Lactobacillus equicursoris sp. nov., isolated from the faeces of a thoroughbred racehorse

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We previously isolated five strains of putative lactobacilli from the faeces of a thoroughbred horse (a 4-year-old male). Of the five strains, four were identified as members of existing *Lactobacillus* species; however, sequence analysis of the 16S rRNA gene revealed that the fifth isolate, DI70^T, showed approximately 97% identity (1325/1366 bp) with the type strain of *Lactobacillus delbrueckii*. Therefore, we considered the possibility that DI70^T represents a novel species of the genus *Lactobacillus*. Cells of strain DI70^T were Gram-stain-positive, catalase-negative, non-spore-forming, non-motile rods. In phylogenetic trees constructed on the basis of 16S rRNA gene sequences, strain DI70^T formed a subcluster in the *L. delbrueckii* phylogenetic group and was closely related to *L. delbrueckii*, *Lactobacillus crispatus* and *Lactobacillus jensenii*. However, analysis of DNA–DNA relatedness showed that DI70^T was genetically distinct from its phylogenetic relatives. The isolate also exhibited distinct biochemical and physiological characteristics when compared with its phylogenetic relatives. It required anaerobic conditions for growth on agar medium. The results indicate that isolate DI70^T indeed represents a novel species of the genus *Lactobacillus*, for which we propose the name *Lactobacillus equicursoris* sp. nov. The type strain is DI70^T (=JCM 14600^T =DSM 19284^T).

The genus *Lactobacillus* includes a large number of species of lactic acid bacteria. Lactobacilli are normal inhabitants of the gastrointestinal tracts of many mammals. The diversity of lactobacilli in the contents of the equine gastrointestinal tract and faeces has been investigated using culturing and molecular profiling methods, respectively (Al Jassim *et al.*, 2005; Endo *et al.*, 2007). The species

Lactobacillus equi, *L. hayakitensis* and *L. equigenerosi* were first isolated from the equine gastrointestinal tract/faeces (Morotomi *et al.*, 2002; Morita *et al.*, 2007b; Endo *et al.*, 2008). *L. equi* and *L. hayakitensis* are placed within the *Lactobacillus salivarius* phylogenetic group and *L. equigenerosi* within the *Lactobacillus reuteri* phylogenetic group. In the present study, isolate DI70^T, from faeces of a thoroughbred horse (Morita *et al.*, 2009), is shown to represent a novel species belonging to the *Lactobacillus delbrueckii* phylogenetic group.

In 2006, we isolated five strains of putative lactobacilli in a study on the strains of lactobacilli inhabiting the faeces of a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DI70^T is AB290830.

16S rRNA gene sequence-based maximum-parsimony and maximum-likelihood trees and details of DNA–DNA hybridization results are available as supplementary material with the online version of this paper.

healthy thoroughbred horse (a 4-year-old male). Of the five strains, four have been identified as strains of *Lactobacillus mucosae*, *L. johnsonii*, *L. equigenersi* and *L. buchneri* (Morita *et al.*, 2009). Although the fifth isolate, DI70^T, formed a subcluster in the *L. delbrueckii* phylogenetic group, this strain was not identified.

Blood liver (BL) agar plates (Eiken Chemical Co.) and anaerobic bacterial culture medium (ABCM broth; Eiken Chemical Co.) were used for cell culture. Strain DI70^T was cultured anaerobically at 37 °C in ABCM broth and on BL agar plates for 24 and 72 h, respectively. *L. delbrueckii* JCM 1002^T, *Lactobacillus crispatus* JCM 1185^T and *Lactobacillus jensenii* DSM 20557^T were obtained from the Japan Collection of Microorganisms and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

The 16S rRNA gene sequence (1486 bp) of strain DI70^T was determined by using previously described methods (Morita *et al.*, 2007b). The closest known relatives of the isolate were determined by performing database searches, and the sequences of closely related species were retrieved from public databases. Multiple alignments of the sequences were carried out using CLUSTAL W (Thompson *et al.*, 1994). Distance matrices for the aligned sequences were calculated using the two-parameter method described by Kimura (1980). We used the neighbour-joining method to construct a phylogenetic tree (Saitou & Nei, 1987) and estimated the robustness of the individual branches by performing bootstrapping analysis with 1000 replicates (Felsenstein, 1985). Phylogenetic trees were also constructed using the maximum-likelihood (Cavalli-Sforza & Edwards, 1967) and maximum-parsimony (Kluge & Farris, 1969) methods with PHYLIP version 3.66. Analysis of the 16S rRNA gene revealed that DI70^T had the highest sequence similarity to the type strains of *L. delbrueckii* (97%; 1325/1366 bp), *L. crispatus* (94%, 1310/1387 bp) and *L. jensenii* (95%, 1230/1288 bp). In the phylogenetic tree constructed by the neighbour-joining method, DI70^T formed a subcluster in the *L. delbrueckii* phylogenetic group (Schleifer & Ludwig, 1996; Hammes & Hertel, 2006) (Fig. 1). We obtained identical tree topologies in the maximum-parsimony and maximum-likelihood analysis (see Supplementary Fig. S1 in IJSEM Online). *L. delbrueckii*, *L. johnsonii*, *L. crispatus* and *Lactobacillus amylovorus*, strains of which have been isolated from the contents of the equine gastrointestinal tract by Bailey *et al.* (2003), Al Jassim *et al.* (2005) and Morita *et al.* (2009), belong to the *L. delbrueckii* phylogenetic group. Although we could not isolate other strains of the same species as DI70^T in this study, we found that strains RA2120, RA2062 and RA2066 (GenBank accession nos AY445128, AY445129 and AY445130; Al Jassim *et al.*, 2005) showed greater identity (97.5–97.6%, 1353–1354/1388 bp) to the 16S rRNA gene sequence of DI70^T than to that of the type strain of *Lactobacillus agilis* (97.0%, 1325/1366 bp). These strains were isolated from the equine gastrointestinal tract (stomach and rectum; Al Jassim *et al.*, 2005) and were thus

Fig. 1. Phylogenetic relationships between strain DI70^T and species in the *L. delbrueckii* phylogenetic group, as determined on the basis of sequence analysis of the 16S rRNA gene. The tree was constructed using the neighbour-joining method. The sequence of *Pediococcus acidilactici* DSM 20284^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

of the same origin as DI70^T. This suggests that members of this subcluster mainly inhabit the equine gastrointestinal tract.

