Bacillus graminis sp. nov., an endophyte isolated from a coastal dune plant

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A Gram-stain-positive endophytic bacterium, designated strain YC6957^T, was isolated from surface-sterilized roots of a halophyte (*Elymus mollis* Trin.) inhabiting coastal tidal flats of Namhae Island, located on the southern coast of Korea, and was subjected to a polyphasic taxonomic study. Cells were facultatively anaerobic, endospore-forming rods to coccoid rods, motile by a single flagellum. Strain YC6957^T was catalase-positive, oxidase-negative and able to grow in the presence of 0–8 % (w/v) NaCl, with optimum growth at 4–5 % (w/v) NaCl. Growth occurred at 15–45 °C (optimal growth at 30–35 °C) and pH 6.0–8.5 (optimal growth at pH 7.0–8.0). The predominant isoprenoid quinone was menaquinone 7 (MK-7). The major cellular fatty acids were C_{16:0} (11.3 %), iso-C_{15:0} (19.2 %) and anteiso-C_{15:0} (36.4 %). The cell-wall peptidoglycan contained meso-diaminopimelic acid. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content was 41.6 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the isolate belonged to the genus *Bacillus*. Strain YC6957^T exhibited high 16S rRNA gene sequence similarity to its closest neighbours, *Bacillus ruris* LMG 22866^T (96.14 %), *Bacillus lentus* NCIMB 8773^T (95.97 %) and *Bacillus galactosidilyticus* LMG 17892^T (95.91 %), and less than 95.84 % similarity to all other type strains in the genus *Bacillus*. On the basis of the phylogenetic, physiological and biochemical data, it is suggested that strain YC6957^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus graminis* sp. nov. is proposed. The type strain is YC6957^T (=KACC 13779^T =DSM 22162^T).

Species of the genus *Bacillus* have been isolated from a wide variety of environments, and their ubiquitous distribution in nature is due to the resistance of their endospores to stressful environmental conditions. Members of the genus *Bacillus* are also common residents of internal tissues of terrestrial and aquatic plants, where these micro-organisms play an important role in plant protection and growth promotion and facilitate plant growth under biotic and abiotic stress conditions (Ji *et al.*, 2008; Sgroi *et al.*, 2009). Although *Bacillus* species have been isolated from a wide range of habitats, to the best of our knowledge, only one *Bacillus* strain belonging to a species with a validly published name, *Bacillus endophyticus* (Reva *et al.*, 2002), has been isolated from inner tissues of a plant root. In the course of a study of the endophytic bacterial diversity of coastal sand-dune halophytes, several endophytes related to

the genus *Bacillus* were isolated from different halophytes, and a novel strain, YC6957^T, belonging to the genus *Bacillus* was isolated from the roots of a dune grass.

Strain YC6957^T was isolated from the roots of a coastal sand-dune plant, *Elymus mollis* Trin., growing on coastal tidal flats of Namhae Island (34° 50' 15.62" N 127° 53' 32.72" E), located midway along the south coast of Korea. Isolation was carried out as described previously (Chung *et al.*, 2008). Root pieces were washed several times with running tap water and surface sterilized by stepwise washing in 70 % ethanol for 5 min, 1.0 % NaOCl for 10 min and 70 % ethanol for about 10 s and finally washed several times with sterile distilled water. To confirm the success of the disinfection process, washed root segments were placed on R2A agar (Difco) for examination of bacterial growth after incubation at 28 °C for 5–6 days. After confirmation of the surface sterility of root segments, 1.0 g dried plant root was ground with a sterile mortar and pestle in 9.0 ml autoclaved filtered seawater (AFS). Serial dilutions were made using AFS and 200 µl aliquots of the 10^{−3} to 10^{−5} dilutions were spread on one-tenth strength

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YC6957^T is GU322908.

Two supplementary figures are available with the online version of this paper.

R2A (1/10 R2A) plates and incubated at 25 °C for 1–2 weeks. The purified strain was maintained on marine agar 2216 (MA; Difco) or half-strength R2A (1/2 R2A) agar.

