Macellibacteroides fermentans gen. nov., sp. nov., a member of the family Porphyromonadaceae isolated from an upflow anaerobic filter treating abattoir wastewaters

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A novel obligately anaerobic, non-spore-forming, rod-shaped mesophilic bacterium, which stained Gram-positive but showed the typical cell wall structure of Gram-negative bacteria, was isolated from an upflow anaerobic filter treating abattoir wastewaters in Tunisia. The strain, designated LIND7HT, grew at 20–45 °C (optimum 35–40 °C) and at pH 5.0–8.5 (optimum pH 6.5–7.5). It did not require NaCl for growth, but was able to grow in the presence of up to 2 % NaCl. Sulfate, thiosulfate, elemental sulfur, sulfite, nitrate and nitrite were not used as terminal electron acceptors. Strain LIND7HT used cellobiose, glucose, lactose, mannose, maltose, peptone, rhamnose, raffinose, sucrose and xylose as electron donors. The main fermentation products from glucose metabolism were lactate, acetate, butyrate and isobutyrate. The predominant cellular fatty acids were anteiso-C15 : 0, C15 : 0, C17 : 0 2-OH and a summed feature consisting of C18 : 2 ω6c and/or anteiso-C18 : 0, and the major menaquinones were MK-9, MK-9(H2) and MK-10. The G+C content of the genomic DNA was 41.4 mol%. Although the closest phylogenetic relatives of strain LIND7HT were Parabacteroides merdae, Parabacteroides goldsteinii and Parabacteroides gordonii, analysis of the hsp60 gene sequence showed that strain LIND7HT was not a member of the genus Parabacteroides. On the basis of phylogenetic inference and phenotypic properties, strain LIND7HT (=CCUG 60892T=DSM 23697T=JCM 16313T) is proposed as the type strain of a novel species in a new genus within the family Porphyromonadaceae, Macellibacteroides fermentans gen. nov., sp. nov.

The phylum Bacteroidetes [formerly the Cytophaga-Flavobacterium–Bacteroides group (Gherna & Woese, 1992)] constitutes the dominant bacterial group of the gastrointestinal microbiota (23 % of the human intestinal microbiota) followed by members of the phylum Firmicutes (Eckburg et al., 2005; Gill et al., 2006). The family Porphyromonadaceae has been proposed to encompass the genera Porphyromonas (type genus), Barnesiella, Dysgonomonas, Paludibacter, Petrimonas, Proteiniphilum, Tannemella and Parabacteroides (Shah & Collins, 1988; Sakamoto et al., 2002). Members of the family Porphyromonadaceae are obligately anaerobic, heterotrophic, non-spore-forming, non-motile, Gram-staining-negative rods that ferment sugars (Krieg, 2011). In this paper, we propose a novel species in a new genus in the family Porphyromonadaceae to accommodate a mesophilic, anaerobic strain, designated LIND7HT1.

Strain LIND7HT1 was isolated from an upflow anaerobic filter treating abattoir wastewaters in Tunisia (Gannoun...
et al., 2009). The digester was operated under both mesophilic (37 °C) and thermophilic (55 °C) conditions. A sample was taken during the mesophilic phase of the process in order to isolate fermentative micro-organisms.

For enrichment and isolation, the basal medium contained (per litre of distilled water) 0.3 g KH₂PO₄, 0.3 g K₂HPO₄, 1 g NH₄Cl, 1 g NaCl, 0.1 g KCl, 0.1 g CaCl₂, 2H₂O, 1 g MgCl₂, 6H₂O, 0.5 g L-cysteine.HCl, 2 g yeast extract (Difco), 10 ml trace mineral element solution (Balch et al., 1979) and 1 ml of 0.1% resazurin (Sigma). The pH was adjusted to 7.2 with 10 M KOH solution. The medium was boiled under a stream of O₂-free nitrogen and cooled to room temperature. Aliquots of 5 ml were dispensed into Hungate tubes, degassed under N₂/CO₂ (80:20, v/v) and subsequently sterilized by autoclaving at 120 °C for 20 min. Prior to inoculation, 0.1 ml of 10% (w/v) NaHCO₃, 0.1 ml of 2% (w/v) Na₂S, 9H₂O and 20 mM glucose from sterile stock solutions were injected into the tubes. The Hungate technique (Hungate, 1969) was used throughout the study.

