Paenibacillus provencensis sp. nov., isolated from human cerebrospinal fluid, and *Paenibacillus urinalis* sp. nov., isolated from human urine

Véronique Roux,¹ Lukas Fenner^{1,2} and Didier Raoult¹

Correspondence

Véronique Roux
vroux91@hotmail.com

¹Laboratoire de Bactériologie–Virologie, Hôpital de la Timone, CNRS UMR 6020, IFR48, 264 rue Saint-Pierre, 13385 Marseille Cedex 05, France

²Microbiology Laboratory, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland

Gram-negative, spore-forming rods were isolated from a human urine sample (strain 5402403^T) and a human cerebrospinal fluid sample (strain 4401170^T). Based on genotypic characteristics, these strains belonged to the genus *Paenibacillus* and were closely related. Phylogenetic analysis based on 16S rRNA gene sequence comparison showed that they clustered with *Paenibacillus massiliensis* 2301065^T (95.9 and 94.3% 16S rRNA gene sequence similarity, respectively, for strains 5402403^T and 4401170^T), *Paenibacillus illinoisensis* NRRL NRS-61356^T (90.6 and 93.8%), *Paenibacillus xylanilyticus* XIL14^T (95.3 and 95.4%), *Paenibacillus barcinonensis* BP-23^T (94.3 and 94.0%), *Paenibacillus pabuli* NCIMB 12781^T (89.1 and 92.3%) and *Paenibacillus amylolyticus* NRRL NRS-290^T (94.2 and 93.8%). The predominant fatty acids were 15:0 anteiso (49.0 and 55.3%, respectively, for strains 5402403^T and 4401170^T), 16:0 iso (15.4 and 13.5%), 16:0 (7.6 and 3.6%), 15:0 (6.3 and 2.8%), 17:0 anteiso (5.7 and 7.5%), 14:0 iso (4.1 and 2.7%) and 15:0 iso (4.1 and 3.4%). 16S rRNA gene sequence similarity between strain 4401170^T and strain 5402403^T was 98.4%, but the DNA–DNA reassociation rate between the two strains was 53.2%. So, considering the recommendations of the ad hoc committee, they do not belong to the same species. On the basis of phenotypic data and genotypic inference, it is proposed that the strains should be assigned to the novel species *Paenibacillus urinalis* sp. nov. (type strain 5402403^T = CIP 109357^T = CCUG 53521^T) and *Paenibacillus provencensis* sp. nov. (type strain 4401170^T = CIP 109358^T = CCUG 53519^T).

Based on phylogenetic studies using the 16S rRNA gene, the so-called rRNA group 3 bacilli were separated to form the novel genus *Paenibacillus* (Ash *et al.*, 1993). Members of the genus *Paenibacillus* are common saprophytic components of soil, water, plants, food, faeces and diseased insect larvae (Daane *et al.*, 2002), but have rarely been considered to be pathogenic for man. Some species have been isolated from human blood samples, but were considered to be contaminants (Noskin *et al.*, 2001; Roux & Raoult, 2004; Teng *et al.*, 2003). A case of bacteraemia due to *Paenibacillus polymyxa* in a patient with cerebral infarction was described (Nasu *et al.*, 2003). *Paenibacillus turicensis* was isolated from the valve of a cerebrospinal

fluid shunt (Bosshard *et al.*, 2002). Karlovsky & Pontari (2002) described *Paenibacillus* species from the genitourinary fluids of men with type III prostatitis.

In this report, we describe two novel species belonging to the genus *Paenibacillus* that were isolated from human urine and cerebrospinal fluid (CSF) and which are closely related to *Paenibacillus massiliensis*, *P. illinoisensis*, *P. xylanilyticus*, *P. barcinonensis*, *P. pabuli* and *P. amylolyticus* (Rivas *et al.*, 2005; Roux & Raoult, 2004; Sánchez *et al.*, 2005; Shida *et al.*, 1997a, b).

Isolation and characterization of strains

A 36-year-old woman, a former intravenous drug user, was admitted to hospital in June 2005 with a suspected tuberculosis infection. She presented emaciation, chronic cough, dysphagia, sweats, inflammatory syndrome and pulmonary nodules. A urine sample was cultivated on a sheep-blood agar plate and incubated for 2 months. The presence of a Gram-negative-staining bacillus was noted, but *Mycobacterium* isolation was negative. The isolate,

Abbreviation: CSF, cerebrospinal fluid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Paenibacillus urinalis* 5402403^T and *Paenibacillus provencensis* 4401170^T are EF212892 and EF212893, respectively.