Thirty-two strains isolated from roots were identified on the basis of 16S rRNA gene sequence similarity and the similarities to related genera were in the range 96.1–100 %. One novel strain designated YC6957^T had the closest similarity (96.14 %) to *Bacillus ruris* LMG 17892^T, while relatively low sequence similarities (95.84–93.09 %) were found to the type strains of other species of the genus *Bacillus* with validly published names.

Cell morphology was observed using transmission electron microscopy (Hitachi model H-600) with cells grown for 1 day at 30 °C in R2A broth (Difco). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Motility was checked using the hanging-drop method (Schaal, 1986). Cells were rods to coccoid rods with a single polar flagellum (Fig. 1). The pH range for growth was tested using 1/2 R2A broth, adjusted to different pH values by using the appropriate biological buffers, as described by Xu *et al.* (2005). The temperature for growth was determined by incubating strain YC6957^T on 1/2 R2A agar at 4 to 50 °C and the optimum temperature for growth was determined in 1/2 R2A broth by turbidity at 600 nm using a spectroscopic method (Optizen 2120UV; Mechasis). Requirement for and tolerance of NaCl were determined in 1/2 R2A broth supplemented with 1–10 % (w/v) NaCl. Catalase and oxidase tests were performed by the procedures outlined by Cappuccino & Sherman (2002). Nitrate reduction was determined according to the method of Lányi (1987). Modified Schaeffer's medium (MSM) was used to induce

sporulation, as described by Kempf *et al.* (2005). The lecithinase test was performed as described previously (Gordon *et al.*, 1973). Anaerobic growth was determined at 28 °C after 2 weeks of incubation in the GasPak EZ Anaerobe Pouch System (BD) on 1/2 R2A agar according to the manufacturer's instructions. Physiological properties of strain YC6957^T and related type strains *B. ruris* KACC 12113^T, *B. lentus* KACC 10930^T and *B. galactosidilyticus* DSM 15595^T were determined using the following methods: hydrolysis of casein, gelatin, tyrosine, starch, aesculin, urea (Lányi, 1987; Smibert & Krieg, 1994), cellulose (Hendricks *et al.*, 1995) and chitin (Singh *et al.*, 1999) and β -1, 3-glucanase (Hong & Meng, 2003). API 20E tests (bioMérieux) were used to determine nitrate reduction, aesculin hydrolysis, β -galactosidase production, fermentation of glucose, the ONPG test (β -galactosidase), urease, gelatinase and indole production. Acid-production profiles from carbohydrates were obtained with the API 50 CH system (bioMérieux) after growth in 50 CHB medium according to the manufacturer's instructions. Phenotypic, physiological and biochemical characteristics of strain YC6957^T are listed in the species description and are given in Table 1.

For measurement of the G + C content of the chromosomal DNA, genomic DNA was extracted and purified as described by Ausubel *et al.* (1995). Purified genomic DNA was then digested enzymically into nucleotides and the G + C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). The G + C content of the genomic DNA of strain YC6957^T was 41.6 mol%. Strain YC6957^T contained MK-7 (97.4 %) as the predominant menaquinone, with MK-8 (2.6 %) present in minor amounts. The 16S rRNA gene was amplified from extracted DNA by using primers 27F and 1492R (Lane, 1991). The purified PCR product was sequenced by GenoTech Co. Ltd (Daejeon, Korea). To ascertain the phylogenetic position of the novel strain, the 16S rRNA gene sequence (1.4 kb) of strain YC6957^T was compared with sequences obtained from NCBI and the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Multiple alignments of sequences were performed by using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed by using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA4 program (Tamura *et al.*, 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985). A phylogenetic tree based on the maximum-likelihood algorithm was also reconstructed using the PHYLIP program, version 3.6 (Felsenstein, 2002). Comparative analysis of 16S rRNA gene sequences revealed that strain YC6957^T belonged to the genus *Bacillus*, exhibiting the highest sequence similarity to *B. ruris* LMG 22866^T (96.14 %), *B. lentus* NCIMB 8773^T (95.97 %) and *B. galactosidilyticus* LMG 17892^T (95.91 %), while relatively

Fig. 1. Transmission electron micrograph of a cell of *Bacillus graminis* sp. nov. YC6957^T, grown in 1/2 R2A broth for 1 day at 30 °C. Bar, 0.5 μ m.