A 0.5 ml aliquot of the sample was inoculated into the Hungate tubes that were subsequently incubated at 37 °C. To obtain pure cultures, the enrichment was subcultured several times under the same growth conditions prior to isolation. For isolation, the culture was serially diluted tenfold in roll tubes (Miller & Wolin, 1974) containing the same culture medium supplemented with 2% (w/v) agar. Several colonies developed after incubation at 37 °C and were picked separately. The process of serial dilution was repeated several times until the isolates were deemed to be axenic. Several strains were isolated; their morphology and metabolic profile were similar and the same phylogenetic inference was obtained for all of them. One strain, designated LIND7Hᵀ, was selected and used for further metabolic and physiological characterization.

Methods for purification of the DNA, PCR amplification and sequencing of the 16S rRNA gene were described previously (Thabet et al., 2004). The partial sequences generated were assembled using BioEdit v. 5.0.9. (Hall, 1999) and the consensus sequence of 1502 nt was corrected manually for assembled using BioEdit v. 5.0.9. (Hall, 1999) and the phylogenetic marker for the classification of Gram-negative anaerobic rods, was performed as described previously (Sakamoto & Ohkuma, 2010).

On the basis of 16S rRNA gene sequence analysis, strain LIND7Hᵀ (HQ020488) formed a separate branch within the family Porphyromonadaceae (Fig. 1). Its closest phylogenetic relatives were micro-organisms of human origin (faeces, intestine and blood), i.e. Parabacteroides merdae (Johnson et al., 1986), Parabacteroides goldsteinii (Song et al., 2005; Sakamoto & Benno, 2006) and Parabacteroides gordonii (Sakamoto et al., 2009), with sequence similarities of 91.4, 91.3 and 91.2% between the type strains of these three species and the novel isolate, respectively. In addition, hsp60 gene sequence similarities between strain LIND7Hᵀ and P. gordonii, P. goldsteinii, Parabacteroides johnsonii, P. merdae and Parabacteroides distasonis were 82.3, 81.4, 78.7, 78.5 and 78.1%, respectively, while sequence similarities with members of other Parabacteroides species were 83.3–97.1% (Table S1 available in IJSEM Online), confirming that the novel isolate did not belong to any of these species.

The Gram reaction was performed with heat-fixed liquid cultures stained with Difco kit reagents. Cellular morphology and purity of the strain were assessed under an Optiphot (Nikon) phase-contrast microscope. For transmission electron microscopy studies, cells were negatively stained with sodium phosphotungstate, as described previously (Fardeau et al., 1997). The presence of spores was assessed: (i) by microscopic observation of cultures; and (ii) by pasteurization tests performed at 80, 90 and 100 °C for 10 and 20 min.

