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Description of *Lysinibacillus sinduriensis* sp. nov., and transfer of *Bacillus massiliensis* and *Bacillus odysseyi* to the genus *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov. with emended description of the genus *Lysinibacillus*

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A Gram-positive, rod-shaped, endospore-forming bacterium, designated strain BLB-1^T, was isolated from samples of tidal flat sediment from the Yellow Sea. 16S rRNA gene sequence analysis demonstrated that the isolate belonged to the *Bacillus* rRNA group 2 and was closely related to *Bacillus massiliensis* CIP 108446^T (97.4 %), *Bacillus odysseyi* ATCC PTA-4993^T (96.7 %), *Lysinibacillus fusiformis* DSM 2898^T (96.2 %) and *Lysinibacillus boronitolerans* DSM 17140^T (95.9 %). Sequence similarities with related species in other genera, including *Caryophanon*, *Sporosarcina* and *Solibacillus*, were <96.1 %. Chemotaxonomic data supported the affiliation of strain BLB-1^T with the genus *Lysinibacillus*. The major menaquinone was MK-7, the cell-wall sugars were glucose and xylose, the cell-wall peptidoglycan type was A4α (L-Lys–D-Asp), the major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and several unknown phospholipids, and the major fatty acids were anteiso-$C_{15:0}$ (35.6 %), iso-$C_{15:0}$ (25.6 %) and anteiso-$C_{17:0}$ (16.5 %). The most closely related species, *Bacillus massiliensis* and *Bacillus odysseyi*, were also assigned to this genus based on phylogenetic analysis and phenotypic data. The results of DNA–DNA hybridizations and phenotypic tests supported the differentiation of all three taxa from species of the genus *Lysinibacillus* with validly published names. Thus, strain BLB-1^T (=KCTC 13296^T =JCM 15800^T) represents a novel species, for which the name *Lysinibacillus sinduriensis* sp. nov. is proposed. It is also proposed that *Bacillus massiliensis* CIP 108446^T (=4400831^T

Abbreviations: AL, aminolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; GBG, gentiobiosyldiacylglycerol; GL, glycolipid; L1, unknown lipid; NPG, ninhydrin-positive phosphoglycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; PS, phosphatidylserine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Lysinibacillus sinduriensis* BLB-1^T is FJ169465.

A supplementary figure is available with the online version of this paper.

=CCUG49529^T =KCTC 13178^T) and *Bacillus odysseyi* NBRC 100172^T (=34hs-1^T =ATCC PTA-4993^T =NRRL B-30641^T =DSM 18869^T =CIP 108263^T =KCTC 3961^T) be transferred to the genus *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov., respectively.

Species of the genus *Bacillus* have been isolated from diverse habitats (Nakamura *et al.*, 2002; La Duc *et al.*, 2004; Heyrman *et al.*, 2005; Glazunova *et al.*, 2006). Based on genotypic analysis and chemotaxonomic data, several spore-forming species in the *Bacillus* rRNA group 2 (Ash *et al.*, 1991) were reclassified into the following novel taxa: *Sporosarcina globispora*, *Sporosarcina psychrophila* and *Sporosarcina pasteurii* (Yoon *et al.*, 2001); *Lysinibacillus fusiformis* and *Lysinibacillus sphaericus* (Ahmed *et al.*, 2007); *Viridibacillus arvi*, *Viridibacillus arenosi* and *Viridibacillus neidei* (Albert *et al.*, 2007); *Solibacillus silvestris* (Krishnamurthi *et al.*, 2009); *Rummeliibacillus pycnus* (Vaishampayan *et al.*, 2009); and *Psychrobacillus insolitus*, *Psychrobacillus psychrotolerans* and *Psychrobacillus psychrodurans* (Krishnamurthi *et al.*, 2010). Several investigators have suggested that some species placed within the *Bacillus* rRNA group 2 do not belong to the genus *Bacillus* and that they should be reclassified as novel genera or transferred to existing genera within the group (Farrow *et al.*, 1994; Yoon *et al.*, 2001; Ahmed *et al.*, 2007; Albert *et al.*, 2007; Krishnamurthi *et al.*, 2009). Based on polyphasic taxonomic data, a new genus, *Lysinibacillus*, was recently proposed for the reclassification of two species assigned to the genus *Bacillus* and a newly described species (Ahmed *et al.*, 2007). At the time of publication, the genus *Lysinibacillus* contained six species with validly published names: *Lysinibacillus boronitolerans*, *Lysinibacillus fusiformis*, *Lysinibacillus sphaericus* (Ahmed *et al.*, 2007), *Lysinibacillus parviboronicapiens* (Miwa *et al.*, 2009), *Lysinibacillus xylanilyticus* (Lee *et al.*, 2010) and *Lysinibacillus macroides* (Coorevits *et al.*, 2012). The taxonomic positions of *Bacillus massiliensis* and *Bacillus odysseyi* indicate that these species are phylogenetically distantly related to *Rummeliibacillus pycnus* or *Solibacillus silvestris*. This issue has been discussed previously (Ahmed *et al.*, 2007; Krishnamurthi *et al.*, 2009) but no proposal has yet been made for transferring these species into new or existing genera. In the present study, we used a polyphasic taxonomic approach to re-evaluate the taxonomic position of *B. massiliensis* and *B. odysseyi*, as well as characterize a strain isolated from tidal flat sediments during this study, identifying it as a novel species.

