Description of *Paralactobacillus selangorensis* gen. nov., sp. nov., a new lactic acid bacterium isolated from chili bo, a Malaysian food ingredient

J. J. Leisner, 1 M. Vancanneyt, 2 J. Goris, 3 H. Christensen 1 and G. Rusul 4

**INTRODUCTION**

During studies on lactic acid bacteria (LAB) isolated from a traditional Malaysian food ingredient, chili bo, isolates belonging to a new group of LAB were obtained (Leisner et al., 1997). These isolates exhibited phenotypic characteristics, including SDS-PAGE whole-cell profiles, which excluded them from any described species of LAB. 16S rRNA sequencing data demonstrated that they represent a new group of LAB distantly related to the *Lactobacillus casei–Pediococcus* group. A phenotypic description that distinguishes *Paralactobacillus selangorensis* from other genera of lactic acid bacteria is presented. The type strain of *Paralactobacillus selangorensis* is LMG 17710T.

**METHODS**

Bacterial strains and growth media. Strains were isolated from two distinct batches of chili bo obtained from a commercial manufacturer located in Pataling Jaya, Selangor DE, Malaysia. The processing of chili bo and the procedure for obtaining the isolates have been described previously (Leisner et al., 1997, 1999). Initially, 22 strains belonging to this group were obtained and characterized by a battery of traditional phenotypic tests. The results of these tests showed that all 22 strains shared very similar phenotypic traits (Leisner et al., 1999; unpublished data). Five strains, three of which (LMG 17710T–17712) were isolated from one batch of chili bo and two (LMG 17713–17714) which were isolated from the other batch, were selected as representative isolates for additional SDS-PAGE whole-cell protein electrophoresis and deposited with the Belgian Coordinated Collections of Microorganisms (BCCM/LMG) (Leisner et al., 1999). These five strains were included in this study.

Biochemical and physiological tests. Strains were tested for the ability to grow in Man–Rogosa–Sharpe broth (MRS; Merck) at 7, 10, 15, 21, 25, 30, 37, 42 and 45 °C. Production of acids from carbohydrates and related compounds was tested by using API 50 CH strips and API CHL medium (bioMérieux). Tests were done according to the manufacturer’s instructions and the results were read after incubation at 30 °C for 2, 4, 7 and 14 d. Production of lactic acid isomers, acetic acid and ethanol was determined enzymically using d- and l-lactate dehydrogenase, acetyl-CoA synthetase and alcohol dehydrogenase (all Boehringer Mannheim), respectively, for cultures grown in MRS broth for 45 h or in All Purpose Tween (APT; Difco) broth for 73 h. Incubation was at 30 °C.

Fatty acid methyl ester analysis. Strains were grown for 24 h on MRS agar plates. A loopful of well grown cells was
harvested. Preparation, separation, identification and numerical comparison of the fatty acid methyl esters was performed using the Microbial Identification System (Microbial ID) as described by Vancanneyt et al. (1996).

**DNA base composition.** DNA was enzymically degraded into nucleosides as described by Mesbah et al. (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters SymmetryShield C8 column with the thermostat set at 37 °C. The solvent was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5% acetonitrile. Non-methylated λ phage DNA (Sigma) was used as the calibration reference.

**DNA–DNA hybridization.** DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as described by Ezaki et al. (1989) using an HTS7000 Bio Assay Reader (Perkin Elmer) for fluorescence measurements. The hybridization temperature was 40 °C.

