**Oribacterium sinus** gen. nov., sp. nov., within the family ‘Lachnospiraceae’ (phylum Firmicutes)

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A hitherto unknown anaerobic bacillus isolated from sinus pus in a young child (strain AIP 354.02T) was characterized by using phenotypic and genotypic methods. 16S rRNA gene sequence analysis indicated that this strain was phylogenetically affiliated with several sequences of cloned 16S rRNA gene inserts previously deposited in the public databases. According to their 16S rRNA gene sequence similarities, these uncultivated bacteria, together with strain AIP 354.02T, formed a separate subgroup belonging to the family ‘Lachnospiraceae’ within the phylum Firmicutes. *Oribacterium* gen. nov. is proposed for this group of organisms and *Oribacterium sinus* gen. nov. sp. nov. for strain AIP 354.02T (=CIP 107991T = CCUG 48084T).

**Culture-independent molecular methods based on 16S rRNA gene cloning and sequence analysis have revealed a vast diversity of micro-organisms in many natural environments (Munson et al., 2002; Paster et al., 2001; Rolph et al., 2001). These recent advances in molecular techniques have permitted new insight into bacterial evolutionary relationships. However, an absence of information regarding the morphology and biochemical properties of unseen bacterial species, phylotypes (Paster et al., 2001), is frustrating for microbiologists. Therefore, isolation and characterization of one of these phylotypes provides an opportunity to describe a novel species.**

In this study we describe the phenotypic characteristics of a strain that was phylogenetically affiliated with several oral clones recently described (Munson et al., 2002; Paster et al., 2001). According to their 16S rRNA gene sequence similarities, these hitherto uncultivated bacteria, together with strain AIP 354.02T, belong to the family ‘Lachnospiraceae’ within the phylum Firmicutes. *Oribacterium* gen. nov. is proposed for this group of organisms and *Oribacterium sinus* gen. nov. sp. nov. for strain AIP 354.02T.

**Bacterial strain and microbiological investigation**

Strain AIP 354.02T was isolated from maxillary sinus pus obtained from a 6-year-old child presenting with a bilateral maxillary sinusitis and hospitalized in a paediatric unit of the University Hospital of Nancy in June 2002. This patient had a history of recurrent infections of the respiratory tract related to a primary humoral immunodeficiency. The pus was streaked on a Wilkins–Chalgren (WC) agar plate (Oxoid) and incubated for 4 days in an anaerobic chamber (Don Whitley Scientific Ltd). A mixed aerobic–anaerobic flora including *Staphylococcus epidermidis*, non-haemolytic streptococcus, *Propionibacterium acnes* and an unidentified rod was recovered. The strain was maintained in trypticase/glucose/yeast extract (TGY) medium consisting of: 3 % (w/v) biotryptace (bioMérieux), 0.5 % glucose (Prolabo), 2 % yeast extract (Difco), 0.05 % L-cysteine hydrochloride (Prolabo) and 5 μg haemin ml−1 (Calbiochem) under anaerobic conditions at 37 °C for 24 h in an anaerobic jar containing 95 % H2 and 5 % CO2 (v/v). Colony morphology and presumptive identification tests (Engelkirk et al., 1992) were observed on WC agar plates.

**Electron microscopy**

For negative staining, bacteria were washed in 0.1 M Tris/HCl buffer (pH 7.4) (Merck), placed on to 200-mesh copper grids, fixed with 2 % glutaraldehyde (Sigma) in 0.1 M Tris/HCl buffer and stained with 2 % uranyl acetate.
For electron microscopy of ultrathin sections, bacteria were fixed with 2.5% glutaraldehyde (Sigma) in 0.1 M cacodylate buffer (pH 7.2), post-fixed with a mixture of 1% osmium tetroxide and treated for 1 h at room temperature with 2% uranyl acetate in Michaelis buffer (pH 6.0). Samples were then dehydrated in increasing concentrations of ethanol and embedded in epoxy resin. Ultrathin sections (70–80 nm) were cut on a Leica Ultracut UCT microtome using a diamond knife, collected on copper grids and stained with 2% uranyl acetate and lead citrate. Samples were examined in a JEOL 1010 transmission electron microscope operating at 80 kV.

