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ORIGINAL ARTICLE

Lysinibacillus composti sp. nov., isolated from compost

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Abstract A Gram-negative, motile, rod-shaped, endospore-forming bacterial strain, designated as NCCP-36^T, was isolated from the compost of fruit and vegetable wastes. The strain NCCP-36^T grew within a temperature range of 10–45 °C (optimum 28 °C) and a pH range of 6.5–8.5 (optimum 7.0), and its cells tolerated <50 mM boron (optimum growth without boron) and 0–5 % NaCl (w/v) in tryptic soya broth medium. Based on comparative analysis of 16S rRNA gene sequence, strain NCCP-36^T showed the highest similarity to *Lysinibacillus sinduriensis* BLB-1^T (97.52 %) and *L. xylanilyticus* XDB9^T (96.96 %), and <97 % similarity with other closely related taxa. However, DNA–DNA relatedness between strain NCCP-36^T and the closely related type strains of genus *Lysinibacillus* was ≤37 %. Phylogenetic and chemotaxonomic analyses [major polar lipids: diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and phospholipids; predominant menaquinone: MK-7; major cellular fatty acids: iso-$C_{15:0}$, antieso-$C_{15:0}$, and iso-$C_{16:0}$; DNA G+C contents: 37 mol %; Lys-Asp (type *A*4α) in cell-wall peptidoglycans as diagnostic amino acids] also support the affiliation of strain NCCP-36^T to genus *Lysinibacillus*. Based upon DNA–DNA relatedness as well as distinctive chemotaxonomic, phylogenetic, and genotypic data, we conclude that strain NCCP-36^T belongs to a novel species of genus *Lysinibacillus*, for which the name *Lysinibacillus composti* sp. nov. is proposed. The type strain is NCCP-36^T (JCM 18777^T=KCTC 13796^T=DSMZ 24785^T).

Keywords *Lysinibacillus composti* sp. nov. · Compost · Cell-wall peptidoglycans

The DDBJ/EMBL/GenBank accession number for the 16S rRNA gene sequence of strain NCCP-36^T (=JCM 18777^T=KCTC 13796 ^{T}; DSMZ 24785 ^{T}) is AB547124.

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Introduction

Bacillus, a genus of Gram-positive, aerobic, endospore-forming motile bacteria, belongs to the family *Bacillaceae* (Claus and Berkeley 1986) and has wide genetic heterogeneity. Over the last decade, the genus *Bacillus* has been taxonomically dissected into five recognized groups (Ash et al. 1991), and several new genera have emerged based on differentiating polyphasic taxonomic features (Wisotzkey et al. 1992; Ash et al. 1993; Shida et al. 1996; Heyndrickx et al. 1998; Wainø et al. 1999; Yoon et al. 2001; Ahmed et al. 2007b; Albert et al. 2007; Krishnamurthi et al. 2009). Ahmed et al. (2007b) transferred two more rRNA group 2 *Bacillus* species, *Bacillus fusiformis* and *B. sphaericus*, into genus *Lysinibacillus* based on the single differentiating character of a cell-wall peptidoglycan containing lysine and aspartate. Although the presence of L-lysine peptidoglycans in *Bacillus* rRNA group 2 inspired researchers to re-evaluate the taxonomy of this group, this

benchmark has remained under continuous criticism/observation since 1990 (Farrow et al. 1994; Rheims et al. 1999; Yoon et al. 2001; Albert et al. 2007; Zhang et al. 2007; Stackebrandt and Swiderski 2008; Jung et al. 2012).

