Lactobacillus delbrueckii subsp. *sunkii* subsp. nov., isolated from sunki, a traditional Japanese pickle

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Although four strains of bacteria isolated from sunki, a traditional Japanese, non-salted pickle, were initially identified as *Lactobacillus delbrueckii*, the molecular and phenotypic characteristics of the strains did not match those of any of the four recognized subspecies of *L. delbrueckii*. Together, the results of phenotypic characterization, DNA–DNA hybridizations (in which the relatedness values between the novel strains and type strains of the recognized subspecies of *L. delbrueckii* were all >88.7%) and 16S rRNA gene sequence, amplified fragment length polymorphism (AFLP) and whole-cell MALDI-TOF/MS spectral pattern analyses indicated that the four novel strains represented a single, novel subspecies, for which the name *Lactobacillus delbrueckii* subsp. *sunkii* subsp. nov. is proposed. The type strain is YIT 11221^T (=JCM 17838^T =DSM 24966^T).

Sunki is a traditional, Japanese, non-salted pickle that is produced by the fermentation of the leaves of red turnips ('otaki-kabu'). The turnips used for the pickle are cultivated in Kiso county, in the Nagano prefecture of Japan. To prepare the pickle, the turnip leaves are harvested in late autumn, parboiled for 10–15 s and then allowed to ferment at a low temperature (10–15 °C) for 1–2 weeks. The parboiled leaves are sometimes inoculated with dried, well-fermented sunki, prepared the previous year, as a starter. During a study on the biodiversity of the lactic acid bacteria (LAB) associated with the fermentation of sunki (Watanabe *et al.*, 2009), four novel species – *Lactobacillus kisonensis*, *Lactobacillus otakiensis*, *Lactobacillus rapi* and *Lactobacillus sunkii* – were identified as the predominant species in 33 samples of pickling solution used in sunki production in Kiso county, while *Lactobacillus delbrueckii* was identified as the subdominant LAB component. In Koso county, which is also in Nagano prefecture, Endo *et al.* (2008) identified *L. delbrueckii*, *Lactobacillus fermentum* and *Lactobacillus plantarum* as the predominant species in the fermentation of sunki samples collected from four families.

L. delbrueckii plays an important part in the production of many fermented foods of both plant and animal origin. It

has been isolated from the fermented brines used in the production of stinky tofu in Taiwan (Chao *et al.*, 2008a). At the time of writing, four subspecies of this species are recognized. Although *Lactobacillus delbrueckii* subsp. *bulgaricus* has been isolated mainly from fermented milk, it has recently also been detected in Bulgarian plants (Michaylova *et al.*, 2007). *Lactobacillus delbrueckii* subsp. *delbrueckii*, which lacks a lactose operon, has been found in fermented plant materials (Germond *et al.*, 2003), while *Lactobacillus delbrueckii* subsp. *indicus* has been isolated from fermented dairy products in India (Dellaglio *et al.*, 2005). Most isolates of the other recognized subspecies of *L. delbrueckii*, *Lactobacillus delbrueckii* subsp. *lactis*, have come from cheeses (Weiss *et al.*, 1983).

Although the reliable identification of *L. delbrueckii* at the subspecies level may be important in the production of several fermented foods, the sugar fermentation tests and other phenotypic characterizations that are frequently employed (Weiss *et al.*, 1983) often give ambiguous results. Since 1999, the potential use of molecular methods to distinguish the subspecies of *L. delbrueckii* has therefore been explored. For example, randomly amplified polymorphic DNA (RAPD) PCR and proline iminopeptidase (*pepIP*) gene sequence analysis have been used to distinguish *L. delbrueckii* subsp. *bulgaricus* from other subspecies of *L. delbrueckii* (Torriani *et al.*, 1999). The analysis of cell-wall proteins by SDS-PAGE was used to distinguish *L. delbrueckii* subsp. *delbrueckii* from *L. delbrueckii* subsp. *bulgaricus* (Gatti *et al.*, 2001), while restriction fragment length polymorphism (RFLP) analysis of bacterial protein-coding genes was used both to characterize *L. delbrueckii* subsp. *lactis* and *L. delbrueckii* subsp. *bulgaricus* and to distinguish between these two subspecies (Giraffa *et al.*,

Abbreviations: AFLP, amplified fragment length polymorphism; LAB, lactic acid bacteria; MALDI-TOF/MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIT 11211^T, YIT 11220, YIT 11446 and YIT 11673 are AB641833, AB641832, AB641834 and AB641835, respectively.

