Journal of Microbiology (2013) Vol. 51, No. 3, pp. 400–404
DOI 10.1007/s12275-013-2664-1

NOTE

Lysinibacillus chungkukjangi sp. nov., Isolated from Chungkukjang, Korean Fermented Soybean Food[§]

Soo-Jin Kim[1], Yun-Hee Jang[1], Moriyuki Hamada[2], Jae-Hyung Ahn[1], Hang-Yeon Weon[1], Ken-ichiro Suzuki[2], Kyung-Sook Whang[3], and Soon-Wo Kwon[1]*

[1]*Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea*
[2]*NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan*
[3]*Institute of Microbial Ecology and Resources, Mokwon University, Daejeon 302-729, Republic of Korea*

(Received November 30, 2012 / Accepted March 6, 2013)

One bacterial strain 2RL3-2^T was isolated from Chungkukjang, a traditional Korean fermented food made from soybeans, and determined to be a Gram-positive, aerobic, spore-forming rod. Growth of the novel strain was optimal at 30°C and pH 7.0. The 16S rRNA gene of strain 2RL3-2^T showed the highest level of sequence similarity to *Lysinibacillus sinduriensis* BLB-1^T (99.0%), *Lysinibacillus massiliensis* 4400831^T (97.1%), *Lysinibacillus xylanilyticus* XDB9^T (97.0%), and *Lysinibacillus odysseyi* 34hs-1^T (96.8%). Phylogenetic analysis showed that strain 2RL3-2^T formed a robust cluster with *L. sinduriensis* BLB-1^T, *L. massiliensis* 4400831^T, and *L. odyssey* 34hs-1^T. The major fatty acids were anteiso-$C_{15:0}$ (47.3%), iso-$C_{16:0}$ (16.3%), and anteiso-$C_{17:0}$ (11.3%), and the only menaquinone was MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine were the major polar lipids, along with an unknown phospholipid and two unknown lipids. The peptidoglycan type was A4*α*, with an interpeptide bridge of l-Lys–d-Asp. DNA-DNA hybridization values between strain 2RL3-2^T and closely related *Lysinibacillus* species were below 43±4%. Therefore, based on phenotypic, chemotaxonomic, and phylogenetic characteristics, it was determined that strain 2RL3-2^T represents a novel species of the genus *Lysinibacillus*, for which the name *Lysinibacillus chungkukjangi* sp. nov. is proposed. The type strain is 2RL3-2^T (=KACC 16626^T =NBRC 108948^T).

*For correspondence. E-mail: swkwon1203@korea.kr; Tel.: +82-31-299-1860; Fax: +82-31-299-1869
[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.
Footnote. The GenBank accession no. for the 16S rRNA gene sequence of strain 2RL3-2^T is JX217747.

Keywords: DNA-DNA hybridization, *Lysinibacillus chungkukjangi*, taxonomy, 16S rRNA

The genus *Lysinibacillus* was proposed for two former *Bacillus* species, *Bacillus fusiformis* and *Bacillus sphaericus*, and a newly described species *Lysinibacillus boronitolerans* (Ahmed *et al*., 2007). Members of this genus have been isolated from environments such as soil, human cerebrospinal fluid, spacecraft surface, and tidal flat sediment (Ahmed *et al*., 2007; Miwa *et al*., 2009; Lee *et al*., 2010; Coorevits *et al*., 2012; Jung *et al*., 2012; Yang *et al*., 2012). The genus is characterized by spore-forming, motile rods, with A4*α*-type (Lys-Asp) cell wall peptidoglycan and iso-$C_{15:0}$ or anteiso-$C_{15:0}$ as the major fatty acids. The dominant respiratory lipoquinone system is MK-7, and polar lipid profiles from this genus contained diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine as the predominant lipids. At present, nine species, *L. boronitolerans*, *L. fusiformis*, *L. sphaericus* (Ahmed *et al*., 2007), *L. parviboronicapiens* (Miwa *et al*., 2009), *L. xylanilyticus* (Lee *et al*., 2010), *L. sinduriensis*, *L. massiliensis*, *L. odysseyi* (Jung *et al*., 2012), and *L. macroides* (Coorevits *et al*., 2012), have been described.

