Bacillus kribbensis sp. nov., isolated from a soil sample in Jeju, Korea

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A Gram-positive, endospore-forming bacterium, designated strain BT080^T, was isolated from a soil sample in Jeju, Korea. Cells of the isolate were strictly aerobic rods that were motile by means of peritrichous flagella. The strain grew optimally at 30–33 °C and pH 5.5–6.5. Chemotaxonomic data (major isoprenoid quinone, MK-7; DNA G+C content, 43.3 mol%; major fatty acids, anteiso-C_{15:0}, iso-C_{14:0}, iso-C_{16:0} and iso-C_{15:0}) supported the affiliation of the isolate to the genus *Bacillus*. Comparative 16S rRNA gene sequence analyses showed that strain BT080^T formed a distinct phyletic line within the genus *Bacillus*. The levels of 16S rRNA gene sequence similarity with respect to related *Bacillus* species were below 96.4 %. On the basis of physiological, biochemical and phylogenetic properties, strain BT080^T represents a novel species within the genus *Bacillus*, for which the name *Bacillus kribbensis* sp. nov. is proposed. The type strain is BT080^T (=KCTC 13934^T=DSM 17871^T).

Since the genus *Bacillus* was first described (Cohn, 1872), the number of *Bacillus* species has fluctuated widely. In particular, 16S rRNA gene sequence analysis by Ash *et al.* (1991) revealed five phylogenetically distinct clusters of species and three ungrouped species from 51 *Bacillus* species studied. Many *Bacillus* species that belonged to these phylogenetic groups have been reclassified as members of novel genera or have been transferred to other genera (Wisotzkey *et al.*, 1992; Shida *et al.*, 1997; Wainø *et al.*, 1999; Nazina *et al.*, 2001; Yoon *et al.*, 2001a; Jeon *et al.*, 2005; Hatayama *et al.*, 2006). Despite the reduction in the number of species in the genus *Bacillus*, the genus is considered as one of the largest genera and additional *Bacillus* species from diverse habitats have been described recently (Heyrman *et al.*, 2005a; Shivaji *et al.*, 2006; Ko *et al.*, 2006). In this study, we describe a novel Gram-positive, endospore-forming species of the genus *Bacillus* that was isolated from agricultural soil used for potato cultivation in Jeju, Korea.

Strain BT080^T was isolated with the serial dilution plating method, using nutrient agar (NA; Difco) at 30 °C for 3 days. Subcultivation was performed on NA at 32 °C for

2–3 days. Physiological characteristics of strain BT080^T were examined by growing the isolate on NA medium at different temperatures and pH values. NA media with different pH values were prepared as described previously (Gomori, 1955). Gram staining was performed using a bioMérieux Gram-stain kit according to the manufacturer's instructions. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon *et al.* (2005). Requirement for and tolerance of NaCl were determined in nutrient broth (Difco) supplemented with NaCl. Oxidase activity was tested using the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by determining the production of oxygen bubbles in a 3 % (v/v) aqueous hydrogen peroxide solution. Endospores were stained using the Schaeffer–Fulton method (Smibert & Krieg, 1981). Hydrolysis of casein, gelatin, starch, Tween 80, L-tyrosine and urea were investigated on NA using methods described previously (Lanyi, 1987; Smibert & Krieg, 1994). Nitrate reduction was determined according to the method of Lanyi (1987). Acid production from carbohydrates was tested as described by Leifson (1963) and additional enzyme activities were determined using the API ZYM system at 32 °C, as recommended by the manufacturer (bioMérieux).

Strain BT080^T formed cream, circular, slightly raised colonies when grown on NA at 32 °C for 2 days. The cells were rods that were motile by means of peritrichous flagella

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BT080^T is DQ280367.

A transmission electron micrograph of a negatively stained cell of strain BT080^T and a thin-layer chromatogram of polar lipids from this strain are available as supplementary figures with the online version of this paper.

(see Supplementary Fig. S1, available with the online version of this paper). Strain BT080^T grew in nutrient broth supplemented with 0–6 % (w/v) NaCl; optimum growth occurred in nutrient broth supplemented with 0–3 % (w/v) NaCl. Cells produced single, oval, terminal endospores in swollen sporangia. Anaerobic growth was not observed after 7 days at 32 °C on NA under anaerobic conditions. Other phenotypic features of strain BT080^T are presented in the description of the novel species.

An analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID) after incubation for 2 days on NA. Isoprenoid quinones and polar lipids were analysed as described by Komagata & Suzuki (1987). The DNA G+C content of strain BT080^T was determined using HPLC apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) using the method of Tamaoka & Komagata (1984). The fatty acid profile of the strain was characterized by the presence of saturated fatty acids such as anteiso-C_{15:0} (46.77 %), iso-C_{14:0} (21.31 %), iso-C_{16:0} (10.95 %) and iso-C_{15:0} (8.05 %) as the major fatty acids, which corresponds with the profiles of related type strains within the genus *Bacillus* (Table 1). The major respiratory lipoquinone of strain BT080^T was MK-7. The polar lipids of strain BT080^T were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and two unknown amino-group-containing glycopospholipids (see Supplementary Fig. S2 in IJSEM Online). The G+C content of the genomic DNA of strain BT080^T was 43.3 mol%. The fatty acid profile, the major lipoquinone, the major polar lipids and the DNA G+C content of strain BT080^T are all in accordance with those of other members of the genus *Bacillus* (Priest *et al.*, 1988; Nielsen *et al.*, 1995; Heyrman *et al.*, 2004, 2005b). Typical phenotypic features of strain BT080^T are summarized and compared with those of the type strains of closely related taxa in Table 2. Some of them are in accordance with the characteristics of members of the genus *Bacillus*, whereas others allow the differentiation of strain BT080^T from closely related *Bacillus* species.

The sequencing of the 16S rRNA gene was carried out as described previously (Lane, 1991). The resultant 16S rRNA gene sequence of strain BT080^T was compared with available 16S rRNA gene sequences from GenBank, using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>), to determine an approximate phylogenetic affiliation. Gene sequences were aligned with those of closely related species using CLUSTAL W software (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different methods, the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms; these methods are available in PHYLIP software, version 3.6 (Felsenstein, 2002). Using the FASTA3 program (of the European Bioinformatics Institute), 16S rRNA gene sequence comparisons were made between the novel strain and members of the genus *Bacillus* in order to obtain similarity calculations. A bootstrap analysis was performed according to the

Table 1. Cellular fatty acid content of strain BT080^T and some related *Bacillus* species

Taxa: 1, strain BT080^T; 2, *B. foraminis* LMG 23174^T (data from Tiago *et al.*, 2006); 3, *Bacillus firmus* DSM 12^T (Kämpfer, 1994); 4, *Bacillus circulans* DSM 11^T (Venkateswaran *et al.*, 2003); 5, *B. jeotgali* YKJ-10^T (Yoon *et al.*, 2001b). Data are expressed as percentages of total fatty acids. –, Not detected. Fatty acids that represented <0.5 % in all strains are omitted.

Fatty acid	1	2	3	4	5
Saturated					
C _{14:0}	0.73	0.8	1.1	2.9	1.3
C _{15:0}	–	–	–	1.0	–
C _{16:0}	2.63	1.4	1.5	2.7	3.2
C _{17:0}	–	0.8	–	–	–
iso-C _{14:0}	21.31	3.8	2.4	4.0	1.9
iso-C _{15:0}	8.05	25.4	45.8	13.9	49.3
iso-C _{16:0}	10.95	4.2	2.9	4.4	2.3
iso-C _{17:0}	–	3.4	2.9	1.3	4.1
anteiso-C _{15:0}	46.77	25.1	19.8	58.4	8.8
anteiso-C _{17:0}	3.56	11.2	3.7	4.8	3.7
Others					
C _{16:1} ω7c alcohol	1.25	4.0	4.5	–	4.5
C _{16:1} ω11c	–	5.6	3.2	2.6	5.0
iso-C _{17:1} ω10c	–	5.5	–	–	7.5
Summed feature*					
3	–	0.5	–	–	–
4	–	6.2	–	–	6.6

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1}ω7c and/or C_{16:1}ω6c; summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B.

algorithm of the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package. An almost-complete 16S rRNA gene sequence (1529 nt) was obtained for strain BT080^T and used for initial BLAST searches in GenBank and in the phylogenetic analysis. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BT080^T formed a distinct phyletic lineage with *Bacillus foraminis* CV53^T and *Bacillus jeotgali* YKJ-10^T within the genus *Bacillus* (Fig. 1). The topologies of phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that the novel isolate belongs to the genus *Bacillus* and that it can be differentiated from the known species of that genus (data not shown). Sequence similarities with respect to other members of the genus *Bacillus* used in the phylogenetic analysis were below 96.38 %, the 16S rRNA gene sequence similarity threshold generally used to defined a novel species (Christensen *et al.*, 2001; Rosselló-Mora & Amann, 2001). Therefore, the physiological, biochemical and phylogenetic properties of strain BT080^T support its description as a novel species within the genus *Bacillus*, for which the name *Bacillus kribbensis* sp. nov. is proposed.