Table 1. Characteristics that differentiate *Bacillus graminis* sp. nov. YC6957^T from type strains of phylogenetically related *Bacillus* species

Strain: 1, *B. graminis* YC6957^T; 2, *B. ruris* KACC 12113^T; 3, *B. galactosidilyticus* DSM 15595^T; 4, *B. lentus* KACC 10930^T. Data were obtained in this study unless indicated. +, Positive; –, negative; w, weak positive reaction.

Characteristic	1	2	3	4
Isolation source	Plant	Dairy farm feed ^{a*}	Raw milk ^b	Soil ^c
Swollen sporangia	+	+	+	–
pH for growth	6.0–8.5	6.5–11.0	5.5–10.5	6.0–9.5
Temperature for growth (°C)	15–45	20–40	20–40	15–35
Oxidase	–	–	–	+
Anaerobic growth	+	+	+	–
Nitrate reduction	+	–	+	+
Citrate utilization	–	–	–	w
Urease production	–	–	–	+
Hydrolysis of:				
Casein	w	–	w	–
Cellulose	w	–	+	+
Acid production from:				
L-Arabinose	w	+	w	–
D-Xylose	w	+	–	w
L-Xylose	–	–	–	w
D-Galactose	–	–	–	w
D-Mannose	+	w	w	–
D-Rhamnose	–	–	–	w
D-Mannitol	w	–	–	w
N-Acetylglucosamine	–	+	+	–
Amygdalin	w	–	+	–
Lactose	–	+	w	w
Melibiose	–	+	+	w
Sucrose	–	+	w	w
Melezitose	–	+	w	w
Raffinose	–	–	–	+
Gentiobiose	–	–	–	w
DNA G+C content (mol%)	41.6	39.2 ^a	37.7 ^b	36.3 ^c

*Data taken from: a, Heyndrickx *et al.* (2005); b, Heyndrickx *et al.* (2004); c, Spanka & Fritze (1993).

low sequence similarities (95.84–93.09 %) were observed to other members of the genus *Bacillus*. The phylogenetic tree reconstructed using the neighbour-joining algorithm further confirmed that strain YC6957^T was phylogenetically related closely to species of the genus *Bacillus* and formed a robust lineage with the type strains of *B. galactosidilyticus* and *B. ruris* (Supplementary Fig. S1a, available in IJSEM Online). The topology was similar to that of the phylogenetic tree constructed by using the maximum-likelihood method (Supplementary Fig. S1b).

Analysis of cellular fatty acid methyl esters was performed for strain YC6957^T and the closely related strains *B. ruris*

KACC 12113^T, *B. lentus* KACC 10930^T and *B. galactosidilyticus* DSM 15595^T from cells grown on TSBA (Difco) for 36 h at 30 °C. Cellular fatty acids were saponified, methylated and extracted according to Sasser (1990). The fatty acids were separated by GC (Hewlett Packard 6890) and identified using the Microbial Identification software package (Microbial ID, Inc.). Whole-cell fatty acid analysis of strain YC6957^T revealed the dominance of C_{16:0} (11.3 %), iso-C_{15:0} (19.2 %) and anteiso-C_{15:0} (36.4 %), which comprised approximately 70 % of the cellular fatty acids extracted. Branched fatty acids of the 14- to 17-carbon iso and anteiso series are typically the major fatty acids found in *Bacillus* cell membranes (Kämpfer, 1994). However, strain YC6957^T and the type strains *B. ruris* KACC 12113^T, *B. lentus* KACC 10930^T and *B. galactosidilyticus* DSM 15595^T could be distinguished clearly from each other based on relative fatty acid concentrations (Table 2).

