***Paenibacillus koreensis* sp. nov., a new species that produces an iturin-like antifungal compound**

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A bacterial strain, YC300^T, that produces an iturin-like antifungal antibiotic was isolated from compost and identified as member of the genus *Paenibacillus*. Gram reaction of the strain was variable depending upon growth stages and culture media. Three different types of colonies were developed on tryptic soy agar. The organism was facultatively anaerobic and grew at 50 °C. The DNA G+C content was 54 mol% and anteiso-C_{15:0} was the major fatty acid. A 0.9 kb fragment was produced by PCR amplification of strain YC300^T DNA using primers PAEN515F and 1377R. Levels of 16S rDNA similarity between strain YC300^T and other *Paenibacillus* species were between 89.8 and 94.8%. Phylogenetically, strain YC300^T formed a significant monophyletic clade with *Paenibacillus validus*. It is clear from polyphasic evidence that the isolate should be classified as *Paenibacillus koreensis* sp. nov., the type strain of which is YC300^T (= KCTC 2393^T, KCCM 40903^T).

Keywords: *Paenibacillus koreensis*, iturin-like antifungal

INTRODUCTION

The genus *Paenibacillus* was created to harbour a phylogenetically coherent group of aerobic or facultatively anaerobic endospore-forming bacilli on the basis of 16S rRNA analysis (Ash *et al.*, 1993; Shida *et al.*, 1997a). The genus currently contains 24 species and is phenotypically related to other genera belonging to the family *Bacillaceae*. A highly specific primer, PAEN515F, was recently designed for the detection of 16S rRNA gene of the genus *Paenibacillus* and used to produce a 0.8 kb PCR amplicon in preparations from the 18 *paenibacilli* by amplifications in conjunction with primer 1377R (Shida *et al.*, 1997a). The method proved to be useful for identifying and differentiating *Paenibacillus* species from other members of the family *Bacillaceae*. More recently, *Paenibacillus campinasensis*, an alkaliphilic species, *Paenibacillus dendritiformis*, forming a distinctive growth pattern, and *Paenibacillus lentimorbus* and *Paenibacillus popilliae*, which cause milky disease in insects, have been introduced as new members of the genus

(Pettersson *et al.*, 1999; Tcherpakov *et al.*, 1999; Yoon *et al.*, 1998).

Some members of the genus, including *Paenibacillus polymyxa* and *Paenibacillus thiaminolyticus*, are known to produce antibacterial compounds such as polymyxin, octopytin and baciphelacin (Slepecky & Hemphill, 1991). However, no antifungal compound has been reported to be produced by *Paenibacillus* species so far, although iturins, cyclic peptide antifungals, are produced by several isolates of *Bacillus subtilis* (Lancini & Lorenzetti, 1993). In addition to the production of antibiotics, several species of *Paenibacillus* are also known to excrete a wide variety of enzymes that degrade natural biopolymers, such as chondroitin, curdlan and chitin (Claus & Berkeley, 1986; Kanzawa *et al.*, 1995; Nakamura, 1987). A chitinolytic bacterial strain, designated YC300^T, was isolated from a compost sample collected from Chinju, Republic of Korea, in the process of screening biological control agents for *Rhizoctonia solani*, a plant-pathogenic fungus. The strain also showed strong antifungal activity against *Fusarium oxysporum*, *Colletotrichum lagenarium*, *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Chung, 1997). The taxonomic status of strain YC300^T was investigated using a combination of phenotypic, chemical and molecular

Abbreviation: TSA, tryptic soy agar.

The GenBank accession number for the 16S rDNA sequence of strain YC300^T is AF130254.

systematic methods. On the basis of polyphasic evidence, we propose that strain YC300^T be classified in the genus *Paenibacillus* as *Paenibacillus koreensis* sp. nov.

METHODS

Bacterial strain. Strain YC300^T was isolated from a compost sample prepared with rice husk and fishery industrial wastes collected from Chinju, Republic of Korea, using the standard dilution agar plating method. Aliquots of serially diluted soil suspension were inoculated onto minimal salt agar medium containing 0.5 g MgSO₄·7H₂O, 0.7 g K₂HPO₄, 0.3 g KH₂PO₄, 0.01 g FeSO₄·H₂O, 0.001 g ZnSO₄ and 0.001 g MnCl₂ per litre distilled water supplemented with 0.2% (w/v) colloidal chitin prepared from crab shell chitin (Sigma) as sole carbon and nitrogen source (Chung *et al.*, 1989). Bacterial colonies were picked up 3 d following incubation at 28 °C and their inhibitory activities against *Rhizoctonia solani* and *Fusarium oxysporum* were determined by paired bioassay on potato dextrose agar (Difco) and 0.1 × tryptic soy agar (TSA; Difco) plates. An isolate, designated YC300^T, showing substantial inhibition of mycelial growth of fungi was selected and studied further. The strain was maintained on TSA at 4 °C.

