Paenibacillus soli sp. nov., a xylanolytic bacterium isolated from soil

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Two novel polysaccharide-degrading bacteria (strains DCY03^T and DCY04) were isolated from a soil sample of a ginseng field in the Republic of Korea and were identified as representing members of the genus *Paenibacillus* on the basis of phenotypic characteristics and phylogenetic inference based on 16S rRNA gene sequences. Cells of the two isolates were Gram-positive, spore-forming, non-motile, straight rods. Based on DNA–DNA relatedness data, the strains were considered to belong to the same species. The DNA G + C content ranged from 56.6 to 57.0 mol%. The predominant cellular fatty acid was anteiso-C_{15:0} (63.8–62.8 %). Levels of 16S rRNA gene sequence similarity between the two novel isolates and the type strains of recognized *Paenibacillus* species were 91.4–96.5 %. Strains DCY03^T and DCY04 could clearly be distinguished from phylogenetically closely related *Paenibacillus* species on the basis of DNA–DNA relatedness data and phenotypic characteristics. Therefore, on the basis of these data, the two isolates are considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus soli* sp. nov. is proposed. The type strain is DCY03^T (=KCTC 13010^T=LMG 23604^T).

Polysaccharide-degrading enzymes such as amylase, cellulase and xylanase are widespread in nature. They can be found in every type of organism, including mammals, plants, algae, moulds, bacteria and phages (Oshima *et al.*, 2002; Sutherland, 1999; Terra & Ferreira, 1994). For the production of polysaccharases, micro-organisms are usually the most convenient sources, and these can be obtained from various natural environments.

During a study of the bacterial community that produces polysaccharases, a large number of novel bacterial strains were isolated from soil samples from different regions of the Republic of Korea (Kang *et al.*, 2003; An *et al.*, 2005). On the basis of 16S rRNA gene sequence data, two of these isolates, designated strains DCY03^T and DCY04, were found to

represent members of the genus *Paenibacillus* and were subjected to polyphasic taxonomic investigation. The results indicated that strains DCY03^T and DCY04 are representative of a novel bacterial species.

Strains DCY03^T and DCY04 were isolated by using the plate technique together with an insoluble chromogenic substrate (Ten *et al.*, 2004). After isolation, strains DCY03^T and DCY04 were cultivated via transfer onto R2A agar (Difco) every month. Gram reaction was performed via the non-staining method as described by Buck (1982). Cell morphology and motility were observed under a Nikon light microscope ($\times 1000$ magnification), with cells grown on R2A agar for 3 days at 30 °C, and via transmission electron microscopy (JEM-1010) after negative staining with 1 % (w/v) phosphotungstic acid (see Supplementary Fig. S1 available in IJSEM Online). Growth at different temperatures (4, 15, 20, 30, 37, 42 and 50 °C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested in an R2A broth supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30 °C. Growth was estimated by monitoring the OD₆₀₀.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains DCY03^T and DCY04 are DQ309072 and DQ309073, respectively.

Transmission electron micrographs of cells of strains DCY03^T and DCY04 and an expanded phylogenetic tree based on 16S rRNA gene sequences showing the relationship of *Paenibacillus soli* sp. nov. to other species of the genus *Paenibacillus* are available as supplementary material in IJSEM Online.

Anaerobic growth was performed in serum bottles by adding a thioglycolate (1 g l⁻¹) to the R2A broth and substituting the upper air layer with nitrogen gas. Substrate utilization as the sole carbon source and some enzyme activities were tested by using the API ZYM, API 50CHB and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Catalase activity was determined by bubble production with 3 % (v/v) H₂O₂, and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Degradation of DNA (using DNA agar from Difco, supplemented with 0.01 % toluidine blue from Merck), casein and starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987) and xylan (Ten *et al.*, 2004) was also investigated; reactions were read after 5 days.

Purified cell-wall preparations were obtained as described by Schleifer & Kandler (1972). Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional TLC on cellulose plates using solvent systems described by Schleifer & Kandler (1972). Menaquinone was extracted from cells grown in an R2A broth (Difco) and was analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Cells for analysis of cellular fatty acids were cultivated on TSA agar at 30 °C for 48 h, then saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed via GC (Hewlett Packard 6890) were identified by the Microbial Identification software package (Sasser, 1990). To determine the G+C content of the chromosomal DNA, the genomic DNA of the strains was first extracted and purified with the Qiagen Genomic-tip system 100/G; this was then enzymically degraded into nucleosides and the G+C content was determined as described by Mesbah *et al.* (1989) by using reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the remaining three values were used to calculate relatedness values. The DNA hybridization values quoted are the means of these three values.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Partial 16S rRNA gene sequences were compiled using SeqMan software; the 16S rRNA gene sequences of the test strains and related taxa (obtained from GenBank) were edited using the BIOEDIT program (Hall, 1999). Multiple alignments were performed by using the CLUSTAL_X program (Thompson *et al.*, 1997). Evolutionary distances were calculated with the Kimura two-parameter model (Kimura, 1983). A phylogenetic tree was constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in MEGA 3 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

The physiological and biochemical characteristics of strains DCY03^T and DCY04 are given in the species description, or are shown in Table 1 together with those of the type strains of related species.