A novel strain, designated BLB-1^T, was isolated from 50 g samples of tidal flat sediment collected at a depth of 20 cm from the Shinduri sand dunes (35° 50′ 09.22″ N 126° 12′ 11.90″ E) at Tae-An on the Yellow Sea coast, Republic of Korea. Samples were individually placed into 50 ml sterile tubes (Corning) and pooled into an anaerobic pouch (GasPak, Becton Dickinson) in an icebox, before transporting them to the laboratory for immediate processing. Each sample was serially diluted in saline solution (0.85 %, w/v, NaCl), spread onto tryptic soy broth medium (TSB; pH 7.3; Difco) solidified with 15.0 g l^{-1} agar (TSBA) and incubated at 30 °C for 48 h. The strain was subcultured several times to obtain a purified culture, before further characterization. Reference strains used in this study included *B. massiliensis* KCTC 13178^T, *B. odysseyi* KCTC 3961^T, *L. boronitolerans* KCTC 13709^T, *L. fusiformis* KCTC 3454^T and *L. sphaericus* KCTC 3346^T. Strains were cultured routinely on TSBA medium under the same conditions before storage at −80 °C as skimmed milk (Difco) suspensions (10 %, w/v).

To determine the differential phenotypic properties of the novel isolate, strain BLB-1^T and several reference strains were subjected to morphological, physiological and biochemical analyses (Chang *et al.*, 2002, 2008). All tests were performed using fresh cultures of purified strains grown under the same conditions. Cell morphology was examined by bright-field microscopy (Nikon Optiphot-2), phase-contrast microscopy (Nikon 80i) and electron microscopy (S4300N, Hitachi). For the electron microscopic analysis, cells were fixed in a 2.5 % (v/v) paraformaldehyde/glutaraldehyde mixture, sputter-coated with gold (SC502, Polaron) and observed using a scanning electron microscope (S4300N, Hitachi). Cells were negatively stained with 1 % (w/v) uranyl acetate and the flagella type was observed using a model CM-20 transmission electron microscope (Philips) (Chang *et al.*, 2002). Growth at 10–60 °C and in 0–7 % (w/v) NaCl (0.5 % increments) was determined over a period of 3–7 days in TSB medium. Growth was assessed by monitoring OD_{600} using a spectrophotometer (Bio-Rad). The pH range for growth was determined in buffered TSB medium at 30 °C using a 500 ml flask (Corning) containing 250 ml medium at pH 4–9 (increments of 0.5 pH units). The medium was buffered using one of three different solutions: 50 mM succinic acid/NaOH (pH 4–6), 100 mM Na_2HPO_4/NaH_2PO_4 (pH 6–8) or 50 mM 2-amino-2-methyl-1,3-propanediol/HCl (pH 8–9). Motility was tested in TSB medium containing 0.4 % agar. Gram reaction, enzyme activity, carbohydrate utilization and hydrolysis reactions were conducted using standard methods (Smibert & Krieg, 1994; Chang *et al.*, 2002). The catalase and oxidase activity, indole production, nitrate reduction, KOH test and endospore staining were conducted as previously described (Smibert & Krieg, 1994). Hydrolysis of casein and starch were tested on skimmed milk agar and starch agar, respectively. Gelatin liquefaction was determined by inoculation on 12 % gelatin followed by incubation for 7 days. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease, utilization

of citrate and amino acids activities, the Voges–Proskauer test and utilization of various carbohydrates were determined as previously described (Smibert & Krieg, 1994; Chang *et al.*, 2002). A variety of tests to determine biochemical characteristics were also performed using API 20 E, API ZYM, and API 50 CH B/E strips (bioMérieux), and the Biolog GP2 MicroPlate system (Biolog).

Strain BLB-1^T was catalase-positive and oxidase-negative. Catalase activity was also present in *B. massiliensis* and *B. odysseyi*, whereas oxidase activity was not detected in strain *B. odysseyi* (La Duc *et al.*, 2004; Glazunova *et al.*, 2006). At the time of publication, *L. parviboronicapiens* was the only oxidase-negative species out of all the species in the genus *Lysinibacillus* (Miwa *et al.*, 2009). No sugars were fermented in the API 50 CH strips with strain BLB-1^T or *B. odysseyi*, which matched the results of tests performed with *B. massiliensis* (Glazunova *et al.*, 2006). Strain BLB-1^T and *B. massiliensis* cells produced spherical endospores whereas *B. odysseyi* produced round endospores in the terminal position. Detailed phenotypic characteristics of strain BLB-1^T are presented in Table 1 and the species description.

A nearly complete 16S rRNA gene sequence (1413 bp) for strain BLB-1^T was obtained using the method of Chang *et al.* (2008). Preliminary sequence comparison was conducted against 16S rRNA gene sequences of species with validly published names retrieved from the EzTaxon and GenBank databases of prokaryotic type strains. The nearly complete 16S rRNA gene sequences of the strains were aligned manually against those of closely related representatives from other genera based on the bacterial 16S rRNA secondary structure model (Woese *et al.*, 1980). The regions available for all sequences (conserved and variable regions) showed unambiguous alignment and they were used to reconstruct phylogenetic trees. Sequences were aligned and neighbour-joining (Saitou & Nei, 1987) analysis was performed using the PHYLIP (Felsenstein, 1993) and jPHYDIT (Jeon *et al.*, 2005) programs. Phylogenetic trees were also reconstructed using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. An evolutionary distance matrix for the neighbour-joining tree was generated according to the model of Jukes & Cantor (1969). The reliability of each tree was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings.

Table 1. Differential characteristics of the strain BLB-1^T and closely related species

Strains: 1, strain BLB-1^T; 2, *B. massiliensis* KCTC 13178^T; 3, *B. odysseyi* KCTC 3961^T; 4, *L. sphaericus* KCTC 3346^T; 5, *L. fusiformis* KCTC 3454^T; 6, *L. boronitolerans* KCTC 13709^T. Symbols: +, positive; −, negative; W, weakly positive. All strains were catalase-positive. All data are from this study. AMP, adenosine monophosphate; UMP, uridine monophosphate.