Amino acids of whole-cell hydrolysates of strain YC6957^T were extracted and analysed according to the method of Stanek & Roberts (1974). Polar lipids were extracted by a modified method of Minnikin *et al.* (1984) and separated by TLC on Merck Kieselgel 60-HPTLC. Aminolipids were detected by spraying the plate with a 0.2 % (w/v) solution of ninhydrin in butanol saturated with water followed by heating at 105 °C for 10 min (Ross *et al.*, 1985). Phospholipids were detected by spraying the plate with Zinzadze reagent (Dittmer & Lester, 1964). Glycolipids were detected with 0.5 % 1-naphthol in methanol/water

Table 2. Fatty acid methyl ester composition of *B. graminis* sp. nov. YC6957^T and type strains of related species

Strain: 1, *B. graminis* YC6957^T; 2, *B. ruris* KACC 12113^T; 3, *B. galactosidilyticus* DSM 15595^T; 4, *B. lentus* KACC 10930^T. Data were obtained in this study and are expressed as percentages of total fatty acids. tr, Less than 1.0 % of total fatty acids; –, not detected.

Fatty acid	1	2	3	4
Straight-chain saturated				
C _{12:0}	2.5	tr	tr	–
C _{14:0}	2.7	4.7	5.6	5.7
C _{15:0}	4.7	9.2	8.1	–
C _{16:0}	11.3	21.4	17.2	16.8
C _{17:0}	–	tr	–	–
C _{18:0}	tr	tr	–	1.1
Branched saturated				
iso-C _{14:0}	6.2	2.6	5.7	6.0
iso-C _{15:0}	19.2	13.3	14.9	11.6
iso-C _{16:0}	7.5	4.9	4.8	5.5
iso-C _{17:0}	1.8	3.0	1.4	1.3
anteiso-C _{15:0}	36.4	34.1	34.2	41.5
anteiso-C _{17:0}	3.8	4.7	3.7	6.8
anteiso-C _{19:0}	1.2	tr	–	–
Mono-unsaturated				
C _{16:1} ω11c	tr	–	tr	–

(1:1, v/v) and then with sulfuric acid/ethanol (1:1, v/v), followed by heating at 120 °C for 5–10 min (Xin *et al.*, 2001). Total lipids were detected by spraying with phosphomolybdic acid solution (Sigma-Aldrich) followed by heating at 150 °C. Analysis of cell-wall hydrolysates of strain YC6957^T revealed the presence of *meso*-diaminopimelic acid as the diagnostic cell-wall diamino acid. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol were predominant in the polar lipid profile; we also detected an unknown phospholipid, an unknown glycolipid and two unknown polar lipids (Supplementary Fig. S2). A similar polar lipid profile, containing the same predominant compounds as well as the unknown phospholipid and the unknown polar lipid, was detected in *B. galactosidilyticus* DSM 15595^T, but the presence of an unknown aminophospholipid and an unknown phospholipid clearly distinguished strain YC6957^T from the related strain *B. galactosidilyticus* DSM 15595^T. The major fatty acid profile, respiratory lipoquinone, major polar lipids and peptidoglycan type of the cell wall of strain YC6957^T were typical of those of members of the genus *Bacillus* (Priest *et al.*, 1988; Wieser *et al.*, 2005; Lim *et al.*, 2006a, b, c).

The results of the phylogenetic analysis and morphological and chemotaxonomic investigations supported the affiliation of strain YC6957^T to the genus *Bacillus*. The characteristics that differentiate strain YC6957^T from related *Bacillus* species are summarized in Table 1. Differences in biochemical characteristics, 16S rRNA gene sequences, isolation source, DNA G + C content, hydrolytic enzyme production and fatty acid composition can be used to distinguish this strain from phylogenetically related taxa (Tables 1 and 2). Therefore, on the basis of these data, according to the minimal standards for describing new taxa of aerobic, endospore-forming bacteria (Logan *et al.*, 2009), we propose that strain YC6957^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus graminis* sp. nov. is proposed.

Description of *Bacillus graminis* sp. nov.