Two supplementary tables and a supplementary figure are available with the online version of this paper.

2003). Germond *et al.* (2003) investigated 16S rRNA gene sequence mutations, expression of β -galactosidase and cell-wall-anchored protease, galactose metabolism and the distribution of insertion sequences (IS elements), as well as characterizing the lactose operon locus and *lacR* gene sequences, in *L. delbrueckii* subsp. *lactis*, *L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *delbrueckii*. Unfortunately, none of these analyses was found useful for distinguishing *L. delbrueckii* subsp. *delbrueckii* from the other subspecies of *L. delbrueckii* that were then recognized, although another type of molecular characterization, amplified-fragment length polymorphism (AFLP) analysis, did allow the identification of *L. delbrueckii* subsp. *indicus* as a fourth subspecies (Dellaglio *et al.*, 2005).

Tanigawa & Watanabe (2011) recently investigated 41 strains of *L. delbrueckii*, by AFLP analysis, the multilocus sequence typing (MLST) of seven housekeeping genes (*fusA*, *gyrB*, *hsp60*, *ileS*, *pyrG*, *recA* and *recG*) and sugar fermentation tests. The results indicated that some reclassification of the subspecies of *L. delbrueckii* might be necessary, since the strains of *L. delbrueckii* subsp. *bulgaricus* formed a cluster that was surprisingly distinct from the cluster formed by strains of *L. delbrueckii* subsp. *indicus*. Furthermore, the type strain of *L. delbrueckii* subsp. *delbrueckii* was clearly separate from the other strains currently classified as members of this subspecies, which appeared more closely related to the investigated strains of *L. delbrueckii* subsp. *lactis*. Tanigawa & Watanabe (2011) also suggested that the four strains derived from sunki that they investigated represented a novel subspecies of *L. delbrueckii*.

In 2004, four strains of bacteria, designated YIT 11220, YIT 11221^T, YIT 11466 and YIT 11673, were isolated from samples of sunki and initially identified as *L. delbrueckii*. Since the molecular and phenotypic characteristics of these strains did not match those of any of the four recognized subspecies of *L. delbrueckii*, the taxonomic positions of the strains were investigated by following a polyphasic approach. For reference, *L. delbrueckii* subsp. *bulgaricus* YIT 0181^T (=ATCC 11842^T), *L. delbrueckii* subsp. *delbrueckii* YIT 0080^T (=ATCC 9649^T), *L. delbrueckii* subsp. *indicus* YIT 11751^T (=DSM 15996^T) and *L. delbrueckii* subsp. *lactis* YIT 0086^T (=ATCC 12315^T) and 19 other strains previously assigned to a subspecies of *L. delbrueckii* were obtained from the culture collection of the Yakult Central Institute in Tokyo, Japan (Table S1, available in IJSEM Online). Prior to testing, unless indicated otherwise, the novel and reference strains were grown for 1 or 2 days, at 37 °C and pH 6.8, in MRS broth (Difco; de Man *et al.*, 1960).

The genomic DNA used in the analysis of 16S rRNA gene sequences and AFLP typing was prepared according to the method of Watanabe *et al.* (2008). The methods used for the amplification and subsequent sequencing of 16S rRNA genes were those described by Chao *et al.* (2008b). The PCR mixture (25 µl) contained 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 200 µM of each deoxyribonucleotide triphosphate, 1 mM MgCl₂, 1 µg BSA, 0.5 U *Taq* DNA

