Lactobacillus faecis sp. nov., isolated from animal faeces

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Three lactic acid bacteria were isolated from faeces of a jackal (Canis mesomelas) and raccoons (Procyon lotor). The isolates formed a subcluster in the Lactobacillus salivarius phylogenetic group, closely related to Lactobacillus animalis, Lactobacillus apodemi and Lactobacillus murinus, by phylogenetic analysis based on 16S rRNA and recA gene sequences. Levels of DNA–DNA relatedness revealed that the isolates belonged to the same taxon and were genetically separated from their phylogenetic relatives. The three strains were non-motile, obligately homofermentative and produced l-lactic acid as the main end-product from d-glucose. The strains metabolized raffinose. The major cellular fatty acids in the three strains were C16:0, C18:1ω9c and C19:1 cyclo 9,10. Based on the data provided, it is concluded that the three strains represent a novel species of the genus Lactobacillus, for which the name Lactobacillus faecis sp. nov. is proposed. The type strain is AFL13-2T (=JCM 17300T=DSM 23956T).

The genus Lactobacillus is the largest genus in a group of lactic acid bacteria and contains over 100 species with validly published names. These organisms are found in diverse environments, including intestinal tracts of animals, plant surfaces, fermented plant materials and dairy products. The species of the genus Lactobacillus in animal intestines are known to be correlated with health of host animals, including humans (Iebba et al., 2011; Isolauri 2012; Rist et al., 2013). Moreover, probiotic strains of species of the genus Lactobacillus have significant effects on the development of allergies and gastrointestinal disorders (Chrzastowska et al., 2009; Isolauri & Salminen, 2008). Despite the importance of species of the genus Lactobacillus in animal intestines, host specificity of the organisms has not been well characterized.

During the study of microbiota of the genus Lactobacillus in herbivores, omnivores and carnivores, three lactic acid bacteria were isolated from the faeces of a jackal (Canis mesomelas) and raccoons (Procyon lotor) (Endo et al., 2010). These isolates formed a subcluster in the Lactobacillus salivarius phylogenetic group, closely related to Lactobacillus animalis, Lactobacillus apodemi and Lactobacillus murinus. Based on DNA–DNA relatedness, the three isolates are genetically distinct from phylogenetic relatives. This paper addresses the taxonomic status of these three isolates.

Strain AFL13-2T was isolated from the faeces of a jackal and strains AFL18-5 and AFL19-3 from faeces of different raccoons kept in animal houses in Cape Town, South Africa, during December 2009. Faeces of each animal was suspended in sterile anaerobic buffer, serially diluted in the same buffer, plated out onto modified LBS agar (Becton Dickinson) and incubated at 37°C under anaerobic conditions (Anaerobic system BR0038B, Oxoid) for three days (Endo et al., 2010). After isolation, the strains were cultured in MRS broth (Biolab Diagnostics) and stored at −80°C in nutrient broth (Becton Dickinson) containing 20% (v/v) glycerol. L. animalis JCM 5670T, L. apodemi JCM 16172T and L. murinus JCM 1717T, used as references in the present study, were cultured in MRS broth.

The 16S rRNA gene sequences of the three isolates were determined according to a method described previously (Endo & Okada, 2005). The closest recognized relatives of strain AFL13-2T were determined by performing database searches, and sequences of closely related species were retrieved from GenBank. Multiple alignments of the sequences were carried out with the program CLUSTAL_X.
version 1.18 (Thompson et al., 1997). Distance matrices for the aligned sequences were calculated by using the two-parameter method of Kimura (1980). Phylogenetic trees were reconstructed by using the neighbour-joining method and the maximum-likelihood method with PHYLIP version 3.65 as described previously (Endo & Okada, 2006). Approximately 1500 bp of 16S rRNA gene sequences of the three strains were determined and 1400 bp of the sequences of the isolates and related species were used to reconstruct phylogenetic trees. The three isolates shared over 99.8 % sequence similarities. The highest sequence similarities of known species to strain AFL13-2T were 98.0, 97.8 and 98.0 % to *L. animalis*, *L. apodemi* and *L. murinus*, respectively. On the other hand, BLAST analysis also indicated that strain AFL13-2T has 99.8 % similarities to uncultured clones (accession numbers HM124081 and HM124086) from human gut specimens, suggesting a possible colonization by this bacterial group in the human gut. The three strains produced a subcluster with the uncultured clones, closely related to *L. animalis*, *L. apodemi* and *L. murinus*, in the *L. salivarius* phylogenetic group on the basis of the neighbour-joining method (Fig. 1). Identical tree topologies were obtained by using maximum-likelihood analysis (Fig. S1 available in IJSEM Online).

