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Lactobacillus pantheris sp. nov., isolated from faeces of a jaguar

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Two novel *Lactobacillus* strains were isolated from the faeces of a jaguar in Beijing Zoo. They were Gram-positive, non-spore-forming, non-motile, rod-shaped organisms that exhibited no catalase activity. The strains fermented cellobiose, D-fructose, galactose, D-glucose, lactose, maltose, D-mannose, salicin, trehalose, N-acetyl glucosamine, β -gentiobiose and D-tagatose. D(–)-Lactic acid was the exclusive product from glucose fermentation. The G+C content of the DNA of strain A24-2-1^T was 52.7 mol%. Phylogenetic analysis based on 16S rDNA sequence similarity indicated that the strains represented a novel member of the genus *Lactobacillus* and were most closely related to *Lactobacillus manihotivorans* LMG 18010^T, with 16S rDNA sequence similarity of 95.2%. The DNA–DNA relatedness between them was 13%. The name *Lactobacillus pantheris* sp. nov. is proposed for the novel strains, with strain A24-2-1^T as the type strain (= AS 1.2826^T = LMG 21017^T).

Keywords: *Lactobacillus pantheris* sp. nov., jaguar faeces, 16S rRNA homology

Since the genus *Lactobacillus* was originally described by Beijerinck, nearly 70 species have been recognized (Kandler & Weiss, 1986). They are a group of rod-shaped, lactic acid-producing, phylogenetically heterogeneous organisms (Collins *et al.*, 1991; Kandler & Weiss, 1986). Lactobacilli are widely distributed in nature and are found frequently in fermented foods such as dairy products, beverages, fish, pork and vegetables and in sewage (Stiles & Holzapfel, 1997; Tanasupawat & Komagata, 1995). Some species are believed to be members of the commensal flora of the intestinal tract of humans and animals (Hammes *et al.*, 1992; Aguirre & Collins, 1993), so that they have been used frequently as probiotics for some years (Fuller, 1989).

During the investigation of lactic acid bacteria in the faeces of different animals in Beijing Zoo, we isolated two *Lactobacillus* strains from a jaguar that exhibited distinguishing phenotypic characteristics, especially in their fermentation patterns and higher G+C content. They could not be assigned to any recognized species of the genus *Lactobacillus*. Phylogenetic analysis based

on 16S rDNA sequence similarity and DNA–DNA hybridization also indicated that they might represent a novel species of the genus *Lactobacillus*.

The two novel *Lactobacillus* strains (A24-2-1^T, A24-1-5) were isolated and purified by streaking on tryptone/peptone/yeast extract/glucose (TPYG) agar (Scardovi, 1986) and were routinely cultivated in liquid TPYG medium in cotton-stopped tubes at 37 °C. Organic acid production from glucose fermentation was detected by GC (GC-7AG, Shimadzu). The configuration of the lactic acid was determined by using a polarimeter (Perkin-Elmer models 241 and 2421 MC). Sugar fermentation patterns were determined by using the API 50 CH system (bioMérieux) and incubation at 37 °C for 24 h.

In order to determine the phylogenetic position of the novel isolates, the 16S rDNA of strain A24-2-1^T was sequenced. The genome DNA was extracted and purified by using a modified method of Marmur (1961) (Dong *et al.*, 2000) and the 16S rDNA was amplified by PCR using the genome DNA as template. The 16S rDNA fragment was sequenced using an ABI Prism Big Dye Terminator cycle sequencing ready reaction kit (Perkin Elmer) and an ABI Prism 377XL DNA sequencer (TaKaRa). The best-matching sequences were retrieved from GenBank and alignment and similarity analysis were performed by using the

Published online ahead of print on 14 March 2002 as DOI 10.1099/ijs.0.02247-0.

The GenBank accession number for the 16S rRNA gene sequence of *Lactobacillus pantheris* LMG 21017^T is AF413523.