Growth experiments were performed in duplicate using Hungate tubes containing the above-mentioned basal medium. The pH of the medium was adjusted to pH 5.0–9.0 (at 0.5 pH unit intervals) by injecting aliquots of anaerobic stock solutions of 1 M HCl, 10% NaHCO₃ or Na₂CO₃ into the Hungate tubes. The pH of the medium was verified after autoclaving. Water baths were used for incubating bacterial cultures at 20–55 °C (at 5 °C intervals). To assess the requirement of and tolerance to NaCl, NaCl (0–10%, at 0.5% intervals) was weighed directly into the tubes before the medium was dispensed. The strain was subcultured at least twice under the same experimental conditions before growth rates were determined. Arabinose, cellobiose, glucose, glyceral, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, xylose, peptone, Casamino acids, acetate, lactate and pyruvate, were tested as electron donors. Each substrate was added to the basal medium at a final concentration of 20 mM. H₂/CO₂ (80:20) alone or in the presence of acetate (2 mM) as a carbon source was also tested as an electron donor at 2 bars (200 kPa). The effects of yeast extract (Panreac), biotrypcase (Panreac) and Balch’s vitamins (Balch et al., 1979) on growth were tested with and without substrate added. Other biochemical tests were performed using the API 20A anaerobe test kit as recommended by the manufacturer (bioMérieux). Resistance to ampicillin, vancomycin and kanamycin was tested between 10 and 500 μg ml⁻¹ in Hungate tubes incubated at 37 °C for 4 days. Elemental sulfur (1% w/v), sodium sulfate
(20 mM), sodium thiosulfate (20 mM), sodium sulfite (2 mM), sodium nitrate (10 mM) and sodium nitrite (2 mM) were tested as terminal electron acceptors. H₂S (2 mM), sodium nitrate (10 mM) and sodium nitrite (20 mM), sodium thiosulfate (20 mM), sodium sulfite (20 mM), sodium molybdatophosphoric acid and heating at 200 °C for 10 min. For fatty acid analysis, the biomass of strain LIND7Hᵀ and P. distasonis CCUG 4941ᵀ were standardized for their physiological age at the point of harvest according to Technical Note 101 of MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). Fatty acids were extracted using the method of Miller (1982) with the modifications of Kuykendall et al. (1988) and analysed by GC (model 6890N; Agilent Technologies) using the Microbial Identification System (MIDI, Sherlock version 6.1; database, TSBA40; Sasser, 1990). DNA was isolated and purified by chromatography on hydroxyapatite using the procedure of Cashon et al. (1977) and the G+C content was determined at the DSMZ by using HPLC as described by Mesbah et al. (1989). The complete fatty acid profile of strain LIND7Hᵀ and P. distasonis CCUG 4941ᵀ is given in Table 1. The major fatty acids of strain LIND7Hᵀ were anteiso-C₁₅:₀ (30.1%), C₁₅:₀ (10.4%), C₁₇:₀ 2-OH (8.0%) and a summed feature consisting of C₁₈:₀ 0:6,9 and/or anteiso-C₁₈:₀ (8.3%). The proportions of several fatty acids differed significantly between the two strains; moreover, the novel isolate contained 3.2% of an unidentified fatty acid, the chain length of which could not be determined. There were also significant differences in the respective proportions of the different menaquinones [especially MK-9(H₂) and MK-10] between strain LIND7Hᵀ and the six Parabacteroides species (Table 2). In addition, the DNA G+C content of strain LIND7Hᵀ was significantly lower than those reported for Parabacteroides species (Table 2). The polar lipid profile of strain LIND7Hᵀ consisted of phosphatidylethanolamine, phosphatidylglycerol, one unidentified glycolipid and several unidentified phospholipids (Fig. 3).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain LIND7Hᵀ and related representatives of the family Porphyromonadaceae. Bootstrap values of 70% or higher (based on 1000 repetitions) are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony and maximum-likelihood algorithms. Bacteroides cellulosolvens DSM 2933ᵀ was used as an outgroup. Bar, 5 substitutions per 100 nt.
Therefore, on the basis of phylogenetic inference and differential characteristics (Tables 1 and 2), we propose that strain LIND7H\textsuperscript{T} represents a novel species in a new genus within the family Porphyromonadaceae, Macellibacteroides fermentans gen. nov., sp. nov.

**Description of Macellibacteroides fermentans**

*Macellibacteroides* (Ma.cell.ii.bac.te.ro'i.des. L. n. macellum a butcher’s stall, meat-market, slaughterhouse; N.L. masc. n. Bacteroides a bacterial genus name; N.L. masc. n. Macellibacteroides a relative of the genus Bacteroides isolated from a slaughterhouse).

Cells stain Gram-positive but have a Gram-negative type of cell wall. Non-motile, non-spore-forming, mesophilic rods with a fermentative and obligately anaerobic type of metabolism. The major respiratory quinones are MK-9 and MK-9(H\textsubscript{2}) and the major fatty acids are anteiso-C\textsubscript{15} : 0 and C\textsubscript{15} : 0. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and several unidentified phospholipids. The type species is *Macellibacteroides fermentans*. A member of the family Porphyromonadaceae, phylum Bacteroidetes, according to 16S rRNA gene sequence analysis.

**Table 1.** Cellular fatty acid composition (%) of strain LIND7H\textsuperscript{T} and *P. distasonis* CCUG 4941\textsuperscript{T}

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>2</th>
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<tbody>
<tr>
<td>iso-C\textsubscript{13} : 0</td>
<td>1.1</td>
<td>Tr</td>
</tr>
<tr>
<td>anteiso-C\textsubscript{13} : 0</td>
<td>2.7</td>
<td>Tr</td>
</tr>
<tr>
<td>ECL 13.566</td>
<td>2.3</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{15} : 0</td>
<td>5.4</td>
<td>8.5</td>
</tr>
<tr>
<td>anteiso-C\textsubscript{15} : 0</td>
<td>30.1</td>
<td>43.5</td>
</tr>
<tr>
<td>C\textsubscript{15} : 0</td>
<td>10.4</td>
<td>6.8</td>
</tr>
<tr>
<td>C\textsubscript{16} : 0</td>
<td>1.8</td>
<td>4.2</td>
</tr>
<tr>
<td>C\textsubscript{15} : 0 3-OH</td>
<td>3.4</td>
<td>Tr</td>
</tr>
<tr>
<td>C\textsubscript{16} : 0 3-OH</td>
<td>1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Summed feature*</td>
<td>8.3</td>
<td>4.8</td>
</tr>
<tr>
<td>iso-C\textsubscript{17} : 0 3-OH</td>
<td>6.3</td>
<td>13.6</td>
</tr>
<tr>
<td>C\textsubscript{17} : 0 2-OH</td>
<td>8.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Unidentified</td>
<td>3.2</td>
<td>–</td>
</tr>
</tbody>
</table>