Chungkukjang is a traditional Korean fermented food made from soybeans. *Bacillus* strains are largely involved in the fermentation process (Lee *et al*., 2005; Choi *et al*., 2008; Seo *et al*., 2009). In this study, Chungkukjang was made with commercially purchased soybeans. To make Chungkukjang, 120 g of soybeans were immersed in water for 12 h, dehydrated for 30 min, and then boiled for 5 h. The boiled soybeans were incubated at 37°C for 30 h without artificial inoculation of *Bacillus* strains. One gram of fermented Chunkukjang was collected for isolation of microorganisms. Chungkukjang samples were suspended in 0.85% NaCl solution, and serially diluted. Several bacterial strains were isolated on R2A (Difco, USA). Among them, one bacterial strain, 2RL3-2^T, was identified as a *Lysinibacillus* species.

The 16S rRNA gene of 2RL3-2^T was amplified using the universal primers 9F and 1512R (Weisburg *et al*., 1991), and was sequenced by Genotec (Korea). The resultant 16S rRNA gene sequence was a continuous stretch of 1,493 bp. The 16S rRNA gene sequence of strain 2RL3-2^T was aligned with those of the closely related reference organisms obtained from GenBank, using the integrated SINA alignment tool from the ARB-silva website (Pruesse *et al*., 2007). Sequence similarities were calculated via the EzTaxon server (http://www.eztaxon.org/; Chun *et al*., 2007). Phylogenetic trees

were constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using MEGA 4 software (Tamura *et al*., 2007), with bootstrap values based on 1,000 replicates (Felsenstein, 1985). Strain 2RL3-2^T showed the greatest sequence similarity to *L. sinduriensis* (99.0%), *L. massiliensis* 4400831^T (97.1%), *L. xylanilyticus* XDB9^T (97.0%), and *L. odysseyi* 34hs-1^T (96.8%). The 16S rRNA sequences from the type strains of all the other species showed <96.0% sequence similarity to that of 2RL3-2^T. Based on the neighbor-joining phylogenetic tree (Fig. 1), strain 2RL3-2^T was determined to be a member of the genus *Lysinibacillus*, and formed a robust cluster with *L. sinduriensis*, *L. massiliensis*, and *L. odyssey*, supported by a high bootstrap value (86%). This cluster was also verified by the maximum parsimony tree (Fig. 1).

Cell morphology and motility were observed by phase-contrast (AXIO; Zeiss) and transmission electron (LEO model 912AB) microscopy using cells grown for 2 days on trypticase soy agar (TSA; Difco) at 30°C. For spore observation, cells were grown on TSA supplemented with $MnSO_4$ (5 mg/L) at 30°C for 7 days, and then observed with phase-contrast microscopy. Gram-staining was performed according to the method of Hucker (Smibert and Krieg, 1994). Catalase activity was assessed by bubble production in 3% (v/v) H_2O_2, and oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization and enzyme activities were examined using API ZYM, API 20NE, API 20E, and API ID 32GN test strips (bioMérieux). API tests were conducted according to the manufacturer's instructions, except that the assimilation reactions embedded in API 20NE, API 20E, and API ID 32GN test strips were observed for 10 days. Growth at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45, and 50°C was assessed following incubation for 14 days in trypticase soy broth (TSB; Difco). Growth at pH 5.0–10.0 (at intervals of 1.0 pH unit) was assessed after 14 days in TSB with the pH adjusted with citrate/phosphate buffer or Tris-hydrochloride buffer (Breznak and Costilow, 1994). Growth in 0–10% (w/v) NaCl (at intervals of 1% NaCl) was assessed following 14 days in TSB. Anaerobic growth was assessed by incubation in a BBL GasPak Anaerobic System (Difco) for 14 days at 30°C on TSA. Casein, starch, and tyrosine degradation were examined on TSA plates containing milk powder (5%, w/v), starch (1%, w/v), and tyrosine (0.1%, w/v), respectively. Hydrolysis of CM-cellulose and Tween 80 was examined using TSA supplemented with 1% (w/v) substrates, respectively, while degradation of chitin, hypoxanthine, pectin, and xanthine was tested according to the methods of Smibert and Krieg (1994). DNase activity was determined with DNase test agar (Difco). For phenotypic comparison, we examined five reference species, including *L. sinduriensis* KACC 16611^T, *L. massiliensis* KACC 14317^T, and *L. odysseyi* KACC 14001^T, which were grouped into one cluster in the phylogenetic tree (Fig. 1), *L. xylanilyticus* KACC 15113^T, which showed a high level of sequence similarity (97.0%) to strain 2RL3-2^T, and the type species of the genus *Lysinibacillus*, *L. boronitolerans* KACC 15323^T. Strain 2RL3-2^T was determined to be a Gram-positive, aerobic, motile rod (1.0 × 2.0–4.5 μm), which formed a terminal spherical spore with a swollen sporangium. Strain 2RL3-2^T colonies grown on TSA medium at 30°C for 2 days were circular, convex, opaque, and dark-brown, and strong growth was observed on TSA, Luria-Bertani agar (LB; Difco), and nutrient agar (NA; Difco). However, strain 2RL3-2^T showed weak growth on R2A and could not grow on MacConkey agar (Difco). Growth were observed in the range of 10–45°C and pH 6–9. The strain survived in media containing up to 3% (w/v) NaCl. Other physiological properties are shown in the species description and Table 1.