Characteristic	1	2	3	4	5	6
pH range for growth	5.0–9.0	5.0–9.0	6.0–10.0	6.0–9.5	6.0–9.5	5.5–9.5
Temperature range for growth	15–45	25–45	25–42	10–40	17–40	16–45
Growth in 7 % NaCl	−	−	−	−	+	−
Oxidase	−	+	−	+	+	+
Voges–Proskauer test	−	+	+	−	−	+
Hydrolysis of:						
Gelatin	+	−	−	+	+	−
Urea	−	+	−	−	+	+
Arginine dihydrolase	−	+	−	−	−	+
Trypsin	+	−	−	W	−	−
α-Chymotrypsin	W	−	+	+	+	W
Acid phosphatase	+	−	−	−	−	+
Naphthol-AS-BI-phosphohydrolase	+	+	−	+	W	+
Tryptophan deaminase	−	+	−	−	−	+
Oxidation of:						
Acetate	−	+	+	+	−	−
Pyruvate	−	+	+	+	+	+
α-Hydroxybutyrate	−	−	+	−	−	+
L-Alanine	−	−	+	+	+	+
Glycyl-L-glutamate	−	−	+	+	+	+
Adenosine	−	−	+	+	+	+
2′-Deoxyadenosine	−	−	−	+	+	+
Inosine	−	−	+	+	+	+
AMP	−	−	+	+	+	+
UMP	−	−	+	+	+	+
Dextrin	−	+	+	+	−	+

Phylogenetic analysis demonstrated that the isolate belonged to the *Bacillus* rRNA group 2, members of which are closely related to *B. massiliensis* CIP 108446^T and *B. odysseyi* NBRC 100172^T. However, strain BLB-1^T could be clearly distinguished from all species with validly published names in the genus *Bacillus*. Strain BLB-1^T, *B. massiliensis* and *B. odysseyi* were closely related and were grouped in a single clade in the neighbour-joining tree containing type strains of species belonging to this group (Fig. 1). Furthermore, all three taxa and species of the genus *Lysinibacillus* were separated into a different clade from members of the genera *Solibacillus*, *Rummeliibacillus* and *Viridibacillus*. The distinct branch formed by the radiation of this group did not depend on the tree-making algorithm used and was supported by a 96 % bootstrap value (Fig. S1, available in IJSEM Online). The phylogenetic analysis strongly indicated that the three taxa represent novel species belonging to the genus *Lysinibacillus*.

The closest phylogenetic relatives to strain BLB-1^T were *B. massiliensis* CIP 108446^T (97.4 % 16S rRNA gene sequence similarity) followed by *B. odysseyi* NBRC 100172^T (96.7 %), *L. fusiformis* DSM 2898^T (96.2 %), *L. sphaericus* DSM 28^T (96.1 %) and *L. boronitolerans* DSM 17140^T (95.9 %). The recently described species *L. parviboronicapiens* (95.7 %), *L. xylanilyticus* (97.2 %; Lee *et al.*, 2010) and *L. macroides* (96.2 %; Coorevits *et al.*, 2012) appeared to be distant phylogenetic relatives of strain BLB-1^T. Sequence similarity alues between strain BLB-1^T and other species in the genera *Caryophanon*, *Sporosarcina*, *Solibacillus*, *Rummeliibacillus* and *Viridibacillus* were <96.1 %. Therefore, additional DNA–DNA hybridization was performed to more accurately differentiate these closely related species (Tindall *et al.*, 2010).

DNA–DNA hybridization was performed as previously described (Ezaki *et al.*, 1989) using photobiotin-labelled probes at 45 °C with a Fluoroskan Ascent Fluorescent plate reader (Thermo Life Sciences). Three replicates of each sample were tested. The DNA–DNA hybridization values between strain BLB-1^T and *B. massiliensis* KCTC 13178^T, *L. sphaericus* KCTC 3346^T, *B. odysseyi* KCTC 3961^T, *L. fusiformis* KCTC 3454^T and *L. boronitolerans* KCTC 13709^T were 24.9, 24.1, 21.0, 20.5 and 18.3 %, respectively. These values are much lower than the suggested threshold value for species delineation (Wayne *et al.*, 1987) and indicate that the isolate represents a novel species that is distinct from closely related species.

To determine the DNA G+C content, DNA was analysed by real-time PCR (Thermocycler, Bio-Rad) with SYBR Green I, according to a previously described fluorometric method (Gonzalez & Saiz-Jimenez, 2002). The relative values were calculated based on *E. coli* KCTC 2441^T DNA (50.8 mol%, T_m) as a standard. The mean DNA G+C contents of strains BLB-1^T, *B. massiliensis* KCTC 13178^T and *B. odysseyi* KCTC 3961^T were 35.9, 36.3 and 35.6 mol%, respectively. These data corresponded more closely with the typical ranges of members of the genera *Lysinibacillus* (35–38 mol%; Ahmed *et al.*, 2007), *Kurthia* (36–38 mol%; Keddie & Jones, 1992), *Viridibacillus* (35–40.4 mol%; Albert

