic data supported the conclusion that strain KIT 00200-70066-1^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus sputi* sp. nov. is proposed; the type strain is KIT 00200-70066-1^T (=KCTC 13252^T =DSM 22699^T).

The genus *Paenibacillus* (type species, *Paenibacillus polymyxa*) was erected by Ash *et al.* (1993) to accommodate the so-called rRNA group 3 bacilli, comprising 11 *Bacillus* species, on the basis of comparative 16S rRNA gene sequence analysis. Since then, eight more *Bacillus* species and one *Clostridium* species have been transferred to the genus, and a large number of novel species have been described; consequently, the genus contains 102 recognized species at the time of writing (Euzéby, 2009). Members of the genus *Paenibacillus* have been isolated from a wide variety of sources including soil, sediment, humus, the plant rhizosphere and phyllosphere, water, food, fodder, faeces, plant materials and diseased insect larvae (Daane *et al.*, 2002). Some species have been reported from human biological samples, e.g. *Paenibacillus turicensis* and *Paenibacillus provencensis* from cerebrospinal fluid samples, *Paenibacillus massiliensis*, *Paenibacillus sanguinis*, *Paenibacillus timonensis* and *Paenibacillus konsidensis* from blood cultures and *Paenibacillus urinalis* from a urine sample,

but they have been considered to be contaminants rather than pathogens (Bosshard *et al.*, 2002; Roux & Raoult, 2004; Ko *et al.*, 2008; Roux *et al.*, 2008). Meanwhile, it has been reported that some pathogenic strains considered to belong to *Paenibacillus alvei* and *Paenibacillus macerans* were isolated from patients with brain abscesses, catheter-associated infections, endophthalmitis, meningitis, prosthetic hip infections and wound infections (Antonello & Weinstein, 1989; Reboli *et al.*, 1989; Bert *et al.*, 1995; Barrero *et al.*, 1996). However, they were classified based only on conventional phenotypic characterization, so their taxonomic affiliation remains uncertain.

In 2006, strain KIT 00200-70066-1^T was co-isolated from the sputum of a 60-year-old male patient during the screening of mycobacteria from patients with non-tuberculous pulmonary diseases. On the basis of preliminary 16S rRNA gene sequence comparison, the strain was found to belong to the genus *Paenibacillus*, but it showed low levels of 16S rRNA gene sequence similarity (<96 %) to all recognized species of this genus. To elucidate the taxonomic status of the isolate further, it was subjected to a polyphasic taxonomic investigation.

A sputum sample from the patient was diluted serially in 0.85 % saline solution. Aliquots of each serial dilution were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KIT 00200-70066-1^T is FN394513.

Transmission electron micrographs, a maximum-parsimony tree and an extended neighbour-joining tree based on 16S rRNA gene sequences and results of 2D TLC of polar lipids are available as supplementary material with the online version of this paper.

spread on Löwenstein–Jensen medium and incubated at 37 °C for 4 weeks. A white and glistening colony, designated KIT 00200-70066-1^T, was isolated. The isolate was subcultured onto tryptic soy agar (TSA; Difco) at 37 °C for 4 days. For most experiments including fatty acid analysis, strain KIT 00200-70066-1^T was cultured on TSA at 30 °C for 2 days. *Paenibacillus nanensis* KCTC 13044^T, *Paenibacillus agaridevorans* KCTC 3849^T and *Paenibacillus alkaliterrae* KCTC 3956^T, grown under identical conditions, were used as reference strains for fatty acid analysis and other phenotypic tests.