To quantify the whole-cell fatty acids, strains 2RL3-2^T, *L. boronitolerans* KACC 15323^T, *L. massiliensis* KACC 14317^T, *L, odyssey* KACC 14001^T, *L. sinduriensis* KACC 16611^T, and *L. xylanilyticus* KACC 15113^T were grown on TSA at 30°C

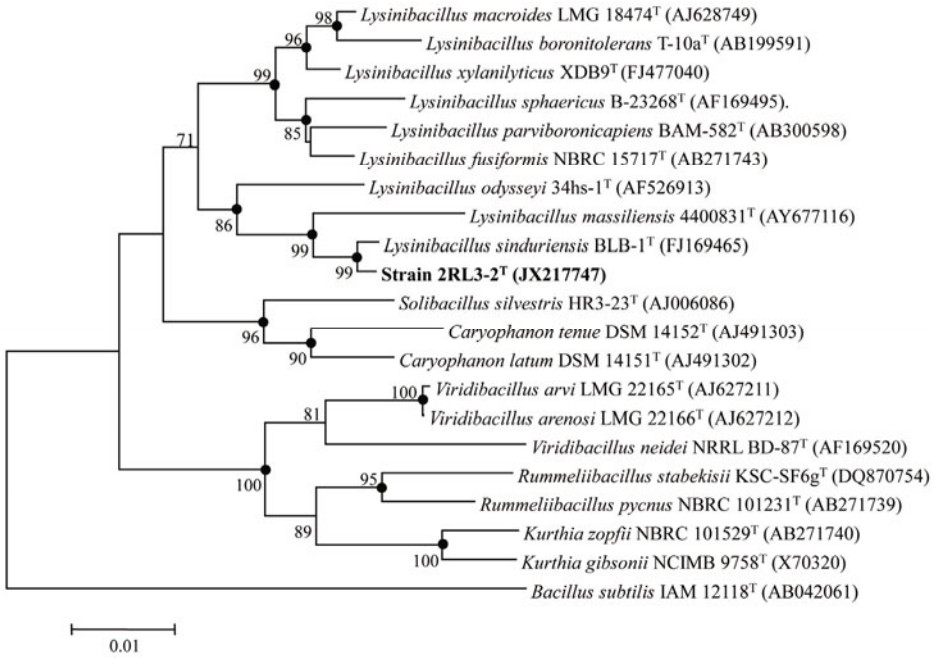

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain 2RL3-2^T. Bootstrap percentages (based on 1,000 replicates) >70% are shown at branching points. Dots indicate that the corresponding branches are also present in the maximum-parsimony tree. Bar, 0.01 accumulated changes per nucleotide.