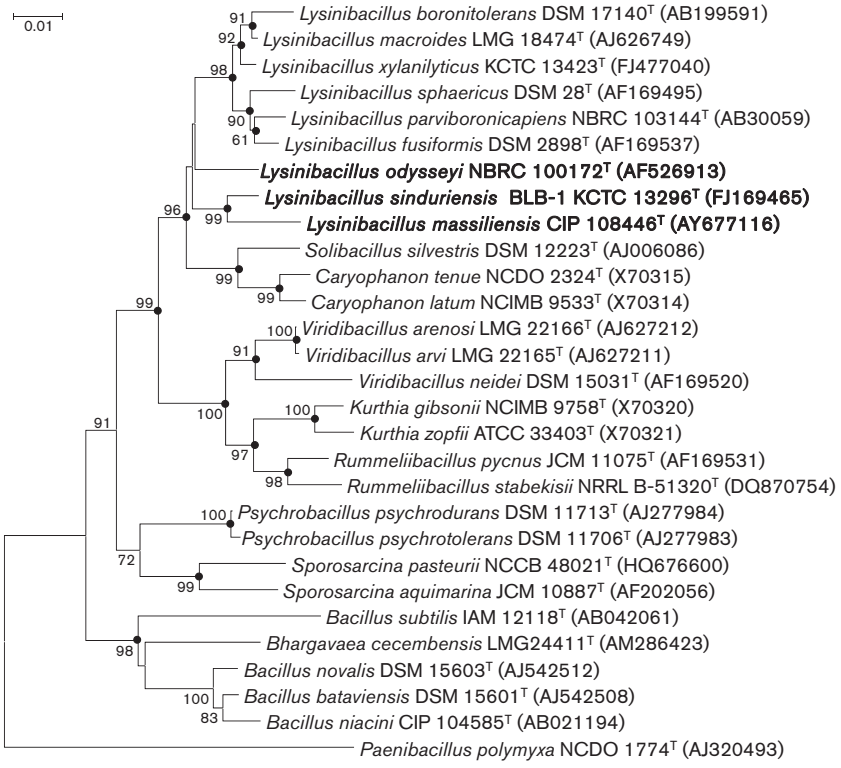

Fig. 1. Phylogenetic tree inferred by neighbour-joining method based on 16S rRNA gene sequences (1413 bp). The tree shows the relationship between strain BLB-1^T and closely related taxa. Bootstrap values >50 % (based on 1000 replications) are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

et al., 2007) and *Bacillus* (32–69 mol%; Heyrman *et al.*, 2005) rather than the genera *Sporosarcina* (40–42 mol%; Yoon *et al.*, 2001) or *Caryophanon* (44–46 mol%; Claus *et al.*, 1992).

The cellular fatty acid profiles of strain BLB-1^T and closely related strains grown on TSBA medium at 28 °C for 48 h were determined according to the Sherlock Microbial Identification System (MIDI). The fatty acids were separated using an automated GC system (model 6890N and 7683 Autosampler; Agilent) and identified with the associated software package version 4.0 (Library TSBA 40, MIDI). The fatty acid profile of strain BLB-1^T (Table 2) was characterized by high proportions of saturated branched fatty acids, such as anteiso-$C_{15:0}$ (35.6 %), iso-$C_{15:0}$ (25.6 %) and anteiso-$C_{17:0}$ (16.5 %). Significant differences in the relative amounts of iso-$C_{15:0}$ and anteiso-$C_{17:0}$ were found between strain BLB-1^T and its closest relatives and these characteristics could be used to differentiate the novel isolate from related species (Kämpfer *et al.*, 1994; Ahmed *et al.*, 2007). The major fatty acids detected in strain BLB-1^T, iso- and anteiso-$C_{15:0}$, have also been detected as major components in *B. subtilis* and members of the genera *Lysinibacillus*, *Viridibacillus*, *Rummeliibacillus* and *Kurthia* (Shaw & Keddie, 1983; Kämpfer *et al.*, 2006; Ahmed *et al.*, 2007; Albert *et al.*, 2007). In contrast, iso-$C_{15:0}$ and iso-$C_{16:1}$ were detected as the major fatty acids in members of the genus *Solibacillus* (Krishnamurthi *et al.*, 2009).

Respiratory quinones were determined as described previously (Komagata & Suzuki, 1987) using TLC and HPLC. The major menaquinone was MK-7 in strains BLB-1^T, *B. massiliensis* KCTC 13178^T and *B. odysseyi* KCTC 3961^T, which corresponded with that found in members of the genus *Lysinibacillus*. However, this characteristic differed in phylogenetically closely related members of the genera *Caryophanon*, which contained MK-6 as the predominant menaquinone (Ahmed *et al.*, 2007; Krishnamurthi *et al.*, 2009), and *Viridibacillus*, which contained MK-8 as the predominant menaquinone (Albert *et al.*, 2007).

Cell-wall sugar analysis of the whole-cell hydrolysates was performed as described by Schleifer & Kandler (1972) using TLC with cellulose plates (Merck). Glucose and xylose were the predominant cell-wall sugars in strain BLB-1^T. The closest relative, *B. massiliensis* KCTC 13178^T, produced similar results to strain BLB-1^T, whereas *B. odysseyi* KCTC 3961^T was different, with only xylose as the predominant cell-wall sugar.

Peptidoglycan structure was determined as described previously (Schleifer & Kandler, 1972; Schleifer, 1985) with modification of the TLC using cellulose plates rather than paper chromatography. Quantitative analysis of amino acids in the peptidoglycan was performed by GC according to the method of MacKenzie (1987). Strain BLB-1^T contained peptidoglycan with alanine, glutamic acid, lysine and aspartic acid as diagnostic amino acids in a molar ratio of 1.3 : 1.0 : 0.9 : 1.0, respectively. The close relatives *B. massiliensis* KCTC 13178^T and *B. odysseyi* KCTC 3961^T had similar amino acid molar ratios. The peptidoglycan type A4α L-Lys–D-Asp (A11.31; DSMZ, 2001) was very similar to that found in species of the genera *Lysinibacillus*, *Kurthia* and *Viridibacillus* (Shaw & Keddie, 1983; Ahmed *et al.*, 2007; Krishnamurthi *et al.*, 2009). By contrast, the type species of the genus *Bacillus*, *B. subtilis*, is known to possess *meso*-diaminopimelic acid in the cell-wall peptidoglycan (Schleifer & Kandler, 1972). These data strongly indicate that *B. massiliensis* KCTC 13178^T and *B. odysseyi* KCTC 3961^T do not belong to the genus *Bacillus* and instead they should be reclassified into new or existing genera. We propose that these species be transferred to the genus *Lysinibacillus*.