polymerase (Takara Bio), 0.1 µM of each primer and 10 ng template DNA. The thermocycler was set to give an initial denaturation step of 2 min at 94 °C, followed by 30 cycles, each of 20 s at 94 °C, 30 s at 55 °C and 20 s at 72 °C, and then a final extension of 3 min at 72 °C. The amplicons were purified, using a Montage PCR filter unit (Millipore) according to the manufacturer's instructions, before being sequenced by using version 3.1 of the ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an ABI PRISM 3130xl genetic analyser (Applied Biosystems). The sequences were assembled using version 2.1 of the Autoassembler package (Applied Biosystems) and version 15.0 of the GENETYX-MAC package (Software Development). The closest known relatives of the novel strains were identified using FASTA (Pearson & Lipman, 1988), before the relevant sequences of the closely related species were retrieved from the GenBank/EMBL/DBJ databases. Multiple alignment of the sequences was carried out with version 2.0.12 of the program CLUSTAL_X (Thompson *et al.*, 1997). The almost-complete 16S rRNA gene sequences of the novel strains and strains of related species were used in the construction of a neighbour-joining phylogenetic tree (Saitou & Nei, 1987). The topology of the tree was evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985). The MEGA4 package (Tamura *et al.*, 2007) was used to construct minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Fitch, 1971) trees, using the same sequences and the Kimura two-parameter model (Kimura, 1980). The novel strain YIT 11221^T appeared to be closely related to the type strains of all four recognized subspecies of *L. delbrueckii* (i.e. *L. delbrueckii* subsp. *bulgaricus* YIT 0181^T, *L. delbrueckii* subsp. *delbrueckii* YIT 0080^T, *L. delbrueckii* subsp. *indicus* YIT 11751^T and *L. delbrueckii* subsp. *lactis* YIT 0086^T), with 16S rRNA gene sequence similarities of at least 98.8%. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, the four novel strains were placed in a cluster formed of members of the species *L. delbrueckii* (Fig. S1). Similar topologies were seen in the corresponding minimum-evolution and maximum-parsimony trees (data not shown).

AFLP analysis was performed by following the method of Tanigawa & Watanabe (2011) and using *EcoRI* and *MseI* ligation/amplification modules (Applied Biosystems). Total DNA was digested with *EcoRI* and *MseI* restriction enzymes (Takara Bio) before the DNA fragments were ligated to double-stranded restriction-site-specific *EcoRI* and *MseI* adaptors. The primers *EcoRI*-A (5'-GACTGCATACCACCAATTCA-3') and *MseI*-CA (5'-AATGAGTCCTGAGTAGCA-3') were used for the preselective and selective PCR [the selective base(s) at the 3'-end of each primer are underlined]. The amplicons were analysed on an ABI PRISM 3130xl genetic analyser in standard fragment-analysis mode, using GENESCAN-500 LIZ (Applied Biosystems) as the size standard. After electrophoresis, the AFLP patterns were recorded and analysed using version 4.0 of the GeneMapper package

Fig. 1. Phylogenetic tree based on AFLP profiles showing the relationships between the novel strains YIT 11220, YIT 11221^T, YIT 11446 and YIT 11673 and closely related strains belonging to the species *Lactobacillus delbrueckii*. Bootstrap values $\geq 95\%$ (based on 100 replications) are shown at branching points. Bar, percentage band similarity.

(Applied Biosystems). A threshold fluorescence value of 100 arbitrary units was used to eliminate background fluorescence, and only DNA fragments of 51–500 bp were analysed. Fragments from different strains that had the same length were assumed to be identical and were classified as the same alleles. Bands of different sizes were treated as independent loci, each with two alleles (present or absent). The data, which were exported in binary format (with 1 and 0 indicating the presence and absence of a particular band, respectively) were analysed in the package MEGA4. For this analysis, an unweighted pair-group method with arithmetic means (UPGMA; Sneath & Sokal, 1973) and Dice coefficient, with a bootstrap analysis involving 100 repetitions, were used. The 27 strains included in the analysis were separated into four major clusters (Fig. 1). Cluster A consisted of two strains of *L. delbrueckii* subsp. *delbrueckii*, nine of *L. delbrueckii* subsp. *lactis* and the four novel strains. Cluster B comprised only a single strain (the type strain of *L. delbrueckii* subsp. *delbrueckii*), cluster C consisted of seven strains of *L. delbrueckii* subsp. *bulgaricus* and cluster D consisted of four strains of *L. delbrueckii* subsp. *indicus*. Within cluster A, the four novel strains, whose band similarities were $>97\%$, formed a distinct subcluster (Fig. 1).

Cell protein for analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) was extracted by the ethanol/formic acid