for 24 h. The fatty acid methyl esters were identified and quantified using the TSBA 6 database (version 6.10) of the Sherlock Microbial Identification System (MIDI). Menaquinones and polar lipids were extracted and analyzed by the method of Minnikin *et al.* (1984). For peptidoglycan analysis, strain 2RL3-2^T was grown in shake flasks containing liquid NBRC medium 802 (1.0% polypeptone, 0.2% yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$; pH 7.0) on a rotary shaker for 24 h at 30°C. Cell wall samples were prepared from approximately 1 g of wet cells by mechanical disruption with an ultrasonic oscillator and glass beads. The cell walls were separated from unbroken cells by differential centrifugation in distilled water, and further purified in boiling 4% SDS (100°C, 40 min), followed by several washings with distilled water. Molar ratios of the amino acids in cell-wall hydrolysates (4 M HCl, 16 h) were determined using the method described by Hamada *et al.* (2010). The amino acid isomers in cell-wall hydrolysates were examined using the method described by Nozawa *et al.* (2007), using a liquid chromatograph-mass spectrometer (LC-MS; model LCMS-2020; Shimadzu). The DNA G+C content was determined using a high-performance liquid chromatography method (Mesbah *et al.*, 1989). The cellular fatty acids of strain 2RL3-2^T were: anteiso-$C_{15:0}$ (47.3%), iso-$C_{16:0}$ (16.3%), anteiso-$C_{17:0}$ (11.3%), iso-$C_{15:0}$ (7.5%), iso-$C_{14:0}$ (6.9%), $C_{16:0}$ (4.5%), $C_{16:1}$ $\omega 7c$ alcohol (2.2%), and $C_{16:1}$ $\omega 11c$ (1.0%) (Table 2). The only menaquinone was MK-7, and the polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown phospholipid, and two unknown lipids (Supplementary data Fig. S1). The peptidoglycan contained alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), and lysine (Lys) in a molar ratio of 1.7:0.6:1.0:0.7. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, L-Ala, D-Asp, D-Glu, and L-Lys. These data suggested that the peptidoglycan type of strain 2RL3-2^T was A4α, with an interpeptide bridge comprising L-Lys–D-Asp, A11.31 (Schleifer and Kandler, 1972; Schumann, 2011). The DNA G+C content of strain 2RL3-2^T was 41.8%, which was higher than those of other *Lysinibacillus* species (35.7–38.7% mol%).

DNA-DNA hybridization was carried out as described by Seldin and Dubnau (1985). Probe labeling was conducted using a nonradioactive DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Molecular Biochemicals, USA). The hybridized DNA was visualized using a DIG luminescent detection kit (Roche Molecular Biochemicals). DNA-DNA relatedness was quantified with a densitometer (Bio-Rad, USA). DNA-DNA hybridization values between

Table 1. Different phenotypic characteristics of strain 2RL3-2^T and closely related species of the genus *Lysinibacillus*

Species: 1, strain 2RL3-2^T; 2, *Lysinibacillus boronitolerans* KACC 15323^T (Ahmed *et al.*, 2007; Jung *et al.*, 2012; this study); 3, *Lysinibacillus massiliensis* KACC 14317^T (*Glazunova et al.*, 2006; Jung *et al.*, 2012; this study); 4, *Lysinibacillus odyssey* KACC 14001^T (La Duc *et al.*, 2004; Jung *et al.*, 2012; this study); 5, *Lysinibacillus sinduriensis* KACC 16611^T (Jung *et al.*, 2012; this study); 6, *Lysinibacillus xylanilyticus* KACC 15113^T (Lee *et al.*, 2010; this study). The results of assimilation and enzymatic activities were obtained using API test strips in this study. All strains were catalase-positive. All strains were negative for glucose fermentation, H_2S production, indole production, and nitrate reduction. None of the strains assimilate adipic acid, L-arabinose, capric acid, D-glucose, D-maltose, D-mannitol, or D-mannose. All strains show negative activity for arginine dihydrolase, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase, ornithine decarboxylase, trypsin, tryptophan deaminase, and valine arylamidase. +, positive; -, negative; w, weak positive.