Polar lipids were extracted from 100 mg freeze-dried cell material and separated using a two-stage method as described previously (Tindall, 1990). Polar lipid analysis was conducted by the DSMZ Identification Service and Dr Brian Tindall (DSMZ Braunschweig, Germany). TLC plates were stained with 5 % molybdophosphoric acid to detect all the lipids. The major polar lipids in strain BLB-1^T and *B. odysseyi* KCTC 3961^T were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE). Moderate amounts of phospholipid (PL2) and minor amounts of two phospholipids (PL1, PL4) and an unknown lipid (L1) were also detected. *B. odysseyi* KCTC 3961^T also contained PL5, L3 and L4 (Fig. 2). By contrast, *B. massiliensis* KCTC 13178^T showed a significantly different profile with major components of DPG and PG, moderate amounts of two glycolipids (GL1, 2) and minor amounts of PL1, 2, 3 and 4 and L1, but no PE was detected. Glycolipids

Table 2. Cellular fatty acid compositions of strain BLB-1^T and closely related species

Strains: 1, strain BLB-1^T; 2, *B. massiliensis* KCTC 13178^T; 3, *B. odysseyi* KCTC 3961^T; 4, *L. sphaericus* KCTC 3346^T; 5, *L. fusiformis* KCTC 3454^T; 6, *L. boronitolerans* KCTC 13709^T. Values are percentages of total fatty acids. t, Trace amount (<1.0 %); −, not detected. All data are from this study.

Fatty acid	1	2	3	4	5	6
iso-$C_{14:0}$	1.9	4.1	2.2	3.5	1.1	2.1
iso-$C_{15:0}$	25.6	54.3	50.4	53.5	59.7	59.9
anteiso-$C_{15:0}$	35.6	10.9	7.1	3.3	10.5	7.4
$C_{15:0}$	1.2	5.4	−	−	−	t
$C_{16:1}\omega7c$ alcohol	4.4	1.8	12.7	17.4	7.8	13.2
iso-$C_{16:0}$	4.3	15.0	11.3	8.4	4.1	3.2
$C_{16:1}\omega11c$	1.1	t	2.1	1.8	2.5	1.5
iso-$C_{17:1}\omega10c$	1.3	t	4.3	4.9	3.9	4.7
iso-$C_{17:0}$	3.1	2.8	5.3	4.2	3.7	2.8
anteiso-$C_{17:0}$	16.5	3.2	1.9	t	2.6	1.2
Summed feature 4*	3.4	−	1.2	1.2	2.6	1.8

*Summed features represent groups of two or three fatty acids that could not be separated by GC using the Microbial Identification System. Summed feature 4 comprises iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