Table 2. Characteristics of strain BT080^T and some related *Bacillus* species

Taxa: 1, strain BT080^T; 2, *B. foraminis* LMG 23174^T (data from Tiago *et al.*, 2006); 3, *B. firmus* DSM 12^T (Pettersson *et al.*, 2000); 4, *B. circulans* DSM 11^T (Pettersson *et al.*, 2000); 5, *Bacillus benzoovorans* DSM 5391^T (Pettersson *et al.*, 2000); 6, *B. fumarioli* LMG 17489^T (Logan *et al.*, 2000); 7, *B. jeotgali* YKJ-10^T (Yoon *et al.*, 2001b). +, Positive; –, negative; v, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Cell shape	Rod	Rod	Rod	Rod	Filamentous	Round-ended rod	Rod
Spore shape	Oval	—	Oval	Oval	Oval	Ellipsoidal/cylindrical	Ellipsoidal
Anaerobic growth	—	—	—	+	—	—	ND
Nitrate reduction	—	+	+	v	+	—	+
Growth with NaCl at:							
5 %	+	—	+	+	—	ND	+
10 %	—	—	+	—	—	ND	+
Growth at 50 °C	—	—	—	—	—	+	+
Acid production from:							
L-Arabinose	—	+	—	+	—	—	—
D-Glucose	+	+	+	+	—	+	+
α -D-Lactose	+	+	—	ND	ND	v	—
D-Mannose	—	+	—	ND	ND	—	—
D-Xylose	+	+	—	—	—	—	—
Hydrolysis of:							
Casein	+	—	+	—	ND	—	—
Gelatin	+	+	+	+	—	+	+
Starch	—	+	+	+	—	ND	+

Description of *Bacillus kribbensis* sp. nov.

Bacillus kribbensis (krib.ben'sis. N.L. masc. adj. *kribbensis* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies on this species were performed).

Cells are aerobic, Gram-positive, spore-forming motile rods that are 1.4–2.0 µm in diameter and 2.0–3.0 µm in

length. Oxidase-negative and catalase-positive. Does not reduce nitrate to nitrite. Grows between 13 and 47 °C (optimum, 30–33 °C) and from pH 4.0 to 9.5 (optimum, pH 5.5–6.5). API ZYM kit gives positive results for esterase (C4), esterase lipase (C8), valine arylamidase, naphthol-AS-BI-phosphohydrolase, β -glucuronidase, α -glucosidase and β -glucosidase. Casein and gelatin are hydrolysed, but Tween 80, starch, urea and L-tyrosine are not hydrolysed. Acids are produced from D-glucose, D-fructose, D-ribose,

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain BT080^T and related taxa. Bootstrap percentages (based on 1000 replicates) greater than 50 % are shown. *Brevibacillus brevis* JCM 2503^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

D-xylose, α -D-lactose, maltose, trehalose, D-melibiose and cellobiose, but not from L-arabinose, adonitol, glycerol, sucrose or D-mannose. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and two unknown amino-group-containing glycerophospholipids. The major isoprenoid quinone is MK-7. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{14:0}, iso-C_{16:0} and iso-C_{15:0}. The DNA G + C content is 43.3 mol% (HPLC).

The type strain, strain BT080^T (=KCTC 13934^T=DSM 17871^T), was isolated from an agricultural field used for potato cultivation in Jeju, Korea.

