Paenibacillus assamensis sp. nov., a novel bacterium isolated from a warm spring in Assam, India

P. Saha,^{1,2} A. K. Mondal,² S. Mayilraj,^{1,2} S. Krishnamurthi,^{1,2}
A. Bhattacharya^{1,2} and T. Chakrabarti^{1,2}

Correspondence

T. Chakrabarti

tapan@imtech.res.in

Microbial Type Culture Collection and Gene Bank (MTCC)¹ and Institute of Microbial Technology², Sector 39A, Chandigarh 160 036, India

A polyphasic approach was used to characterize a bacterium, GPTSA 11^T, isolated from a warm spring located in a reserve forest in Assam, India. The cells are Gram-variable, strictly aerobic, sporulating motile rods. The major fatty acids of the strain are C_{15:0} anteiso (48.42%), C_{16:0} iso (11.59%), C_{16:1ω11c} (6.16%), C_{15:0} iso (6.03%), C_{17:0} anteiso (5.68%) and C_{16:1ω7c} alcohol (5.01%). The presence of the fatty acid C_{16:1ω7c} alcohol distinguishes this strain from other closely related species of the genus *Paenibacillus*. The strain contains MK-7 as the diagnostic menaquinone. The G+C content of the genomic DNA is 41.2 mol%. Analysis of the 16S rRNA gene sequence (1466 nt) revealed the presence of signature sequences PAEN 515F (5'-GAGTAACTGCTCTCGGAATGACGGTACTTGAGAAGAAAGCCCC-3') and PAEN 862F (5'-TCGATACCCCTTGGTGCCGAAGT-3'), which were found in the species of the genus *Paenibacillus* surveyed by Shida *et al.* [Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). *Int J Syst Bacteriol* **47**, 289–298]. The sequence shows closest similarity (95.85%) to that of *Paenibacillus apiarius*, followed by *Paenibacillus alvei* (94.34%), *Paenibacillus cineris* (93.87%), *Paenibacillus favisporus* (93.80%), *Paenibacillus chibensis* (93.47%) and *Paenibacillus azoreducens* (93.40%). Biochemical, physiological, chemotaxonomic and phylogenetic analyses justify placement of the strain in the genus *Paenibacillus* but not within any existing species. It should, therefore, be considered as representing a novel species, for which the name *Paenibacillus assamensis* sp. nov. is proposed. The type strain is GPTSA 11^T (=MTCC 6934^T=JCM 13186^T).

The genus *Bacillus* was created by Cohn in 1872 and accommodates Gram-positive, aerobic or facultatively anaerobic, endospore-forming rods (Claus & Berkeley, 1986). On the basis of 16S rRNA-based molecular analysis it became apparent that the genus is very heterogeneous, consisting of different phylogenetic groups (Ash *et al.*, 1991). Later, Ash *et al.* (1993) moved the members of *Bacillus* group 3 to the genus *Paenibacillus*. Thereafter, its description was emended by Shida *et al.* (1997). At present, more than 60 species (<http://www.bacterio.cict.fr/p/paenibacillus.html>) are recognized as members of the genus *Paenibacillus*. Members of this genus produce ellipsoidal endospores in swollen sporangia, possess C_{15:0} anteiso as the major cellular fatty acid and have genomic DNA G+C contents in the range 39–54 mol% (Shida *et al.*, 1997; Montes *et al.*, 2004; Takeda *et al.*, 2005). Species belonging

to the genus *Paenibacillus* have been isolated from various ecological niches such as soil, rhizospheres, water, diseased insect larvae, food (Daane *et al.*, 2002), cow faeces (Velázquez *et al.*, 2004), blood cultures (Roux & Raoult, 2004), Antarctic sediment (Montes *et al.*, 2004) and recently from the phyllosphere of *Phoenix dactylifera* (Rivas *et al.*, 2005). Examination of such habitats has resulted in the discovery of 23 novel species in the last 3 years (2002–2004).

