Lactobacillus kisonensis sp. nov., *Lactobacillus otakiensis* sp. nov., *Lactobacillus rapi* sp. nov. and *Lactobacillus sunkii* sp. nov., heterofermentative species isolated from sunki, a traditional Japanese pickle

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Thirty six Gram-positive, rod-shaped, non-spore-forming, non-motile bacterial strains were isolated from the non-salted pickle solution used in producing sunki products, a traditional Japanese pickle. The novel strains were discriminated and separated into four groups by amplified fragment length polymorphism profiling, and by analysis based on *recA* gene sequences. The strains were classified into four species groups belonging to the *Lactobacillus buchneri* species group, which consists of *L. buchneri*, *Lactobacillus diolivorans*, *Lactobacillus hilgardii*, *Lactobacillus kefir*, *Lactobacillus parabuchneri* and *Lactobacillus parakefir*. The phenotypic and genotypic features of the four groups demonstrated that they represented four novel species, for which the names *Lactobacillus kisonensis* sp. nov. (type strain YIT 11168^T=NRIC 0741^T=JCM 15041^T=DSM 19906^T), *Lactobacillus otakiensis* sp. nov. (type strain YIT 11163^T=NRIC 0742^T=JCM 15040^T=DSM 19908^T), *Lactobacillus rapi* sp. nov. (type strain YIT 11204^T=NRIC 0743^T=JCM 15042^T=DSM 19907^T) and *Lactobacillus sunkii* sp. nov. (type strain YIT 11161^T=NRIC 0744^T=JCM 15039^T=DSM 19904^T) are proposed.

Sunki is a traditional Japanese non-salted pickle product prepared from the spontaneous fermentation of the parboiled leaves and stems of red turnips, called 'otaki-kabu'. The red turnips are cultivated in Kiso county, Nagano prefecture, Japan. To prepare the pickle, the red turnips are parboiled and

sometimes inoculated with dried, well-fermented sunki that was prepared the previous year as a starter. The turnips are then allowed to ferment at a low temperature during late autumn at around 10–15 °C for 1–2 months.

During a study on the biodiversity of lactic acid bacteria (LAB) associated with the fermentation of sunki, 36 strains were isolated from 33 samples of the non-salted pickle solution used in sunki production. The samples were collected in December 2004 in Kiso county, Nagano prefecture, Japan. The novel strains were classified as belonging to the *Lactobacillus buchneri* species group, which consists of *L. buchneri*, *Lactobacillus diolivorans*, *Lactobacillus hilgardii*, *Lactobacillus kefir*, *Lactobacillus parabuchneri* and *Lactobacillus parakefir*, by a phylogenetic analysis based on 16S rRNA gene sequences. Samples were taken aseptically, stored at 4 °C and pelleted by centrifugation at 20 000 g for 10 min. The pellet was resuspended with Nutrient Broth (BD; Difco) containing 10 % (v/v) DMSO and stored at –80 °C until use.

Thawed samples were serially diluted with sterile saline (0.85 % NaCl) before being inoculated onto MRS (BD;

Abbreviations: AFLP, amplified fragment length polymorphism; LAB, lactic acid bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains *L. buchneri* YIT 0077^T, *L. diolivorans* YIT 10368^T, *L. hilgardii* YIT 0269^T, *L. kefir* YIT 0222^T, *L. parabuchneri* YIT 0272^T, *L. parakefir* YIT 10382^T, *L. sunkii* sp. nov. YIT 11161^T, *L. otakiensis* sp. nov. YIT 11163^T, YIT 11167, *L. kisonensis* sp. nov. YIT 11168^T, *L. rapi* sp. nov. YIT 11204^T, YIT 11442, YIT 11452, YIT 11505, YIT 11510, YIT 11539, YIT 11549, YIT 11557, YIT 11635, YIT 11661 and YIT 11688 are AB429368–AB429373 and AB366385–AB366399, respectively, and for the *recA* gene sequences AB430354–AB430374, respectively.