The nearly complete 16S rRNA gene sequences of strains DCY03^T and DCY04 (1486 and 1438 bp, respectively) were determined and subjected to comparative analysis. The 16S rRNA gene sequence of strain DCY03^T showed high similarity (more than 99.0 %) to that of strain DCY04, and the level of DNA–DNA relatedness between the two strains was 93 %. The two novel strains are therefore considered to represent the same species. The phylogenetic tree (Fig. 1) shows that these strains are clustered within the genus *Paenibacillus* of the class ‘*Bacilli*’ (an expanded tree is shown in Supplementary Fig. S2 in IJSEM Online). Levels of

Table 1. Selective phenotypic characteristics of strains DCY03^T and DCY04 and the type strains of related species of the genus *Paenibacillus*

Strains: 1, DCY03^T; 2, DCY04; 3, *P. ehimensis* DSM 11029^T (Kuroshima *et al.*, 1996); 4, *P. elgii* KCTC 10016BP^T (Kim *et al.*, 2004); 5, *P. chinjuensis* DSM 15045^T (Yoon *et al.*, 2002); 6, *P. koreensis* YC300^T (Chung *et al.*, 2000); 7, *P. naphthalenovorans* DSM 14203^T (Daane *et al.*, 2002); 8, *P. validus* DSM 3037^T (Heyndrickx *et al.*, 1995). +, Positive reaction; –, negative reaction; W, weakly positive reaction; V, variable reaction; ND, not determined. All species are positive for the production of catalase and acid production from glucose, and negative for H₂S production.

Characteristic	1	2	3	4	5	6	7	8
Motility	–	–	+	+	+	+	+	+
Oxidase	–	–	+	–	+	+	ND	–
α-Glucosidase	–	–	+	+	+	+	V	W
β-Glucosidase	–	–	ND	+	ND	+	–	+
Protease	–	–	+	ND	+	ND	–	–
Urease	–	–	ND	ND	–	–	+	–
Growth at 50 °C	–	W	+	–	–	+	–	+
Growth at 5 % (w/v) NaCl	–	–	ND	–	–	+	–	–
Production of indole	+	+	ND	+	ND	–	–	–
Fermentation of:								
L-Arabinose	+	+	+	–	–	+	–	–
Lactose	+	–	ND	ND	–	+	–	–
Maltose	+	–	ND	+	+	+	+	+
D-Raffinose	+	–	ND	ND	–	+	–	+
L-Rhamnose	–	–	ND	ND	–	–	–	–
D-Xylose	+	+	+	V	–	–	–	+
Glycerol	–	–	ND	V	–	ND	–	+
Inositol	–	–	ND	ND	ND	+	–	+
Mannitol	–	–	+	+	–	+	+	+
Inulin	–	–	ND	ND	–	–	–	–
Salicin	+	–	ND	ND	–	–	–	–
DNA G+C content (mol%)	56.6	57	54.9	51.7	53	54	49	51.7

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships among members of the genus *Paenibacillus*. Dots indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bootstrap values are expressed as percentages of 1000 replications at the branch points. Bar, 0.02 substitutions per nucleotide position.

16S rRNA gene sequence similarity between DCY03^T and *Paenibacillus ehimensis* DSM 11029^T, *Paenibacillus elgii* KCTC 10016BP^T and *Paenibacillus chinjuensis* DSM 15045^T were 96.5, 96.2 and 96.1 %, respectively. Levels of DNA–DNA relatedness between strain DCY03^T and *P. ehimensis* DSM 11029^T, *P. elgii* KCTC 10016BP^T and *P. chinjuensis* DSM 15045^T were 30, 25 and 22 %, respectively. These results suggest that strains DCY03^T and DCY04 should be classified as representing a novel species in the genus *Paenibacillus*, based on the recommendations of Wayne *et al.*

(1987) and of Stackebrandt & Goebel (1994) for the delineation of novel species.