**Colony and cell morphology**

Colonies appeared on WC blood agar after 2 days incubation. They were circular, convex, about 1–1.5 mm in diameter, non-pigmented and non-haemolytic. Cells were elongated ovoid rods, about 1.7–2.2 μm long and 0.8–1 μm wide, usually single, in pairs or occasionally in short chains. Bacteria were highly motile with laterally inserted flagella (Fig. 1a), Gram-negative after staining but structurally Gram-positive (Fig. 1b). Spore formation was never observed.

**Biochemical characteristics**

Biochemical reactions were assessed according to the procedures described by Holdeman et al. (1977). Metabolic end products were assayed by quantitative GC as described by Carlier (1985). A Rapid ID 32A kit (API bioMérieux) was used for enzymic profile determination as recommended by the manufacturer.

Strain AIP 354.02T was a strictly anaerobic organism. Abundant gas was produced in glucose broth cultures. Catalase activity and nitrate reduction were not detected. Gelatin was not liquefied and milk was not modified. Indole was produced. Acid was produced from glucose, galactose, raffinose and sucrose, but not from aesculin, arabinose, cellobiose, fructose, glycerol, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, trehalose or xylene. Aesculin was not hydrolysed. The major metabolic end products were acetic acid (9.3 mmol l⁻¹) and lactic acid (28.6 mmol l⁻¹). By presumptive identification tests, the strain was resistant to 1 mg kanamycin and 10 μg colistin discs, but susceptible to 5 μg vancomycin, 4 μg metronidazole and bile discs. The enzymic activity of this bacterium gave a positive reaction for α-galactosidase, β-galactosidase, α-glucosidase, indole, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, leucine arylamidase, alanine arylamidase, glycine arylamidase and serine arylamidase (API code 4500 2726 04). Table 1 provides the primary characteristics that can be used to differentiate *Oribacterium sinus* from its closest relatives.

**Chemotaxonomic analyses**

The G+C content of the DNA of strain AIP 354.02T was 42.4 mol%, as determined by HPLC at the Identification Service of the DSMZ. Cellular fatty acid composition was analysed by GC according to Veys et al. (1989). Briefly, strains were grown anaerobically in 10 ml TGYH for 48–72 h and methyl esters were chromatographed on a fused-silica capillary column (25 m x 0.25 mm i.d.) coated with a 5% methyl phenyl silicone. Analysis of fatty acid methyl esters showed that strain AIP 354.02T contained C14:0 (22%), anteiso-C15:0 (14%), C15:0 (7.4%), C16:1ω9c (9%) and C16:0 (34%) as the major fatty acids, minor amounts of anteiso-C17:0 (4.3%) and C18:1ω9t (3.6%), and trace amounts of C12:0 (0.7%), C13:0 (0.5%), iso-C14:0 (0.5%), C17:0 (0.7%), C18:1ω9c (0.6%) and C18:0 (1.8%).

**16S rRNA gene sequencing and phylogenetic analysis**

The 16S rRNA gene was selectively amplified from genomic DNA by PCR using 5′-AGAGTTTGATCATGGCTCAG-3′ (8–27f in the *Escherichia coli* numbering system) as the
forward primer and 5′-TACGGCTACCTTGTTACGACTT-3′ (1492–1513r E. coli numbering) as the reverse primer. PCR was carried out in 50 μl reaction mixture containing approximately 0.5 μg DNA template, 200 μM each primer, 200 μM each dNTPs and 1 U Taq polymerase (Roche) in the appropriate reaction buffer. Temperature cycling was performed using 30 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C. The 1.5 kb PCR product was directly sequenced on an Applied Biosystems Automatic Sequencer (Genome Express) in both directions by using forward and reverse primers. A partial sequence of the 16S rRNA genes of strain AIP 354.02T was determined. This almost complete (1374 nt) 16S rRNA gene sequence was compared with all eubacterial 16S rRNA gene sequences available in the GenBank database using the multisequence Advanced BLAST comparison software from the National Center for Biotechnology Information (Altschul et al., 1997). Alignment was via CLUSTAL W (Thompson et al., 1994). The phylogenetic tree shown in Fig. 2 was constructed from 1374 positions by applying the correction of Jukes & Cantor.