Antimicrobial susceptibility profiles of the novel strains and an extended phylogenetic tree are available as supplementary material with the online version of this paper.

strain 5402403^T, was identified as a representative of the genus *Paenibacillus* based on 16S rRNA gene sequence comparison, and was considered to be a contaminant.

A 54-year-old man with cerebellar syndrome and diarrhoea episodes was diagnosed with Whipple's disease in January 2004 by PCR amplification of *Tropheryma whippelii* DNA from CSF. The patient was treated with ceftriaxone in association with trimethoprim-sulfamethoxazole. After 4 months of antibiotic therapy, a sample of CSF was taken and culture was performed. The presence of a Gram-negative-staining bacillus was noted. The isolate, strain 4401170^T, could not be identified using a biochemical approach, but was identified as a representative of the genus *Paenibacillus* based on 16S rRNA gene sequence comparison and was considered to be a contaminant.

Antimicrobial susceptibility was determined according to the CLSI (formerly National Committee for Clinical Laboratory Standards; NCCLS) criteria and the results are presented in Supplementary Table S1 (available in IJSEM Online).

Surface colonies on sheep-blood agar (bioMérieux) were circular, greyish, smooth and shiny, 5 mm in diameter after 24 h. Growth and haemolytic activity were tested at 37 °C on Columbia agar with 5 % sheep blood. Growth was tested in anaerobic and microaerobic atmospheres, which were created using GENbag anaer and GENbag microaer (bioMérieux), respectively. Growth was also tested in the presence of air or 5 % CO₂ and at different temperatures (25, 30, 37, 44 and 50 °C). Optimum growth was obtained at 30–37 °C, but growth occurred between 25 and 44 °C. Strain 5402403^T grew under aerobic and anaerobic conditions, but strain 4401170^T did not grow in an anaerobic atmosphere. The bacteria were non-haemolytic. The size and ultrastructure of cells were determined by transmission electron microscopy. Cells were grown on agar with 5 % sheep blood for 24 h. Bacterial suspension was pre-fixed in 5 % (v/v) glutaraldehyde in phosphate buffer (Gibco) for at least 1 h at room temperature, washed in the same buffer and stained with 1 % (w/v) phosphotungstic acid. The samples were examined on a Morgagni 268D (Philips) electron microscope at an operating voltage of 60 kV. The rods were 1.9–4.2 µm long and 0.75–1.1 µm wide for strain 5402403^T and 1.8–4.6 µm long and 0.75–1.2 µm wide for strain 4401170^T.

Catalase activity determined by the ID colour catalase test kit (bioMérieux) was positive. Oxidase activity was assayed by applying the cells to moistened discs that were impregnated with dimethyl-*p*-phenylenediamine (bioMérieux) and was negative.

The commercially available API ZYM, API 50 CH strips combined with API 50CHB/E and API 20NE were used to characterize the biochemical properties of the strains according to the manufacturer's instructions (bioMérieux) and incubation was performed at 30 °C. Tolerance to NaCl was measured in trypticase soy broth that contained 1–5 %

(w/v) NaCl. Phenotypic characteristics were compared with those of *P. massiliensis* CIP 107939^T, *P. illinoisensis* CIP 105253^T, *P. xylanilyticus* CIP 109086^T and *P. pabuli* CIP 103119^T, which were the closest related species when 16S rRNA gene sequences were compared. Characteristic traits are presented in Table 1. Acid production for strain 4401170^T was weak because it did not grow in an anaerobic atmosphere. Results of other tests for the type strain are given in the species description.

Preparation and identification of cellular fatty acids were carried out by following the procedures given for the Sherlock Microbial Identification System (MIDI). The fatty acid composition was significantly different from those of other members of the genus *Paenibacillus*. The predominant cellular fatty acids found are shown in Table 2 and compared with values that are available for closely related *Paenibacillus* species.