Table 1. Differential characteristics of *Lactobacillus pantheris* sp. nov. and closely related species

Strains: 1, *L. pantheris* A24-2-1^T; 2, *L. pantheris* A24-1-5; 3, *L. manihotivorans* OND32^T (data from Morlon-Guyot *et al.*, 1998); 4, *Paralactobacillus selangorensis* (Leisner *et al.*, 2000); 5, *L. casei* (Kandler & Weiss, 1986); 6, *Lactobacillus brevis* (Kandler & Weiss, 1986). +, Positive; −, negative; +w, weakly positive; ND, no data.

Characteristic	1	2	3	4	5	6
Acid production from:						
Amygdalin	−	−	+	+	−	−
Arabinose	−	−	−	−	+	+
Cellobiose	+	+	+	+	+	−
Gluconate	−	−	−	−	+	+
Lactose	+	+w	+	−	+	+
Maltose	+	+w	+	+	+	+
Mannitol	−	−	−	−	+	−
D-Mannose	+	+	+	+	+	−
Melezitose	−	−	−	−	+	+
Melibiose	−	−	+	−	−	−
Raffinose	−	−	+	−	−	−
Rhamnose	−	−	−	+	−	−
Ribose	−	−	−	−	+	+
Salicin	+	+	+	+	+	−
Sorbitol	−	−	−	−	+	−
Sucrose	−	−	+	+	+	+
Trehalose	+	+	+	+	+	−
Xylose	−	−	−	−	−	+
NH ₃ production from arginine	−	−	ND	−	−	+
Lactic acid isomer	D	D	L	DL	L	DL
Growth at 45 °C	−	−	+	−	ND	ND
DNA G+C content (mol %)	52.7	52.8	48.4	46	45–47	44–47

DNAMAN program (version 4.0; Lynnon Biosoft). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) as implemented within the DNAMAN program. The clustering stability of the tree was evaluated by bootstrap analysis of 1000 datasets. DNA relatedness was determined at 67 °C on the basis of the DNA–DNA liquid reassociation rate (De Ley *et al.*, 1970) and the G+C content was determined from the thermal denaturation temperature (Marmur & Doty, 1962). A UV spectrophotometer with a thermal controller (Shanghai 3rd Analytic Factory) was used for the determinations.

The two isolates grew optimally at 30–37 °C and even at as low as 10 °C; however, no growth occurred at 42 °C. They grew at pH values ranging from 4.5 to 7.5 and grew optimally at pH 6.0–6.4. Product determination showed that the two strains produced exclusively D(−)-lactic acid from glucose fermentation and produced no gas, suggesting that they were members

Fig. 1. Phylogenetic tree based on 1439 bp fragments of 16S rDNA sequences. The tree, rooted with *Bacillus subtilis*, was constructed by the neighbour-joining method with bootstrap values calculated from 1000 trees. Numbers at branch points are percentages supported by bootstrap analysis. GenBank accession numbers are given in parentheses. *L.*, *Lactobacillus*; *Pa.*, *Paralactobacillus*; *Pe.*, *Pediococcus*. Bar, 5% sequence divergence.

of the homofermentative group. By using the API 50 CH system, the two strains showed fermentation profiles that differed from those of related species (Table 1). They rarely fermented disaccharides or trisaccharides such as sucrose, raffinose and melezitose and did not ferment pentoses, such as arabinose, ribose, xylose, adonitol, rhamnose and mannitol, at all.