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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.
- Christensen, H., Bisgaard, M., Frederiksen, W., Mutters, R., Kuhnert, P. & Olsen, J. E. (2001). Is characterization of a single isolate sufficient for valid publication of a new genus or species? Proposal to modify recommendation 30b of the Bacteriological Code (1990 Revision). *Int J Syst Evol Microbiol* **51**, 2221–2225.
- Cohn, F. (1872). Untersuchungen über Bakterien. *Beiträge zur Biologie der Pflanzen*, 1875 1 (Heft 2) 1, 127–224.
- Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.
- Hatayama, K., Shoun, H., Ueda, Y. & Nakamura, A. (2006). *Tuberibacillus calidus* gen. nov., sp. nov., isolated from a compost pile and reclassification of *Bacillus naganensis* Tomimura *et al.* 1990 as *Pullulanibacillus naganensis* gen. nov., comb. nov. and *Bacillus laevolacticus* Andersch *et al.* 1994 as *Sporolactobacillus laevolacticus* comb. nov. *Int J Syst Evol Microbiol* **56**, 2545–2551.
- Heyrman, J., Vanparys, B., Logan, N. A., Balcaen, A., Rodríguez-Díaz, M., Felske, A. & De Vos, P. (2004). *Bacillus novalis* sp. nov., *Bacillus vireti* sp. nov., *Bacillus soli* sp. nov., *Bacillus bataviensis* sp. nov. and *Bacillus drenensis* sp. nov., from the Drentse A grasslands. *Int J Syst Evol Microbiol* **54**, 47–57.
- Heyrman, J., Rodríguez-Díaz, M., Devos, J., Felske, A., Logan, N. A. & De Vos, P. (2005a). *Bacillus arenosi* sp. nov., *Bacillus arvi* sp. nov. and *Bacillus humi* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **55**, 111–117.
- Heyrman, J., Logan, N. A., Rodríguez-Díaz, M., Scheldeman, P., Lebbe, L., Swings, J., Heyndrickx, M. & De Vos, P. (2005b). Study of mural painting isolates, leading to the transfer of '*Bacillus maroccanus*' and '*Bacillus carotarum*' to *Bacillus simplex*, emended description of *Bacillus simplex*, re-examination of the strains previously attributed to '*Bacillus macroides*' and description of *Bacillus muralis* sp. nov. *Int J Syst Evol Microbiol* **55**, 119–131.
- Jeon, C. O., Lim, J.-M., Lee, J. M., Xu, L. H., Jiang, C. L. & Kim, C.-J. (2005). Reclassification of *Bacillus haloalkaliphilus* Fritze 1996 as *Alkalibacillus haloalkaliphilus* gen. nov., comb. nov. and the description of *Alkalibacillus salilacus* sp. nov., a novel halophilic bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* **55**, 1891–1896.
- Kämpfer, P. (1994). Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* **17**, 86–98.
- 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.
- Ko, K. S., Oh, W. S., Lee, M. Y., Lee, J. H., Lee, H., Peck, K. R., Lee, N. Y. & Song, J. H. (2006). *Bacillus infantis* sp. nov. and *Bacillus idriensis* sp. nov., isolated from a patient with neonatal sepsis. *Int J Syst Evol Microbiol* **56**, 2541–2544.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- 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: John Wiley.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, M., Murray, B. L., Syme, N., Wynn-Williams, D. D. & De Vos, P. (2000). Aerobic endospore-forming bacteria from geothermal environments in northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich archipelago, with the proposal of *Bacillus fumarioli* sp. nov. *Int J Syst Evol Microbiol* **50**, 1741–1753.
- Nazina, T. N., Tourova, T. P., Poltarau, A. B., Novikova, E. V., Grigoryan, A. A., Ivanova, A. E., Lysenko, A. M., Petrunka, V. V., Osipov, G. A. & other authors (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzoniensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. *Int J Syst Evol Microbiol* **51**, 433–446.
- Nielsen, P., Fritze, D. & Priest, F. G. (1995). Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. *Microbiology* **141**, 1745–1761.
- Pettersson, B., de Silva, S., Uhlén, M. & Priest, F. G. (2000). *Bacillus siralis* sp. nov., a novel species from silage with a higher order structural attribute in the 16S rRNA genes. *Int J Syst Evol Microbiol* **50**, 2181–2187.
- Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* **134**, 1847–1882.
- Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.
- 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.
- Shivaji, S., Chaturvedi, P., Suresh, K., Reddy, G. S., Dutt, C. B., Wainwright, M., Narlikar, J. V. & Bhargava, P. M. (2006). *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp.

nov. and *Bacillus altitudinis* sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. *Int J Syst Evol Microbiol* **56**, 1465–1473.

Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Microbiology*, pp. 409–443. 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.

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.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Tiago, I., Pires, C., Mendes, V., Morais, P. V., da Costa, M. S. & Veríssimo, A. (2006). *Bacillus foraminis* sp. nov., isolated from a non-saline alkaline groundwater. *Int J Syst Evol Microbiol* **56**, 2571–2574.

Venkateswaran, K., Kempf, M., Chen, F., Satomi, M., Nicholson, W. & Kern, R. (2003). *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are γ -radiation resistant. *Int J Syst Evol Microbiol* **53**, 165–172.

Wainø, M., Tindall, B. J., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.

Wisotzkey, J. D., Jurtshuk, P., Jr, Fox, G. E., Deinhard, G. & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* **42**, 263–269.

Yoon, J. H., Weiss, N., Lee, K. C., Lee, I. S., Kang, K. H. & Park, Y. H. (2001a). *Jeotgalibacillus alimentarius* gen. nov., sp. nov., a novel bacterium isolated from jeotgal with L-lysine in the cell wall, and reclassification of *Bacillus marinus* Ruger 1983 as *Marinibacillus marinus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 2087–2093.

Yoon, J. H., Kang, S. S., Lee, K. C., Kho, Y. H., Choi, S. H., Kang, K. H. & Park, Y. H. (2001b). *Bacillus jeotgali* sp. nov., isolated from jeotgal, Korean traditional fermented seafood. *Int J Syst Evol Microbiol* **51**, 1087–1092.