A table detailing the DNA G + C contents and DNA–DNA relatedness of the novel strains as compared with type strains of species in the *L. buchneri* group is available with the online version of this paper. Additional phylogenetic trees based on 16S rRNA and *RecA* gene sequences and constructed with the neighbour-joining, maximum-parsimony and maximum-likelihood methods are also available.

Difco) agar supplemented with 0.001 % sodium azide and 0.001 % cycloheximide and incubated anaerobically at 30 °C for 3 days. The total cell counts for the 36 LAB strains in the 33 samples ranged from 5.40 to 8.71 log₁₀ c.f.u. ml⁻¹ (7.19 ± 0.94 log₁₀ c.f.u. ml⁻¹; mean ± SD). This study describes the morphological, biochemical and molecular characterization of the 36 novel strains, which could not be clearly placed within any recognized species in the *L. buchneri* species group.

The following strains were obtained from the culture collection of the Yakult Central Institute (YIT; Tokyo, Japan) and were used as reference strains: *L. buchneri* YIT 0077^T, *L. diolivorans* YIT 10368^T, *L. hilgardii* YIT 0269^T, *L. kefir* YIT 0222^T, *L. parabuchneri* YIT 0272^T and *L. parakefir* YIT 10382^T. The strains used for further experiments were cultivated and maintained in MRS broth (pH 7.0) at 30 °C for 1 or 2 days, unless indicated otherwise.

Chromosomal DNA was used for an amplified fragment length polymorphism (AFLP) analysis and the sequence amplification of the 16S rRNA and *recA* genes from the novel isolates was conducted according to the method of Watanabe *et al.* (2008). For discrimination of the strains, the AFLP analysis method was performed as described previously (Thompson *et al.*, 2001), with the following modifications. Total DNA was digested with *EcoRI* and *MseI* restriction enzymes and the DNA fragments were ligated to double-stranded restriction site-specific adaptors, *EcoRI*-adaptors (5'-CTCGTAGACTGCGTACC-3', 5'-CATCTGACGCATGGTTAA-3') and *MseI*-adaptors (5'-TACTCAGGACTCAT-3', 5'-GAGTCCTGAGTAGCAG-3'). For the preselective and selective PCR amplification, primers *EcoRI*-A (5'-GACTGCATACCACCAATTCA-3') and *MseI*-CA (5'-AATGAGTCCTGAGTAGCA-3') (selective bases at the 3'-end are underlined) were used. The 5' end of *EcoRI* primer was labelled with 6-carboxy-fluorescein (FAM). PCR products were analysed on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) in standard fragment analysis mode protocol with a GENESCAN-500LIZ size standard (Applied Biosystems). After electrophoresis, the AFLP patterns were analysed and extracted with GeneMapper software v4.0 (Applied Biosystems). A threshold fluorescence value of 250 arbitrary units was used to eliminate the background and DNA fragments of between 51–500 bp were analysed. Bands that were positioned at the same length (DNA size) in different individual strains were assumed to be homologous and to represent the same allele. Bands of different sizes were treated as independent loci with two alleles (present or absent). Data were exported in a binary format with '1' representing the presence of a band/peak and '0' representing its absence. The data were analysed phylogenetically with InforBIO software v5.26 (Sugawara *et al.*, 2003) by using the unweighted pair group method with arithmetic mean clustering using the Pearson correlation coefficient. The 36 strains were separated into four groups. Group A comprised strains YIT 11161^T, YIT 11436, YIT 11442, YIT 11539, YIT 11547, YIT 11549, YIT 11557, YIT

11574, YIT 11575, YIT 11635 and YIT 11675. Group B comprised strains YIT 11168^T, YIT 11510, YIT 11515, YIT 11554, YIT 11566 and YIT 11661. The strains in group C were YIT 11204^T and YIT 11688 and group D comprised strains YIT 11163^T, YIT 11167, YIT 11194, YIT 11218, YIT 11224, YIT 11452, YIT 11461, YIT 11505, YIT 11512, YIT 11532, YIT 11535, YIT 11548, YIT 11556, YIT 11571, YIT 11663, YIT 11674 and YIT 11687 (Fig. 1).