For most of the *Bacillus* species in rRNA group 2 information is lacking on one or more of the chemotaxonomic standards (Krishnamurthi et al. 2009) required for describing new taxa (Kämpfer et al. 2006; Logan et al. 2009). La Duc et al. (2004) and Glazunova et al. (2006) described novel species *Bacillus massiliensis* and *B. odysseyi*, respectively, based on phenotypic characteristics and the 16S rRNA gene sequence. However, these authors did not report any data on cell-wall peptidoglycans, polar lipids, and menaquinone for these species. *Bacillus massiliensis* and *B. odyssey* are phylogenetically more closely related to *Bacillus pycnus* and *Solibacillus silvestris* (Albert et al. 2007; Krishnamurthi et al. 2009), but analysis of the 16S rRNA gene sequence phyletic line delineated that *B. odyssey* is closely related to *Lysinibacillus boronitolerans* KCTC 13709^T sharing a sequence similarity of 96.1 % (Ahmed et al. 2007b). Recently, a proposal has been accepted to transfer *Bacillus massiliensis* and *B. odyssey* into the genus *Lysinibacillus* as *L. massiliensis* and *L. odyssey*, respectively, along with classification of *L. sinduriensis* through detailed examination of their fatty acid profile, polar lipids, cell-wall peptidoglycans, and menaquinone systems (Jung et al. 2012).

The genus *Lysinibacillus* was proposed based on lysine and aspartate (Lys-Asp) as diagnostic amino acids in peptidoglycans rather than *meso*-diaminopimelic acid, which is a specific characteristic of the genus *Bacillus*. All members of genus *Lysinibacillus* are characterized as strictly aerobic, rod-shaped, and spore-forming, which are positive for catalase but negative for the production of indole and H_2S. Chemotaxonomically, these are characterized by the presence of diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) as the major polar lipids, MK-7 as the predominant menaquinone, iso-$C_{15:0}$, antieso-$C_{15:0}$, and iso-$C_{16:0}$ as the major cellular fatty acids, and DNA G+C contents of 35–38.7 mol %. To date, the genus *Lysinibacillus* contains 13 validly recognized species, including *Lysinibacillus boronitolerans*, *L. fusiformis*, *L. sphaericus* (Ahmed et al. 2007b), *L. parviboronicapiens* (Miwa et al. 2009), *L. xylanilyticus* (Lee et al. 2010), *L. sinduriensis*, *L. massiliensis*, *L. odyssey* (Jung et al. 2012), *L. macroides* (Coorevits et al. 2012), *L. mangiferihumi* (Yang et al. 2012), *L. contaminans* (Kämpfer et al. 2013), and *L. manganicus* (Liu et al. 2013), and *L. meyeri* (Seiler et al. 2013). However, one new species, *L. pakistanensis* (Hayat et al. 2013), has recently been proposed. In this study, we describe a novel strain NCCP-36^T in the genus *Lysinibacillus* as *Lysinibacillus composti* sp. nov. based on its phenotypic, phylogenetic, and chemotaxonomic relationship to the genus *Lysinibacillus*.

Materials and methods

Isolation, morphology, and phenotypic characterization

A bacterial strain, designated as NCCP-36^T, was isolated from the compost of fruit and vegetable wastes prepared aerobically in a 1 m × 1 m × 1 m - (L × W × D) pit at the research farm of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. The waste was turned over on a weekly basis to maintain aerobic conditions at all depths. Following the required time for composting (approx. 100 days), we collected a sample of the compost and isolated bacteria by the dilution plate technique using a phosphate-buffered saline solution on tryptic soya agar (TSA; Difco Laboratories, Detroit, MI) and incubation at 28 °C. The purified strain was obtained through sub-culturing and was stored in glycerol (final concentration 35 % w/v) at −80 °C for further characterization. The reference strains used in these studies were *Lysinibacillus massiliensis* KCTC13178^T (Glazunova et al. 2006), *L. xylanilyticus* KCTC13423^T (Lee et al. 2010), *L. odyssey* KCTC3961^T (La Duc et al. 2004), *L. sinduriensis* KCTC13296^T (Jung et al. 2012), *L. fusiformis* KCTC3454^T (Priest et al. 1988), *L. parviboronicapiens* KCTC13154^T (Miwa et al. 2009), *L. boronitolerans* KCTC13709^T (Ahmed et al. 2007b), and *L. sphaericus* KCTC3346^T (Claus and Berkeley 1986). All strains were routinely grown on TSA at 28 °C, unless otherwise mentioned.