As an additional genetic marker, the *recA* gene sequences of the isolates were determined and used for phylogenetic analysis. Amplification, purification and partial sequencing of the *recA* gene were performed according to methods described previously (Endo & Okada, 2008), except that primers *recA*-ampF (5'-GCCCTAAAAARATYGAAAA-GAAHTTYGGTAAAGG-3') and *recA*-ampR (5'-AATG-GTGGCGCYACYTGGTTTTHACAACTTT-3') were used for PCR amplification and sequencing. Phylogenetic analysis was conducted as described above and a phylogenetic tree was reconstructed by the neighbour-joining method. Approximately 550 bp of the *recA* gene sequences of the isolates and related species were used in the analysis. *Oenococcus oeni* PSU-1 was used as an outgroup. The three isolates shared sequence similarities ranging from 97.4 to 97.8 %. Based on amino acid sequences, the three strains shared 100 % similarities. The highest similarities of the *recA* sequence of strain AFL13-2T were 82.0 and 79.4 % to sequences from *L. animalis* and *L. murinus*, respectively.
Phylogenetic analysis of the three isolates and related taxa based on \textit{recA} gene sequences indicated an independent phylogenetic position of the three strains (Fig. 2).

Levels of DNA–DNA relatedness between the isolates and the phylogenetic relatives, i.e. \textit{L. animalis} JCM 5670\textsuperscript{T}, \textit{L. apodemi} JCM 16172\textsuperscript{T} and \textit{L. murinus} JCM 1717\textsuperscript{T}, and the G+C contents of the isolates, were determined according to the methods described by Kitahara et al., (2001). AFL13-2\textsuperscript{T}, \textit{L. animalis} JCM 5670\textsuperscript{T}, \textit{L. apodemi} JCM 16172\textsuperscript{T} and \textit{L. murinus} JCM 1717\textsuperscript{T} were used as probe DNA. Extraction of bacterial DNA was performed by using a Qiagen Genomic-tip 100/G kit following the manufacturer’s instructions. The isolates showed high levels of DNA–DNA relatedness, ranging from 92 to 100 %. This indicated that the isolates belonged to the same taxon. In contrast, DNA–DNA relatedness of the strains to \textit{L. animalis}, \textit{L. apodemi} and \textit{L. murinus} were 16–20 %, 15–19 % and 16–18 %, respectively. The G+C content of the isolates ranged from 40.4 to 42.3 mol\% (type strain 41.1 mol\%).

To differentiate the isolates, repetitive element (rep)-PCR fingerprinting was performed as described by Endo et al. (2012). The (GTG)\textsubscript{5} primer (5’-GTGGTGGTGGTGGTG-3’) and a primer set REP1R-I (5’-IIIICGICGICATCICGC-3’) and REP2-I (5’-IIICGNCGNCATCNGGC-3’), described by Versalovic et al. (1994), were used. The products produced by primer set REP1R-I/REP2-I clearly differentiated the isolates at the strain level (Fig. 3). On the other hand, the (GTG)\textsubscript{5} primer produced similar profiles for all three isolates, although they were isolated from different hosts. This may be the reason why the (GTG)\textsubscript{5} primer is more suitable for identification at the species level (Adimpong et al., 2012; Švec et al., 2011). Similar results have been obtained for strains of \textit{Lactobacillus rhamnosus} (Endo et al., 2012).

Morphological, physiological and biochemical characteristics were determined by using the methods described previously (Endo & Okada, 2005), except that API 50CHL galleries (bioMérieux) were used for determination of acid production from carbohydrates. Morphological characteristics were also determined by using a scanning electron microscope (SEM) as described previously by Endo et al. (2008). Cellular fatty acids were extracted from the three novel strains and reference strains. These strains were grown in MRS broth under static conditions overnight.
The composition of the fatty acids was determined according to the method described by Sakamoto et al. (2002). The detailed characteristics of the strains are listed in the species description and a SEM image is shown in Fig. S2. The characteristics were further compared with those of the phylogenetic relatives L. animalis, L. apodemi and L. murinus (Table 1). The strains are obligately homofermentative lactic acid bacteria and produce l-lactic acid as the main end product from D-glucose. Interestingly, the three strains and the phylogenetic relatives metabolize raffinose, which is sometimes used as a prebiotic in humans (Dinoto et al., 2006; Fernando et al., 2010). Several species in the L. salivarius phylogenetic group are known to possess unique characteristics among members of the genus Lactobacillus, which include motility, extrapoly saccharide production from sucrose and diaminopimelic acid in the peptidoglycan (Endo & Okada, 2005; Chao et al., 2008; Irisawa & Okada, 2009). The three strains, however, did not possess these characteristics. The major cellular fatty acids in the three strains were $\text{C}_{16:0}$, $\text{C}_{18:1\,\text{cis}3}$ and $\text{C}_{19:1\,\text{cyclo}9,10}$, and they were similar to those recorded for the phylogenetic relatives (Table 2).

