Lactobacillus xiangfangensis sp. nov., isolated from Chinese pickle

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A Gram-positive bacterial strain, 3.1.1^T, was isolated from traditional pickle in Heilongjiang Province, China. The bacterium was characterized by a polyphasic approach, including 16S rRNA gene sequence analysis, *pheS* gene sequence analysis, *rpoA* gene sequence analysis, *dnaK* gene sequence analysis, fatty acid methyl ester analysis, determination of DNA G+C content, DNA–DNA hybridization and an analysis of phenotypic features. Based upon the data obtained in the present study, a novel species, *Lactobacillus xiangfangensis* sp. nov., is proposed with the type strain 3.1.1^T (=LMG 26013^T =NCIMB 14687^T).

The genus *Lactobacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae* (Garrrity *et al.*, 2004). *Lactobacilli* are usually found in dairy products, fermented meat, sour doughs, vegetables, fruits, beverages, sewage, plant material, and in respiratory, GI and genital tracts of humans and animals (Felis & Dellaglio, 2007).

In the present study, a Gram-positive bacterial strain, 3.1.1^T, was isolated from traditional pickle in Heilongjiang Province, China. The bacterium was characterized by a polyphasic approach. The strains used in this study are listed in Table 1. All strains were incubated aerobically at 30 °C on MRS medium (De Man *et al.*, 1960).

Amplification of the 16S rRNA gene was performed using the primers of An *et al.* (2006). The *pheS* and *rpoA* genes were amplified using the primers of De Bruyne *et al.* (2007) and the protocols of Naser *et al.* (2005). The *dnaK* gene was amplified using the primers and protocol of Huang *et al.* (2010). Purification and sequencing of PCR products were carried out by the Sheng Gong Company in Shanghai, China. The resulting sequences, together with those of related strains obtained from the GenBank database were aligned by using CLUSTAL W. A phylogenetic tree was reconstructed

using the neighbour-joining method with the maximum composite likelihood model. Bootstrap analysis was performed based on 1000 replicates. The MEGA4 package (Tamura *et al.*, 2007) was used for all analyses. 16S rRNA gene sequence analysis of strain 3.1.1^T indicated that it belonged to the *Lactobacillus plantarum* species group, showing sequence similarities of 98.8, 98.7, 98.9, 98.8 and 98.5 % to the type strains of *L. plantarum* subsp. *plantarum*, *L. plantarum* subsp. *argenteratensis*, *Lactobacillus pentosus*, *Lactobacillus paraplantarum* and *Lactobacillus fabifermentans*, respectively (Fig. 1). A large phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain 3.1.1^T and the type strains of all defined species within the genus *Lactobacillus* is given in Supplementary Fig. S1 (available in IJSEM Online). Strain 3.1.1^T showed 75.1–84 % *pheS* gene sequence similarities (Fig. 2), 92.8–93.6 % *rpoA* gene sequence similarities (Fig. 3) and 83.9–87.9 % *dnaK* gene sequence similarities (Fig. 4) to the type strains of all species in the *L. plantarum* species group, respectively. *pheS*, *rpoA* and *dnaK* gene sequence analyses (Figs 2–4) clearly show a higher resolution than the 16S rRNA gene sequence

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *pheS*, *rpoA* and *dnaK* gene sequences of strain 3.1.1^T are HM443954, HM443974, HM443977 and HM443963, respectively. The accession number for the *rpoA* gene sequence of *Lactobacillus fabifermentans* LMG 24284^T is HM443978.

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

Table 1. Bacterial strains used in this study

Strain	Isolation source
<i>L. xiangfangensis</i> sp. nov. 3.1.1 ^T	Pickle, China
<i>L. fabifermentans</i> LMG 24284 ^T	Cocoa bean fermentation, Ghana
<i>L. paraplantarum</i> LMG 16673 ^T	Beer, France
<i>L. pentosus</i> LMG 10755 ^T	Sawdust fermentation
<i>L. plantarum</i> LMG 6907 ^T	Pickled cabbage

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships of strain 3.1.1^T and related reference strains based on 16S rRNA gene sequences. Numbers at nodes are bootstrap values based on 1000 replicates. Bar, 0.2 % substitutions per site.

Fig. 2. Neighbour-joining tree showing the phylogenetic relationships of strain 3.1.1^T and related reference strains based on *pheS* gene sequences. Numbers at nodes are bootstrap values based on 1000 replicates. Bar, 2 % substitutions per site.

Fig. 3. Neighbour-joining tree showing the phylogenetic relationships of strain 3.1.1^T and related reference strains based on *rpoA* gene sequences. Numbers at nodes are bootstrap values based on 1000 replicates. Bar, 1 % substitutions per site.

analysis and suggest that strain 3.1.1^T represents a novel species within the genus *Lactobacillus*.

The API 50 CH system (bioMérieux) was used to determine the carbohydrate fermentation profile of strain 3.1.1^T. Test preparations were incubated at 30 °C, and readings were made after 48 h. The results are given in the species description below. Characteristics that differentiate strain 3.1.1^T from its closest relatives are summarized in Table 2.

Whole-cell fatty acids were analysed as fatty acid methyl esters with the MIDI Microbial Identification system. Cultures were incubated for 2 days at 30 °C on MRS solid medium. Fatty acid methyl esters were extracted and prepared according to the protocol of Sasser (1990).

Comparative fatty acid compositions of strain 3.1.1^T and phylogenetically related reference strains are given in Table 3.