Morphological and physiological characteristics. The cells were grown at 28 °C using a fermenter (51; Korea Fermentation; aeration: 31 min⁻¹, 300 r.p.m.) containing soybean meal media (SM; 2 g yeast extract, 1 g beef extract, 20 g soluble starch, 25 g soybean meal, 5 g dextrose, 0.5 g K₂HPO₄, 2 g NaCl, 0.5 g MgSO₄·7H₂O, 6 g CaCO₃ per litre distilled water) and sampled after 4, 12, 24 and 48 h. The morphological properties were examined by light microscopy after Gram staining. Vegetative cells and spores were fixed with glutaraldehyde/osmium and after sectioning and staining the specimens were observed by using a transmission electron microscope (Hitachi model H-600). The methods described by Smbert & Krieg (1981) were used for the following physiological tests: oxidase, catalase, indole production, Voges-Proskauer reaction, nitrate reduction, motility and hydrolysis of casein, starch, chitin and chitosan. Carbohydrate utilization and other tests described below were performed with GNI and BAC cards according to the instructions of the VITEK system (AMS 60; bioMérieux). Growth in the presence of 7% (w/v) NaCl and 0.001% lysozyme, and the temperature range for growth were determined using 0.1 × TSA as basal medium.

Chemotaxonomy. The biomass for cellular fatty acid analysis was prepared from a 1-d-old culture grown on a TSA plate at 28 °C. Fatty acid methyl esters were prepared using the method described in the manual of the MIDI Microbial Identification System (Hewlett Packard). The resultant esters were separated using a gas chromatograph (model 5890; Hewlett Packard) fitted with a phenylmethyl silicone fused silica capillary column (25 m × 0.2 mm; Hewlett Packard).

DNA base composition. The G + C content of the DNA was determined from the midpoint value of the thermal denaturation profile using a spectrophotometer (Ultrospec 2000; Pharmacia Biotech) equipped with programmable peltier temperature control unit according to the equation of Marmur & Doty (1962) as modified by De Lay (1970).

16S rDNA sequencing. 16S rDNA was enzymically amplified using two oligonucleotide primers as described previously (Chung *et al.*, 1999). The sequences of the two primers are 5'-

TATGGATCCTTCTACGGAGAGTTTGATCC-3' and 5'-TATGGATCCCACCTTCCGGTACGGCTACC-3' (*Bam*HI sites are indicated by underlining). The temperatures for denaturation, annealing and extension were 94, 55 and 72 °C, respectively. The PCR products were digested with *Bam*HI and cloned into linearized pBluescript II SK(+) vector. Overlapping subclones were generated by exonuclease III digestion of the insert DNA. The nucleotide sequences of these clones were sequenced using an automatic sequencer (ABI), according to the manufacturer's protocol.

Phylogenetic analysis. The resultant 16S rDNA sequence of strain YC300^T was manually aligned with representative sequences of paenibacilli and related taxa obtained from the Ribosomal Database Project (Maidak *et al.*, 1997) and GenBank databases, using known 16S rRNA secondary structure information. Phylogenetic trees were inferred by using three treeing algorithms, namely the Fitch-Margoliash (Fitch & Margoliash, 1967), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the neighbour-joining and Fitch-Margoliash methods were generated according to the model of Jukes & Cantor (1969). The trees were rooted using *Alicyclobacillus acidocaldarius* (accession no. X60742) and *Brevibacillus laterosporus* (D16271) as outgroups. The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant unrooted tree topology was evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings.

***Paenibacillus* specific PCR.** The *Paenibacillus*-specific primer PAEN515F (5'-GCTCGGAGAGTGACGGTACCTGAGA-3') and universal primer 1377R (5'-GGCATGCTGATCCGCGATTACTAGC-3') were applied to isolate YC300^T following the method of Shida *et al.* (1996, 1997a). The universal forward and reverse primers, 27FC and 1377R, were also used for PCR amplification.