The Gram reaction was assessed as described by Gerhardt *et al.* (1994). Cell morphology and motility were observed under a light microscope (×1500 magnification; Nikon Optiphot-2) with cells grown for 1–7 days. Motility was tested using the hanging-drop technique (Skerman, 1967). For the observation of flagella and endospores, cells were negatively stained with 1% (w/v) uranyl acetate and examined using a model H-7600 transmission electron microscope (Hitachi); thin sections of cells were prepared as described by Paster & Canale-Parola (1982). Oxidase activity was tested using Bactident oxidase strips (Merck) and catalase activity was tested using 3% H₂O₂. Growth was investigated at 5, 10, 15, 20, 25, 30, 37, 42, 45 and 50 °C, in the presence of 1, 2, 3, 5, 6.5 and 10% NaCl and at pH 5–11 (in increments of 1 pH unit). Anaerobic growth was examined on TSA or on TSA supplemented with nitrate incubated in a jar with the GasPak Anaerobic System (BBL). Growth on MacConkey agar and cetrimide agar (Difco) was also investigated. Hydrolysis of casein, DNA, starch and tyrosine was tested on casein agar, DNase test agar, starch agar and tyrosine agar (BBL), respectively. Hydrolysis of Tween 80 was tested on Tween 80 agar (DSMZ medium no. 884). The production of lecithinase and lipase was detected on egg-yolk agar (McClung & Toabe, 1947). Haemolysis was studied on TSA supplemented with 5% (v/v) sheep blood. H₂S production was tested in tryptic soy broth (Difco) supplemented with 0.01% (w/v) cysteine, with a strip of lead acetate paper (Fluka) as an indicator. The methyl red and Voges–Proskauer tests were performed in MR-VP broth (BBL). Single carbon source assimilation tests were performed in mineral salts basal medium (Stanier *et al.*, 1966) supplemented with 1% (v/v) vitamin solution (Staley, 1968). Filter-sterilized substrate (0.1%, w/v) was added to this medium, with the exception of carbohydrates, which were used at 0.2% (w/v). The organic compounds tested as sole sources of carbon and energy were acetate, *N*-acetylglucosamine, *L*-alanine, amygdalin, *L*-arabinose, *L*-arabitol, cellobiose, citrate, *D*-fructose, *L*-fucose, *D*-galactose, gentiobiose, gluconate, *D*-glucose, glycerol, glycogen, *L*-histidine, inositol, *DL*-lactate, lactose, malate, malonate, maltose, *D*-mannitol, *D*-mannose, melezitose, melibiose, *L*-proline, propionate, raffinose, *L*-rhamnose, *D*-ribose, *D*-salicin, *L*-serine, *D*-sorbitol, starch, sucrose, trehalose, turanose and *D*-xylose. Acid production tests, enzyme activity tests and additional phenotypic tests were performed using API 20E, API 20NE,

API 50CH (with CHB/E medium) and API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Duplicate antibiotic-susceptibility tests were performed using Sensi-Discs (6 mm; BBL) containing the following: ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), neomycin (30 µg), penicillin G (10 IU), rifampicin (5 µg), streptomycin (10 µg), tetracycline (30 µg), ticarcillin/clavulanic acid (75 µg/10 µg) and vancomycin (30 µg); zone diameter interpretive standards for staphylococci of the NCCLS (2003) were used for interpretation.

Fatty acid methyl esters were prepared and analysed as described by Klatte *et al.* (1994) by using the standard Microbial Identification System (MIDI Inc.) for automated gas chromatographic analysis (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by using TLC on Kieselgel 60 F₂₅₄ plates (20 × 20 cm, 0.5 mm thickness; Merck) with petroleum ether/diethyl ether (9:1, v/v) as the solvent. The quinones were identified by using reversed-phase HPLC analysis, as described by Shin *et al.* (1996). The diamino acid of the peptidoglycan was determined by using TLC as described by Staneck & Roberts (1974). Polar lipids were extracted, examined by using two-dimensional TLC and identified according to published procedures (Minnikin *et al.*, 1977).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Rainey *et al.* (1996). The 16S rRNA gene sequence was aligned with published sequences retrieved from GenBank/EMBL/DDBJ using CLUSTAL_X (Thompson *et al.*, 1997) and edited via the BioEdit program (Hall, 1999). Phylogenetic trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods; distances were estimated by the method of Jukes & Cantor (1969) using MEGA version 4.0 (Tamura *et al.*, 2007). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. DNA G + C contents were determined by using HPLC, following hydrolysis, as described by Tamaoka & Komagata (1984), and non-methylated λ phage DNA (Sigma) was used as a standard.