In this paper, we report the taxonomic characterization of a novel aerobic, mesophilic, Gram-variable, endospore-forming bacterial strain, GPTSA 11^T, which produces spreading colonies. Strain GPTSA 11^T was isolated from a warm-spring water sample by dilution-plating on tryptic soy broth agar (TSBA; tryptic soy broth, 3%; agar, 1.5%). The warm spring is located in a reserve forest inhabited by wild elephants and other animals and is rarely disturbed by human activities. As elephant dung was found in and around the spring, it can be assumed that this is a water hole used by animals living in the forest. The temperature of the spring was 38 °C and the pH was 7.2. The colony

Published online ahead of print on 16 September 2005 as DOI 10.1099/ijs.0.63846-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Paenibacillus assamensis* GPTSA 11^T is AY884046.

morphology, cell morphology, motility and Gram reaction of the strain were determined by using standard methods (Smibert & Krieg, 1994; Murray *et al.*, 1994; Powers, 1995). Physiological characteristics were examined by growing the isolate on basal TSBA medium at different temperatures, pH values and NaCl concentrations. After 36 h growth at 30 °C on TSBA, colonies were round and yellowish white in colour. Within 48 h, the entire plate was occupied by single colonies apparently produced as a result of 'jumping' from the primary inoculum. The isolate was able to grow at temperatures between 20 and 37 °C, at pH 6.8–12.0 and was able to tolerate NaCl concentrations of up to 2.5 %. Various biochemical tests were done at 30 °C using standard methods (Claus & Berkeley, 1986; Smibert & Krieg, 1994). Degradation of chitin and xylan was checked on nutrient agar (HiMedia) medium supplemented with 0.4 % (w/v) each compound. Acid production from various carbohydrates were checked on basal medium (beef extract, 0.3 %; tryptose, 1 %; NaCl, 0.5 %; phenol red as acid–base indicator) supplemented with 0.5 % carbohydrate. The biochemical and physiological characteristics of the strain are given in the species description.

For cellular fatty acid analysis, the strain was grown on TSBA medium at 30 °C for 24 h. Extraction and analysis of cellular fatty acids were done using the Sherlock Microbial Identification System (MIDI), as described previously (Pandey *et al.*, 2002). The major fatty acid was found to be C_{15:0} anteiso, followed by C_{16:0} iso, C_{16:1}ω11c, C_{15:0} iso, C_{17:0} anteiso and C_{16:1}ω7c alcohol (Table 1). The diagnostic cell-wall amino acid was found to be *meso*-diaminopimelic acid, as determined by TLC (Staneck & Roberts, 1974). Menaquinones were extracted from 200 mg dry cell mass with a 10 % aqueous solution of 0.3 % (w/v) NaCl in methanol and petroleum ether (60–80 °C boiling point) at a ratio of 1 : 1. The upper phase was collected and dried in a Turbo Vap LV evaporator (Zymark). The residue was dissolved in 100 µl acetone. The extract was developed on a TLC plate (20 × 20 cm Silica gel 60 F₂₅₄; Merck) using petroleum ether (boiling point 60–80 °C) and diethyl ether (85 : 15, v/v). Purified menaquinones were dissolved in 2-propanol and analysed by reverse-phase TLC according to Collins & Jones (1980). The diagnostic menaquinone of strain GPTSA 11^T was found to be MK-7. The genomic DNA of GPTSA 11^T was isolated by using Marmur's protocol (Johnson, 1994). The G + C content of the genomic DNA was determined spectrophotometrically (Lambda 35 spectrophotometer; Perkin Elmer), using the thermal denaturation method (Mandel & Marmur, 1968), and was found to be 41.2 mol%. A fragment comprising the full-length 16S rRNA gene from strain GPTSA 11^T was amplified by a PCR using primers 8-27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1500r (5'-AGAAAGGAGGTGATCCAGGC-3') (*Escherichia coli* numbering). The amplification reaction and the purification of amplicons were performed as described previously (Pandey *et al.*, 2002). The purified PCR product was sequenced by using the dideoxy chain terminator method with the Big Dye Terminator kit

Table 1. Cellular fatty acid composition of strain GPTSA 11^T and phylogenetically related *Paenibacillus* species

Values are percentages of total fatty acids. Species/strains: 1, GPTSA 11^T; 2, *P. apiarius* MTCC 1497^T (data from this study); 3, *P. alvei* (data from Shida *et al.*, 1997); 4, *P. cineris* (Logan *et al.*, 2004); 5, *P. favisporus* (Velázquez *et al.*, 2004); 6, *P. thiaminolyticus* (Shida *et al.*, 1997); 7, *P. popilliae* (Pettersson *et al.*, 1999; only major fatty acid data were available); 8, *Paenibacillus polymyxa* (Yoon *et al.*, 2003). Summed feature 4 comprises C_{17:1} iso I and/or C_{17:1} anteiso B. ND, Not detected; NM, not mentioned.