The phylogenetic position of the 36 strains was initially investigated using the 16S rRNA gene sequence analysis as described by Miyake *et al.* (1998). Next, the taxonomic positions were analysed using *recA* gene sequence analysis. The sequences of the forward (*recA*-90F) and reverse (*recA*-500R) primers used for the PCR amplification of the *recA* genes were 5'-TAYGGVCCNGAAAGTTCDGG-3' and 5'-CATVACVCCVACTTTTTCACG-3', respectively. These primers were designed from the consensus sequences of the *recA* genes of *L. buchneri* GenBank accession no. AJ621626, *L. brevis* DQ080023, *L. diolivorans* AJ621635, *L. hilgardii* AJ621647, *L. kefir* AJ621650, *L. parabuchneri* AJ621661, *L. parakefir* AJ621665, *L. delbrueckii* subsp. *bulgaricus* AJ586864 and *L. fermentum* AJ579534. The PCR mixture (25 µl) contained 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 200 µM of each dNTP, 1 mM MgCl₂, 1 µg BSA, 0.5 U *Taq* DNA polymerase (Takara Bio Inc.), 0.1 µM each primer and 10 ng template DNA. The amplification program consisted of one cycle of 94 °C for 2 min; 30 cycles of 94 °C for 20 s, 55 °C for 30 s, 72 °C for 20 s; and finally one cycle of 72 °C for 3 min. The PCR-amplified 16S rRNA and *recA* genes were purified using a Montage PCR Filter unit (Millipore) and were subsequently sequenced using the ABI PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and an ABI PRISM 3130xl Genetic Analyzer. The closest known relatives of the novel strains were determined by FASTA and sequences of closely related species were retrieved from the DDBJ/GenBank/EMBL databases. Multiple alignments of the sequences were carried out with the CLUSTAL_X program (version 1.82) (Thompson *et al.*, 1997). Approximately 1470 bp of the 16S rRNA gene sequences (approx. 380 bp for the *recA* gene) of the 36 strains and related species were used for the construction of phylogenetic trees with the neighbour-joining method (Saitou & Nei, 1987). The statistical reliability of the tree was evaluated by bootstrap analysis of 1000 replicates (Felsenstein, 1985) and the tree topology was also confirmed with the maximum-parsimony (Fitch, 1972) and maximum-likelihood (Cavalli-Sforza & Edwards, 1967) methods, by using PHYLIP v3.67 (Felsenstein, 2007).

The 16S rRNA gene sequence similarities between the type strains of groups A, B, C and D and the closest six neighbouring species, *L. buchneri* YIT 0077^T, *L. diolivorans* YIT 10368^T, *L. hilgardii* YIT 0269^T, *L. kefir* YIT 0222^T, *L. parabuchneri* YIT 0272^T and *L. parakefir* YIT 10382^T, were 98.0–99.6 %. These values are considerably higher than the recommended value for species differentiation (97 %;

Fig. 1. Cluster analysis of digitalized AFLP fragment patterns of 36 selected LAB strains isolated from sunki and closest phylogenetic relative strains. The corresponding dendrogram was constructed from the UPGMA linkage of Dice genetic distance coefficients. Bar, 10% sequence divergence.