The results obtained from chemotaxonomic analyses were in agreement with the results of phylogenetic inference showing that strains DCY03^T and DCY04 belong within the genus *Paenibacillus*. The strains contained MK-7 as the major menaquinone and *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. Whole-cell fatty acid compositions of the two isolates and

Table 2. Cellular fatty acid profiles of strains DCY03^T and DCY04 and other species in the genus *Paenibacillus*

Strains: 1, DCY03^T; 2, DCY04; 3, *P. ehimensis* DSM 11029^T (Kuroshima *et al.*, 1996); 4, *P. elgii* KCTC 10016BP^T (Kim *et al.*, 2004); 5, *P. chinjuensis* DSM 15045^T (Yoon *et al.*, 2002); 6, *P. koreensis* YC300^T (Chung *et al.*, 2000); 7, *P. naphthalenovorans* DSM 14203^T (Daane *et al.*, 2002); 8, *P. validus* DSM 3037^T (Heyndrickx *et al.*, 1995). Strains DCY03^T and DCY04 were cultured on TSA agar at 30 °C for 48 h. Values are percentages of total fatty acids. Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system.

Fatty acid	1	2	3	4	5	6	7	8
Saturated								
C _{14:0}	1.7	1.7	1.0	1.9	0.8	2.5	1.2	1.7
C _{15:0}	6.9	4.9	1.2	1.2	4.5		0.8	1.5
C _{16:0}	10.5	3.5	7.1	9.7	5.3	14.4	8.5	7.2
C _{18:0}	4.0	3.5						0.3
Branched								
iso-C _{14:0}	2.3	3.0	1.8	2.1	2.2		1.6	2.3
iso-C _{15:0}	3.6	6.6	8.1	10.6	5.0	5.3	6.0	15.1
anteiso-C _{15:0}	57.6	64.0	52.9	54.1	66.1	51.8	43.4	52.7
iso-C _{16:0}	5.6	6.7	8.6	6.5	7.0	5.6	12.2	6.3
iso-C _{17:0}			3.3	3.2	1.6	3.2	5.5	3.3
anteiso-C _{17:0}	2.7	2.5	8.0	6.2	6.1	7.9	11.9	4.6
Unsaturated								
C _{16:1ω7c} alcohol			1.2				1.4	0.9
C _{16:1ω11c}			5.2	4.5		9.3	5.4	2.6
C _{18:1ω9c}	2.3							0.3
Summed feature 4 (C _{16:1ω7c} /iso-C _{15:0} 2-OH)	1.4	1.7						0.3
Summed feature 7 (C _{18:1ω7c} /ω9t/ω12t)	1.5	2.0						

related species are given in Table 2. The fatty acid profiles of the two novel strains were similar to those of recognized *Paenibacillus* species (Shida *et al.*, 1997; Rivas *et al.*, 2005a, b, c; Takeda *et al.*, 2005; Sánchez *et al.*, 2005). The DNA G + C content of the strains was 56.6–57.0 mol%.

The phenotypic characteristics of strains DCY03^T and DCY04 were compared with those of some *Paenibacillus* species that form a coherent phylogenetic cluster. As shown in Table 1, the physiological characteristics of the two novel isolates are different from those of the recognized *Paenibacillus* species investigated.

Therefore, on the basis of the data presented, strains DCY03^T and DCY04 are considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus soli* sp. nov. is proposed.

Description of *Paenibacillus soli* sp. nov.

Paenibacillus soli (so'li. L. neut. gen. n. *soli* of soil, the source of the organism).

Cells are Gram-positive, aerobic, non-motile and rod-shaped, about 0.5–0.6 µm in width and 1.1–1.2 µm in length. Colonies grown on R2A agar (Difco) for 2 days are smooth, circular and white. Growth occurs at 20–42 °C, pH 8–9 and in the presence of up to 2 % (w/v) NaCl. No growth occurs on MacConkey agar. Catalase-positive and oxidase-negative. Positive for aerobic nitrate reduction and indole production. H₂S is not produced. Aesculin, starch and xylan are hydrolysed, but gelatin, cellulose, olive oil and DNA are not. Acid is produced from DL-arabinose, ribose, D-xylose, methyl β-D-xylose, galactose, glucose, fructose, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, gentiobiose, D-turanose, glycogen, methyl α-D-glucoside, 5-ketogluconate and salicin. Acid is not produced from L-xylose, mannose, sorbose, rhamnose, D-lyxose, D-tagatose, DL-fucose, glycerol, erythritol, adonitol, dulcitol, inositol, mannitol, sorbitol, xylitol, DL-arabitol, methyl α-D-mannoside, gluconate, 2-ketogluconate, N-acetylglucosamine, amygdalin, arbutin or inulin. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acid is anteiso-C_{15:0}. The DNA G + C content is 56.6–57.0 mol%.

The type strain, DCY03^T (=KCTC 13010^T =LMG 23604^T), and reference strain, DCY04 (=KCTC 13011 =LMG 23602), were isolated from a soil sample from a ginseng field in the Republic of Korea.