Strain KIT 00200-70066-1^T formed visible colonies (about 1–2 mm in diameter) within 2 days on TSA incubated at 30 °C. Good growth occurred at 25–37 °C. The colonies were colourless to white, translucent and circular to slightly irregular and became mucoid after prolonged incubation. Cells were Gram-variable, facultatively anaerobic, motile rods with peritrichous flagella. Ellipsoidal subterminal spores were formed in swollen sporangia. Transmission electron micrographs of cells of strain KIT 00200-70066-1^T are available as Supplementary Fig. S1 in IJSEM Online.

An almost-complete 16S rRNA gene sequence (1457 nt) of strain KIT 00200-70066-1^T was determined and compared with those of all known species of the genus *Paenibacillus*.

Strain KIT 00200-70066-1^T showed the highest levels of sequence similarity with *P. nanensis* MX2-3^T, *P. agaridevorans* DSM 1355^T and *P. alkaliterrae* KSL-134^T, with values of 95.4, 95.2 and 94.8%, respectively. In the neighbour-joining tree (Fig. 1), strain KIT 00200-70066-1^T formed a separate lineage within the genus *Paenibacillus*, while it formed a clade with *P. nanensis* MX2-3^T and *P. agaridevorans* DSM 1355^T in the maximum-parsimony tree (Supplementary Fig. S2), which was poorly supported by bootstrap analysis. Consequently, strain KIT 00200-70066-1^T occupied quite a distinct position with respect to its phylogenetic neighbours within the genus *Paenibacillus*. An extended 16S rRNA gene sequence-based neighbour-joining tree is available as Supplementary Fig. S3.

The morphological, physiological, biochemical and chemotaxonomic characteristics of strain KIT 00200-70066-1^T are given in the species description; those that serve to differentiate it from related taxa are listed in Table 1. Strain KIT 00200-70066-1^T was distinguished from related taxa by differences in colony colour, Gram stain reaction, cell size, temperature range for growth, lipase activity on egg-yolk agar, hydrolysis of L-leucyl 2-naphthylamide and 2-naphthyl phosphate (pH 5.4) and susceptibility to kanamycin as well as by differences in acid production and substrate utilization profiles. Strain KIT 00200-70066-1^T showed a fatty acid profile similar to those of related taxa, i.e. the fatty acids anteiso-C_{15:0}, iso-C_{16:0} and C_{16:0} were predominant (Table 2), and contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and several unknown lipids in the polar lipid profile (Supplementary Fig. S4) and menaquinone MK-7 as the major isoprenoid quinone.

Strain KIT 00200-70066-1^T was isolated from the sputum of a patient suffering from pulmonary disease. However, its clinical significance could not be determined because no sign or symptom of serious infection developed in the patient. Whether it was an opportunistic pathogen or a

contaminant, strain KIT 00200-70066-1^T was worth studying in that, to our knowledge, it was the first *Paenibacillus* strain to be isolated from human sputum.

On the basis of the 16S rRNA gene dissimilarity with respect to related taxa, together with the phylogenetically distinct position and unique phenotypic characteristics, strain KIT 00200-70066-1^T merits recognition as a representative of a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus sputi* sp. nov. is proposed.

Description of *Paenibacillus sputi* sp. nov.

Paenibacillus sputi (spu'ti. L. gen. n. *sputi* of sputum).