Stackebrandt & Goebel, 1994) (see Supplementary Fig. S1 in IJSEM Online). The similarities among the *recA* gene sequences of the 36 novel strains in groups A, B, C, D and the closest six neighbouring species ranged from 79.2 to 84.5%. The similarity values between the strains in groups A, B, C and D were 99.7–100%, 100%, 100% and 100%, respectively. Interspecies gaps within the genus *Lactobacillus* based on *recA* gene sequences normally exceed 10–15% (Torriani *et al.*, 2001), which suggested that these 36 novel strains should be classified into four novel *Lactobacillus* species. On the basis of the neighbour-joining analysis of the *recA* gene sequences, the novel strains did not belong to any recognized phylogenetic groups and formed four distinct subclusters, groups A, B, C and D, in the *L. buchneri* cluster (Fig. 2). Similar topologies were obtained by the maximum-parsimony and maximum-likelihood methods (see Supplementary Figs S2 and S3 in IJSEM Online).

For the determination of DNA–DNA relatedness and the DNA G+C content, chromosomal DNA was extracted according to the method of Marmur (1961). DNA–DNA hybridization analyses were performed between the novel strains YIT 11161^T, YIT 11168^T, YIT 11204^T and YIT 11163^T and *L. buchneri* YIT 0077^T, *L. diolivorans* YIT 10368^T, *L. hilgardii* YIT 0269^T, *L. kefiri* YIT 0222^T, *L. parabuchneri* YIT 0272^T and *L. parakefiri* YIT 10382^T. The microdilution well technique was used as described by Ezaki *et al.* (1989) using a Fluoskan II microplate reader

(Labsystems) for fluorescence measurement. Reciprocal hybridization experiments were performed for every pair of strains at 41 °C for 15 min in the presence of 50% formamide, using biotinylated DNA and unlabelled ssDNA, which was bonded non-covalently to microplate wells. The data were calculated as the mean values of four replicate wells for each experiment. The levels of DNA–DNA relatedness between strain YIT 11161^T and *L. buchneri* YIT 0077^T, *L. diolivorans* YIT 10368^T, *L. hilgardii* YIT 0269^T, *L. kefiri* YIT 0222^T, *L. parabuchneri* YIT 0272^T and *L. parakefiri* YIT 10382^T were 6–35%; those between YIT 11168^T and these reference strains were 9–36%; those between YIT 11204^T and these reference strains were 8–17% and those between YIT 11163^T and the reference strains were 9–27%. The values among the four novel strains, YIT 11161^T, YIT 11168^T, YIT 11204^T and YIT 11163^T, were 8–25% (see Supplementary Table S1 in IJSEM Online). The values among the reference strains were well below the 70% cut-off value that indicates separate species (Stackebrandt & Goebel, 1994).

To assess the DNA base composition (G+C content), DNA was enzymically degraded into nucleosides as previously described (Mesbah *et al.*, 1989) and then separated by HPLC. The DNA G+C contents of strains YIT 11168^T, YIT 11204^T, YIT 11163^T and YIT 11161^T were 38.8, 40.3, 39.6 and 39.2 mol%, respectively.

Morphological, cultural and biochemical tests were performed according to standard techniques at 30 °C unless

Fig. 2. Phylogenetic tree based on *recA* gene sequences showing the relationship of the novel strains with closely related species. The tree was constructed by the neighbour-joining method on the basis of a comparison of approximately 380 nt. *Bacillus subtilis* was used as an outgroup. Bootstrap values (%) based on 1000 replications are given at nodes. Bar, 10% sequence divergence.

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 MRS soft agar (0.15 %). Catalase activity was determined by 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 glucose was replaced with 2 % (w/v) sucrose. The methods of Barrow & Feltham (1993) were used to determine growth at various temperatures and pH and in the presence of NaCl and the reduction of nitrate and production of ammonia from arginine. Carbohydrate fermentation tests were conducted by using the API 50 CHL system (bioMérieux) according to the manufacturer's instructions. The isomers of lactic acid formed from glucose were determined with a D-/L-lactic acid enzymic kit (R-Biopharm AG).