*The summed feature comprises C\textsubscript{18} : 2\textsuperscript{ω6,ω9}c and/or anteiso C\textsubscript{18} : 0.*

Fig. 2. Cell morphology of strain LIND7H\textsuperscript{T}. Phase-contrast micrograph (a) and thin-section electron micrographs (b, c) showing the Gram-negative cell wall type. Bars: a, 10 \( \mu \)m; b, 1 \( \mu \)m; c, 0.2 \( \mu \)m.
Table 2. Comparison of the menaquinone composition and DNA G+C content of strain LIND7H\textsuperscript{T} and Parabacteroides species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Menaquinone (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-8</td>
<td>0</td>
<td>1–5 (4)</td>
</tr>
<tr>
<td>MK-9</td>
<td>52</td>
<td>10–54 (24)</td>
</tr>
<tr>
<td>MK-9(H\textsubscript{2})</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>MK-10</td>
<td>15</td>
<td>37–72 (67)</td>
</tr>
<tr>
<td>MK-11</td>
<td>0</td>
<td>tr*–13 (4)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>41.4</td>
<td>Approx. 44–48 (47.1)</td>
</tr>
</tbody>
</table>

*tr, Trace amount.

Displays the following characteristics in addition to those listed in the genus description. Cells are approximately 2.0–3.0 \( \mu \text{m} \) in length and 0.5–1.0 \( \mu \text{m} \) in diameter, occurring singly or in pairs. Colonies are yellowish and circular with entire edges, 1.0–2.0 mm in diameter after 3–5 days of incubation at 37 °C. Growth occurs at 20–45 °C (optimum 35–40 °C) and at pH 5.0–8.5 (optimum pH 6.5–7.5). Does not require NaCl for growth, but tolerates up to 2 % NaCl. Yeast extract is required for growth. Catalase-negative. Cellobiose, glucose, lactose, mannose, rhamnose, sorbitol, rhamnose, sucrose and xylose are used as electron donors, but not arabinose, glycerol, mannitol, Casamino acids, acetate, lactate, pyruvate, H\textsubscript{2}/CO\textsubscript{2} or H\textsubscript{2}/CO\textsubscript{2} in the presence of acetate. Sodium sulfate, sodium thiosulfate, elemental sulfur, sodium sulfite, sodium nitrate and sodium nitrite are not used as terminal electron acceptors. The main fermentation products from glucose metabolism are lactate, acetate, butyrate and isobutyrate. In the API 20A system, the following reactions are positive: hydrolysis of gelatin and aesculin and production of acid from glycerol, glucose, mannitol, lactose, sucrose, maltose, xylose, arabinose, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose and trehalose. The following reactions in the API 20A system are negative: urease activity and production of acid from salicin. In addition to the major fatty acids and respiratory quinones listed in the genus description, significant amounts (>5 %) of iso-C\textsubscript{17:0} 3-OH, iso-C\textsubscript{15:0}, a summed feature consisting of C\textsubscript{18:0} 7c and/or anteiso C\textsubscript{17:1} a, and MK-10 are also present. In addition to the major polar lipids listed in the genus description, one unidentified glycolipid and several unidentified phospholipids are present. Susceptible to ampicillin (10 \( \mu \text{g ml}^{-1} \)) and vancomycin (10 \( \mu \text{g ml}^{-1} \)) but resistant to kanamycin (400 \( \mu \text{g ml}^{-1} \)).

The type strain, LIND7H\textsuperscript{T} (=CCUG 60892\textsuperscript{T}=DSM 23697\textsuperscript{T}=JCM 16313\textsuperscript{T}), was isolated from an upflow anaerobic filter treating abattoir wastewaters in Tunisia. The DNA G+C content of the type strain is 41.4 mol%.

Acknowledgements

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References