Cells are Gram-variable, facultatively anaerobic rods (0.5–0.6 × 2.0–3.0 µm). Motile by means of peritrichous flagella. Ellipsoidal subterminal spores are formed in swollen sporangia. Forms colourless to white, translucent, mucoid colonies on TSA. Catalase- and oxidase-positive. Growth occurs at 15–42 °C (optimum 30–37 °C) and at pH 6–9 (optimum pH 7). Growth occurs in the presence of up to 3% NaCl, but not with 5% NaCl. Anaerobic growth is observed, regardless of the presence of nitrate. Indole is not produced. No growth is observed on MacConkey agar or cetrimide agar. H₂S is produced from L-cysteine, but not from thiosulfate. Nitrate is not reduced. The methyl red test is negative, but the Voges–Proskauer test is positive. On 5% sheep blood agar, α-haemolysis is observed. Aesculin, starch and Tween 80 are hydrolysed, but casein, DNA, gelatin and tyrosine are not. β-Galactosidase is produced, but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase and urease are not. On egg-yolk agar, lipase activity is present, but lecithinase activity is absent. L-Arabinose, cellobiose, D-galactose, D-glucose, glycogen, lactose, maltose, L-rhamnose, D-salicin, starch and D-xylose are utilized as sole sources of carbon and energy, but acetate, N-acetylglucosamine, L-alanine, amygdalin, L-arabitol, citrate, D-fructose,

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, constructed with the neighbour-joining method, showing the position of strain KIT 00200-70066-1^T among members of the genus *Paenibacillus*. Percentages at nodes are levels of bootstrap support (≥50%) based on 1000 resamplings. Bar, 0.01 substitutions per nucleotide position.

Table 1. Phenotypic characteristics that differentiate strain KIT 00200-70066-1^T from the type strains of related *Paenibacillus* species

Strains: 1, *Paenibacillus sputi* sp. nov. KIT 00200-70066-1^T; 2, *P. nanensis* KCTC 13044^T; 3, *P. agaridevorans* KCTC 3849^T; 4, *P. alkaliterrae* KCTC 3956^T. Data were obtained in this study unless indicated. All strains were positive for catalase and oxidase activity and showed α -haemolysis. +, Positive; –, negative; V, variable; NA, no data available.

Characteristic	1	2	3	4
Colony colour*	C/WH	WH	WH	I
Gram stain	V	+	V/–	+
Cell length (μ m)	2.0–3.0	3.0–8.0 ^{a†}	2.0–5.0 ^b	1.5–3.0 ^c
Cell width (μ m)	0.5–0.6	1.0–1.2 ^a	0.6–0.8 ^b	0.4–0.5 ^c
Flagellar arrangement‡	Pe	Pe ^a	NA	P ^c
Anaerobic growth	+	+	–	–
Temperature range for growth (°C)	15–42	20–45	10–42	10–37
Voges–Proskauer test	+	+	–	–
Nitrate reduction	–	+	–	+
Lipase on egg-yolk agar	+	–	–	–
Acid production from:				
<i>N</i> -Acetylglucosamine	+	–	–	–
D-Arabinose	+	–	–	+
L-Fucose	+	–	–	–
D-Ribose	+	–	–	–
Sucrose	–	+	+	+
Trehalose	–	+	+	+
Growth on:				
D-Fructose	–	+	–	+
Gentiobiose	–	+	+	–
Glycerol	–	+	+	–
D-Mannose	–	+	–	+
Melibiose	–	+	+	+
L-Rhamnose	+	–	–	–
Hydrolysis of:				
L-Leucyl 2-naphthylamide	+	–	–	–
2-Naphthyl phosphate (pH 5.4)	+	–	–	–
6-Bromo-2-naphthyl α -D-galactopyranoside	–	+	–	+
2-Naphthyl α -D-glucopyranoside	–	+	–	+
6-Bromo-2-naphthyl β -D-glucopyranoside	–	+	–	+
Susceptibility to:				
Chloramphenicol	+	–	–	+
Kanamycin	–	+	+	+
Streptomycin	+	–	–	+
DNA G + C content (mol%)	48.1	53.5	50.1	49.8

*C, Colourless; I, ivory; WH, white.

†Data taken from: *a*, Khianngam *et al.* (2009); *b*, Uetanabaro *et al.* (2003); *c*, Yoon *et al.* (2005).