Colonial morphology of the isolated strain NCCP-36^T was observed on well-isolated colonies grown on TSA for 2 days. Cell morphology and motility were examined by light microscopy (model E600; Nikon, Tokyo, Japan). For pH optimization, various pH levels (4.0–10.0) were adjusted in tryptic soya broth (TSB; Difco) using Na_2CO_3 and HCl. The pH levels were confirmed after the media had been autoclaved. For NaCl tolerance, strain NCCP-36^T was inoculated in TSB (pH 7.0) containing different NaCl concentrations from 0 to 10 % (w/v). Optimum growth temperature for NCCP-36^T was determined by streaking bacterial strains on TSA plates (pH 7.0) and incubation at temperatures of 4, 10, 16, 22, 28, 32, 37, 45, and 50 °C. The boron tolerance of strain NCCP-36^T was determined in TSB (pH 7.0) containing different levels of boron ranging from 0 to 150 mM (Ahmed et al. 2007b). Physiological and biochemical characteristics were examined using API kits (AP50CH, API 20E, API-ZYM; bioMérieux, Marcy l'Etoile, France) and the Biolog GP system (Biolog Inc., Hayward, CA) to determine the different metabolic features of the strains in accordance with the manufacturers' instructions. Gram staining and the KOH reaction test were also performed by standard procedures as described earlier (Chang et al. 2002).

Genotypic and chemotaxonomic analyses

To identify the strain, the nearly complete 16S rRNA gene was amplified by the PCR as described by Ahmed et al. (2007a) using forward (9F; 5′-GAGTTTGATCCTGGCTCAG-3′) and reverse (1510R; 5′-GGCTACCTTGTTACGA-3′) primers. The PCR product was purified using the PureLink PCR purification kit (Invitrogen, Carlsbad, CA) and sequenced by Macrogen Inc., Korea (www.dna.macrogen.com/eng) using four universal 16S rRNA gene primers, namely, 9F, 515F (5′-GTGCCAGCAGCCGCGGT-3′), 926R (5′-CCGTCAATTCCTTTGAGTTT-3′), and 1510R.

Table 1 Characteristics that differentiate strain NCCP-36^T from its closely related taxa

Characteristics	Strains[a]						
	1	2	3	4	5	6	7
Growth temperature range (°C) (optimum)	10–45 (28)	15–45 (30)	25–45 (30–37)	25–42 (30–35)	10–40 (30)	10–37 (30)	16–45 (37)
pH range (optimum)	6.5–8.5 (7.0)	5.0–9.0 (7.0)	5.0–9.0[b] (7.0)	6.0–10.0 (7.0)	5.0–9.0 (7.5)	6.0–8.0 (7.0)	5.5–9.5 (7.5)
Growth in 6 % (w/v) NaCl	–	–	–	–	–	+	–
Boron tolerance (mM)	<50	ND	ND	ND	0	50	150
Oxidase	–	–	+	–[b]	+	–	+
Arginine dihydrolase	–	–	+	–	–	–	+
Lysine decarboxylase	–	–	+	–	–	–	–
Ornithine decarboxylase	–	–	+	–	–	–	–
Citrate utilization	–	–	+	–	+	–	+
Urease	–	–	+	–	-	+	+
Tryptophan deaminase	w+	–	+	–	ND	w+	+
Voges-Proskauer test	+	–	+	+	–	+	+
Hydrolysis of gelatin	–	+	–	–	+	–	–
Enzyme activity:							
Alkaline phosphatase	+	–	+[b]	–[b]	+	ND	w+
Esterase (C4)	–	+	+[b]	+[b]	–	–	+
Esterase lipase (C8)	–	+	+[b]	+[b]	–	+	w+
Leucine arylamidase	–	+	+[b]	+[b]	–	–	w+
Valine arylamidase	–	–	–	–[b]	–	–	w+
Trypsin		+	–	–[b]	–	–	ND
α-Chymotrypsin	–	+	–	+[b]	–	+	+
Acid phosphatase	+	+	–	–[b]	+	–	w+
Naphthol-AS-BI-phosphohydrolase	–	+	+[b]	–[b]	+	–	w+
α-Glucosidase	–	–	+[b]	–[b]	–	–	ND
Oxidation/reduction of:							
Acetic acid	+	–	+	+	–	ND	+
α-Ketovaleric acid	–	+	+	+	+	ND	+
Pyruvatic acid methyl ester	–	+	+	+	–	ND	ND
Pyruvic acid	–	–	+	+	–	–	+
L-Alanine	–	–	–	+	–	–	+
L-Glutamic acid	–	–	–	ND	–	–	+
Adenosine	–	–	–	-	–	–	+
Dextrin	+	–	+	+	+	ND	ND
β-Hydroxybutyric acid	–	–	–	+	–	–	–
G+C content (mol%)	37	35.9	36.3[b]	35.6[b]	37.2	38.7	36.5