RESULTS

Morphological and physiological characteristics

Cells grown vegetatively were Gram-positive and rod-shaped, measuring 0.5–0.9 × 2.3–4.5 µm. Ellipsoidal endospores were produced in swollen sporangia in a fermenter at 28 °C (Fig. 1). During the early and

Fig. 1. Electron micrograph of vegetative cell (a), endospore in swollen sporangia (b) and endospore (c) of strain YC300^T. Bar, 1 µm.

Fig. 2. Various colony morphologies of strain YC300^T. The organism was grown on TSA at 28 °C for 10 d.

Table 1. Acid production from carbohydrates as sole carbon source by strain YC300^T

+, −, positive or negative for carbohydrate fermentation; (+), positive for carbohydrate oxidation.

Carbohydrate	Reaction
<i>N</i> -Acetyl-D-glucosamine	+
Amygdalin	−
Amylopectin	+
L-Arabinose	+
Adonitol	−
Arabitol	+
D-Galactose	+
D-Glucose	+(+)
Inositol	+
Inulin	−
Lactose	+(+)
Maltose	+(+)
Mannitol	+(+)
Palatinose	+
D-Raffinose	+
L-Rhamnose	−
D-Ribose	−
Salicin	−
Sorbitol	+
Sucrose	+
Tagatose	−
D-Trehalose	+
D-Xylose	−(+)

exponential growth stages under well aerated conditions, cells were Gram-positive, but with the onset of spore formation, the cells became Gram-negative. The

cells were motile by peritrichous flagella. Strain YC300^T formed three types of colonies on 0.1 × TSA: (i) circular, flat, smooth and opaque; (ii) circular, convex, glistening and translucent; and (iii) circular or irregular, wrinkled hollow and opaque (Fig. 2). The first type was mainly developed. The strain was facultatively anaerobic and grew at 10–50 °C with an optimum growth temperature of 38–40 °C. This strain grew in the presence of 0.001 % lysozyme, but not in the presence of 7 % NaCl. Oxidase, catalase, arginine dihydrolase and ornithine decarboxylase activities were observed, but not urease or lysine decarboxylase activities. Casein, chitin, chitosan, aesculin and starch were hydrolysed. Sodium acetate and malonate were utilized, but acetamide and citrate were not. Nitrate was reduced and tests for indole production and Voges–Proskauer reaction were negative. Reactions for the oxidation and fermentation of carbohydrates as sole carbon sources are shown in Table 1. Strain YC300^T inhibited growth of several plant and human fungal pathogens, such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Colletotrichum lagenarium*, *Magnaporthe grisea*, *Botryosphaeria dothidea*, *Candida albicans* and *Trichophyton mentagrophyte*, by producing iturin-like anti-fungal compounds. The active substance is composed of seven amino acids (Glu, Tyr, Ser, Pro, Asp1, Asp2, Asp3) and one β-amino acid, typical components of the iturins (S. S. Moon, personal communication).

Chemotaxonomy

The predominant fatty acids in whole-cell methanolysates of strain YC300^T were anteiso-15:0 (51.1 %), 16:0 (28.3 %) and iso-16:0 (20.6 %). The G+C content of the DNA was 54 mol %.

16S rDNA analysis

The almost complete sequence of the 16S rDNA of strain YC300^T was determined (1536 bp). The preliminary phylogenetic analysis based on a large data set consistently placed strain YC300^T in a clade corresponding to the genus *Paenibacillus* (data not shown). The detailed evolutionary relationships between strain YC300^T and all of the validly described *Paenibacillus* species were examined using the neighbour-joining method (Fig. 3). Strain YC300^T was recovered in a monophyletic clade containing the type strains of *Paenibacillus alginolyticus*, *Paenibacillus chondroitinus*, *Paenibacillus larvae* and *Paenibacillus validus*, the relationship supported by a high bootstrap value of 96 %. The reconstruction of this clade was also confirmed in the Fitch–Margoliash and maximum-parsimony analyses. Furthermore, the isolate formed a monophyletic subclade with *P. validus* with 86 % bootstrap support. Comparative analysis of 16S rDNA primary structure indicated that strain YC300^T is distantly related to all of the validly described *Paenibacillus* species as pairwise sequence

Fig. 3. A rooted neighbour-joining tree based on 1396 unambiguously aligned nucleotide positions, including positions 45–70, 98–1133 and 1141–1431 (*E. coli* numbering; Brosius *et al.*, 1981). The numbers at the nodes exhibit the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled data sets. The scale bar represents 0.1 nt substitutions per position.

similarity values range from 94.8 (*P. validus*) to 89.8% (*P. dendritiformis*).

