Lactobacillus ingluviei sp. nov., isolated from the intestinal tract of pigeons

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Lactic acid bacteria were isolated from the crop and intestines of pigeons. One group of strains, showing similar genomic patterns after screening with tRNA intergenic spacer PCR, could not be identified to the species level. Sequencing of the 16S rRNA gene of one representative strain revealed about 96% similarity to sequences from Lactobacillus fermentum and Lactobacillus mucosae. Determination of the DNA base composition, DNA–DNA hybridization experiments, SDS-PAGE of whole-cell proteins and biochemical testing confirmed that the seven strains studied constitute a single novel Lactobacillus species, for which the name Lactobacillus ingluviei sp. nov. is proposed. The type strain is strain KR3T (= LMG 20380T = CCUG 45722T).
Table 1. Strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pigeon</th>
<th>Origin</th>
<th>Isolation medium</th>
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</thead>
<tbody>
<tr>
<td>KR10 (=LMG 20381)</td>
<td>A Crop</td>
<td>Slanetz &amp; Bartley</td>
<td></td>
</tr>
<tr>
<td>KR21 (=LMG 20978)</td>
<td>B Crop</td>
<td>Slanetz &amp; Bartley</td>
<td></td>
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<tr>
<td>KR47 (=LMG 20384)</td>
<td>B Intestines</td>
<td>Rogosa SL</td>
<td></td>
</tr>
<tr>
<td>KR36 (=LMG 20383)</td>
<td>C Crop</td>
<td>Rogosa SL</td>
<td></td>
</tr>
<tr>
<td>KR45 (=LMG 20979)</td>
<td>C Intestines</td>
<td>Rogosa SL</td>
<td></td>
</tr>
<tr>
<td>KR37 (=LMG 20380T)</td>
<td>D Crop</td>
<td>Slanetz &amp; Bartley</td>
<td></td>
</tr>
<tr>
<td>KR16 (=LMG 20382)</td>
<td>E Crop</td>
<td>Slanetz &amp; Bartley</td>
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DNA base composition. Strains LMG 20380\(^T\) and LMG 20383 were grown on MRS broth and incubated for 24 h at 37 °C under anaerobic conditions. High-molecular-mass native DNA was extracted from 0.75–1.25 g wet weight of cells by using the protocol described by Pitcher et al. (1989), with the following modifications: the washed cell pellet was resuspended and lysed in a buffer (10 mM Tris/HCl, 100 mM EDTA, pH 8) containing RNase (200 μg ml\(^{-1}\); Sigma), mutanolysin (100 U ml\(^{-1}\); Sigma) and lysozyme (25 mg ml\(^{-1}\); Serva) for 1 h at 37 °C. Before the addition of GES reagent, protease K (200 μg ml\(^{-1}\); Merck) was added to the mixture for 15 min.

For determination of the DNA base composition, DNA was enzymically degraded into nucleosides and then separated by HPLC as described previously (Vancoeyene et al., 2001).

DNA–DNA hybridization experiments. High-molecular-mass native DNA was prepared as described above for determination of the DNA base composition. DNA–DNA hybridizations were performed as described by Vancoeyene et al. (2001) by using a microplate method and fluorescence measurements for calculation of the binding values. Hybridizations were performed at 41 °C.

PAGE analysis of whole-cell protein. Cells were cultivated as indicated for determination of DNA base composition. Whole-cell protein extracts were prepared and PAGE was then performed as described by Pot et al. (1994). Registration of the protein patterns, normalization of the densitometric traces, pattern storage and grouping of the strains using Pearson’s product-moment correlation coefficient (r) and UPGMA analysis were performed as described by Pot et al. (1994) by using the software GelCompar (Applied Maths).

Biochemical activity and growth characteristics. All seven strains were tested biochemically as described previously. Growth was tested on MRS agar at 25, 30, 37 and 42 °C under anaerobic conditions. The influence of the gas atmosphere was determined: growth yield under aerobic and anaerobic (H\(_2\) + CO\(_2\), GasPak Plus; BBL) conditions and in a 5% CO\(_2\)-enriched atmosphere were compared. Carbohydrate acidification tests were carried out with API 50 CH galleries according to the instructions of the manufacturer (bioMérieux).