ND, No data available; +, positive; w+, weakly positive; –, negative results

[a] 1, NCCP- 36^T (*Lysinibacillus composti* sp. nov.); 2, *L. sinduriensis* KCTC 13296^T (Jung et al. 2012); 3, *L. massiliensis* KCTC 13178^T (Glazunova et al. 2006); 4, *L. odysseyi* KCTC 3961^T (La Duc et al. 2004); 5, *L. xylanilyticus* KCTC 13423^T (Lee et al. 2010); 6, *L. parviboronicapiens* KCTC 13154^T (Miwa et al. 2009); 7, *L. boronitolerans* KCTC 13709^T (Ahmed et al. 2007b)

[b] Data from Jung et al. (2012)

The strain NCCP-36^T was identified using the sequence of 16S rRNA gene on the EzTaxon Server (http://eztaxon-e.ezbiocloud.net). To explore the exact taxonomic position of strain NCCP-36^T, we performed phylogenetic analyses with all of the published species of genus *Lysinibacillus* as described previously (Roohi et al. 2012). Sequences of closely related type strains that had been validated were retrieved from the database of the EzTaxon Server (http://eztaxon-e.ezbiocloud.net) for constructing the phylogenetic tree. Molecular evolutionary analyses were performed using MEGA ver. 5.20 (Tamura et al. 2011), and phylogenetic trees were constructed using three algorithms: neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (MLH). The stability of the relationship was assessed by bootstrap analysis (Felsenstein 2005) using 1,000 resampling for the tree topology of NJ data.

For DNA–DNA hybridization, genomic DNA of strain NCCP-36^T and of closely related reference species were isolated using a previously described procedure (Marmur 1963; Ahmed et al. 2007a). DNA–DNA hybridization was performed by the microplate method, as previously described (Ezaki et al. 1989) with five replications for each sample. The plate was pre-hybridized for 30 min and then hybridized with photobiotin-labeled probes at 45 °C. The fluorescence intensity was measured by a Flouroskan Ascent Fluorescent plate reader (Thermo Life Sciences, Basingstoke, UK). The highest and lowest values were excluded from each sample, and the means of the remaining three values were taken as the DNA relatedness value (Chang et al. 2008). The G+C content of the extracted DNA was determined by high-performance liquid chromatography at column temperature of 40 °C and wavelength of 270 nm using the mobile phase as 0.2 M ammonium phosphate: acetonitrile in the ratio of 40:1 (Mesbah et al. 1989).

Cellular fatty acid profiles of strain NCCP-36^T and of the closely related reference species were determined by growing strains on TSA medium at 28 °C for 48 h. The analysis was carried out according to a standard protocol (Sherlock Microbial Identification System; MIDI, Microbial ID, Newark, DE). The fatty acids were separated on an automated gas chromatography system (model 6890 N and 7683 Autosampler; Agilent Technologies, Santa Clara, CA) and identified by the associated software package ver. 4.0 (Library TSBA 40; MIDI, Microbial ID). Respiratory quinones were analyzed as described by Xie and Yokota (2003). To determine the peptidoglycan structure, 2 g of wet cells grown in TSB for 24 h was harvested and the cell walls purified as described previously (Kawamoto et al. 1981). The purified cell walls were hydrolyzed and their amino acids quantitatively analyzed on an automatic amino acid analyzer (Hitachi, Tokyo, Japan). Polar lipids were extracted and separated from 100 mg freeze-dried cell material by the two-stage method described by Tindall (1990).