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References

- An, D.-S., Im, W.-T., Yang, H.-C., Kang, M.-S., Kim, K.-K., Jin, L., Kim, M.-K. & Lee, S.-T. (2005). *Cellulomonas terrae* sp. nov., a cellulolytic and xylanolytic bacterium isolated from soil. *Int J Syst Evol Microbiol* **55**, 1705–1709.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*, pp. 196, 843. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Chung, Y. R., Kim, C. H., Hwang, I. & Chun, J. (2000). *Paenibacillus korensis* sp. nov., a new species that produces an iturin-like antifungal compound. *Int J Syst Evol Microbiol* **50**, 1495–1500.
- Daane, L. L., Harjono, I., Barns, S. M., Launen, L. A., Palleroni, N. J. & Haggblom, 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.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limit 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.
- 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.
- Heyndrickx, M., Vandemeulebroecke, K., Scheldeman, P., Hoste, B., Kersters, K., De Vos, P., Logan, N. A., Aziz, A. M., Ali, N. & Berkeley, R. C. (1995). *Paenibacillus* (formerly *Bacillus*) *gordonae* (Pichinoty et al. 1986) Ash et al. 1994 is a later subjective synonym of *Paenibacillus* (formerly *Bacillus*) *validus* (Nakamura 1984) Ash et al. 1994: emended description of *P. validus*. *Int J Syst Bacteriol* **45**, 661–669.
- Kang, M.-S., Im, W.-T., Ten, L. N. & Lee, S.-T. (2003). Isolation and diversity of bacteria having extracellular degrading enzymes of xylan and/or amylose and/or cellulose. In *Proceedings of the International Meeting of the Federation of Korean Microbiological Societies 2003*, abstract B4022, p. 165. Seoul: The Microbiological Society of Korea.
- Kim, D. S., Bae, C.-Y., Jeon, J.-J., Chun, S.-J., Oh, H. W., Hong, S. G., Baek, K.-S., Moon, E. Y. & Bae, K. S. (2004). *Paenibacillus elgii* sp. nov., with broad antimicrobial activity. *Int J Syst Evol Microbiol* **54**, 2031–2035.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Komagata, K. & Suzuki, K. I. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

- Kouker, G. & Jaeger, K.-E. (1987). Specific and sensitive plate assay for bacterial lipase. *Appl Environ Microbiol* **53**, 211–213.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kuroshima, K.-I., Sakane, T., Takata, R. & Yokota, A. (1996). *Bacillus ehimensis* sp. nov. and *Bacillus chitinolyticus* sp. nov., new chitinolytic members of the genus *Bacillus*. *Int J Syst Bacteriol* **46**, 76–80.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Oshima, H., Miyazaki, R., Ohe, Y., Hayashi, H., Kawamura, K. & Kikuyama, S. (2002). Isolation and sequence of a novel amphibian pancreatic chitinase. *Comp Biochem Physiol Part B Biochem Mol Biol* **132**, 381–388.
- Rivas, R., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2005a). *Paenibacillus xylanilyticus* sp. nov., an airborne xylanolytic bacterium. *Int J Syst Evol Microbiol* **55**, 405–408.
- Rivas, R., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2005b). *Paenibacillus phyllosphaerae* sp. nov., a xylanolytic bacterium isolated from phyllosphere of *Phoenix dactylifera*. *Int J Syst Evol Microbiol* **55**, 743–746.
- Rivas, R., Gutiérrez, C., Abril, A., Mateos, P. F., Martínez-Molina, E., Ventosa, A. & Velázquez, E. (2005c). *Paenibacillus rhizosphaerae* sp. nov., isolated from the rhizosphere of *Cicer arietinum*. *Int J Syst Evol Microbiol* **55**, 1305–1309.
- 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., Díaz, 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.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. MIDI Technical Note 101. Newark, DE: MIDI.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- 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.
- 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.
- Sutherland, I. W. (1999). Polysaccharases for microbial exopolysaccharides. *Carbohydr Polym* **38**, 319–328.
- Takeda, M., Suzuki, I. & Koizumi, J. (2005). *Paenibacillus hodogayensis* sp. nov., capable of degrading the polysaccharide produced by *Sphaerotilus natans*. *Int J Syst Evol Microbiol* **55**, 737–741.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004). Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.
- Terra, W. R. & Ferreira, C. (1994). Insect digestive enzymes: properties, compartmentalization and function. *Comp Biochem Physiol Part B Biochem Mol Biol* **109**, 1–62.
- 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* **24**, 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). 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., Seo, W.-T., Shin, Y. K., Kho, Y. H., Kang, K. H. & Park, Y.-H. (2002). *Paenibacillus chinjuensis* sp. nov., a novel exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **52**, 415–421.