In order to ascertain the phylogenetic position of strain A24-2-1^T, its 16S rDNA sequence was compared with the most similar sequences retrieved from GenBank. A phylogenetic tree (Fig. 1) was constructed based on the comparison of a 1439 bp consensus length of 16S rDNA. It was shown that the novel strain was phylogenetically related to *Lactobacillus manihotivorans* LMG 18010^T (Morlon-Guyot *et al.*, 1998), *L. manihotivorans* YAM 1, *Paralactobacillus selangorensis* LMG 17710^T (Leisner *et al.*, 2000) and *Lactobacillus casei* ATCC 334^T, with 16S rDNA

similarities of 95.2, 93.9, 93.1 and 92.7%, respectively, all lower than the generally accepted criterion (97.5%) for the discrimination of species. This indicated that the novel strains were different from the closely related *Lactobacillus* species. Although the novel strains shared some phenotypic characteristics with *Lactobacillus delbrueckii*, *Lactobacillus jensenii*, *Lactobacillus vitulinus* and *Lactobacillus coryniformis*, such as production of D(–)-lactic acid and ammonia, the 16S rDNA similarity between the novel strain and these species was lower than 90% in each case. Furthermore, strain A24-2-1^T also shared quite high 16S rDNA similarities with *Pediococcus* species, a group of coccus-shaped lactic acid bacteria; 94.7% with *Pediococcus pentosaceus* DSM 20336^T, 94.4% with *Pediococcus damnosus* DSM 20331^T and 92.9% with *Pediococcus inopinatus* DSM 20285^T. However, it was easy to discriminate the novel strains from *Pediococcus* by their cell morphology. Therefore, the phylogenetic analysis confirmed that strain A24-2-1^T represents a novel *Lactobacillus* species, belonging to the *L. casei*–*Pediococcus* phylogenetic group.

The DNA G+C content of strain A24-2-1^T was 52.7 mol%, which is within the range of the genus *Lactobacillus* (32–55 mol%); however, this value is about 4 mol% higher than that of its phylogenetic closest relative, *L. manihotivivans* LMG 18010^T (48.4 mol%). It is generally agreed that organisms that exhibit differences in G+C content of more than 2% may be members of different species (Johnson, 1973); the novel strains should therefore be assigned to a different species.

Since *L. manihotivivans* LMG 18010^T was the closest species to strain A24-2-1^T based on 16S rDNA sequence similarity analysis, the DNA–DNA reassociation rate between the two was determined. The resulting DNA relatedness between them was as low as 13%, apparently lower than the accepted level to define species (> 70% DNA–DNA relatedness), thus verifying that the novel strains and *L. manihotivivans* represent different species.

By combining the analyses of phenotypic characteristics, phylogenetic relationships and DNA–DNA relatedness, the novel species *Lactobacillus pantheris* sp. nov. is proposed.

Description of *Lactobacillus pantheris* sp. nov.

Lactobacillus pantheris (pan'ther.is. L. gen. n. *pantheris* of the panther, referring to the isolation of the strains from jaguar faeces).

Cells are Gram-positive, non-spore-forming, non-motile rods, 0.5 × 1.5–2.5 µm in size after 24 h incubation in TPYG liquid medium. Catalase-negative and facultatively anaerobic. D(–)-Lactic acid but no gas is produced from glucose fermentation. Colonies are convex, opaque and rough with irregular edges and less than 1 mm in diameter after 24 h cultivation on TPYG plates. Growth occurred at 10 °C but not at

45 °C. The optimum pH for growth is 6.0–6.4 and growth can occur at pH 4.5, but not at pH values above 7.5. Ammonia is produced from arginine and aesculin is hydrolysed. Acid is produced from galactose, D-glucose, D-fructose, D-mannose, N-acetyl glucosamine, arbutin, salicin, cellobiose, maltose, lactose, trehalose, β-gentiobiose and D-tagatose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-xyloside, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-raffinose, starch, glycogen, xylitol, D-turanose, D-lyxose, D-fucose, L-fucose, D-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. The DNA G+C content of strain A24-2-1^T is 52.7 mol%.

The type strain and a second strain, A24-1-5, were isolated from the faeces of a jaguar in Beijing Zoo. The type strain is strain A24-2-1^T (= AS 1.2826^T = LMG 21017^T).

Acknowledgements

This study was supported by grants from the China National Foundation of Sciences under grant no. 3007001 and the Chinese Academy of Sciences.

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