Results and discussion

The optimum pH for the growth of strain NCCP-36^T was 7.0 (range 6.5–8.5). The strain did not show any growth at pH 6.0, whereas its closest species, Lysinibacillus sinduriensis, even grew at pH 5.0. Strain NCCP-36^T tolerated up to 5 % (w/v) NaCl. Slight growth was observed at 10 °C after 3 days of incubation, but it could not grow at ≥50 °C. The optimum growth temperature was 28 °C. Strain NCCP-36^T could not tolerate ≥50 mM boron, whereas the reference *Lysinibacillus boronitolerans* can tolerate up to 150 mM boron (Ahmed et al. 2007b). Strain NCCP-36^T was negative for oxidase (bioMérieux) and positive for catalase activity. The characteristics of strain NCCP-36^T which differentiate it from closely related strains are given in Table 1.

The comparative analysis of the 16S rRNA gene sequence of NCCP-36^T with that of its closely related strains indicated that strain NCCP-36 ^{T} belongs to *Bacillus* rRNA group 2. The highest sequence similarity of the 16S rRNA gene of strain NCCP-36^T was 97.52 % with *Lysinibacillus sinduriensis* KCTC13296^T (FJ169465); its sequence similarity with other closely related taxa was <97 % (Table 2). Strain NCCP-36^T clustered with species of genus *Lysinibacillus* and was found

Table 2 16S rRNA gene sequence similarity and DNA–DNA relatedness between strain NCCP-36^T and closely related reference strains of genus *Lysinibacillus*

Strains	*Lysinibacillus composti* NCCP-36^T (AB547124)	
	16S rRNA gene sequence similarity (%)	DNA–DNA hybridization (%)
Lysinibacillus composti NCCP-36^T (AB547124)	100.0	100
L. sinduriensis KCTC13296^T (FJ169465)	97.52	31
L. xylanilyticus KCTC 13423^T (FJ477040)	96.96	33
L. massiliensis KCTC13178^T (AY677116)	96.32	37
L. contaminans KCTC33155^T (KC254732)	96.02	–
L. sphaericus KCTC 3346^T (CP000817)	95.93	31
L. fusiformis KCTC 3454^T (AB271743)	95.91	23
L. manganicus Mn1-7^T (JX993821)	95.86	–
L. meyeri WS4626 (HE577173)	95.86	–
L. odyssey KCTC 3961^T (AF526913)	95.79	30
L. macroides LMG18474^T (AJ628749)	95.77	–
L. mangiferihumi M-GX18^T (JF731238)	95.51	–
L. parviboronicapiens KCTC 13154^T (AB300598)	95.28	27
L. boronitolerans KCTC 13709^T (AB199591)	95.05	25

to be closely associated to *L. sinduriensis* BLB-1^T (FJ169465) with a high bootstrap value (82 %) in the NJ phylogenetic tree inferred from 16S rRNA gene sequences (Fig. 1). This coherent association of NCCP-36^T with species of genus *Lysinibacillus* was also confirmed by MLH and MP algorithms.

The DNA–DNA hybridization values between strain NCCP-36^T and the reference species were <37 % (Table 2). These values are below the threshold (70 %) for species delineation (Stackebrandt and Goebel 1994) and thus allow the strain to be classified as a new species. The DNA G+C contents of strain NCCP-36^T were 37 mol% (Table 1). These data are in agreement with the values reported previously for genus *Lysinibacillus* (range 35–38.7 %; Ahmed et al. 2007b).

The predominant cellular fatty acids of strain NCCP-36^T were iso-$C_{15:0}$ (45.02 %) and anteiso-$C_{15:0}$ (16.74 %) (Table 3), and MK-7 (88 %) was identified as the predominant menaquinone system. The whole cell hydrolysate of strain NCCP-36^T contained Lys-Asp as diagnostic amino acids. The presence of Lys-Asp in cell-wall peptidoglycans corresponds to type *A*4α (Schleifer and Kandler 1972), which is in agreement with the description of genus *Lysinibacillus* (Ahmed et al. 2007b). The phylogenetic and chemotaxonomic analyses [major polar lipids: DPG, PG, phosphatidylethanolamine (PE), and phospholipids (PL); predominant menaquinone: MK-7;