Characteristics	1	2	3	4	5	6
Isolation source	Fermented soybeans	Soil	Human CSF	Spacecraft	Tidal flat sediment	Forest humus
Temperature range	10–45	16–45	25–45	25–42	15–45	10–40
Growth in 5% NaCl	-	+	+	+	+	+
Oxidase	+	+	+	+	-	ND
Voges-Proskauer	w	+	+	+	-	+
Hydrolysis of:						
Aesculin	+	+	-	+	+	-
Gelatin	+	-	-	+	+	+
Urea	-	+	-	-	-	+
Assimilation of (API test strips)[a]:						
N-Acetylglucosamine	-	+	-	-	-	+
Potassium gluconate	+	+	+	-	+	-
Malic acid	+	+	+	+	-	+
Trisodium citrate	-	+	-	-	-	+
Phenylacetic acid	-	-	-	-	-	+
Enzymatic activity of (API test strips)[a]:						
Alkaline phosphatase	+	-	-	-	-	+
Esterase (C4)	-	-	+	+	-	+
Esterase lipase (C8)	-	-	+	+	-	-
Leucine arylamidase	-	+	+	-	-	+
α-Chymotrypsin	+	+	-	+	-	-
Acid phosphatase	+	+	+	+	-	+
Naphthol-AS-BI-phosphohydrolase	-	+	+	-	+	+
DNA G+C content (mol%)	41.8	36.5	36.3	35.7	35.9	37.2

[a] Data from this study.

Table 2. Cellular fatty acid composition of strain 2RL3-2^T and type strains of closely related *Lysinibacillus* species
Strains: 1, 2RL3-2^T; 2, *Lysinibacillus boronitolerans* KACC 15323^T; 3, *Lysinibacillus massiliensis* KACC 14317^T; 4, *Lysinibacillus odyssey* KACC 14001^T; 5, *Lysinibacillus sinduriensis* KACC 16611^T; 6, *Lysinibacillus xylanilyticus* KACC 15113^T. All data were obtained in this study using cells retrieved following growth on TSA at 30°C for 24 h. -, <1.0% or not detected.

Fatty acids	1	2	3	4	5	6
iso-$C_{14:0}$	6.9	3.0	2.2	12.7	2.8	3.9
$C_{14:0}$	-	-	-	-	-	2.4
iso-$C_{15:0}$	7.5	32.7	51.4	20.0	25.9	31.4
anteiso-$C_{15:0}$	47.3	21.6	18.0	9.1	23.8	5.0
$C_{16:1}$ *ω7c* alcohol	2.2	9.2	3.2	14.1	6.2	16.2
iso-$C_{16:0}$	16.3	12.0	7.1	31.7	10.0	12.4
$C_{16:1}$ *ω11c*	1.0	3.4	2.0	2.7	2.0	3.4
$C_{16:0}$	4.5	2.2	1.8	1.8	1.6	3.6
iso-$C_{17:1}$ *ω10c*	-	1.2	-	-	1.7	3.3
iso-$C_{17:0}$	-	3.1	4.5	2.2	5.7	4.6
anteiso-$C_{17:0}$	11.3	7.6	7.2	2.4	17.5	2.6
$C_{18:1}$ *ω9c*	-	-	-	-	-	1.2
$C_{18:0}$	-	-	-	-	-	2.7
anteiso-$C_{19:0}$	-	-	-	-	-	-
Summed feature 4[a]	-	2.8	-	-	2.6	2.5

[a] Summed Feature 4 means iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

2RL3-2^T and *L. sinduriensis* KACC 16611^T, *L. massiliensis* KACC 14317^T, and *L. xylanilyticus* KACC 15113^T were 43±4% (reciprocal, 40±5%), 34±6%, and 26±2%, respectively (Supplementary data Table S1). The recommended criteria for delineating bacterial species (Wayne *et al*., 1987) state that <70% DNA-DNA relatedness indicates that a strain belongs to a separate species. Therefore, strain 2RL3-2^T was determined to be a novel species.