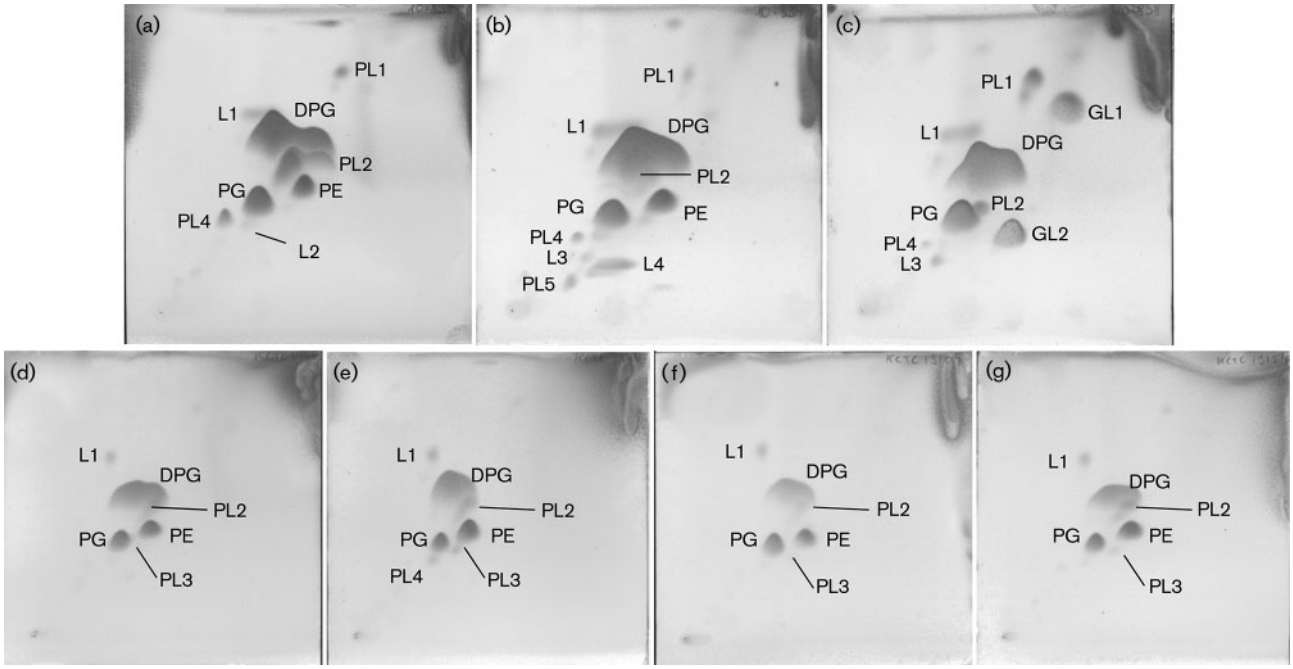

Fig. 2. Polar lipid profiles after separation by two-dimensional TLC. (a) Strain BLB-1^T; (b) *L. odysseyi* KCTC 3961^T; (c) *L. massiliensis* KCTC 13178^T; (d) *L. fusiformis* KCTC 3454^T; (e) *L. sphaericus* KCTC 3346^T; (f) *L. boronitolerans* KCTC 13709^T; (g) *L. parviboronicapiens* KCTC 13154^T. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL1–5, unidentified phospholipids; GL1–2, unidentified glycolipids; L1–4, unidentified lipid.

have also been reported in members of the genera *Bacillus*, *Paenibacillus* and *Cohnella* (Kämpfer *et al.*, 2006), where the absence of PE is used to differentiate species. The profiles of three strains were compared with *B. subtilis* (Kämpfer *et al.*, 2006) and significant differences were observed, i.e. the presence of gentiobiosyldiacylglycerol (GBG) and aminophospholipids (APL) in *B. subtilis*. The phylogenetic relative, *Solibacillus silvestris* (Krishnamurthi *et al.*, 2009) differed from the three strains in terms of the presence of phosphatidylserine (PS). In addition, the presence of aminolipids (AL) was reported in the genus *Viridibacillus* (Albert *et al.*, 2007), whereas AL and APL are also found in the genus *Rummeliibacillus* (Vaishampayan *et al.*, 2009). The closest phylogenetic relatives, species of the genus *Lysinibacillus*, matched the lipid profiles of strains BLB-1^T, *B. odysseyi* KCTC 3961^T and *B. massiliensis* KCTC 13178^T because they shared DPG and PG as major components (Ahmed *et al.*, 2007). However, the ninhydrin-positive phosphoglycolipids (NPG) that are typical of members of the genus *Lysinibacillus* were not detected in the strains investigated in this study.

This investigation had to be expanded to include more detailed polar lipid profiles for the type species of the genus *Lysinibacillus*. Thus, comparative analyses of *L. boronitolerans* KCTC 13709^T (=DSM 17140^T), *L. fusiformis* KCTC 3454^T (=DSM 2898^T) and *L. sphaericus* KCTC 3346^T (=DSM 28^T) were performed to better understand their lipid profiles. The major profiles of these three species contained DPG, PG and PE, as well as minor amounts of PL2, PL3 and L1 (Fig. 2). *L. sphaericus* KCTC 3346^T also possessed PL4. NPG was not detected in these three species, which was in agreement with the lipid profile of BLB-1^T determined in the current study. No spots had the same R_f value as the major component NPG reported by Ahmed *et al.* (2007). *L. xylanilyticus* and *L. macroides* also lacked the previously reported NPG (Lee *et al.*, 2010; Coorevits *et al.*, 2012). Thus, the affiliation of these species differs from the description of the genus *Lysinibacillus* in terms of their polar lipid profiles (Ahmed *et al.*, 2007), which suggests that the original description of the genus needs to be amended based on the current analysis. The profiles of strains BLB-1^T, *B. odysseyi* KCTC 3961^T and *B. massiliensis* KCTC 13178^T were far more complex than those of previously reported species of the genus *Lysinibacillus* (Ahmed *et al.*, 2007; Miwa *et al.*, 2009; Lee *et al.*, 2010). Furthermore, the presence of GL and the lack of PE in *B. massiliensis* KCTC 13178^T showed that it had a significantly different profile from its closest relative strain, as well as from BLB-1^T and species of the genus *Lysinibacillus*. This difference could be used to differentiate the species from closely related taxa but was insufficient to exclude it from the genus *Lysinibacillus*. In conclusion, the polar lipid profiles indicate that the genus *Lysinibacillus* is as heterogeneous as the genera *Bacillus* and *Paenibacillus* (Kämpfer *et al.*, 2006).

In conclusion, the novel isolate BLB-1^T, *B. massiliensis* KCTC 13178^T, and *B. odysseyi* KCTC 3961^T all possess

peptidoglycan type A4α (L-Lys–D-Asp) and they share this characteristic with members of the genus *Lysinibacillus* (Ahmed *et al.*, 2007). The phylogenetic analysis (Fig. 1) also showed that all three taxa, along with species of the genus *Lysinibacillus*, belong to the same clade within the *Bacillus* rRNA group 2. Based on these data, it is reasonable to assign the three taxa to the genus *Lysinibacillus*. The data presented in this study also show that the isolated strain BLB-1^T can be differentiated from other members of the genus *Lysinibacillus* and represents a novel species, for which the name *Lysinibacillus sinduriensis* sp. nov. is proposed. The chemotaxonomic and phylogenetic analyses also demonstrated that *B. massiliensis* and *B. odysseyi* should be transferred to the genus *Lysinibacillus* with the names *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov., respectively.

Emended description of the genus *Lysinibacillus* Ahmed *et al.* 2007

The description is as given previously (Ahmed *et al.*, 2007) with the following amendments. Oxidase activity is variable. Major cellular fatty acids iso-$C_{15:0}$ or anteiso-$C_{15:0}$ are present. The polar lipid profiles contain DPG, PG and PE as predominant lipids, with varying numbers of unidentified polar lipids and amino-group-containing lipids. Some species do not contain PE and instead contain unidentified glycolipids as major components.