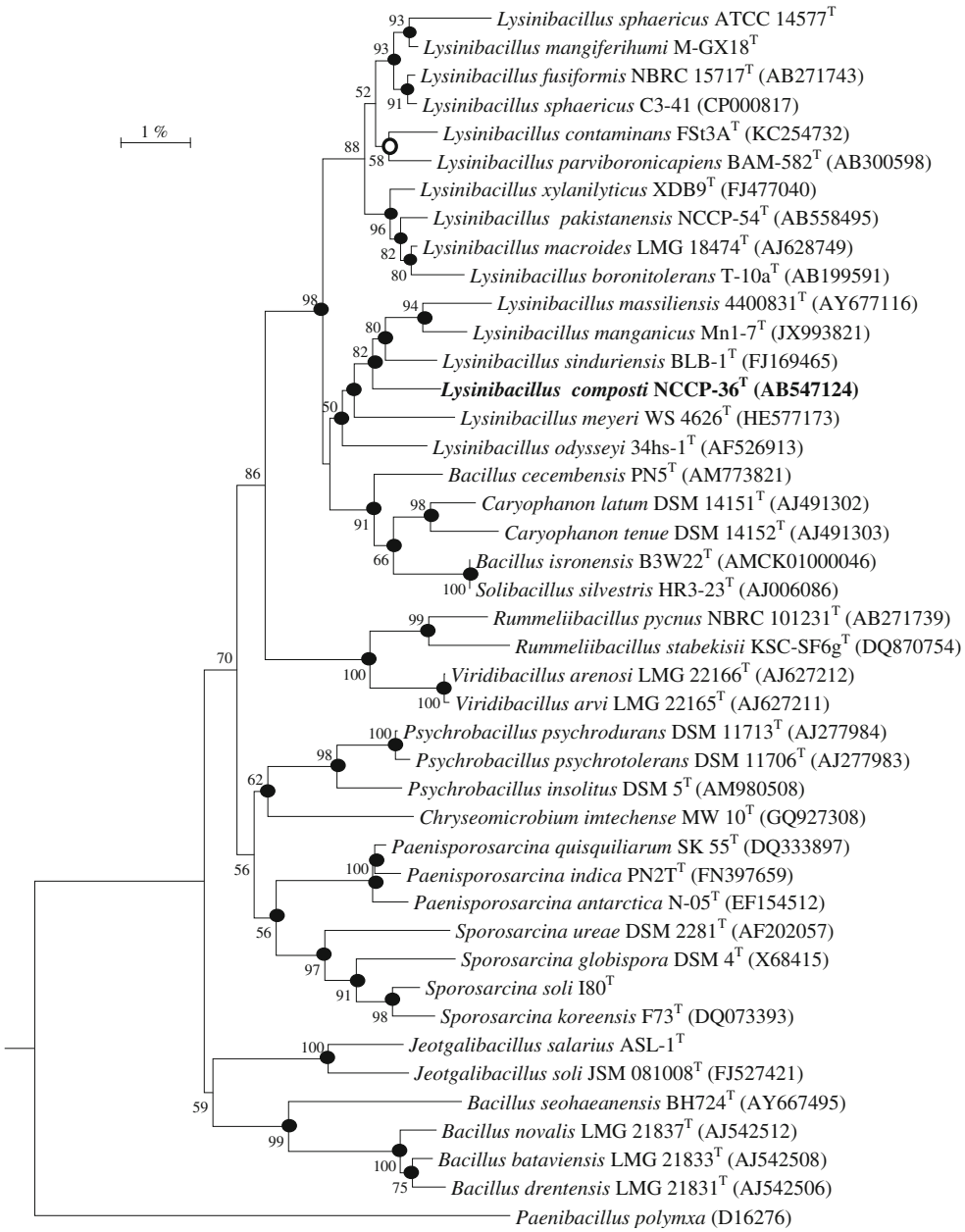

Fig. 1 Phylogenetic tree showing interrelationships of strain NCCP-36^T with closely related species of *Lysinibacillus* and other related genera inferred from 16S rRNA gene sequences. The tree was generated using the neighbor-joining (NJ) method based on a comparison of approximately 1,324 nucleotides and was rooted using *Paenibacillus polymyxa* (D16276) as an outgroup. Bootstrap values (>50 %), expressed as percentage of 1,000 replications, are indicated at the nodes. Nodes denoted by *open circles* were recovered by at least two algorithms, whereas nodes denoted with *filled circles* were recovered by three algorithms (NJ, maximum parsimony and maximum likelihood). Number in parenthesis is the accession number of type strain