Strain 2RL3-2^T can be differentiated from *L. sinduriensis* KACC 16611^T, its closest relative, on the basis of growth temperature range, oxidase activity, esculin hydrolysis, urease activity, assimilation of malic acid, various enzymatic activities, and the quantitative amount of fatty acids (Tables 1 and 2). The differential properties among strain 2RL3-2^T and other closely related *Lysinibacillus* species is also shown in Tables 1 and 2. Based on the phylogenetic, genotypic, and phenotypic data, strain 2RL3-2^T represents a novel species in the genus *Lysinibacillus*, for which the name *Lysinibacillus chungkukjangi* sp. nov. is proposed.

Description of *Lysinibacillus chungkukjangi* sp. nov.

Lysinibacillus chungkukjangi (Chung.kuk.jan'gi. N.L. gen. n. *chungkukjangi*, of chungkukjang, a traditional Korean fermented food).

The strain is a Gram-positive, strictly aerobic, motile rod (1.0 × 2.0–4.5 μm) with a terminal spherical spore within a swollen sporangium. Colonies grown on TSA medium are circular, convex, opaque, and dark-brown. It grows on TSA, LB, and NA, and weakly on R2A, but does not grow on MacConkey agar. Growth occurs in the range of 10–45°C (optimum, 30°C), pH 6–9 (optimum, pH 7.0), and 0–3% (w/v) NaCl (optimum, 0%). It hydrolyzes DNA but not chitin, CM-cellulose, hypoxanthine, pectin, starch, Tween 80, tyrosine, or xanthine. Catalase- and oxidase-positive. The strain is positive for esculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, urease and arginine dihydrolase (API 20NE test strip). It assimilates potassium gluconate and malic acid but not *N*-acetylglucosamine adipic acid, L-arabinose, capric acid, D-glucose, D-maltose, D-mannitol, D-mannose, phenylacetic acid, or trisodium citrate (API 20NE test strip). We found positive activity for acid phosphatase, alkaline phosphatase, and *α*-chymotrypsin, but negative for *N*-acetyl-*β*-glucosaminidase, cystine arylamidase, esterase (C4), esterase lipase (C8), *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *β*-glucuronidase, leucine arylamidase, lipase (C14), *α*-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase (API ZYM test strip). The major cellular fatty acids (>10%) are anteiso-$C_{15:0}$, iso-$C_{16:0}$, and anteiso-$C_{17:0}$. MK-7 is the only menaquinone. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown phospholipid, and two unknown lipids. The peptidoglycan type is A4*α*, with an interpeptide bridge comprising L-Lys–D-Asp. The DNA G+C content of the type strain is 41.8%.

The type strain, 2RL3-2^T (=KACC 16626^T =NBRC 108948^T), was isolated from Chungkukjang, a traditional Korean fermented food made of soybeans.

This study was supported by the "Research Program for Agricultural Science & Technology Development (Project No. PJ008676)", National Academy of Agricultural Science, Rural Development Administration, Republic of Korea. The authors thank Professor J. P. Euzéby of the Ecole Nationale Vétérinaire in Toulouse for advice concerning the naming.

References

Ahmed, I., Yokota, A., Yamazoe, A., and Fujiwara, T. 2007. Proposal of *Lysinibacillus boronitolerans* gen. nov., sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. *Int. J. Syst. Evol. Microbiol.* **57**, 1117–1125.

Breznak, J.A. and Costilow, R.N. 1994. Physicochemical factors in growth, Methods for General and Molecular Bacteriology, pp. 137–154. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.). American Society for Microbiology, Washington, D.C., USA.

Choi, J.M., Yi, N.R., Seo, K.C., Han, J.S., Song, Y.O., and Cho, E.J. 2008. Protective effect of Chungkukjang from Sunchang province against cellular oxidative damage. *J. Food Sci. Nutr.* **13**, 90–94.

Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and 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.

Coorevits, A., Dinsdale, A.E., Heyrman, J., Schumann, P., Van Landschoot, A., Logan, N.A., and De Vos, P. 2012. *Lysinibacillus macroides* sp. nov., nom. rev. *Int. J. Syst. Evol. Microbiol.* **62**, 1121–1127.

Felsenstein, J. 1985. Confidence limits 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.

Glazunova, O.O., Raoult, D., and Roux, V. 2006. *Bacillus massiliensis* sp. nov., isolated from cerebrospinal fluid. *Int. J. Syst. Evol. Microbiol.* **56**, 1485–1488.