DNA was prepared using Power-Microbial Maxi DNA isolation kits (Mo Bio Laboratories). DNA G+C content was measured using the thermal melting protocol of De Ley (1970) with *Escherichia coli* K-12 as the standard. DNA–DNA hybridizations were performed by the initial renaturation rate method (De Ley *et al.*, 1970). The DNA G+C content of strain 3.1.1^T was 46.6 mol%. DNA–DNA hybridization values between strain 3.1.1^T and *L. plantarum* LMG 6907^T, *L. pentosus* LMG 10755^T, *L. paraplantarum* LMG 16673^T and *L. fabifermentans* LMG 24284^T were 27, 26, 41 and 42 %, respectively, confirming that strain 3.1.1^T represents a novel species within the genus *Lactobacillus*.

Fig. 4. Neighbour-joining tree showing the phylogenetic relationships of strain 3.1.1^T and related reference strains based on *dnaK* gene sequences. Numbers at nodes are bootstrap values based on 1000 replicates. Bar, 1 % substitutions per site.

Table 2. Distinctive features of the carbohydrate fermentation profiles of strain 3.1.1^T and phylogenetically related species determined using API 50 CH

Strains: 1, *L. xiangfangensis* sp. nov. 3.1.1^T (data from this study); 2, *L. fabifermentans* LMG 24284^T (De Bruyne *et al.*, 2009); 3, *L. paraplantarum* (Curk *et al.*, 1996); 4, *L. pentosus* (Zanoni *et al.*, 1987); 5, *L. plantarum* subsp. *plantarum* (Bringel *et al.*, 2005; Zanoni *et al.*, 1987); 6, *L. plantarum* subsp. *argenteratensis* (Bringel *et al.*, 2005). +, 90 % or more strains positive; –, 90 % or more strains negative; d, 11–89 % of strains positive; D, delayed reaction (positive after incubating at 30 °C for 4 days).

Characteristic	1	2	3 (n=4)*	4 (n=3)	5 (n=15)	6 (n=14)
Glycerol	–	–	–	+	d	d
L-Arabinose	–	+	d	+	d	d
D-Xylose	+	+	–	+	–	–
Adonitol	+	–	–	–	–	–
Rhamnose	–	–	d	d	d	d
Dulcitol	–	–	–	d	–	d
Sorbitol	+	–	d	+	d	d
Methyl α-D-mannopyranoside	–	–	–	–	d	–
Methyl α-D-glucopyranoside	–	–	–	d	d	d
Amygdalin	–	+	+	+	+	+
Arbutin	–	+	+	+	+	+
Lactose	–	–	+	+	+	+
Melibiose	–	–	+	+	+	+
Melezitose	–	–	+	d	d	–
Raffinose	–	–	d	+	d	d
Starch	–	–	–	–	d	d
Glycogen	–	–	–	–	d	d
Turanose	–	–	d	d	d	d
D-Arabitol	–	–	d	–	d	d
Gluconate	–	–	+	–	d	d
Aesculin	D	+	+	+	+	+

*n, Number of strains tested.

On the basis of the results presented here, strain 3.1.1^T is considered to represent a novel species of the genus *Lactobacillus*, for which the name *Lactobacillus xiangfangensis* sp. nov. is proposed.

Description of *Lactobacillus xiangfangensis* sp. nov.

Lactobacillus xiangfangensis (xi.ang.fang.en'sis. N.L. masc. adj. *xiangfangensis* pertaining to xiangfang district, located in Harbin, Heilongjiang Province, where the type strain was isolated).

Gram-positive. Non-spore-forming rods. Catalase is not produced. Facultatively anaerobic. Optimum growth temperature is 30 °C; can grow at 37 °C. Acid is produced from ribose, D-xylose, adonitol, galactose, glucose, fructose, mannose, mannitol, sorbitol, N-acetylglucosamine, salicin, cellobiose, maltose, sucrose, trehalose and gentiobiose. Acid

Table 3. Comparative fatty acid contents (%) of strain 3.1.1^T and phylogenetically related reference strains

Strains: 1, *L. xiangfangensis* sp. nov. 3.1.1^T; 2, *L. fabifermentans* LMG 24284^T; 3, *L. paraplantarum* LMG 16673^T; 4, *L. pentosus* LMG 10755^T; 5, *L. plantarum* LMG 6907^T. –, Not detected.

Fatty acid	1	2	3	4	5
10:0	–	–	–	0.4	–
12:0	–	–	–	0.3	–
14:0	–	2.3	–	2.5	–
16:0	–	38.4	–	30.4	–
16:1ω5c	–	0.3	–	–	–
17:0	–	0.2	–	0.1	–
17:0 iso 3-OH	29.2	–	–	–	–
17:0 2-OH	27.6	–	–	–	–
18:0	–	5.6	6.0	2.3	–
18:0 2-OH	–	–	–	–	7.7
18:0 10-methyl, TBSA	–	–	–	–	43.9
18:1 2-OH	4.4	–	–	–	–
18:1ω5c	–	–	–	–	6.1
18:1ω9c	–	20.1	22.1	12.0	–
19:0 iso	–	–	–	3.8	–
19:1 iso I	–	1.1	1.3	–	42.3
16:1ω6c and/or 16:1ω7c	–	2.5	2.4	4.4	–
18:2ω6,9c and/or 18:0 anteiso	–	0.7	0.8	0.6	–
19:1ω6c and/or 19:0 cyclo ω10c	38.9	6.8	8.1	7.1	–
18:1ω6c and/or 18:1ω7c	–	22.1	59.3	36.1	–

is not produced from glycerol, erythritol, D-arabinose, L-arabinose, L-xylose, methyl β-D-xylopyranoside, sorbose, rhamnose, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, lyxose, tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-keto-gluconate. Aesculin is hydrolysed.

The type strain is 3.1.1^T (=LMG 26013^T =NCIMB 14687^T). The DNA G + C content of strain 3.1.1^T is 46.6 mol%.

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