PCR amplifications and molecular systematics

PCR amplification using primers PAEN515F and 1377R produced a 0.9 kb PCR fragment in the preparation of strain YC300^T. A 1.3 kb fragment was also amplified in the strain tested with bacterial universal primers 27FC and 1377R.

DISCUSSION

It is evident from the results of *Paenibacillus*-specific PCR amplification and comprehensive phylogenetic

analyses based on 16S rDNA sequence data that strain YC300^T belongs to the genus *Paenibacillus*. Shida *et al.* (1997a) reported that a 0.8 kb fragment was produced from *paenibacilli* using the same primers under the reaction conditions used in the present study. Careful examination of 16S rDNA sequences of all *Paenibacillus* type strains clearly indicated that the primer pair set should yield a fragment in the size range 895 (*Paenibacillus apiarius*) to 907 bp (*P. dendritiformis*). Therefore, the size of the *Paenibacillus*-specific PCR amplicon should be 0.9 instead of 0.8 kb. The genetic uniqueness of our isolate among the *paenibacilli* was evident, as none of the validly described species showed 95% or greater 16S rDNA sequence similarity, a level of similarity far less than the borderline for defining bacterial genomic species, i.e. 97%, proposed by Stackebrandt & Goebel (1994).

The chemotaxonomic data, i.e. G + C content of DNA (54 mol%) and the anteiso-15:0 fatty acid as the major cellular fatty acid (51.1%), also fall within the ranges exhibited by *Paenibacillus* species (Shida *et al.*, 1997a, b; Yoon *et al.*, 1998). In a comparison of phenotypic characteristics of strain YC300^T with those of phylogenetically closely related *Paenibacillus* species, *P. validus*, *P. chondroitinus* and *P. alginolyticus*, strain YC300^T exhibited several differential properties. Strain YC300^T grew anaerobically and had oxidase activity in opposition to those related species. Xylose was not fermented by the isolate. The Gram reaction of YC300^T was variable, depending on the growth stage and culture medium, a phenomenon often observed in *Bacillus* species (Claus & Berkeley, 1986). The appearance of colonies was variable depending on culture media and three different colony types were developed on TSA. Each colony produced these various colony types in many repeated experiments. We could not find the culture conditions necessary to induce colonial variation of strain YC300^T. Such variations in colony morphology can also be observed in many *Bacillus* species (Claus & Berkeley, 1986). Like other *Paenibacillus* species, including *Paenibacillus alvei*, *Paenibacillus macerans*, *Paenibacillus polymyxa* and *P. thiaminolyticus*, strain YC300^T has the ability to degrade many natural biopolymers, such as chitin (Nielson & Sorensen, 1997; Shida *et al.*, 1997a). The isolate has been used as the biological control agent of soil-borne pathogens because of its ability to degrade chitin, a major component of the fungal cell wall (Singh *et al.*, 1999). YC300^T produces iturin-like antifungal agents, the structures of which were elucidated by a series of chromatographic and spectroscopic analyses. Some *Bacillus* and *Paenibacillus* species are known to produce antifungal and antibacterial substances, such as bacillomycin and polymyxin (Slepecky & Hemphill, 1991). However, no production of an antifungal by *Paenibacillus* species has been reported to date. To our knowledge, this is the first report of the production of an iturin-like antifungal by *Paenibacillus* species.

On the basis of polyphasic evidence, we propose that

strain YC300^T be assigned in the genus *Paenibacillus* as *Paenibacillus koreensis* sp. nov.

Description of *Paenibacillus koreensis* sp. nov.

Paenibacillus koreensis (ko.re.en'sis, M.L. masc. adj. *koreensis* indicating Korea, the geographical origin of isolation).