### RESULTS AND DISCUSSION

All five strains of *Paralactobacillus selangorensis* produced acid from D-fructose, D-mannose, N-acetylglucosamine and salicin within 4 d of incubation in API 50 CH medium at 30 °C. Some strains also produced acid within 4 d or more (up to 2 weeks) from galactose, rhamnose, methyl α-D-glucoside, amygdalin, arbutin, cellobiose, maltose, sucrose, trehalose, β-gentiobiose, D-turanose and D-tagatose. Aesculin was hydrolysed. No acid was produced by any of the five strains from glyceral, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methylxylloside, L-sorbose, mannitol, dulcitol, inositol, sorbitol, methyl α-D-mannoside, lactose, melibiose, melizitose, inulin, D-raffinose, starch, glycerogen, xylitol, D-lxylose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. API 50 CH reactions useful in differentiating *Paralactobacillus selangorensis* from homofermentative *Lactobacillus* spp. of the *Lactobacillus casei–Pediococcus* group are shown in Table 1 and from homofermentative *Lactobacillus* spp. of the *Lactobacillus delbrueckii* group in Table 2.

All of the strains, excluding LMG 17711, were tested for production of lactic acid isomers, acetic acid and ethanol. Both D- and L-lactic acid were produced from MRS broth in the approximate ratio 1:6–2:1 (0:1). The yield of D- and L-lactic acid was 50–1–76 and 30–41–2 mM, respectively, after 44 h incubation at 30 °C in MRS broth. Only trace amounts of acetic acid (<3 mM) and ethanol (<0.35 mM) were produced under these conditions. LMG 17710 was examined for production of acetic acid, ethanol and lactic acid during growth in APT broth. Both D- and L-lactic acid were produced, whereas only trace amounts of acetic acid and ethanol were observed (results not shown).

All five strains exhibited visible growth within 2–3 d in MRS broth incubated at 21–30 °C. Variation in growth was observed at 37 °C. Visible growth at 15 °C was observed only after 5–10 d incubation. No strains grew above 37 °C or below 15 °C.

The G+C content of the DNA was 46.1 ± 0.3 mol% for LMG 17710 and 17112, and 46.2 mol% for LMG 17714. DNA–DNA hybridization gave similarity

### Table 1. Differential phenotypic characteristics of *Paralactobacillus* and obligatory homofermentative *Lactobacillus* spp. of the *Lactobacillus casei–Pediococcus* group

Data for homofermentative *Lactobacillus* spp. are from Hammes & Vogel (1995) except for *Lactobacillus aviarus* (inulin data from Fujisawa et al., 1984), *Lactobacillus manihotivorans* (all data from Morlon-Guyot et al., 1998), *Lactobacillus farciminis* (some of the data from Reuter, 1983), *Lactobacillus mali* (some of the data from Kaneuchi et al., 1988), and *Lactobacillus salivarius* and *Lactobacillus ruminis* (data for anaerobic growth from Fujisawa et al., 1990). For data given by Hammes & Vogel (1995): +, ≥ 90% of strains are positive, –, ≥ 90% of strains are negative, d, 11–89% of strains are positive.

<table>
<thead>
<tr>
<th><em>P. selangorensis</em> or <em>Lactobacillus</em> spp.</th>
<th>G+C (mol%)</th>
<th>Growth at 45 °C</th>
<th>Strictly anaerobic</th>
<th>Acid from:</th>
<th>Lactic acid isomer*</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Raffinose</td>
<td>Lactose</td>
</tr>
<tr>
<td><em>P. selangorensis</em></td>
<td>45–47</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>L. aviarus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subsp. aviarus</td>
<td>39–43</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>subsp. araffinosus</td>
<td>39–43</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>L. farciminis</em></td>
<td>34–36</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>L. mali</em></td>
<td>32–34</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>L. manihotivorans</em></td>
<td>48–49</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>L. ruminis</em></td>
<td>44–47</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>*L. salivarius†</td>
<td>34–36</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>*L. sharpeae††</td>
<td>53</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Isomers in parentheses indicate <15–20% of total acid.
†† Includes both subspecies: *Lactobacillus salivarius* subsp. salicinii and subsp. salivarius.
Table 2. Differential phenotypic characteristics of Paralactobacillus and obligatory homofermentative Lactobacillus spp. of the Lactobacillus delbrueckii group

Data for homofermentative Lactobacillus spp. are from Hammes & Vogel (1995) except for Lactobacillus amyloiticus, Lactobacillus iners and Lactobacillus kefirgranum for which the data are from Bohak et al. (1998), Falsen et al. (1999) and Takizawa et al. (1994), respectively. For data given by Hammes & Vogel (1995): +, ≥ 90% of strains are positive, −, ≤ 90% of strains are negative, d, 11–89% of strains are positive.