Genotypic characterization

Bacterial DNA was extracted using the MagNA Pure LC DNA isolation kit III (Roche) with the MagNA Pure LC instrument as described by the manufacturer. PCR amplification of the 16S rRNA gene was performed using the universal primer pair fd1 and rp2 (Weisburg *et al.*, 1991). PCR products were purified using MultiScreen PCR (Millipore) and sequencing reactions were carried out using a DNA sequencing kit (BigDye Terminator Cycle Sequencing v1.1 Ready Reactions; Applied Biosystems) according to the manufacturer's instructions. Sequencing products were purified and electrophoresis was performed with the 3100 Genetic Analyzer (Applied Biosystems). The sequences obtained were compared with sequences deposited in the GenBank database by using the BLAST program through the NCBI server. Gene sequences were aligned using the multiple alignment program CLUSTAL_X version 1.8 (Thompson *et al.*, 1997). Phylogenetic relationships with closely related species were determined by using MEGA version 2.1 (Kumar *et al.*, 2001). Distance matrices were determined following the assumptions described by Kimura (1980) and were used to elaborate a dendrogram using the neighbour-joining method (Saitou & Nei, 1987). The maximum-parsimony algorithm was also used to infer phylogenetic analysis. A bootstrap analysis (bootstrap values were obtained for a consensus tree based on 100 randomly generated trees) was performed to investigate the stability of the trees obtained. The clustering of the new isolates was the same with the two methods. The phylogenetic analysis demonstrated that our isolates are members of the genus *Paenibacillus*. They clustered with the type strains of *P. massiliensis* (Fig. 1 and Supplementary Fig. S1), a species described recently in our laboratory, and five other species, *P. illinoisensis*, *P. xylanilyticus*, *P. barcinonensis*, *P. pabuli* and *P. amylolyticus*. Percentage similarities between 16S rRNA gene sequences were determined by using NALIGN in the PC/GENE software package (IntelliGenetics). Similarities between strains

Table 1. Diagnostic traits of the type strains tested in this study

Strains: 1, strain 4401170^T (*P. provencensis* sp. nov.); 2, strain 5402403^T (*P. urinalis* sp. nov.); 3, *P. massiliensis* CIP 107939^T; 4, *P. illinoisensis* CIP 105253^T; 5, *P. xylanilyticus* CIP 109086^T; 6, *P. pabuli* CIP 103119^T. w, Weakly positive. Test results were recorded after 24 h unless indicated.

Test	1	2	3	4	5	6
Acid production from:						
Glycerol	—	—	+	+	+	+
L-Arabinose	w (48 h)	+	+	+	+	+
D-Ribose	w (48 h)	+	+	+	+	+
D-Xylose	w (48 h)	+	—	+	+	+
Methyl β -D-xylopyranoside	w (48 h)	+	—	+	+ (48 h)	+
D-Galactose	+	+	+	+	+	+
D-Fructose	+	—	+	+	+	+
D-Mannose	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+
Methyl α -D-mannopyranoside	—	—	+	—	—	—
Methyl α -D-glucopyranoside	w (48 h)	w (48 h)	—	+ (48 h)	+ (48 h)	+
N-Acetylglucosamine	+	+	+ (48 h)	+	+	+
Amygdalin	+	+	+	+	+	+
Arbutin	w (48 h)	+	+	+	+	+
Sucrose	w (48 h)	+	+	+	+	+
Trehalose	—	—	+	+	+	+
Inulin	—	—	+	+	+	+
Melezitose	—	—	—	—	—	+
Glycogen	+	—	+	+	+	+
Gentiobiose	w (48 h)	w (48 h)	+	+	+	+
Turanose	—	—	—	+ (48 h)	+ (48 h)	+
Potassium gluconate	w (48 h)	w (48 h)	—	—	—	—
Enzyme activities (after 4 h)						
Esterase (C4)	—	—	—	+	—	—
Esterase lipase (C8)	—	—	—	+	+	+
Leucine arylamidase	—	—	—	+	+	—
α -Chymotrypsin	—	—	—	+	—	—
α -Galactosidase	—	—	—	+	+	+
β -Galactosidase	+	+	—	+	+	+
α -Glucosidase	—	—	—	+	+	+
β -Glucosidase	—	+	+	+	+	+
Reduction of nitrates to nitrites	—	—	+	—	+	+
Assimilation of:						
D-Glucose	—	+	+	+	+	+
L-Arabinose	—	+	+	+	+	+
D-Mannose	—	—	+	—	—	—
D-Mannitol	—	+	+	+	+	+
N-Acetylglucosamine	—	+	—	+	+	+
Maltose	—	+	+	+	+	+
Potassium gluconate	—	+	—	+	+	+
Capric acid	—	—	—	—	—	—
Adipic acid	—	—	—	—	—	—
Malic acid	—	—	—	—	—	—
Trisodium citrate	—	—	—	—	—	—
Phenylacetic acid	—	—	—	—	—	—

5402403^T and 4401170^T and the type strains of *P. massiliensis*, *P. illinoisensis*, *P. xylanilyticus*, *P. barcinonensis*, *P. pabuli* and *P. amylolyticus* were 95.9 and 94.3 %, 90.6 and 93.8 %, 95.3 and 95.4 %, 94.3 and 94.0 %, 89.1 and

92.3 % and 94.2 and 93.8 %, respectively. All these values are below 97 % (which is the suggested threshold for determining the species level), indicating that strains 4401170^T and 5402403^T belong to novel species.