method. Briefly, 4 ml culture (incubated overnight) was centrifuged at 14 000 *g* for 5 min. The resultant cell pellet was washed twice in double-distilled water before the cells were resuspended in 500 μ l of 70% (v/v) ethanol and then centrifuged down at 10 000 *g* for 2 min. The pellet produced was mixed with 10 μ l of 70% (v/v) formic acid, by vortexing, and then gently mixed with 10 μ l acetonitrile. The resulting mixture was centrifuged, again at 10 000 *g* for 2 min, before 0.5 μ l supernatant solution was applied to a FlexiMass-DS disposable MALDI target (Shimadzu), air-dried and then overlaid with 1 μ l matrix solution, which comprised α -cyano-4-hydroxycinnamic acid (Shimadzu) at a concentration of 20 mg ml⁻¹ (3% trifluoroacetic acid/33% acetonitrile/33% ethanol). MALDI mass spectra in the range of 2000–20 000 molecular masses (*m/z*) were recorded, in positive linear mode, by averaging 500 individual laser shots made in an Axima Performance MALDI-TOF/TOF spectrometer (Shimadzu) equipped with a pulsed N₂ laser ($\lambda=337.1$ nm; pulse width=3 ns; frequency=50 Hz). The spectra were calibrated externally by using *Escherichia coli* DH5 α electro cells (Takara Bio) as the reference standards. MALDI-TOF/MS peaks from different strains that showed the same *m/z* value were assumed to be identical and were classified as the same alleles. Peaks of different *m/z* were treated as independent loci with two alleles (present or absent). Data were exported in binary format and analysed like those from

Fig. 2. Phylogenetic tree based on MALDI-TOF/MS patterns showing the relationships between the novel strains YIT 11220, YIT 11221^T, YIT 11446 and YIT 11673 and closely related strains belonging to the species *Lactobacillus delbrueckii*. Bootstrap values $\geq 95\%$ (based on 100 replications) are shown at branching points. Bar, proportion of peaks that are different.

the AFLP analysis. The 27 *L. delbrueckii* strains included in the MALDI-TOF/MS analysis were separated into three major clusters (Fig. 2). Cluster A consisted of three strains of *L. delbrueckii* subsp. *delbrueckii*, nine of *L. delbrueckii* subsp. *lactis* and the four novel strains. Cluster B consisted of seven strains of *L. delbrueckii* subsp. *bulgaricus* while cluster C consisted of four strains of *L. delbrueckii* subsp. *indicus*. In the tree based on MALDI-TOF/MS patterns (Fig. 2), as in the tree based on AFLP patterns, the four novel strains formed a tight, separate subcluster in cluster A.

The genomic DNA used to determine the DNA G+C contents of the novel and reference strains and to investigate DNA–DNA relatedness values among the same strains was extracted according to the method of Marmur (1961). To assess its G+C content, DNA was enzymically degraded into nucleosides (Mesbah *et al.*, 1989) and then separated by HPLC. The genomic DNA G+C contents of the four novel strains (50.9–51.7 mol%) were very similar to the corresponding values for the type strains of the four recognized subspecies of *L. delbrueckii* (50.5–54.3 mol%).

DNA–DNA hybridizations were performed in microdilution wells; photobiotin was used for the DNA labelling (Ezaki *et al.*, 1989). Reciprocal hybridization experiments were carried out at 45 °C for 2 h in the presence of 50 % (v/v) formamide, using biotinylated DNA and unlabelled, single-strand DNA that had been bonded, non-covalently, to microplate wells. For each hybridization, the highest and lowest values from eight replicate wells were excluded before the means of the six remaining values were calculated and recorded as a relatedness value. The DNA–DNA relatedness values among the four novel strains were high (at least 94.8%), whereas those between strain YIT 11221^T and *L. delbrueckii* subsp. *bulgaricus* YIT 0181^T (91.6 %), *L. delbrueckii* subsp. *delbrueckii* YIT 0080^T (88.7 %), *L. delbrueckii* subsp. *indicus* YIT 11751^T (90.1 %) and *L. delbrueckii* subsp. *lactis* YIT 0086^T (89.0 %) were slightly lower. These values indicate that the four novel strains belong to a single taxon within the species *L. delbrueckii* (Table S2).

Morphological, cultural and biochemical tests were performed, according to standard techniques, at 37 °C unless

Table 1. Major characteristics that can be used to distinguish the novel strains YIT 11220, YIT 11221^T, YIT 11446 and YIT 11673 from the type strains of the four established subspecies of *Lactobacillus delbrueckii*

Strains: 1, YIT 11220; 2, YIT 11221^T; 3, YIT 11446; 4, YIT 11673; 5, *L. delbrueckii* subsp. *bulgaricus* YIT 0181^T; 6, *L. delbrueckii* subsp. *delbrueckii* YIT 0080^T; 7, *L. delbrueckii* subsp. *indicus* YIT 11751^T; 8, *L. delbrueckii* subsp. *lactis* YIT 0086^T. All data are from this study. All eight strains produced acid from D-glucose, D-fructose and D-mannose. None of the strains produced acid from glycerol, erythritol, D- or L-arabinose, D-ribose, D- or L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, inulin, melezitose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. +, Positive; –, negative; w, weakly positive.