DNA–DNA relatedness between DI70^T and three closely related type strains was determined by previously described methods (Ezaki *et al.*, 1990). We used the method described by Morita *et al.* (2007a) to isolate the genomic DNA of strain DI70^T, *L. delbrueckii* JCM 1002^T, *L. crispatus* JCM 1185^T and *L. jensenii* DSM 20557^T. DNA–DNA relatedness values are shown in Supplementary Table S1. Each value was less than the 70% cut-off point recommended for species differentiation (Stackebrandt & Goebel, 1994).

The G + C content of strain DI70^T was determined in three separate trials by using previously described methods (Morita *et al.*, 2007b). The DNA G + C content of strain DI70^T was 48.3 mol%; this differed from the values of 35.0 and 36.0 mol% obtained for the closely related *L. crispatus* JCM 1185^T and *L. jensenii* DSM 20557^T (Table 1). However, the DNA G + C content of DI70^T was similar to that of *L. delbrueckii* JCM 1002^T (50.3 mol%).

The biochemical and physiological characteristics of strain DI70^T were determined by using previously described methods (Mitsuoka, 1969). The detailed characteristics of the isolate are provided in the species description, and these characteristics were compared with those of the phylogenetic relatives *L. delbrueckii* JCM 1002^T, *L. crispatus* JCM 1185^T and *L. jensenii* DSM 20557^T (Table 1). The isolate was an obligately homofermentative lactic acid bacterium that could produce D-lactic acid from D-glucose. Although the three phylogenetic relatives formed colonies under aerobic conditions, DI70^T did not. Strain DI70^T was distinguished from *L. delbrueckii* JCM 1002^T by its pattern

Table 1. Physiological characteristics of strain DI70^T, *L. delbrueckii* JCM 1002^T, *L. crispatus* JCM 1185^T and *L. jensenii* DSM 20557^T

Strains: 1, DI70^T (data from this study); 2, *L. delbrueckii* JCM 1002^T (Rogosa & Hansen, 1971); 3, *L. crispatus* JCM 1185^T (Cato *et al.*, 1983); 4, *L. jensenii* DSM 20557^T (Gasser *et al.*, 1970; Kandler & Weiss, 1986). All strains are obligately homofermentative, produce no gas from D-glucose and possess L-Lys-D-Asp peptidoglycan type of the cell wall. All strains are negative for the following characters: growth at 15 °C, catalase activity and fermentation of arabinose, D-ribose, D-xylose, rhamnose, sorbitol and melezitose. G+C contents were determined by HPLC. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4
Lactic acid isomer(s)	D	D	DL	D
Aerobic growth	–	+	+	+
Growth at 45 °C	w	+	+	–
Fermentation of:				
Gluconate	–	–	ND	ND
Fructose	+	w	+	+
Galactose	+	w	+	+
Mannose	+	w	+	+
Cellobiose	+	–	+	+
Lactose	+	+	+	–
Salicin	+	–	+	+
Melibiose	w	–	w	–
Trehalose	–	–	–	+
Raffinose	w	–	+	–
Mannitol	+	–	–	+
Starch	w	–	+	ND
DNA G+C content (mol%)	48.3	50.3	35.0	36.1

of acid production from carbohydrates. Using the HPLC method of Komagata & Suzuki (1987), the peptidoglycan type in the cell wall of strain DI70^T was L-Lys–L-Asp, as was the case with the three phylogenetic relatives. Anaerobic conditions were essential for growth of strain DI70^T.

The experimental data suggest that the isolate can be assigned to the genus *Lactobacillus* on the basis of its phylogenetic position; however, the unique physiological and biochemical characteristics of the isolate and DNA–DNA relatedness results distinguish it from its phylogenetic relatives. Thus, the isolate represents a novel species, for which we propose the name *Lactobacillus equicursoris* sp. nov.

Description of *Lactobacillus equicursoris* sp. nov.

Lactobacillus equicursoris (e'qui.cur'so.ris. L. n. *equus* a horse; L. gen. n. *cursoris* of a runner or racer; N.L. gen. n. *equicursoris* of a racing horse, referring to the isolation of the type strain from faeces of a thoroughbred racehorse).

Cells are Gram-stain-positive, non-motile, curved rods, 0.5–1.0 × 10–30 µm, and they usually grow singly. Colonies on BL agar after incubation under anaerobic conditions for

2 days at 37 °C are beige, smooth and approximately 0.7–1.0 mm in diameter. Homofermentative, obligately anaerobic and catalase-negative. Produces D-lactic acid from D-glucose. Produces acid from glucose, fructose, galactose, mannose, cellobiose, lactose, salicin and mannitol, and produces acid weakly from melezitose, raffinose and starch. Grows well at 30–40 °C and slowly at 45 °C; does not grow at 15 or 50 °C. The peptidoglycan type of the cell wall is L-Lys–L-Asp and the DNA G+C content of the type strain is 48.3 mol%.

The type strain is DI70^T (=JCM 14600^T =DSM 19284^T), isolated from faeces of a thoroughbred racehorse collected in 2006.

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