Bacillus graminis (gra'mi.nis. L. gen. neut. n. *graminis* of grass).

Cells are Gram-stain-positive, facultatively anaerobic rods to coccoid rods, 0.7–1.0 × 1.2–2.2 µm. They bear an ellipsoidal endospore that lies in a subterminal position. Motile by a single polar flagellum. After 2 days on 1/2 R2A, colonies are semi-transparent and whitish in colour with opaque centres. Cells usually occur singly or in pairs; tetrad-like cells are common. Cells are catalase-positive and oxidase-negative. Grows at 15–45 °C (optimum 30–35 °C), but not at 10 or 50 °C. Grows at pH 6.0–8.5 but not at pH 5.5 or 9.0. Growth is observed in R2A broth containing 8% (w/v) NaCl but not 9.0% (w/v) NaCl. Nitrate is reduced to nitrite. Hydrolysis of aesculin is positive; weakly positive for hydrolysis of casein and cellulose. Does not hydrolyse gelatin, chitin or urea. Citrate is not utilized. Indole production and egg-yolk reaction are negative. In API 20E tests, nitrate is reduced to nitrite; negative for

ONPG reaction, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease, citrate utilization, hydrogen sulfide production, the Voges–Proskauer reaction, indole production and gelatin hydrolysis. Positive for acid production from L-arabinose, D-xylose, D-mannose, D-mannitol and amygdalin, but negative for acid production from erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, N-acetylglucosamine, arbutin, salicin, cellobiose, maltose, inulin, melezitose, gentiobiose, raffinose, D-lyxose, D-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. Positive for utilization of D- and L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, sucrose, D-mannose, D-mannitol and amygdalin. The major respiratory menaquinone is MK-7. The diagnostic diamino acid of the cell-wall peptidoglycan is *meso*-diaminopimelic acid. Major fatty acid components (>10% of the total) are C_{16:0}, iso-C_{15:0} and anteiso-C_{15:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol; an unknown phospholipid, an unknown glycolipid and two unknown lipids are minor components. The G + C content of the genomic DNA of the type strain is 41.6 mol%.

The type strain, YC6957^T (=KACC 13779^T =DSM 22162^T), was isolated from internal tissue of roots of *Elymus mollis* Trin. growing on the coast of Namhae Island, Korea.

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References

- Ausubel, F. W., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1995). *Current Protocols in Molecular Biology*. New York: Wiley.
- Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin/Cummings.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57, 2259–2261.
- Chung, B. S., Aslam, Z., Kim, S. W., Kim, G. G., Kang, H. S., Ahn, J. W. & Chung, Y. R. (2008). A bacterial endophyte, *Pseudomonas brassicaevarum* YC5480 isolated from the root of *Artemisia* sp. producing antifungal and phytotoxic compounds. *Plant Pathol J* 24, 461–468.
- Dittmer, J. C. & Lester, R. L. (1964). A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. *J Lipid Res* 15, 126–127.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.

- Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gordon, R. E., Haynes, W. C. & Pang, C. H.-N. (1973). *The Genus Bacillus*. Agriculture Handbook no. 427. Washington, DC: United States Department of Agriculture.
- 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.
- Hendricks, C. W., Doyle, J. D. & Hugley, B. (1995). A new solid medium for enumerating cellulose-utilizing bacteria in soil. *Appl Environ Microbiol* 61, 2016–2019.
- Heyndrickx, M., Logan, N. A., Lebbe, L., Rodríguez-Díaz, M., Forsyth, G., Goris, J., Scheldeman, P. & De Vos, P. (2004). *Bacillus galactosidilyticus* sp. nov., an alkali-tolerant beta-galactosidase producer. *Int J Syst Evol Microbiol* 54, 617–621.
- Heyndrickx, M., Scheldeman, P., Forsyth, G., Lebbe, L., Rodríguez-Díaz, M., Logan, N. A. & De Vos, P. (2005). *Bacillus ruris* sp. nov., from dairy farms. *Int J Syst Evol Microbiol* 55, 2551–2554.
- Hong, T. Y. & Meng, M. (2003). Biochemical characterization and antifungal activity of an endo-1,3-beta-glucanase of *Paenibacillus* sp. isolated from garden soil. *Appl Microbiol Biotechnol* 61, 472–478.
- Ji, X., Lu, G., Gai, Y., Zheng, C. & Mu, Z. (2008). Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. *FEMS Microbiol Ecol* 65, 565–573.
- Kämpfer, P. (1994). Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* 17, 86–98.
- Kempf, M. J., Chen, F., Kern, R. & Venkateswaran, K. (2005). Recurrent isolation of hydrogen peroxide-resistant spores of *Bacillus pumilus* from a spacecraft assembly facility. *Astrobiology* 5, 391–405.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* 5, 2359–2367.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 19, 1–67.
- Lim, J.-M., Jeon, C. O., Lee, J. C., Ju, Y. J., Park, D. J. & Kim, C.-J. (2006a). *Bacillus koreensis* sp. nov., a spore-forming bacterium, isolated from the rhizosphere of willow roots in Korea. *Int J Syst Evol Microbiol* 56, 59–63.
- Lim, J.-M., Jeon, C. O., Lee, S.-M., Lee, J. C., Xu, L. H., Jiang, C. L. & Kim, C. J. (2006b). *Bacillus salarius* sp. nov., a halophilic, spore-forming bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* 56, 373–377.
- Lim, J.-M., Jeon, C. O. & Kim, C.-J. (2006c). *Bacillus taeanensis* sp. nov., a halophilic Gram-positive bacterium from a solar saltern in Korea. *Int J Syst Evol Microbiol* 56, 2903–2908.
- Logan, N. A., Berge, O., Bishop, A. H., Busse, H. J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Rabinovitch, L. & other authors (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* 59, 2114–2121.
- 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.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2, 233–241.
- Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* 134, 1847–1882.
- Reva, O. N., Smirnov, V. V., Pettersson, B. & Priest, F. G. (2002). *Bacillus endophyticus* sp. nov., isolated from the inner tissues of cotton plants (*Gossypium* sp.). *Int J Syst Evol Microbiol* 52, 101–107.
- Ross, H. N. M., Grant, W. D. & Harris, J. E. (1985). Lipids in archaeobacterial taxonomy. In *Chemical Methods in Bacterial Systematics*, pp. 289–300. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI Technical Note 101. Newark, DE: MIDI, Inc.
- Schaal, K. P. (1986). Genus *Actinomyces* Harz 1877, 133^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1383–1418. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Sgro, V., Cassán, F., Masciarelli, O., Del Papa, M. F., Lagares, A. & Luna, V. (2009). Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strobilifera*. *Appl Microbiol Biotechnol* 85, 371–381.
- Singh, P. P., Shin, Y. C., Park, C. S. & Chung, Y. R. (1999). Biological controls of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* 89, 92–99.
- 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.
- Spanka, R. & Fritze, D. (1993). *Bacillus cohnii* sp. nov., a new, obligately alkaliphilic, oval-spore-forming *Bacillus* species with ornithine and aspartic acid instead of diaminopimelic acid in the cell wall. *Int J Syst Bacteriol* 43, 150–156.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 28, 226–231.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.
- Wieser, M., Worliczek, H., Kämpfer, P. & Busse, H.-J. (2005). *Bacillus herbersteinensis* sp. nov. *Int J Syst Evol Microbiol* 55, 2119–2123.
- Xin, H., Itoh, T., Zhou, P., Suzuki, K. & Nakase, T. (2001). *Natronobacterium nitratreducens* sp. nov., a haloalkaliphilic archaeon isolated from a soda lake in China. *Int J Syst Evol Microbiol* 51, 1825–1829.
- Xu, P., Li, W. J., Tang, S. K., Zhang, Y. Q., Chen, G. Z., Chen, H. H., Xu, L. H. & Jiang, C. L. (2005). *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family 'Oxalobacteraceae' isolated from China. *Int J Syst Evol Microbiol* 55, 1149–1153.