Fatty acid	1	2	3	4	5	6	7	8
C _{10:0}	0.64	ND	NM	NM	NM	NM	NM	NM
C _{11:0} 2-OH	ND	ND	NM	NM	NM	NM	NM	0.8
C _{14:0}	1.80	1.15	2.3	2.96	1.2	0.9	NM	0.7
C _{15:0}	ND	ND	1.6	1.93	3.6	0.6	6.8	0.5
C _{16:0}	3.49	5.9	15.3	18.73	8.7	10.5	31	9.1
C _{17:0}	1.56	1.25	NM	NM	NM	NM	NM	ND
C _{18:0}	ND	ND	NM	NM	NM	NM	NM	1.1
C _{14:0} iso	4.74	0.97	0.3	1.19	0.9	0.6	NM	0.6
C _{15:0} anteiso	48.42	49.96	53.9	43.15	48.8	42.4	32	49.9
C _{15:0} iso	6.03	13.55	12.3	6.25	6.5	10.5	3.2	5.5
C _{16:0} iso	11.59	6.52	2.3	9.09	11.1	5.8	NM	7.7
C _{17:0} anteiso	5.68	8.80	3.3	10.72	15.0	11.6	NM	16.7
C _{17:0} iso	1.59	6.39	3.3	2.99	NM	6.5	NM	7.0
C _{16:1} ω11c	6.16	2.18	1.3*	NM	NM	5.2*	NM	ND
C _{16:1} ω7c alcohol	5.01	ND	NM	NM	NM	NM	NM	NM
C _{17:1} ω10c iso	1.18	1.35	0.5*	NM	NM	1.7*	NM	NM
C _{17:1} ω6c	ND	ND	NM	NM	NM	NM	NM	0.7
Summed feature 4	1.64	0.92	NM	NM	NM	NM	NM	ND

*The *cis/trans* isomeric nature of the fatty acids was not specified; rather, these were reported as C_{16:1}ω11 and C_{17:1}ω10 iso, respectively.

followed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems). An almost-complete (1466 nt continuous stretch) 16S rRNA gene sequence of the strain was used as a query to search for homologous sequences in the GenBank database and in the Ribosomal Database Project library (Project II, release 9; <http://rdp.cme.msu.edu/index.jsp>). Analysis of the sequence indicated that GPTSA 11^T contains the consensus signature sequence stretches PAEN 515F (5'-GAGTAACTGCTCTCGGAATGACGGTACTTGA-GAAGAAAGCCCC-3') (Shida *et al.*, 1997) and PAEN 862F (5'-TCGATACCCCTTGGTGCCGAAGT-3') (Pettersson *et al.*, 1999), which are mostly found among different species within the genus *Paenibacillus*. Strain GPTSA 11^T showed the highest degree of sequence similarity with *Paenibacillus apiarius* DSM 5581^T (95.85 %), followed by *Paenibacillus alvei* IAM 1258^T (94.34 %), *Paenibacillus cineris* LMG 18439^T (93.87 %), *Paenibacillus favisporus* GMP 01^T (93.80 %), *Paenibacillus chibensis* JCM 11200^T (93.47 %) and *Paenibacillus azoreducens* CMI^T (93.40 %). Sequences from type strains of these six and 13 other species of *Paenibacillus* showing more than 92.50 % similarity were used for phylogenetic analysis. These sequences

were aligned with that of strain GPTSA 11^T by the CLUSTAL X program (Thompson *et al.*, 1997) and edited manually. Aligned sequences were analysed by the PHYLIP software package, version 3.5c (Felsenstein, 1993). Computation of pairwise evolutionary distances for the aligned sequences was done using the DNADIST program with the Kimura two-parameter model (Kimura, 1980). To obtain the confidence value for the aligned sequence dataset, bootstrap analysis of 100 replications were done using SEQBOOT. Phylogenetic trees showing the relationships between GPTSA 11^T and other reference strains were constructed using the neighbour-joining method (Saitou & Nei, 1987) and the unweighted pair group arithmetic averages linkage algorithm. Distance-matrix data obtained from DNADIST were also used to construct a phylogenetic tree by using KITSCH. Consensus trees for each of these methods were generated using CONSENSE from the PHYLIP package. Irrespective of the different tree-generating methods used, strain GPTSA 11^T always occurred as a separate clade within the *Paenibacillus thiaminolyticus*–*Paenibacillus popilliae*–*P. alvei*–*P. apiarius* cluster (Fig. 1). The 16S rRNA gene sequence similarity of these strains with respect to GPTSA 11^T lies between 92.98 and 95.85 %. The overall genomic relatedness of GPTSA 11^T with respect to these species may not be high because, as Stackebrandt & Goebel (1994) observed, strains with less than 97 % 16S rRNA gene sequence similarity have DNA–DNA relatedness values below 70 %. The latter percentage of genomic relatedness is considered as the ‘gold standard’ for bacterial species definition (Wayne *et al.*, 1987). Thus, in the absence of a close relative with significant 16S rRNA gene sequence similarity, strain GPTSA 11^T can be considered as a novel species. The presence of ellipsoidal endospores, C_{15:0} anteiso