Description of *Lysinibacillus sinduriensis* sp. nov.

Lysinibacillus sinduriensis (sin.du.ri.en′sis. N.L. masc. adj. *sinduriensis* pertaining to the Sinduri, Republic of Korea, geographical origin of the type strain of the species).

Cells are Gram-positive, strictly aerobic, terminal spherical spore-forming, straight and club-shaped rods (0.5–1.2 × 0.7–3.5 μm) with swollen sporangium and are motile by peritrichous flagella. Colonies grown on TSBA medium are circular, convex, undulate, greyish white and 3 mm in diameter. Cells grow at 15–45 °C (optimum 30 °C), at pH 5–9 (optimum pH 7) and in 0–5 % (w/v) NaCl but not in 6 % (w/v) NaCl. Negative for nitrate reduction, oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities, citrate utilization, Voges–Proskauer test, production of H_2S, indole and acetoin and hydrolysis of casein and starch; positive for gelatinase and catalase. No sugar is fermented in the API 50 CH strips, using CHB/E suspension medium. Oxidizes α-ketovaleric acid and pyruvic acid methyl ester but not acetate, pyruvate, α- and β-hydroxybutyrate, methyl pyruvate, L-alanine, glycyl-L-glutamate, adenosine, 2′-deoxyadenosine, inosine, adenosine monophosphate, uridine monophosphate or dextrin. Negative for alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities; positive for esterase, esterase lipase, leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase. The major polar lipids are DPG, PG, PE and three unknown phospholipids, PL1, PL2 and PL3. The predominant fatty acids are anteiso-$C_{15:0}$ (35.6 %), iso-$C_{15:0}$ (25.6 %) and anteiso-$C_{17:0}$ (16.5 %). The major cell-wall sugars are glucose and xylose. The cell-wall peptidoglycan contains L-Lys–D-Asp (type A4α). The major menaquinone is MK-7.

The type strain, BLB-1^T (=KCTC 13296^T =JCM 15800^T), was isolated from tidal flat sediment in the Shinduri sand dunes of the Yellow Sea, Republic of Korea. The DNA G+C content of the type strain is 35.9 mol%.

Description of *Lysinibacillus odysseyi* comb. nov.

Lysinibacillus odysseyi (o.dys.se′yi. L. n. *Odyssea* the Odyssey; N.L. gen. n. *odysseyi* pertaining to the Mars Odyssey spacecraft, from which the organism was isolated).

Basonym: *Bacillus odysseyi* La Duc *et al.* (2004).

In addition to the description of the type strain given by La Duc *et al.* (2004), cells are negative for alkaline phosphatase, valine arylamidase, cystine arylamidase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, trypsin, acid phospatase and naphthol-AS-BI-phosphohydrolase activities; positive for esterase, esterase lipase, leucine arylamidase and α-chymotrypsin activities. Oxidizes α-ketovaleric acid, pyruvic acid methyl ester and dextrin. Negative for oxidase, citrate utilization, urease and tryptophan deaminase activity, hydrolysis of casein and starch and indole production and positive for Voges–Proskauer test and acetoin production. No sugar is fermented in API 50 CH strips using CHB/E suspension medium. The cell-wall peptidoglycan contains L-Lys–D-Asp (type A4α). The major polar lipids are DPG, PG, PE, four unknown phospholipids, PL1, PL2, PL3 and PL4, and an unknown lipid (L1). The predominant fatty acids are iso-$C_{15:0}$ (50.4 %), iso-$C_{16:0}$ (11.3 %) and $C_{16:1}\omega7c$ alcohol (12.7 %). The major cell-wall sugar is xylose. The major menaquinone is MK-7. The type strain, 34hs-1^T (=ATCC PTA-4993^T =NRRL B-30641^T =NBRC 100172^T =DSM 18869^T =CIP 108263^T =KCTC 3961^T), was isolated from the surface of the Mars Odyssey spacecraft. The DNA G+C content of the type strain is 35.6 mol%.

Description of *Lysinibacillus massiliensis* comb. nov.

Lysinibacillus massiliensis (mas.si.li.en′sis. L. masc. adj. massiliensis of *Massilia*, the ancient Greek and Roman name for Marseille, France, where the type strain was isolated).

Basonym: *Bacillus massiliensis* Glazunova *et al.* 2006.

In addition to the description of the type strain given by Glazunova *et al.* (2006), cells are negative for trypsin, α-chymotrypsin, acid phospatase, lipase, valine arylamidase,

cystine arylamidase, α- and β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities; positive for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acetoin production, α-glucosidase, and naphthol-AS-BI-phosphohydrolase activities. Negative for nitrate reduction, and hydrolysis of casein and starch. Oxidizes α-ketovaleric acid, pyruvic acid methyl ester and dextrin. The cell-wall peptidoglycan contains L-Lys–D-Asp (type A4α). The major polar lipids are DPG, PG, three unknown phospholipids, PL1, PL2 and PL3, and two unknown glycolipids, GL1 and GL2. The major cell-wall sugars are glucose and xylose. The major menaquinone is MK-7.

The type strain, 4400831^T (=CIP 108446^T =CCUG 49529^T =KCTC 13178^T), was isolated from human cerebrospinal fluid. The DNA G+C content of the type strain is 36.3 mol%.

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References

Ahmed, I., Yokota, A., Yamazoe, A. & 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.

Albert, R. A., Archambault, J., Lempa, M., Hurst, B., Richardson, C., Gruenloh, S., Duran, M., Worliczek, H. L., Huber, B. E. & other authors (2007). Proposal of *Viridibacillus* gen. nov. and reclassification of *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei* as *Viridibacillus arvi* gen. nov., comb. nov., *Viridibacillus arenosi* comb. nov. and *Viridibacillus neidei* comb. nov. *Int J Syst Evol Microbiol* **57**, 2729–2737.