Table 3. Mean values of fatty acid profiles ± so for Paralactobacillus selangorensis strains LMG 17710T–17714

Table 3. Mean values of fatty acid profiles ± so for Paralactobacillus selangorensis strains LMG 17710T–17714

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Incidence (%)</th>
</tr>
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<tbody>
<tr>
<td>14:0</td>
<td>4.1±1.1</td>
</tr>
<tr>
<td>16:0</td>
<td>12.1±2.0</td>
</tr>
<tr>
<td>16:1o7c</td>
<td>6.1±0.8</td>
</tr>
<tr>
<td>17:1o8c</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>18:1o9c</td>
<td>5.7±3.2</td>
</tr>
<tr>
<td>Summed feature 7*</td>
<td>7.9±0.3</td>
</tr>
<tr>
<td>Summed feature 9*</td>
<td>8.1±1.8</td>
</tr>
</tbody>
</table>

*Summed feature 7 contained one or more of the following isomers: 18:1 47c, 18:1 49r and/or 18:1 412r (cis and trans isomers are indicated by the suffixes c and t, respectively). Summed feature 9 contained one or more of the following fatty acids: 19:0 cyclo 410c, unknown 18:846 and/or unknown 18:858.

Values of 99±5% between LMG 17710T and 17712, 100±6% between LMG 17710T and 17714, and 104±11% between LMG 17712 and 17714.

The cellular fatty acid composition for the five strains examined is shown in Table 3. All of the strains possessed predominantly straight-chain saturated and monounsaturated types. This combination of major fatty acids is similar to that for the genus Lactobacillus (Kandler & Weiss, 1986).

Recently, Paralactobacillus selangorensis was found to be distantly related to other LAB taxa by phylogenetic analysis of 16S rRNA sequences (Leisner et al., 1999). In Fig. 1 the phylogenetic relationship between Paralactobacillus selangorensis and a representative collection of low-G+C Gram-positive bacteria has been established on the basis of 16S rRNA sequence comparison by maximum-likelihood analysis as described previously (Leisner et al., 1999). Thirty-nine taxa were aligned, resulting in 1457 nt positions being compared covering the region 33–1472 of the 16S rRNA sequence (Escherichia coli numbering). The phylogenetic relationship between Paralactobacillus selangorensis and the Lactobacillus casei–Pediococcus group was repeatedly verified by maximum-likelihood analysis. The position of the node joining Paralactobacillus selangorensis with the Lactobacillus casei–Pediococcus group was, however, not well supported by bootstrap analysis (48%). The highest similarity in 16S rRNA sequences between Paralactobacillus selangorensis and the Lactobacillus casei–Pediococcus group was between 90.1 and 91.7%. These values are similar to or lower than those reported for similarities in 16S rRNA sequences for other LAB taxa.
genera described recently and accepted LAB genera. Examples include comparisons of 16S rRNA sequences for Vagococcus and Enterococcus (Collins et al., 1989), Weissella and Leuconostoc (Collins et al., 1993) and Lactosphaera and Carnobacterium (Janssen et al., 1995). It requires a combination of several phenotypical tests to differentiate Paralactobacillus selangorensis from homofermentative Lactobacillus spp., including species of the Lactobacillus casei–Pediococcus group (Table 1 and 2), similar to what has been demonstrated for the phenotypic differentiation of Weissella and Leuconostoc (Collins et al., 1993). However, 16S rRNA sequences clearly demonstrate that Paralactobacillus selangorensis represents a new group of LAB, distantly related to the Lactobacillus casei–Pediococcus group (Leisner et al., 1999). It is therefore formally proposed that these organisms are given independent genus rank within the LAB. Analyses of G + C content, DNA–DNA similarity and fatty acid content all demonstrate that the new taxon is very homogeneous and constitutes a single genomic species.