Fig. 1. Phylogenetic tree of representatives of the genus *Paenibacillus* inferred from 16S rRNA gene sequence comparison (1320 nt fragment). Numbers at nodes are proportions of 100 resamplings that support the topology shown. *Listeria monocytogenes* NCTC 10357^T (GenBank accession no. X65153) was used as the outgroup (not shown). Bar, 0.02 nucleotide changes per nucleotide position. An extended tree containing a wider selection of reference sequences is available in Supplementary Fig. S1 in IJSEM Online.

To perform DNA–DNA hybridization, DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). DNA–DNA reassociation between strains 5402403^T and 4401170^T was 53.2 %. So, according to the recommendations of the ad hoc committee, strains 5402403^T and 4401170^T did not belong to the same species, as the value found for DNA–DNA relatedness was lower than the threshold value of 70 % (Wayne *et al.*, 1987).

Based on results described above, we propose the names *Paenibacillus urinalis* sp. nov., with strain 5402403^T as the type strain, and *Paenibacillus provencensis* sp. nov., with strain 4401170^T as the type strain.

Description of *Paenibacillus urinalis* sp. nov.

Paenibacillus urinalis (u.ri.na'lis. L. masc. adj. *urinalis* pertaining to urine, urinary).

Cells are facultatively anaerobic, Gram-negative straight rods, forming endospores in swollen sporangia. Motile by means of peritrichous flagella. Catalase-positive but

oxidase-negative. Optimal growth occurs at 30–37 °C; growth occurs in the presence of 5 % (w/v) NaCl. After 24 h growth on sheep-blood agar, surface colonies are circular, greyish, smooth, shiny and up to 5 mm in diameter. Rods are 1.8–4.6 µm long and 0.75–1.2 µm in diameter (estimated by electronic microscopy). Voges–Proskauer test (API 20E) is negative. Using API ZYM, activities of β-galactosidase and β-glucosidase are detected; activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not detected. Using API 50CH strips after an incubation time of 24 h, acid is produced from L-arabinose, D-ribose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-mannose, D-mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, D-lactose, melibiose, sucrose, raffinose and starch. After an incubation time of 48 h, acid is produced weakly from methyl α-D-glucopyranoside, gentiobiose and potassium gluconate. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, trehalose, inulin, melezitose, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium 2-ketogluconate or potassium 5-ketogluconate. Using the API 20NE strip, reduction of nitrates to nitrites, indole production, arginine dihydrolase, urease and hydrolysis of gelatin are negative. Hydrolysis of aesculin is positive. Assimilation of D-glucose, L-arabinose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate is positive. Assimilation of D-mannose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid is negative. The fatty acid profile is characterized by the predominance of 15:0 anteiso (49.0 %), 16:0 iso (15.4 %), 16:0 (7.6 %), 15:0 (6.3 %), 17:0 anteiso (5.7 %), 14:0 iso (4.1 %) and 15:0 iso (4.1 %).

The type strain is strain 5402403^T (=CIP 109357^T =CCUG 53521^T), which was isolated from a human urine sample.

Description of *Paenibacillus provencensis* sp. nov.

Paenibacillus provencensis (pro.ven.cen'cis. N.L. masc. adj. *provencensis* pertaining to Provence, the region of France where the type strain was isolated).

Cells are Gram-negative straight rods that form endospores in swollen sporangia. Grows in an aerobic atmosphere but not under anaerobic conditions. Motile by means of peritrichous flagella. Catalase-positive but oxidase-negative. Optimal growth occurs at 30–37 °C; growth occurs in the presence of 5 % (w/v) NaCl. After 24 h growth on sheep-blood agar, surface colonies are circular, greyish, smooth, shiny and up to 5 mm in diameter. Rods are 1.9–4.2 µm long and 0.8–1.1 µm in diameter (estimated by

Table 2. Whole-cell fatty acid compositions (%) of the novel *Paenibacillus* isolates and related type strains

Strains: 1, *P. urinalis* 5402403^T; 2, *P. provencensis* 4401170^T; 3, *P. massiliensis* CIP 107939^T; 4, *P. illinoisensis* CIP 105253^T (data from Shida *et al.*, 1997b); 5, *P. xylanilyticus* CIP 109086^T (Rivas *et al.*, 2005); 6, *P. pabuli* CIP 103119^T (Shida *et al.*, 1997a); 7, *P. amylolyticus* NRRL NRS-290^T (Shida *et al.*, 1997b). Data were obtained in this study unless indicated. ND, Not detected/reported.