The three isolates are phylogenetically and biochemically different from species in the genus Lactobacillus with validly published names, and they represent a novel species for which the name Lactobacillus faecis sp. nov. is proposed.

### Description of Lactobacillus faecis sp. nov.

*Lactobacillus faecis* (fae‘cis. L. n. *faex* faeces; L. gen. n. faecis of faeces).

Cells are Gram-stain-positive, non-motile rods, measuring 0.8 $\times$ 2–8 $\mu$m. Cells usually occur singly or in pairs. Facultatively anaerobic and catalase-negative. Grows under aerobic and anaerobic conditions. Colonies on MRS agar are white, smooth and approximately 1–2 mm in diameter when incubated anaerobically for 2 days and approximately 1–1.5 mm in diameter when incubated aerobically for 3 days. Obligately homofermentative and produces lactic acid as the main end product. Gas is not produced from D-glucose. D-Lactate and L-lactate are produced at a ratio of 2:98. Nitrate is not reduced. Acid is produced from the fermentation of D-glucose, D-fructose, D-mannose, cellobiose, maltose, sucrose and raffinose). Acid production from D-galactose, D-mannitol, N-acetyl-D-glucosamine, lactose, melibiose and β-gentiobi ose is variable among the strains. Acid is not produced from L-arabinose, D-arabinose, L-arabitol, D-arabitol, ribose, adonitol, amygdalin, arbutin, dulcitol, aesculin, erythritol, D-fucose, L-fucose, 2- and 5-ketogluconate, potassium gluconate, methyl α-D-glucoside, glyc erol, glycogen, inositol, inulin, D-lyxose, methyl α-D-mannoside, melezitose, rhamnose, salicin, starch, sorbitol, L-sorbitose, D-tagatose, trehalose, turanose, xylitol, methyl β-xylo side, L-xylose and D-xylose. Dextran is not produced from sucrose. Growth occurs at 45 $^\circ$C, but weakly at 20 $^\circ$C and not at 15 or 50 $^\circ$C. Grows at pH 5.0–8.0 and in the presence of 5% (w/v) NaCl. *meso*-Diaminopimelic acid is not present in peptidoglycan. The major cellular fatty acids are $\text{C}_{16:0}$, $\text{C}_{18:1\,\text{cis}9}$ and $\text{C}_{19:1\,\text{cyclo}9,10}$. The DNA G+C content ranges from 40.4 to 42.3 mol% (type strain 41.1 mol%).

The type strain is AFL13-2T (=JCM 17300T=DSM 23956T). The type strain was isolated from faeces of a jackal (*Canis mesomelas*), collected at Cape Town, Western Cape, South Africa in 2009.
Table 2. Cellular fatty acid profiles of L. faecis sp. nov. strains and phylogenetically related species

Strains: 1, L. faecis AFL13-2T; 2, L. faecis AFL18-5; 3, L. faecis AFL19-3; 4, L. animalis JCM 5670; 5, L. apodenii JCM 16172; 6, L. murinus JCM 17172. Values are percentages of total fatty acids. TR, Trace amount (<0.5%); ND, not detected; DMA, dimethylacetal; ECL, equivalent chain length; FAME, fatty acid methyl ester. All strains were analysed under identical conditions.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td></td>
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<tr>
<td>C14:0</td>
<td>0.51</td>
<td>0.61</td>
<td>TR</td>
<td>3.15</td>
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<td>ND</td>
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<td>ND</td>
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<tr>
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<td>0.79</td>
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<tr>
<td>C9:1 cyclo 9,10 FAME</td>
<td>22.77</td>
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<td>ND</td>
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<td>C12-OH</td>
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<td>ND</td>
<td>ND</td>
<td>15.23</td>
<td>4.28</td>
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<td>10</td>
<td>8.10</td>
<td>7.50</td>
<td>8.99</td>
<td>9.22</td>
<td>7.79</td>
<td>4.20</td>
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<tr>
<td>12</td>
<td>1.94</td>
<td>2.08</td>
<td>2.04</td>
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<td>1.46</td>
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<td>1.87</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</table>

*Summed features represent groups of two or three fatty acids that could not be separated in the study. Summed feature 10 contains one or more of an unknown fatty acid of ECL 17.834 and/or C18:1o11c9t6t fatty acid methyl ester. Summed feature 12 contains one or more of an unknown fatty acid of ECL18.622 and/or C19:0.

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Reference