Various phenotypic characteristics of the novel strains differed from those of *L. buchneri* (Kandler & Weiss, 1986), *L. diolivorans* (Krooneman *et al.*, 2002), *L. hilgardii* (Kandler & Weiss, 1986), *L. kefir* (Kandler & Kunath, 1983), *L. parabuchneri* (Farrow *et al.*, 1988) and *L. parakefiri* (Takizawa *et al.*, 1994), the phylogenetically closest related species (Table 1). According to the data

obtained, the new strains are genetically distinguishable from the recognized species of LAB and thus represent novel species. The names *Lactobacillus kisonensis* sp. nov., *Lactobacillus otakiensis* sp. nov., *Lactobacillus rapi* sp. nov. and *Lactobacillus sunkii* sp. nov. are suggested for strains YIT 11168^T, YIT 11163^T, YIT 11204^T and YIT 11161^T, respectively.

Description of *Lactobacillus kisonensis* sp. nov.

Lactobacillus kisonensis (ki.so.nen'sis. N.L. masc. adj. *kisonensis* pertaining to Kiso, the name of the county located in the south-west area of Nagano in Japan, from where the type strain was isolated).

Cells are rod-shaped, 0.4–0.6 × 0.8–3 µm and occur singly, in pairs or in short chains comprising three or four cells. Cells are Gram-positive, catalase-negative, non-motile, non-spore-forming and facultatively anaerobic. After anaerobic growth at 30 °C for 48 h, colonies on MRS agar are beige, with a smooth to rough surface, circular to slightly irregular and approximately 1–2 mm in diameter. In MRS broth, growth occurs at 10 °C but not at 45 °C. Growth occurs at pH 4.0 and pH 8.5. Some strains grow at

Table 1. Differential phenotypic characteristics among the novel species and closely related lactobacilli

Strains/species: 1, YIT 11168^T, YIT 11510 and YIT 11566 (*L. kisonensis* sp. nov.); 2, YIT 11163^T, YIT 11167, YIT 11452 and YIT 11505 (*L. otakiensis* sp. nov.); 3, YIT 11204^T and YIT 11688 (*L. rapi* sp. nov.); 4, YIT 11161^T, YIT 11442, YIT 11539, YIT 11549, YIT 11557 and YIT 11635 (*L. sunkii* sp. nov.); 5, *L. buchneri* YIT 0077^T; 6, *L. diolivorans* YIT 10368^T; 7, *L. hilgardii* YIT 0269^T; 8, *L. kefir* YIT 0222^T; 9, *L. parabuchneri* YIT 0272^T; 10, *L. parakefir* YIT 10382^T. +, Positive; -, Negative; w, weakly positive; v, variable; ND, no data available. All strains isolated from the non-salted pickle solution used in sunki production are able to ferment L-arabinose, ribose and maltose. None of the strains are able to ferment glycerol, erythritol, D-arabinose, L-xylose, adonitol, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, trehalose, inulin, starch, glycogen, xylitol, β -gentiobiose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol or 2-ketogluconate.

Characteristic	1	2	3	4	5	6	7	8	9	10
Growth at 10 °C	+	+	+	+	ND*	-†	ND*	+‡	+§	ND
Acid production from:										
D-Fructose	-	+	+	+	+	+	+	w	+	-
Galactose	-	+	-	v	-	+	+	+	+	+
Gluconate	-	w	w	w	w	w	w	w	w	-
D-Glucose	-	+	+	+	+	+	+	+	+	+
5-Ketogluconate	-	-	w	v	+	w	w	-	w	-
Melezitose	-	-	+	v	+	-	-	-	+	+
Melibiose	-	+	+	+	+	+	+	+	+	+
Methyl α -D-glucoside	-	-	+	-	-	+	-	-	-	-
Methyl β -xyloside	+	-	+	v	-	+	-	-	-	-
Raffinose	-	-	+	+	-	w	-	-	+	-
Sucrose	+	-	+	+	+	-	+	-	+	-
Turanose	v	-	+	-	-	-	-	-	-	-
D-Xylose	+	-	+	v	+	+	+	+	-	-
Lactate isomer	L	DL	L	DL	DL	DL	DL	DL	DL	L

*Data from Kandler & Weiss (1986).