Characteristics	1	2	3	4	5	6	7	8
Growth on MRS at/in:								
15 °C	w	w	w	w	–	–	–	–
pH 4.0	+	+	+	+	w	w	–	w
4 % (w/v) NaCl	w	w	w	w	w	w	–	+
NH ₃ production from L-arginine	+	+	+	+	–	–	–	–
Fermentation of:								
D-Galactose	–	–	w	–	–	–	+	–
D-Mannitol	+	+	+	+	–	–	–	–
N-Acetyl-D-glucosamine	+	+	+	+	–	+	+	+
Amygdalin	+	+	w	+	–	–	+	+
Arbutin	+	+	w	+	–	–	+	+
Salicin	+	+	+	+	–	–	–	+
Cellobiose	+	+	w	+	–	–	+	+
Maltose	+	+	+	+	–	–	–	+
Lactose	–	–	–	–	+	–	+	+
Melibiose	+	+	+	+	–	–	–	–
Sucrose	+	+	+	+	–	+	+	+
D-Trehalose	+	+	+	+	–	–	–	+
Raffinose	+	+	+	+	–	–	–	w
Gentiobiose	w	w	w	w	–	–	–	–
Aesculin hydrolysis	+	+	+	+	–	–	+	–
DNA G + C content (mol%)	50.9	51.0	51.7	51.5	51.6	54.3	50.5	51.6

otherwise stated. Cell shape, cell size and Gram staining were determined by using cultures grown in MRS broth at 30 °C for 16 h. Motility was tested in soft MRS agar that contained 0.15 % (w/v) agar. Catalase activity was also determined using cells grown on MRS agar. Gas production from glucose was measured with a Durham tube in MRS broth. Production of dextran was assessed on MRS agar in which the usual glucose had been replaced with 2 % (w/v) sucrose. The methods of Barrow & Feltham (1993) were used to determine growth at various temperatures and pH values and growth with various concentrations of NaCl, and to determine if the novel strains reduced nitrate and/or produced ammonia from arginine. Carbohydrate fermentation tests were conducted by using the API 50 CHL system (bioMérieux) according to the manufacturer's instructions. The presence of diaminopimelic acid in the cell-wall peptidoglycan was investigated by the method of Kandler & Weiss (1986). Cell-wall peptidoglycan was prepared and hydrolysed according to the method of Schleifer & Kandler (1972) so that the amino acids could be analysed, by HPLC, in an Alliance 2695 system (Waters) equipped with a fluorescence detector (model 474; Waters) and an AccQ-Tag column (3.9 × 150 mm; Waters). The

AccQ-Fluor reagent (Waters), 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, was used for the derivatization.

Cellular fatty acid methyl esters were obtained from cells grown on MRS agar at 30 °C (YIT 11221^T) or 37 °C (other strains) for 3 days by saponification, methylation and extraction using the method of Miller (1982) with minor modifications (Kuykendall *et al.*, 1988). Fatty acids were then identified by following the protocol of the Sherlock Microbial Identification System (MIDI) and using the MOORE5 library. The isomers of lactic acid formed from glucose were determined by using the TC D-/L-lactic acid test kit (Boehringer Mannheim).

The four novel strains differed from the type strains of the four recognized subspecies of *L. delbrueckii* in several phenotypic characteristics. For example, all four novel strains were positive for acid production from D-mannitol, melibiose and gentiobiose, growth at 15 °C and ammonia production from L-arginine whereas all of the type strains of the recognized subspecies of *L. delbrueckii* were negative for all of these characteristics (Table 1). Based on the genotypic, proteomic and phenotypic evidence, the four novel strains represent a single novel subspecies of *L.*

Table 2. Cellular fatty acid contents (%) of the novel strain YIT 11221^T and the type strains of the four established subspecies of *Lactobacillus delbrueckii*

Strains: 1, YIT 11221^T; 2, *L. delbrueckii* subsp. *bulgaricus* YIT 0181^T; 3, *L. delbrueckii* subsp. *delbrueckii* YIT 0080^T; 4, *L. delbrueckii* subsp. *indicus* YIT 11751^T; 5, *L. delbrueckii* subsp. *lactis* YIT 0086^T. All data are from this study. –, Not detected; DMA, dimethylacetal; ECL, equivalent chain-length. C_{16:1}ω5c, C_{18:1}ω9c DMA, iso-C_{17:0} and C_{18:0} 12-OH were not detected in any strains.