as a major fatty acid, *meso*-diaminopimelic acid as a cell-wall amino acid, MK-7 as the diagnostic menaquinone and PAEN 515F and PAEN 862F signature sequences in the 16S rRNA gene suggest that the strain belongs to the genus *Paenibacillus*. However, it differs from closely related species in terms of several phenotypic characteristics and the DNA G + C content (Table 2). In addition, unlike *P. cineris*, strain GPTSA 11^T cannot produce acid from L-arabinose, *myo*-inositol, D-melezitose, arbutin, D-fructose, galactose or lactose. Close phylogenetic relatives also differ from GPTSA 11^T in terms of cellular fatty acid composition, both qualitatively and quantitatively (Table 1). On the basis of the phenotypic, chemotaxonomic and phylogenetic data, it is evident that GPTSA 11^T represents a novel species within the genus *Paenibacillus*, for which we propose the name *Paenibacillus assamensis* sp. nov.

Description of *Paenibacillus assamensis* sp. nov.

Paenibacillus assamensis (as.sam.en'sis. N.L. masc. adj. *assamensis* pertaining to Assam, a north-eastern state in India, where the type strain was isolated).

Gram-variable, strictly aerobic, mesophilic, sporulating, motile rods occurring as single cells or in pairs. Cells measure 1.0–2.5 µm in length and 0.5–0.6 µm in width. Colonies are round, convex with undulated margins and light yellowish-white in colour, spreading as single colonies over the entire plate. Endospores are ellipsoidal and subterminal, occurring in bulging sporangia. The strain can grow at temperatures between 20 and 37 °C, at pH values in the range 6.8–12.0 and can tolerate NaCl at concentrations of up to 2.5 %. It is oxidase-, catalase-, gelatinase- and arginine dihydrolase-positive but negative for urease, DNase, phenylalanine deaminase, lysine and ornithine decarboxylase activities. Negative for indole and H₂S production, nitrate reduction, in the Voges–Proskauer test and for gas production from glucose but gives a positive result in the methyl red test. Cannot utilize acetate, citrate or propionate. Hydrolyses starch, aesculin and casein but not tyrosine, ONPG or Tweens 20, 40 and 80. Produces acid from D-glucose, glycerol, gentiobiose, glycogen, D-maltose, D-mannose, D-ribose, produces acid weakly from D-amygdalin, cellobiose, sucrose and trehalose, but not from adonitol, L-arabinose, D-arabinose, L-arabitol, arbutin, D-fructose, D-galactose, *myo*-inositol, inulin, D-lactose, D-mannitol, D-melibiose, D-melezitose, D-raffinose, L-rhamnose, salicin, sorbitol, L-sorbose, xylitol or D-xylose. Can grow in the presence of 0.001 % lysozyme but not 0.01 % lysozyme. Cannot degrade chitin or xylan. The major fatty acids are C_{15:0} anteiso, followed by C_{16:0} iso, C_{16:1ω11c}, C_{15:0} iso, C_{17:0} anteiso and C_{16:1ω7c} alcohol. The cell-wall amino acid is *meso*-diaminopimelic acid. The diagnostic menaquinone is MK-7. The DNA G + C content is 41.2 mol%.

The type strain is GPTSA 11^T (=MTCC 6934^T=JCM 13186^T), isolated from a warm-spring water sample from Assam, India.

Fig. 1. Phylogenetic relationships of strain GPTSA 11^T and other closely related *Paenibacillus* species based on 16S rRNA gene sequences. The tree was generated using the neighbour-joining method and displayed with TREECON software (Van de Peer & De Wachter, 1997). Bootstrap values as percentages of 100 replications are shown at branches. The sequence from *Paenibacillus hodogayensis* SG^T is taken as the outgroup. Bar, 0.02 substitutions per site.