**Description of Paralactobacillus gen. nov.**

*Paralactobacillus* (pa.ra.lac.to.ba.cill’us. Gr. prep. *para* resembling; M.L. n. *Lactobacillus* a bacterial genus; *Paralactobacillus* resembling the genus *Lactobacillus*).

Surface colonies on MRS agar after 3 d aerobic incubation at 30 °C are <2–3 mm in diameter, round and with smooth surfaces. Non-spore-forming, straight, slender rods 2:5–6:5 µm long and 1:0 µm wide, usually occurring singly or as pairs but sometimes in older cultures as short chains. Cells are Gram-positive and non-motile. Homofermentative, producing D–(−)– as well as L–(+)–lactic acid from glucose. No gas is produced from glucose and growth is not observed with gluconic acid as substrate. Acid is produced from...
mannose and salicin but not from lactose, melibiose, raffinose, ribose or xylose. No ammonia is produced from arginine (Leisner et al., 1999). Growth is slow in sterilized chili bo (Leisner et al., 1997) as well as in MRS broth at 30°C (unpublished data). Growth occurs in MRS broth at 15°C but not at 45°C. No growth with 6-5% NaCl. Able to grow on acetate agar and to lower pH to <4.15 in La-broth (Leisner et al., 1999). Resistant towards 30 mg vancomycin g⁻¹ (Leisner et al., 1999). Catalase-negative. Nitrate is not reduced (Leisner et al., 1999). G+C content of DNA is 46 mol%. Isolated from a Malaysian food ingredient, chili bo (Leisner et al., 1997). Readily distinguished from obligatory heterofermentative Lactobacillus, Weissella and Leuconostoc by lack of production of gas from glucose and from facultative heterofermentative Lactobacillus by lack of production of gas and acid from gluconic acid and lack of production of acid from ribose or xylose. Two species of facultative heterofermentative Lactobacillus, Lactobacillus acetotolerans and Lactobacillus homohiochii, may contain strains that also show negative reactions in all three tests (Hammes & Vogel, 1995). Lactobacillus acetotolerans may be differentiated from Paralactobacillus selangorensis by its G+C content of 35–37 mol% and lack of growth at 15°C, and Lactobacillus homohiochii may be differentiated by a G+C content of 35–38 mol% and lack of growth in MRS broth (Hammes & Vogel, 1995). Paralactobacillus may be distinguished from the homofermentative rod-shaped Carnobacterium by the ability to grow on acetate agar and the lowering of pH to below 4·15 during growth in La-broth, and from the homofermentative Lactococcus, Enterococcus, Streptococcus, Pediococcus, Lactosphaera, Vogococcus and Tetragenococcus by possessing a distinctive rod-shaped cell morphology. The differentiation of Paralactobacillus from homofermentative Lactobacillus is more problematic and requires the use of a combination of characters for particular species. The differentiation of Paralactobacillus from homofermentative Lactobacillus spp. of the Lactobacillus casei–Pediococcus group is given by the tests listed in Table 1 and from homofermentative Lactobacillus spp. of the Lactobacillus delbrueckii group by the tests listed in Table 2.

**Description of Paralactobacillus selangorensis sp. nov.**

Paralactobacillus selangorensis (sel.an.gor’en.sis. M. L. adj. selangorensis belonging to the province of Selangor, Malaysia).

Description of species as for genus. The type strain is LMG 17710T. The characteristics of LMG 17710T correspond to those of the species except that acid is produced from D-tagatose, in contrast to other strains.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