Fatty acid	1	2	3	4	5
Saturated					
C _{12:0}	0.49	1.19	1.31	1.03	2.05
C _{14:0}	2.31	1.65	1.38	2.34	1.67
C _{15:0}	0.11	—	—	—	—
C _{16:0}	47.58	5.54	32.66	38.94	6.20
C _{17:0}	0.28	1.53	0.14	0.10	0.24
C _{18:0}	—	—	4.46	3.29	3.47
C _{20:0}	0.16	—	—	—	—
Unsaturated					
C _{16:1} ω7c	12.83	20.90	1.76	8.63	1.03
C _{18:1} ω5c	0.65	—	—	—	—
C _{18:1} ω6c	32.66	—	—	—	—
C _{18:1} ω9c	—	31.60	47.37	14.17	44.67
C _{18:1} ω7c DMA	1.30	0.25	0.27	0.23	0.87
C _{18:2} ω6,9c	—	—	—	—	0.36
C _{20:1} ω10c	—	0.14	—	—	—
Cyclo					
C _{17:0} cyclo	—	0.89	—	1.71	—
C _{19:0} cyclo 9,10	—	24.21	—	17.06	34.52
C _{19:0} cyclo 11,12	—	—	—	5.00	—
C _{19:0} cyclo 11,12 DMA	—	—	—	0.21	—
Branched-chain					
anteiso-C _{17:0}	0.11	0.54	—	—	—
Hydroxy					
C _{15:0} 3-OH	0.31	0.21	—	—	—
Unknown					
ECL 18.199	—	0.67	0.51	0.47	1.44
Summed features*					
10	—	10.41	9.73	6.32	2.44
12	1.22	0.26	0.42	0.50	1.05

*Summed features represent groups of two or more fatty acids that could not be separated by GLC with the MIDI system. Summed feature 10 contained C_{18:1}ω7c and/or an unknown with an ECL of 17.834; summed feature 12 contained an unknown with an ECL of 18.622 and/or iso-C_{19:0}.

delbrueckii, for which the name *Lactobacillus delbrueckii* subsp. *sunkii* subsp. nov. is proposed.

Description of *Lactobacillus delbrueckii* subsp. *sunkii* subsp. nov.

Lactobacillus delbrueckii subsp. *sunkii* (sun'ki.i. N.L. gen. n. *sunkii* of sunki, the name of the non-salted, traditional, Japanese pickle from which the type strain was isolated).

Cells are rod-shaped (0.4–0.6 × 2.0–5.0 µm) and occur singly, in pairs or in short chains of three or four cells. Gram-staining-positive, non-motile, non-spore-forming and facultatively anaerobic. Negative for catalase and pseudocatalase activities. After anaerobic growth for 48 h at 30 °C, colonies on MRS agar are beige, with a smooth to rough surface, circular or slightly irregular in outline and approximately 2–4 mm in diameter. In MRS broth, weak growth occurs at 15 °C but no growth occurs at 45 °C. Growth occurs at pH 4.0. Weak growth occurs with 4 % (w/v) NaCl. Gas is not produced from glucose. The main end-product of glucose fermentation is D-lactate. Ammonia is produced from L-arginine. Nitrate is not reduced. Acid is produced from D-glucose, D-fructose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, melibiose, sucrose, D-trehalose, raffinose and gentiobiose but not from glycerol, erythritol, D- or L-arabinose, D-ribose, D- or L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, lactose, inulin, melezitose, starch, glycogen, xylitol, turanose, D-lyxose, D- or L-fucose, D- or L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Acid production from D-galactose is strain-dependent. Aesculin is hydrolysed. Dextran is not produced from sucrose. Cells do not contain *meso*-diaminopimelic acid in their cell-wall peptidoglycan. In the presence of Lys, Glu, Ala and Asp, peptidoglycan structure is of the L-Lys–D-Asp type. The major cellular fatty acids are the saturated fatty acid C_{16:0} and the unsaturated fatty acid C_{18:1}ω6c (Table 2). No cyclopropane is present.

The type strain, YIT 11221^T (=JCM 17838^T =DSM 24966^T), was isolated from sunki, a traditional, Japanese pickle, in the Nagano prefecture of Japan. The genomic DNA G + C content of the type strain is 51.0 mol%.

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