Fatty acid	1	2	3	4	5	6	7
14:0 iso	4.1	2.7	8.0	2.0	2.3	0.7	2.3
15:0 iso	4.1	3.4	5.4	1.5	7.1	2.2	2.0
15:0 anteiso	49.0	55.3	42.8	56.6	47.9	73.7	46.2
15:0	6.3	2.8	1.6	1.0	ND	0.1	0.5
16:0 iso	15.4	13.5	13.6	6.3	4.7	4.8	9.0
16:1 ω 11c	1.3	3.6	0.3	ND	8.1	ND	0.1
16:0	7.6	3.6	14.1	24.7	16.6	10.1	12.8
17:0 iso	2.9	2.4	1.0	1.4	4.5	1.3	3.5
17:0 anteiso	5.7	7.5	2.9	4.9	4.9	4.1	2.2

electronic microscopy). Voges–Proskauer test (API 20E) is negative. Using API ZYM, β -galactosidase activity is detected; activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase tests are not detected. Using API 50CH strips after an incubation time of 24 h, acid is produced from D-glucose, D-fructose, aesculin ferric citrate, salicin, cellobiose, maltose, D-lactose, melibiose, raffinose, starch and glycogen. After an incubation time of 48 h, acid is produced from D-galactose, D-mannose, D-mannitol, N-acetylglucosamine and amygdalin. Acid is produced weakly from L-arabinose, D-ribose, D-xylose, methyl β -D-xylopyranoside, methyl α -D-glucopyranoside, arbutin, sucrose, gentiobiose and potassium gluconate. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α -D-mannopyranoside, trehalose, inulin, melezitose, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium 2-ketogluconate or potassium 5-ketogluconate. Using the API 20NE strip, reduction of nitrates to nitrites, indole production, arginine dihydrolase, urease and hydrolysis of gelatin are negative. Hydrolysis of aesculin is positive. Tests for assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are negative. The fatty acid profile is characterized by the predominance of 15:0 anteiso (55.3%), 16:0 iso (13.5%), 16:0 (3.6%), 15:0 (2.8%), 17:0 anteiso (7.5%), 14:0 iso (2.7%) and 15:0 iso (3.4%).

The type strain is strain 4401170^T (=CIP 109358^T =CCUG 53519^T), which was isolated from human cerebrospinal fluid.

Acknowledgements

We are grateful to Bernard Campagna and Nicolas Aldrovandi for their technical assistance in electronic microscopy and to Kent Molin

(University of Göteborg, Sweden) for his technical assistance in cellular fatty acid composition analysis.

References

- 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. Proposals for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.
- 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.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- 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.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- HuB, 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.
- Karlovsky, M. E. & Pontari, M. A. (2002). Theories of prostatitis etiology. *Curr Urol Rep* **3**, 307–312.
- 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.
- Kumar, S., Tamura, K., Jakobsen, I.-B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Nasu, Y., Nosaka, Y., Otsuka, Y., Tsuruga, T., Nakajima, M., Watanabe, Y. & Jin, M. (2003). A case of *Paenibacillus polymyxa* bacteraemia in a patient with cerebral infarction. *Kansenshogaku Zasshi* **77**, 844–848 (in Japanese).
- Noskin, G. A., Suriano, T., Collins, S., Sesler, S. & Peterson, L. R. (2001). *Paenibacillus macerans* pseudobacteremia resulting from contaminated blood culture bottles in a neonatal intensive care unit. *Am J Infect Control* **29**, 126–129.

- Rivas, R., Mateos, P. F., Martinez-Molina, E. & Velazquez, E. (2005). *Paenibacillus xylanilyticus* sp. nov., an airborne xylanolytic bacterium. *Int J Syst Evol Microbiol* **55**, 405–408.
- 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.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sánchez, M. M., Fritze, D., Blanco, A., Spröer, C., Tindall, B. J., Schumann, P., Kroppenstedt, R. M., Diaz, P. & Pastor, F. I. J. (2005). *Paenibacillus barcinonensis* sp. nov., a xylanase-producing bacterium isolated from a rice field in the Ebro River delta. *Int J Syst Evol Microbiol* **55**, 935–939.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997a). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997b). Emended description of *Paenibacillus amylolyticus* and description of *Paenibacillus illinoisensis* sp. nov. and *Paenibacillus chibensis* sp. nov. *Int J Syst Bacteriol* **47**, 299–306.
- Teng, J. L. L., Woo, P. C. Y., Leung, K. W., Lau, S. K. P., Wong, M. K. M. & Yuen, K. Y. (2003). Pseudobacteraemia in a patient with neutropenic fever caused by a novel *paenibacillus* species: *Paenibacillus hongkongensis* sp. nov. *Mol Pathol* **56**, 29–35.
- 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.
- 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). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.