‡P, Polar; Pe, peritrichous.

L-fucose, gentiobiose, gluconate, glycerol, L-histidine, *myo*-inositol, DL-lactate, malate, malonate, D-mannitol, D-mannose, melezitose, melibiose, L-proline, propionate, raffinose, D-ribose, L-serine, D-sorbitol, sucrose, trehalose and turanose are not. In the API 50CH gallery, acid is produced from *N*-acetylglucosamine, amygdalin, D- and L-arabinose, cellobiose, aesculin, L-fucose, D-galactose, D-glucose, glycogen, lactose, maltose, D-mannose, melibiose, methyl α -D-glucoside, methyl β -D-xylose, raffinose, L-

rhamnose, D-ribose, D-salicin, starch and D-xylose, but not from D-adonitol, D- or L-arabitol, arbutin, dulcitol, erythritol, D-fructose, D-fucose, gentiobiose, gluconate, glycerol, inositol, inulin, 2- or 5-ketogluconate, D-lyxose, D-mannitol, melezitose, methyl α -D-mannoside, D-sorbitol, L-sorbose, sucrose, D-tagatose, trehalose, turanose, xylitol or L-xylose. According to the results from API ZYM tests, L-leucyl 2-naphthylamide, 2-naphthyl butyrate, 2-naphthyl β -D-galactopyranoside and 2-naphthyl phosphate

Table 2. Cellular fatty acid compositions (%) of strain KIT 00200-70066-1^T and the type strains of related *Paenibacillus* species

Strains: 1, *P. sputi* sp. nov. KIT 00200-70066-1^T; 2, *P. nanensis* KCTC 13044^T; 3, *P. agaridevorans* KCTC 3849^T; 4, *P. alkaliterrae* KCTC 3956^T. Data were obtained in this study. Fatty acids are listed using standard abbreviations (number of carbon atoms : number of double bonds). ND, Not detected; tr, trace (<0.5 %).

Fatty acid	1	2	3	4
Saturated straight-chain				
C _{14:0}	0.9	0.7	1.0	1.6
C _{15:0}	8.5	2.1	4.4	1.4
C _{16:0}	15.7	13.0	9.3	16.8
C _{17:0}	1.9	0.6	0.7	tr
C _{18:0}	ND	tr	ND	0.5
Saturated branched				
iso-C _{14:0}	3.7	1.9	6.0	3.9
iso-C _{15:0}	6.4	5.6	7.3	7.8
iso-C _{16:0}	16.5	17.9	19.8	15.2
iso-C _{17:0}	3.7	5.5	3.4	3.4
iso-C _{18:0}	tr	tr	ND	ND
anteiso-C _{15:0}	37.7	42.8	43.4	43.4
anteiso-C _{17:0}	4.1	9.2	4.2	5.5
Unsaturated				
C _{16:1} ω11c	0.8	tr	tr	ND

(pH 5.4) are hydrolysed, but *N*-benzoyl-DL-arginine 2-naphthylamide, 6-bromo-2-naphthyl α-D-galactopyranoside, 6-bromo-2-naphthyl β-D-glucopyranoside, 6-bromo-2-naphthyl α-D-mannopyranoside, L-cystyl 2-naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI-β-D-glucuronide, naphthol-AS-BI-phosphate, 1-naphthyl *N*-acetyl-β-D-glucosaminide, 2-naphthyl caprylate, 2-naphthyl α-L-fucopyranoside, 2-naphthyl α-D-glucopyranoside, 2-naphthyl myristate, 2-naphthyl phosphate (pH 8.5) and L-valyl 2-naphthylamide are not. Susceptible to ampicillin, chloramphenicol, erythromycin, neomycin, penicillin G, rifampicin, streptomycin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin. Resistant to kanamycin. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The major isoprenoid quinone is MK-7. The fatty acids anteiso-C_{15:0}, iso-C_{16:0} and C_{16:0} are predominant. The major cellular phospholipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The G + C content of the DNA of the type strain is 48.1 mol%.

