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 17171T, 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-
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 similar-
ities 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 coloniza-
tion 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-
GAHHTYGGTAAAGG-3') and recA-ampR (5'-AATG-
GTGGCCGACYTTGTTTTTHACAACTTT-3') were used
for PCR amplification and sequencing. Phylogenetic
analysis was conducted as described above and a phylo-
genetic 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.

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**Fig. 1.** Phylogenetic relationships of Lactobacillus faecis sp. nov. AFL13-2T, AFL18-5 and AFL19-3 to related species based on 16S rRNA gene sequences. The tree was reconstructed by the neighbour-joining method. Lactobacillus delbrueckii subsp. delbrueckii ATCC 9649T was used as an outgroup. Bootstrap percentages above 70 % are given at branching points. Bar, 1 % sequence divergence.
Phylogenetic analysis of the three isolates and related taxa based on 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. L. animalis JCM 5670\(^T\), L. apodemi JCM 16172\(^T\) and L. murinus JCM 1717\(^T\), and the G+C contents of the isolates, were determined according to the methods described by Kitahara et al. (2001). AFL13-2\(^T\), L. animalis JCM 5670\(^T\), L. apodemi JCM 16172\(^T\) and L. murinus JCM 1717\(^T\) 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 L. animalis, L. apodemi and 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)\(_5\) primer (5'-GTGGTGGTGGTGGTGG-3') and a primer set REP1R-I (5'-IIICGICGICATCIGGC-3') and REP2-I (5'-IIICGNCGNCATCNGGC-3'), described by Versalovic et al. (1994), were used. The profiles produced by primer set REP1R-I/REP2-I clearly differentiated the isolates at the strain level (Fig. 3). On the other hand, the (GTG)\(_5\) primer produced similar profiles for all three isolates, although they were isolated from different hosts. This may be the reason why the (GTG)\(_5\) 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 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.

![Fig. 2. Phylogenetic relationships of L. faecis sp. nov. AFL13-2\(^T\), AFL18-5 and AFL19-3 to related species based on recA gene sequences. The tree was reconstructed by the neighbour-joining method. Oenococcus oeni PSU-1 was used as an outgroup. Bootstrap percentages above 70 % are given at branching points. Bar, 10 % sequence divergence.](image-url)
Fig. 3. Repetitive element (rep)-PCR fingerprints of *L. faecis* sp. nov. strains. (GTG)₅ primer (lanes 1–3) and a primer set consisting of REP1R-I and REP2-I (lanes 4–6) were used. Lanes: M, size marker (200 kb DNA ladder, Takara Bio); 1 and 4, AFL13-2 T; 2 and 5, AFL18-5; 3 and 6, AFL19-3.

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, extraplycrosaccharide 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 C₁₆:₀, C₁₈:₁ω9c and C₁₉:₁ 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.

### Table 1. Differential characteristics between *Lactobacillus faecis* sp. nov. and phylogenetically related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at 20 °C</td>
<td>W</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+/D</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+/D/−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>N-acetyl-D-glucosamine</td>
<td>+/W</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arbutin</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+/D</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Melibiose</td>
<td>+/D</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
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<td>−</td>
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<td>−</td>
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<tr>
<td>Gentiose</td>
<td>+/D/−</td>
<td>D</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Turanose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>D</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>40.4–42.3</td>
<td>42.5</td>
<td>41.0</td>
<td>42.2</td>
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</tbody>
</table>

*Description of *Lactobacillus faecis* sp. nov.*

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

Cells are Gram-stain-positive, non-motile rods, measuring 0.8 x 2–8 μm. 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-gentiobiose 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, glycerol, glycogen, inositol, inulin, D-lyxose, methyl α-D-mannoside, melezitose, rhamnose, salicin, starch, sorbitol, L-sorbose, D-tagatose, trehalose, turanose, xylitol, methyl β-xyloside, L-xylose and D-xylose. Dextran is not produced from sucrose. Growth occurs at 45 °C, but weakly at 20 °C and not at 15 or 50 °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 C₁₆:₀, C₁₈:₁ω9c and C₁₉:₁ 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 AFL13-2 T (=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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
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<th>5</th>
<th>6</th>
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<td>Saturated straight-chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>0.51</td>
<td>0.61</td>
<td>TR</td>
<td>3.15</td>
<td>4.64</td>
<td>1.58</td>
</tr>
<tr>
<td>C16:0</td>
<td>21.79</td>
<td>22.63</td>
<td>18.95</td>
<td>36.99</td>
<td>45.11</td>
<td>28.06</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.38</td>
<td>2.37</td>
<td>2.64</td>
<td>ND</td>
<td>1.60</td>
<td>ND</td>
</tr>
<tr>
<td>C20:0</td>
<td>2.96</td>
<td>5.11</td>
<td>4.96</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unsaturated straight-chain</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C16:1t7c</td>
<td>0.82</td>
<td>0.78</td>
<td>0.79</td>
<td>2.46</td>
<td>1.51</td>
<td>1.69</td>
</tr>
<tr>
<td>C18:1t9c</td>
<td>35.30</td>
<td>30.47</td>
<td>38.21</td>
<td>12.07</td>
<td>12.68</td>
<td>28.95</td>
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<tr>
<td>C18:1t7c DMA</td>
<td>1.96</td>
<td>1.51</td>
<td>2.49</td>
<td>1.99</td>
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</tr>
<tr>
<td>Cyclopropane</td>
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</tr>
<tr>
<td>C19:1 cyclo 9,10 FAME</td>
<td>22.77</td>
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<td>C19:1 cyclo 11,12 FAME</td>
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<td>ND</td>
<td>ND</td>
<td>7.48</td>
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<tr>
<td>C18:0 12-OH</td>
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<td>ND</td>
<td>ND</td>
<td>15.23</td>
<td>4.28</td>
<td>11.26</td>
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<td>Summed features*</td>
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<tr>
<td>10</td>
<td>8.10</td>
<td>7.50</td>
<td>8.99</td>
<td>9.22</td>
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<td>1.94</td>
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<td>2.04</td>
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<tr>
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<td>1.01</td>
<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 C18:1t11c9t6t fatty acid methyl ester. Summed feature 12 contains one or more of an unknown fatty acid of ECL18.622 and/or iso-C19:0.

### Acknowledgements

We are grateful to J. P. Ezéby (Société de Bactériologie Systématique et Vétérinaire and École Nationale Vétérinaire de Toulouse) for advice on the naming of the novel species. We are also thankful to the World of Birds (Cape Town, South Africa) for their assistance with the collection of samples.

### Reference