Table 3 Cellular fatty acid profile of NCCP-36^T in comparison with the closely related reference species of genus *Lysinibacillus*

Fatty acid	Strains[a]								
	1	2	3	4	5	6	7	8	9
iso-$C_{14:0}$	2.78	4.80	1.87	2.61	1.24	–	1.77	3.66	1.05
$C_{14:0}$	–	–	1.01	–	–	–	0.57	–	–
iso-$C_{15:0}$	45.02	40.55	49.01	36.18	48.58	33.51	39.27	49.85	27.39
anteiso-$C_{15:0}$	16.74	11.48	9.25	8.71	9.27	11.81	16.22	3.34	20.15
$C_{15:0}$	–	1.45	–	–	–	–	–	–	–
$C_{16:1}\omega 7c$ alcohol	4.8	3.66	7.93	16.78	8.29	5.11	9.25	14.46	2.45
iso-$C_{16:0}$	7.44	24.83	5.51	13.17	5.15	4.66	12.70	12.12	7.60
$C_{16:1}\omega 11c$	2.80	–	2.20	2.58	3.54	4.38	2.35	1.72	1.27
$C_{16:0}$	2.84	1.31	–	3.11	1.02	3.04	1.68	–	3.03
iso-$C_{17:1}\omega 10c$	2.87	–	5.87	1.37	5.92	7.33	1.33	3.23	1.34
iso-$C_{17:0}$	6.06	4.39	7.22	6.28	4.70	12.08	5.79	8.07	10.12
anteiso-$C_{17:0}$	6.24	5.86	4.81	6.51	3.38	9.25	6.86	1.07	24.01
Summed feature 4 [b]	1.01	–	3.21	–	3.61	6.26	2.22	1.00	1.60

–, Not detected

All data were obtained in this study. Values are percentages of total fatty acid detected

[a] 1, NCCP-36^T (*Lysinibacillus composti* sp. nov.); 2, *Lysinibacillus massiliensis* KCTC 13178^T; 3, *L. xylanilyticus* KCTC 13423^T; 4, *L. odysseyi* KCTC 3961^T ; 5, *L. fusiformis* KCTC 3454^T; 6, *L. parviboronicapiens* KCTC 13154^T; 7, *L. boronitolerans* KCTC 13709^T; 8, *L. sphaericus* KCTC 3346^T; 9, *L. sinduriensis* KCTC 13296^T

[b] Summed feature contains iso-$C_{17:1}$ I or anteiso-$C_{17:1}$ B; these could not be separated by gas chromatography using the microbial Identification System (Microbial ID) software

major cellular fatty acids: iso-$C_{15:0}$, antieso-$C_{15:0}$, and iso-$C_{16:0}$; DNA G+C contents: 37 mol %; Lys-Asp (type *A*4α) in cell-wall peptidoglycans as diagnostic amino acids] also support the affiliation of strain NCCP-36^T with genus *Lysinibacillus*.

Strain NCCP-36^T shared a similar polar lipid profile (Electronic Supplementary Material Fig. 1; Table 4) with *Lysinibacillus massiliensis* KCTC 13178^T *L. xylanilyticus* KCTC13423^T, *L. odyssey* KCTC3961^T, *L. fusiformis* KCTC3454^T, *L. parviboronicapiens* KCTC13154^T, *L. boronitolerans* KCTC13709^T, and *L. sphaericus* KCTC3346^T, which consisted of predominantly DPG, PG and PE (Table 4).