RESULTS AND DISCUSSION

Genotypic studies

Screening of the isolates by tDNA-PCR revealed an atypical pattern different from all profiles of lactic acid bacteria available in the database. Capillary electrophoresis of the amplicons revealed mean peak positions of 162.5, 176.5, 185.5 and 255.2 bp. Numerical analysis clearly confirmed the aberrant position of the strains (Fig. 1).

To determine their phylogenetic position, one representative strain, LMG 20380\(^T\), was selected for 16S rDNA sequence analysis. Highest similarities, of 96 and 95.4%, were obtained to the sequences of Lactobacillus fermentum and Lactobacillus mucosae, respectively (Fig. 2). These values clearly indicate that strain LMG 20380\(^T\) belongs...
to the genus Lactobacillus, but the similarities are too low for possible relatedness at the species level with one of the currently described Lactobacillus species (Stackebrandt & Goebel, 1994).

Two strains, LMG 20380T and LMG 20383, were subsequently subjected to DNA–DNA hybridization analysis. A relatedness value of 77% confirmed that the two strains constitute a single genomic species. Determination of the DNA G+C content of the two strains revealed respective values of 49.4 and 49.2 mol%.

Phenotypic studies

SDS-PAGE analysis of whole-cell proteins yielded highly similar patterns in the seven strains, confirming that they represent a single species. The profiles were different from all patterns of lactic acid bacteria in the database (data not shown), confirming their separate species status. The patterns of three of the strains and of L. fermentum and L. mucosae, their phylogenetically closest neighbours, are shown in Fig. 3.

The growth characteristics of all seven strains were compared. Six strains showed smooth colonies on MRS agar, but strain LMG 20383 showed dry, crumbly colonies. On Columbia blood agar, the strains appear streptococcus-like and without further testing, might be confused with the latter taxon. Other growth characteristics and differentiating phenotypic features between the novel taxon and its phylogenetic neighbours, L. fermentum and L. mucosae, are described below and are summarized in Table 2.

The results of the present study allowed us to assign the strains to a novel species, for which we propose the name Lactobacillus ingluviei sp. nov.

Description of Lactobacillus ingluviei sp. nov.

Lactobacillus ingluviei (in.glu’vi.ei. L. n. ingluvies crop sac; L. gen. n. ingluviei of a crop sac).

Cells are Gram-positive, non-motile, very short, plump rods, rapidly decolorizing in the Gram-stain procedure. Cells mostly occur singly or in pairs, and some appear to be slightly longer than others. They are non-sporulating and catalase-negative. Colonies are white and smooth or crumbly and dry. Growth is enhanced under anaerobic conditions and also slightly in the presence of 5% CO2, compared with aerobic growth. Better growth is obtained at 42°C than at 37°C. No growth occurs at 25°C and growth is poor at 30°C. The strains grow as non-haemolytic streptococcus-like colonies on Columbia blood agar with...
diameters of up to 0.5 mm. Acid is produced from L-arabinose, D-fructose, methyl β-xyloside, ribose, sucrose and D-xylose. No acid is produced from adonitol, amygdalin, D-arabinose, D- or L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- and L-fucose, galactose, β-gentiobiose, 2-ketogluconate, 5-ketogluconate, N-acetylglucosamine, glycerol, glycogen, methyl α-D-glycoside, inositol, inulin, lactose, D-lyxose, mannanol, methyl α-D-mannoside, melezitose, melibiose, rhamnose, salcin, sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, xylitol or L-xylose. Acidification of aesculin, gluconate (weak reaction), D-glucose, maltose, D-mannose and D-raffinose is variable.

The DNA G+C content is 49 mol% and the characteristic tDNA-PCR fingerprint is composed of fragments with lengths of 162±5, 176±5, 185±5 and 255±2 bp, as determined by fluorescent capillary electrophoresis. The habitat is pigeon crop and intestines. The type strain is strain KR3T (=LMG 20380T = CCUG 45722T).

ACKNOWLEDGEMENTS

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Table 2. Characteristics that differentiate *L. ingluviei* sp. nov. from closely related species

<table>
<thead>
<tr>
<th>Production of acid from</th>
<th><em>L. ingluviei</em></th>
<th><em>L. fermentum</em></th>
<th><em>L. mucosae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
<td>+</td>
<td>D+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>–</td>
<td>+</td>
<td>D+</td>
</tr>
</tbody>
</table>

REFERENCES