The type strain is KIT 00200-70066-1^T (=KCTC 13252^T =DSM 22699^T), isolated from the sputum of a patient.

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References

- Antonello, A. & Weinstein, G. W. (1989). Successful treatment of *Bacillus alvei* endophthalmitis. *Am J Ophthalmol* **108**, 454–455.
- Ash, C., Priest, F. G. & Collins, D. C. (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.
- Barrero, F., Galán, F., Marín, P., García-Martos, P. & Capote, F. J. (1996). Catheter associated infection by *Bacillus macerans* in a patient with acute leukemia. *Enferm Infecc Microbiol Clin* **14**, 628–629.
- Bert, F., Ouahes, O. & Lambert-Zechovsky, N. (1995). Brain abscess due to *Bacillus macerans* following a penetrating periorbital injury. *J Clin Microbiol* **33**, 1950–1953.
- Bosshard, P. P., Zbinden, R. & Altwegg, M. (2002). *Paenibacillus turicensis* sp. nov., a novel bacterium harbouring heterogeneities between 16S rRNA genes. *Int J Syst Evol Microbiol* **52**, 2241–2249.
- Daane, L. L., Harjono, I., Barns, S. M., Launen, L. A., Palleroni, N. J. & Häggblom, M. M. (2002). PAH-degradation by *Paenibacillus* spp. and description of *Paenibacillus naphthalenovorans* sp. nov., a naphthalene-degrading bacterium from the rhizosphere of salt marsh plants. *Int J Syst Evol Microbiol* **52**, 131–139.
- Euzéby, J. P. (2009). *List of Prokaryotic Names with Standing in Nomenclature*. <http://www.bacterio.cict.fr/>
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Khianngam, S., Akaracharanya, A., Tanasupawat, S., Lee, K. C. & Lee, J.-S. (2009). *Paenibacillus thailandensis* sp. nov. and *Paenibacillus nanensis* sp. nov., xylanase-producing bacteria isolated from soil. *Int J Syst Evol Microbiol* **59**, 564–568.
- Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiensis* comb. nov. and *Gordona amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.
- Ko, K. S., Kim, Y.-S., Lee, M. Y., Shin, S. Y., Jung, D. S., Peck, K. R. & Song, J.-H. (2008). *Paenibacillus konsidensis* sp. nov., isolated from a patient. *Int J Syst Evol Microbiol* **58**, 2164–2168.
- McClung, L. S. & Toabe, R. (1947). The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. *J Bacteriol* **53**, 139–147.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- NCCLS (2003). *Performance standards for antimicrobial disk susceptibility tests*, 8th edn. Approved Standard, M2-A8. Wayne, PA: National Committee for Clinical Laboratory Standards.

- Paster, B. J. & Canale-Parola, E. (1982).** Physiological diversity of rumen spirochetes. *Appl Environ Microbiol* **43**, 686–693.
- 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 *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Reboli, A. C., Bryan, C. S. & Farrar, W. E. (1989).** Bacteremia and infection of a hip prosthesis caused by *Bacillus alvei*. *J Clin Microbiol* **27**, 1395–1396.
- 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.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shin, Y. K., Lee, J.-S., Chun, C. O., Kim, H.-J. & Park, Y.-H. (1996).** Isoprenoid quinone profiles of *Leclercia adecarboxylata* KCTC 1036^T. *J Microbiol Biotechnol* **6**, 68–69.
- Skerman, V. B. D. (1967).** *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Staley, J. T. (1968).** *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.
- Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Appl Microbiol* **28**, 226–231.
- Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966).** The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- 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.
- Yoon, J.-H., Kang, S.-J., Yeo, S.-H. & Oh, T.-K. (2005).** *Paenibacillus alkaliterrae* sp. nov., isolated from an alkaline soil in Korea. *Int J Syst Evol Microbiol* **55**, 2339–2344.