On the basis of genotypic (DNA–DNA relatedness and DNA G+C contents) and phylogenetic analysis along with the phenotypic data presented in this paper, we assigned the isolated strain NCCP-36^T to a novel species in the genus *Lysinibacillus* as *Lysinibacillus composti* sp. nov. This strain is described in detail in the following section.

Description of *Lysinibacillus composti* sp. nov.

Lysinibacillus composti (com.pos′ti. N.L. gen. n. *composti*, of compost, from which the organism was isolated).

Cells are Gram-negative, aerobic, endospore-forming, and motile. The colonies are smooth with a shiny surface and opaque; the texture is butyrous and elevation is convex. The colony has a diameter of 1–2 mm after 24 h of

Table 4 Comparison of polar lipids profile of NCCP-36^T with the closely related reference species of genus *Lysinibacillus*

Strains[a]								
1	2	3	4	5	6	7	8	9
DPG	DPG	DPG	DPG	DPG	DPG	DPG	DPG	DPG
PG	PG	PG	PG	PG	PG	PG	PG	PG
PE		PE	PE		PE	PE		PE
PL1–PL3	PL1–PL2–PL3		PL1–PL2–PL3–PL4					PL1–PL2–PL3–PL4
	GL1–GL2			GL1	GL1	GL1	GL1	
UL1–UL2			UL1		UL1		UL1	UL1

DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL1–PL4, phospholipids 1–4; GL1–GL2, ninhydrin-positive phosphoglycolipids; UL1–UL2, unidentified polar lipid

[a] 1, NCCP-36^T (*Lysinibacillus composti* sp. nov.); 2, *Lysinibacillus massiliensis* KCTC 13178^T; 3, *L. xylanilyticus* KCTC 13423^T; 4, *L. odysseyi* KCTC 3961^T ; 5, *L. fusiformis* KCTC 3454^T; 6, *L. parviboronicapiens* KCTC 13154^T; 7, *L. boronitolerans* KCTC 13709^T; 8, *L. sphaericus* KCTC 3346^T; 9, *L. sinduriensis* KCTC 13296^T

incubation 28 °C on TSA, and it has a circular form with an entire margin. The pigmentation is light yellow. The temperature range and pH range for cell growth are 10–45 °C (optimum 28 °C) and 6.5–8.5 (optimum pH 7.0), respectively. Cells tolerate up to 5 % (w/v) NaCl but not 6 %, and they tolerate 0–50 mM boron in TSA but boron is not required for growth. Negative for nitrate reduction, oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, urease, geletinase, production of H_2S, indole, hydrolysis of casein, and starch. Positive for reduction of NO_3 to N_2, Voges–Proskauer test, and catalase activity; however, only weakly positive for tryptophane deaminase. No sugar is fermented in the API 50 CH strips using CHB/E suspension medium and in the API 20E strips (bio-Merieux). The cells show strong enzyme activity for alkaline phosphatase and acid phosphatase, but weak activity for esterase lipase (C8) and naphtol-AS-BI-phosphohydrolase and no activity for esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase (API-ZYM; bio-Merieux). Biolog results revealed that the strain is positive for acetic acid dextrin, but it is negative for α-ketovaleric acid, pyruvatic acid methyl ester, pyruvic acid, *L*-alaninamide, *L*-alanine, *L*-glutamate, adenosine, thymidine, thymidine-5′-monophosphate, β-hydroxybutyric acid, D-lactic acid methyl ester, and succinic acid mono-methyl ester. The dominant polar lipids are DPG, PG, PE, and PL. Cell-wall peptidoglycan contains Lys-Asp as the diagnostic amino acids, corresponding to peptidoglycan type *A*4α. The predominant cellular fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{17:0}$, $C_{16:1}$ ω7c alcohol, iso-$C_{17:1}$ ω10c, $C_{16:0}$, $C_{16:1}$ ω11c, iso-$C_{14:0}$. The major menaquinone is MK-7. The G+C contents of the type strain is 37 mol%.

The strain NCCP-36^T (=JCM 18777^T=KCTC 13796^T; DSMZ 24785^T) was isolated from compost of fruit and vegetable wastes prepared aerobically.

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