†Data from Krooneman *et al.* (2002).

‡Data from Kandler & Kunath (1983).

§Data from Farrow *et al.* (1988).

||Data from Takizawa *et al.* (1994).

30 °C in the presence of 5 % NaCl but none grow in the presence of 8 % NaCl. Gas is produced from glucose. Both L- (85 %) and D-lactate (15 %) are produced as the end products from glucose. Ammonia is produced from arginine. Nitrate is not reduced. Acid is produced from L-arabinose, ribose, D-xylose, methyl β -xyloside, maltose and sucrose. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin,

cellobiose, lactose, melibiose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, β -gentiobiose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate. Acid production from turanose is strain dependent. Aesculin is hydrolysed. Dextran is not produced from sucrose. Cells do not contain *meso*-diaminopimelic acid in their cell-wall peptidoglycan. Phylogenetic analysis of the *recA* gene sequence places the species in the *L. buchneri* species group of lactobacilli.

The type strain, YIT 11168^T (=NRIC 0741^T=JCM 15041^T=DSM 19906^T), and the reference strains YIT 11510 and YIT 11661 were isolated from non-salted pickle solution used in producing sunki products in Kiso county, Nagano prefecture, Japan, in 2004. The DNA G+C content of the type strain is 38.8 mol%.

Description of *Lactobacillus otakiensis* sp. nov.

Lactobacillus otakiensis (o.ta.ki.en'sis. N.L. masc. adj. *otakiensis* pertaining to Otaki, the name for the village located in the west part of Kiso county in Nagano prefecture, Japan, from where the type strain was isolated).

Cells are rod-shaped, 0.4–0.6 $\times$ 0.8–2 μ m, and occur singly, in pairs or in short chains comprising three or four cells. Cells are Gram-positive, catalase-negative, non-motile, non-spore-forming and facultatively anaerobic. After anaerobic growth at 30 °C for 48 h, colonies on MRS agar are beige, with a smooth to rough surface, circular to slightly irregular and approximately 2–3 mm in diameter. In MRS broth, growth occurs at 10 °C but not at 45 °C. Growth occurs at pH 4.0 and pH 8.5. Grows at 30 °C in the presence of 5 % NaCl but not in the presence of 8 % NaCl. Gas is produced from glucose. Both L- (40 %) and D-lactate (60 %) are produced as the end products from glucose. Ammonia is produced from arginine. Nitrate is not reduced. Acid is produced from L-arabinose, ribose, galactose, D-glucose, D-fructose, maltose, melibiose, sucrose and gluconate. Acid is not produced from glycerol, erythritol, D-arabinose, D- or L-xylose, adonitol, methyl β -xyloside, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, trehalose, inulin, melezitose, starch, glycogen, xylitol, β -gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol or 2- or 5-ketogluconate. Acid production from lactose and raffinose is strain-dependent. Aesculin is weakly hydrolysed. Dextran is not produced from sucrose. Cells do not contain *meso*-diaminopimelic acid in their cell-wall peptidoglycan. Phylogenetic analysis of the *recA* gene sequence places the species in the *L. buchneri* species group of lactobacilli.

The type strain, YIT 11163^T (=NRIC 0742^T=JCM 15040^T=DSM 19908^T), and the reference strains YIT 11167, YIT 11452 and YIT 11505 were isolated from non-salted pickle solution used in producing sunki

products in Kiso county, Nagano prefecture, Japan, in 2004. The DNA G+C content of strain YIT 11163^T is 39.6 mol%.

Description of *Lactobacillus rapi* sp. nov.

Lactobacillus rapi (ra'pi. L. gen. n. *rapi* of a turnip, used in making sunki products).