Table 2. Comparison of phenotypic properties and G+C content of GPTSA 11^T and closely related *Paenibacillus* species

Species/strains: 1, GPTSA 11^T; 2, *P. apiarius* MTCC 1497^T (data from this study); 3, *P. alvei* (data from Shida *et al.*, 1997); 4, *P. cineris* (Logan *et al.*, 2004) 5, *P. favisporus* (Velázquez *et al.*, 2004). 6, *P. thiaminolyticus* (Shida *et al.*, 1997); 7, *P. popilliae* (Pettersson *et al.*, 1999); 8, *P. polymyxa* MTCC 122^T (data from this study unless indicated). +, Positive; –, negative; V, variable; W, weak; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Spreading colonial growth	+	–	+	–	–	–	–	–
Anaerobic growth	–	+	+	+	+	+	+	+
Nitrate reduction	–	+	–	+	+	+	–	+
Production of:								
Acetylmethylcarbinol	–	–	+	V/W	–	–	–	+
Indole	–	–	+	–	–	+	–	–
Dihydroxyacetone	–	–	+	ND	–	–	ND	+
Decomposition of tyrosine	–	+	V	ND	–	+	–	–
Hydrolysis of:								
Casein	+	+	+	W	–	+	–	+
Urea	–	+	ND	–	+	–	ND	–
Growth at pH 5.6	–	+	–	+	+	–	–	+
Growth in 5% NaCl	–	+	V	–	W	V	ND	–
Acid production from:								
Mannitol	–	–	–	+	+	V	–	+
D-Xylose	–	–	–	+	+	–	–	+
G+C content (mol%)	41.2	51.3	45–47	51.5	53	52–54	ND	43–46*

*Data from Shida *et al.* (1997).

Acknowledgements

We are grateful to Dr K. Ganesan for his help with DNA sequencing and Dr G. S. Prasad and Dr K. Suresh for their advice and for fruitful discussions. The help of Dr T. C. Bora (Regional Research Laboratory, Jorhat, India) is gratefully acknowledged. We also thank the Editor and referees for making useful suggestions. Financial assistance from DBT, Government of India, and CSIR is duly acknowledged. P. S., S. K. and A. B. are recipients of CSIR fellowships. This is IMTECH communication number 011/2005.

References

- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small subunit ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.
- Ash, C., Priest, F. G. & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.
- Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycan based on 2,4-diamino butyric acid (DAB). *J Appl Bacteriol* **48**, 459–470.
- 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.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*, pp. 656–682. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- 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.
- Logan, N. A., De Clerck, E., Lebbe, L., Verhelst, A., Goris, J., Forsyth, G., Rodríguez-Díaz, M., Heyndrickx, M. & De Vos, P. (2004). *Paenibacillus cineris* sp. nov. and *Paenibacillus cookii* sp. nov. from Antarctic volcanic soils and a gelatin-processing plant. *Int J Syst Evol Microbiol* **54**, 1071–1076.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.

- Montes, J. M., Mercadé, 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.
- Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Pandey, K. K., Mayilraj, S. & Chakrabarti, T. (2002). *Pseudomonas indica* sp. nov., a novel butane-utilizing species. *Int J Syst Evol Microbiol* **52**, 1559–1567.
- Pettersson, B., Rippere, K. E., Yousten, A. A. & Priest, F. G. (1999). Transfer of *Bacillus lentimorbus* and *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov. *Int J Syst Bacteriol* **49**, 531–540.
- Powers, E. M. (1995). Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Appl Environ Microbiol* **61**, 3756–3758.
- Rivas, R., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2005). *Paenibacillus phyllosphaerae* sp. nov., a xylanolytic bacterium isolated from the phyllosphere of *Phoenix dactylifera*. *Int J Syst Evol Microbiol* **55**, 743–746.
- 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.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). 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.
- 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.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Takeda, M., Suzuki, I. & Koizumi, J. (2005). *Paenibacillus hodo-gayensis* sp. nov., capable of degrading the polysaccharide produced by *Sphaerotilus natans*. *Int J Syst Evol Microbiol* **55**, 737–741.
- 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.
- Van de Peer, Y. & De Wachter, R. (1997). Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput Appl Biosci* **13**, 227–230.
- Velázquez, E., de Miguel, T., Poza, M., Rivas, R., Rosselló-Mora, R. & Villa, G. T. (2004). *Paenibacillus favisporus* sp. nov., a xylanolytic bacterium isolated from cow faeces. *Int J Syst Evol Microbiol* **54**, 59–64.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yoon, J.-H., Oh, H.-M., Yoon, B.-D., Kang, K. H. & Park, Y.-H. (2003). *Paenibacillus kribbensis* sp. nov. and *Paenibacillus terrae* sp. nov., biofloculants for efficient harvesting of algal cells. *Int J Syst Evol Microbiol* **53**, 295–301.