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.

Chang, Y. H., Han, J. I., Chun, J. S., Lee, K. C., Rhee, M. S., Kim, Y. B. & Bae, K. S. (2002). *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea. *Int J Syst Evol Microbiol* **52**, 377–381.

Chang, Y. H., Jung, M. Y., Park, I. S. & Oh, H. M. (2008). *Sporolactobacillus vineae* sp. nov., a spore-forming lactic acid bacterium isolated from vineyard soil. *Int J Syst Evol Microbiol* **58**, 2316–2320.

Claus, D., Fritze, D. & Kocur, M. (1992). Genera related to the genus *Bacillus* – *Sporolactobacillus*, *Sporosarcina*, *Planococcus*, *Filibacter* and *Caryophanon*. In *The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn, vol. 2, pp. 1769–1791. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

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

DSMZ (2001). Catalogue of Strains, 7th edn, p. 617. Braunschweig: DSMZ. http://www.dsmz.de/fileadmin/Bereiche/Microbiology/Dateien/Key_to_Murein2.pdf.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1994). Phylogenetic interrelationships of round-spore-forming bacilli containing cell walls based on lysine and the non-spore-forming genera *Caryophanon*, *Exiguobacterium*, *Kurthia*, and *Planococcus*. *Int J Syst Bacteriol* **44**, 74–82.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

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

Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c, Seattle: Department of Genetics, University of Washington.

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. & Roux, V. (2006). *Bacillus massiliensis* sp. nov., isolated from cerebrospinal fluid. *Int J Syst Evol Microbiol* **56**, 1485–1488.

Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

Heyrman, J., Rodríguez-Díaz, M., Devos, J., Felske, A., Logan, N. A. & De Vos, P. (2005). *Bacillus arenosi* sp. nov., *Bacillus arvi* sp. nov. and *Bacillus humi* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **55**, 111–117.

Jeon, Y. S., Chung, H., Park, S., Hur, I., Lee, J. H. & Chun, J. (2005). jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kämpfer, P., Blasczyk, K. & Auling, G. (1994). Characterization of *Aeromonas* genomic species by using quinone, polyamine, and fatty acid patterns. *Can J Microbiol* **40**, 844–850.

Kämpfer, P., Rosselló-Mora, R., Falsen, E., Busse, H. J. & Tindall, B. J. (2006). *Cohnella thermotolerans* gen. nov., sp. nov., and classification of '*Paenibacillus hongkongensis*' as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 781–786.

Keddie, R. M. & Jones, D. (1992). The genus *Kurthia*. In *The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn, vol. 2, pp. 1654–1662. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Krishnamurthi, S., Chakrabarti, T. & Stackebrandt, E. (2009). Re-examination of the taxonomic position of *Bacillus silvestris* Rheims *et al.* 1999 and proposal to transfer it to *Solibacillus* gen.

nov. as *Solibacillus silvestris* comb. nov. *Int J Syst Evol Microbiol* **59**, 1054–1058.

Krishnamurthi, S., Ruckmani, A., Pukall, R. & Chakrabarti, T. (2010). *Psychrobacillus* gen. nov. and proposal for reclassification of *Bacillus insolitus* Larkin & Stokes, 1967, B. *psychrotolerans* Abd-El Rahman *et al.*, 2002 and B. *psychrodurans* Abd-El Rahman *et al.*, 2002 as *Psychrobacillus insolitus* comb. nov., *Psychrobacillus psychrotolerans* comb. nov. and *Psychrobacillus psychrodurans* comb. nov. *Syst Appl Microbiol* **33**, 367–373.

La Duc, M. T., Satomi, M. & 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. & Yoon, J.-H. (2010). *Lysinibacillus xylanilyticus* sp. nov., a xylan-degrading bacterium isolated from forest humus. *Int J Syst Evol Microbiol* **60**, 281–286.

MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.

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

Nakamura, L. K., Shida, O., Takagi, H. & Komagata, K. (2002). *Bacillus pycnus* sp. nov. and *Bacillus neidei* sp. nov., round-spored bacteria from soil. *Int J Syst Evol Microbiol* **52**, 501–505.

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

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

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

Shaw, S. & Keddie, R. M. (1983). A numerical taxonomic study of the genus *Kurthia* with a revised description of *Kurthia zopfii* and a description of *Kurthia gibsonii* sp. nov. *Syst Appl Microbiol* **4**, 253–276.

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.

Tindall, B. J. (1990). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.

Vaishampayan, P., Miyashita, M., Ohnishi, A., Satomi, M., Rooney, A., La Duc, M. T. & Venkateswaran, K. (2009). Description of *Rummeliibacillus stabekisii* gen. nov., sp. nov. and reclassification of *Bacillus pycnus* Nakamura *et al.* 2002 as *Rummeliibacillus pycnus* comb. nov. *Int J Syst Evol Microbiol* **59**, 1094–1099.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Woese, C. R., Magrum, L. J., Gupta, R., Siegel, R. B., Stahl, D. A., Kop, J., Crawford, N., Brosius, J., Gutell, R. & other authors (1980). Secondary structure model for bacterial 16S ribosomal RNA: phylogenetic, enzymatic and chemical evidence. *Nucleic Acids Res* **8**, 2275–2293.

Yoon, J. H., Lee, K. C., Weiss, N., Kho, Y. H., Kang, K. H. & Park, Y. H. (2001). *Sporosarcina aquimarina* sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., and emended description of the genus *Sporosarcina*. *Int J Syst Evol Microbiol* **51**, 1079–1086.