Cells are rod-shaped, 0.4–0.6 × 0.8–3 µm and occur singly, in pairs or in short chains comprising three or four cells. Cells are Gram-positive, catalase-negative, non-motile, non-spore-forming and facultatively anaerobic. After anaerobic growth at 30 °C for 48 h, colonies on MRS agar are beige, with a smooth to rough surface, circular to slightly irregular and approximately 2–3 mm in diameter. In MRS broth, growth occurs at 10 °C but not at 45 °C. Growth occurs at pH 4.0 and pH 8.5. Some strains grow at 30 °C in the presence of 5 % NaCl but none grow in the presence of 8 % NaCl. Gas is produced from glucose. Both L- (80 %) and D-lactate (20 %) are produced as the end products from glucose. Ammonia is produced from arginine. Nitrate is not reduced. Acid is produced from L-arabinose, ribose, D-xylose, methyl β-xyloside, D-glucose, D-fructose, methyl α-D-glucoside, maltose, melibiose, sucrose, melezitose, raffinose, turanose, gluconate and 5-ketogluconate. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, galactose, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, trehalose, inulin, starch, glycogen, xylitol, β-gentiobiose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol and 2-ketogluconate. Aesculin is hydrolysed. Dextran is not produced from sucrose. Cells do not contain meso-diaminopimelic acid in their cell-wall peptidoglycan. Phylogenetic analysis of the *recA* gene sequence places the species in the *L. buchneri* species group of lactobacilli.

The type strain, YIT 11204^T (=NRIC 0743^T=JCM 15042^T=DSM 19907^T), and the reference strain YIT 11688 were isolated from non-salted pickle solution used in producing sunki products in Kiso county, Nagano prefecture, Japan, in 2004. The DNA G+C content of strain YIT 11204^T is 40.3 mol%.

Description of *Lactobacillus sunkii* sp. nov.

Lactobacillus sunkii (sun'kii. N.L. gen. n. *sunkii* of sunki, the name of a non-salted Japanese traditional pickle).

Cells are rod-shaped, 0.4–0.8 × 1–4 µm and occur singly, in pairs or in short chains comprising three or four cells. Cells are Gram-positive, catalase-negative, non-motile, non-spore-forming and facultatively anaerobic. After anaerobic growth at 30 °C for 48 h, colonies on MRS agar are beige, with a smooth to rough surface, circular to slightly irregular and approximately 1–3 mm in diameter. In MRS broth, growth occurs at 10 °C but not at 45 °C.

Growth occurs at pH 4.0 and pH 8.5. Some strains grow at 30 °C in the presence of 5 % NaCl but none grow in the presence of 8 % NaCl. Gas is produced from glucose. Both L- (40 %) and D-lactate (60 %) are produced as the end products from glucose. Ammonia is produced from arginine. Nitrate is not reduced. Acid is produced from L-arabinose, ribose, D-glucose, D-fructose, maltose, melibiose, sucrose, raffinose, and gluconate. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, trehalose, inulin, starch, glycogen, xylitol, β-gentiobiose, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol or 2-ketogluconate. Acid production from D-xylose, methyl β-xyloside, galactose, melezitose and 5-ketogluconate is strain-dependent. Aesculin is weakly hydrolysed. Dextran is not produced from sucrose. Cells do not contain meso-diaminopimelic acid in their cell-wall peptidoglycan. Phylogenetic analysis of the *recA* gene sequence places the species in the *L. buchneri* species group of lactobacilli.

The type strain, YIT 11161^T (=NRIC 0744^T=JCM 15039^T=DSM 19904^T), and the reference strains YIT 11442, YIT 11539, YIT 11549, YIT 11557 and YIT 11635 were isolated from non-salted pickle solution used in producing sunki products in Kiso county, Nagano prefecture, Japan, in 2004. The DNA G+C content of strain YIT 11161^T is 39.2 mol%.

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