Hamada, M., Iino, T., Iwami, T., Harayama, S., Tamura, T., and Suzuki, K. 2010. *Mobilicoccus pelagius* gen. nov., sp. nov. and *Piscicoccus intestinalis* gen. nov., sp. nov., two new members of the family *Dermatophilaceae*, and reclassification of *Dermatophilus chelonae* (Masters *et al*. 1995) as *Austwickia chelonae* gen. nov., comb. nov. *J. Gen. Appl. Microbiol.* **56**, 427–436.

Jung, M.Y., Kim, J.S., Paek, W.K., Styrak, I., Park, I.S., Sin, Y., Paek, J., Park, K.A., Kim, H., Kim, H.L., Tindall, B.J., and Chang, Y.H. 2012. Description of *Lysinibacillus sinduriensis* sp. nov., and transfer of *Bacillus massiliensis* and *Bacillus odysseyi* to *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov. with emended description of the genus. *Int. J. Syst. Evol. Microbiol.* **62**, 2347–2355.

La Duc, M.T., Satomi, M., and Venkateswaran, K. 2004. *Bacillus odysseyi* sp. nov., a round-spore-forming bacillus isolated from the Mars Odyssey spacecraft. *Int. J. Syst. Evol. Microbiol.* **54**, 195–201.

Lee, C.S., Jung, Y.T., Park, S., Oh, T.K., and Yoon, J.H. 2010. *Lysinibacillus xylanilyticus* sp. nov., a xylan-degrading bacterium isolated from forest humus. *Int. J. Syst. Evol. Microbiol.* **60**, 281–286.

Lee, M.Y., Park, S.Y., Jung, K.O., Park, K.Y., and Kim, S.D. 2005. Quality and functional characteristics of Chungkukjang prepared with various *Bacillus* sp. isolated from traditional Chungkukjang. *J. Food Sci.* **70**, M195–M196.

Mesbah, M., Premachandran, U., and 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., and Parlett, J.H. 1984. Anintegrated procedure for the extraction of bacterial isoprenoid quinones and polarlipids.*J. Microbiol. Methods* **2**, 233–241.

Miwa, H., Ahmed, I., Yokota, A., and Fujiwara, T. 2009. *Lysinibacillus parviboronicapiens* sp. nov., a low-boron-containing bacterium isolated from soil. *Int. J. Syst. Evol. Microbiol.* **59**, 1427–1432.

Nozawa, Y., Sakai, N., Arai, K., Kawasaki, Y., and Harada, K. 2007. Reliable and sensitive analysis of amino acids in the peptidoglycan of actinomycetes using the advanced Marfey's method. *J. Microbiol. Methods* **70**, 306–311.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. 2007. Silva: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188–7196.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Schleifer, K.H. and Kandler, O. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**, 407–477.

Schumann, P. 2011. Peptidoglycan Structure. *In* Rainey, F. and Oren, A. (eds.) Taxonomy of Prokaryotes, vol. 38, pp. 101–129. Methods in Microbiology. Academic Press, London, UK.

Seldin, L. and Dubnau, D. 1985. Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int. J. Syst. Bacteriol.* **35**, 151–154.

Seo, H.R., Kim, J.Y., Kim, J.H., and Park, K.Y. 2009. Identification of *Bacillus cereus* in a Chungkukjang that showed high anticancer effects against AGS human gastric adenocarcinoma cells. *J. Med. Food* **12**, 1274–1280.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. *In* Gerhart, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.) Methods for General and Molecular Bacteriology, pp. 607–654. American Society for Microbiology, Washington, D.C., USA.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596–1599.

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., Stackebrandt, E., and *et al.* 1987. Report of the ad hoc committee on reconciliation of approaches of bacterial systematics. *Int. J. Syst. Bacteriol.* **37**, 463–464.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.

Yang, L.L., Huang, Y., Liu, J., Ma, L., Mo, M.H., Li, W.J., and Yang, F.X. 2012. *Lysinibacillus mangiferahumi* sp. nov., a new bacterium producing nematicidal volatiles. *Antonie van Leeuwenhoek* **102**, 53–59.