Cells are rod-shaped, measuring 0.5–0.9 × 2.3–4.5 µm and motile by peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Three types of colonies are formed when grown on 0.1 × TSA: circular, flat, smooth and opaque; circular, convex, glistening and translucent; and circular or irregular, wrinkled hollow and opaque. Facultatively anaerobic, growing at 10–50 °C with an optimum growth temperature of 38–40 °C. Growth occurs in the presence of 0.001 % lysozyme, but not in the presence of 7 % NaCl. Positive for oxidase, catalase, arginine dihydrolase, ornithine decarboxylase and nitrate reduction. Negative for urease, lysine decarboxylase, indole production and Voges–Proskauer reaction. Casein, chitin, chitosan, aesculin and starch are hydrolysed. Utilizes sodium acetate and malonate as sole carbon sources, but not acetamide and citrate. Acid is produced from glucose, lactose, maltose, mannitol and xylose oxidatively, and fermentatively from arabinose, arabinol, amylopectin, *N*-acetyl-D-glucosamine, galactose, glucose, inositol, lactose, maltose, mannitol, palatinose, raffinose, sorbitol and sucrose. Carbohydrates not utilized by the species are shown in Table 1. The major fatty acid is anteiso-15:0. The G+C content is 54 mol %. A 16S rDNA fragment (0.9 kb) is amplified by PCR with primers PAEN515F and 1377R. Inhibits the growth of fungi by producing an iturin-like antifungal compound. The type strain is *Paenibacillus koreensis* YC300^T and has been deposited in the Korean Collection for Type Cultures and the Korean Culture Center of Microorganisms under the accession numbers KCTC 2393^T and KCCM 40903^T, respectively.

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REFERENCES

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 Leeuwenhoek* **64**, 253–260.

Brosius, J., Dull, T. J., Sleeter, D. D. & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* **148**, 107–127.

Chung, M. H. (1997). *Biological control of Rhizoctonia damping-off of radish by antagonistic bacteria with chitinolytic activity in commercial bed soils*. MSc thesis, Gyeongsang National University, Chinju, Korea (in Korean).

Chung, Y. R., Ohh, S. H. & Chung, H. S. (1989). Antagonistic activity of *Streptomyces* species against *Fusarium solani* causing ginseng root rot. *Korean J Microbiol* **27**, 56–62.

Chung, Y. R., Sung, K. C., Mo, H. K., Son, D. Y., Nam, J. S., Chun, J. & Bae, K. S. (1999). *Kitasatospora cheerisanensis* sp. nov., a new species of the genus *Kitasatospora* that produces an antifungal agent. *Int J Syst Bacteriol* **49**, 753–758.

Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1140. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

De Ley, J. (1970). Re-examination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5.1. Seattle: Department of Genetics, University of Washington.

Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.

Fitch, W. M. (1972). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kanzawa, Y., Harada, A., Takeuchi, A., Yokota, A. & Harada, T. (1995). *Bacillus curdlanolyticus* sp. nov. and *Bacillus kobensis* sp. nov., which hydrolyze resistant curdlan. *Int J Syst Bacteriol* **45**, 515–521.

Lancini, G. & Lorenzetti, R. (1993). *Biotechnology of Antibiotics and Other Bioactive Microbial Metabolites*. New York: Plenum.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1997). The RDP (Ribosomal Database Project). *Nucleic Acids Res* **25**, 109–111.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Nakamura, L. K. (1987). *Bacillus alginolyticus* sp. nov. and *Bacillus chondroitinus* sp. nov., two alginate-degrading species. *Int J Syst Bacteriol* **37**, 284–286.

Nielson, P. & Sorensen, J. (1997). Multi-target and medium-independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. *FEMS Microb Ecol* **22**, 183–192.

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 description of *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov. *Int J Syst Bacteriol* **49**, 531–540.

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. & Komagata, K. (1996). Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol* **46**, 939–946.

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.

Singh, P. P., Shin, Y. C., Park, C. S. & Chung, Y. R. (1999). Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* **89**, 92–99.

Slepecky, R. A. & Hemphill, H. E. (1991). The genus *Bacillus* – nonmedical. In *The Prokaryotes*, pp. 1663–1696. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–433.

Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

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.

Tcherpakov, M., Ben-Jacob, E. & Gutnick, D. L. (1999). *Paenibacillus dendritiformis* sp. nov., proposal for a new pattern-forming species and its localization within a phylogenetic cluster. *Int J Syst Bacteriol* **49**, 239–246.

Yoon, J. H., Yim, D. K., Lee, J. S., Shin, K. S., Sato, H. H., Lee, S. T., Park, Y. K. & Park, Y. H. (1998). *Paenibacillus campinasensis* sp. nov., a cyclodextrin-producing bacterium isolated in Brazil. *Int J Syst Bacteriol* **48**, 833–837.