Table 1. Characteristics that differentiate Oribacterium sinus gen. nov., sp. nov. from other closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Oribacterium sinus</th>
<th>Lachnospira multipara</th>
<th>Lachnospira pectinoschiza</th>
<th>Butyrivibrio crosstus</th>
<th>Butyrivibrio fibrisolvens</th>
<th>Clostridium clostridioforme</th>
<th>Shuttleworthia satelles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Ovoid</td>
<td>Curved</td>
<td>Straight</td>
<td>Curved</td>
<td>Curved</td>
<td>Straight</td>
<td>Slightly curved</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Spore formation</td>
<td>–</td>
<td>–</td>
<td>Spore-like structures</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>V</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>42.4</td>
<td>ND</td>
<td>51.4</td>
<td>36–37</td>
<td>41</td>
<td>47–49</td>
<td>51</td>
</tr>
<tr>
<td>Habitat</td>
<td>Human oral cavity</td>
<td>Rumen of ruminants</td>
<td>Pig intestine</td>
<td>Human faeces</td>
<td>Rumen of ruminants; human, rabbit, horse faeces</td>
<td>Isolated from humans and animals</td>
<td>Human oral cavity</td>
</tr>
</tbody>
</table>

*F, Formate; A, acetate; B, butyrate; L, lactate. Products in parentheses are produced variably. Capital letters indicate major products.

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1374 aligned bases indicating the relationships between clinical isolate AIP 354.02T and related species within the phylum Firmicutes. The sequence of Propionibacterium acnes was used as an outgroup. Numbers on the tree designate bootstrap values. Bar, 5% sequence divergence. Accession numbers are given in parentheses.
(1969) and neighbour-joining methods (Felsenstein, 1993) with 1000 bootstrap resamplings.

Comparative sequence analysis revealed that the DNA sequence of strain AIP 354.02T was closely related to that of a Eubacterium sp. oral clone JN088 (98.5% sequence similarity). These two sequences grouped together and then grouped with an oral strain (FTB 41) described by Paster et al. (2001) as Firmicutes sp. (98% sequence similarity) and an oral clone MCE 10.236 described by Munson et al. (2002) as a Lachnospiraceae oral clone. Following this, this subgroup branched together with two other Lachnospiraceae oral clones, MCE 7.60 and MCE 9.31 (Munson et al., 2002). These 16S rRNA gene sequences formed a separate group within the phylum Firmicutes (similarities ranging from 89 to 99%) (Fig. 2). On the basis of a sequence divergence of 3% as cut-off, strain AIP 354.02T together with the oral clones JN088 and MCE 10.236 and strain FTB 41 can be considered to represent the same genus, for which we propose the name Oribacterium gen. nov. Strain AIP 354.02T represents a novel species, for which we propose the name Oribacterium sinus sp. nov. This is the only species characterized within this genus and serves as the type species. The other two phylogenotypes, MCE 7.60, MCE 9.31, which are more distantly related to each other (sequence similarity of 89–92%), could represent other species within the genus Oribacterium.

Description of Oribacterium gen. nov.

Oribacterium (O.ri.bac.te’ri.um). L. gen. n. oris of the mouth; N.L. neut. n. bacterium from Gr. n. bakterion a small rod; N.L. neut. n. Oribacterium small rod from the mouth, because all clone sequences originated from the mouth).

Elongated ovoid rods, about 1.7–2.2 μm long and 0.8–1 μm wide, usually single, in pairs or, occasionally, in short chains. Motile with laterally inserted flagella. Gram-positive but may appear Gram-negative after staining. Strictly anaerobic. Do not form spores. Weakly fermentative. Major metabolic end products are acetic and lactic acid. Minor fatty acids are C14:0 anteiso-C15:0 C15:0 C16:0 9c and C16:0 present in substantial amounts. Smaller amounts of anteiso-C17:0 and C18:1ω9t are also detected. DNA G+C content is 42.4 mol%. Phylogenetically related to members of the family ‘Lachnospiraceae’. The type species is Oribacterium sinus.

Description of Oribacterium sinus sp. nov.

Oribacterium sinus (sin’us. L. gen. n. sinus of the sinus, referring to the anatomical site from where the type strain was isolated).

Cell morphology is as described for the genus. Colonies circular, convex, 1–1.5 mm in diameter, non-pigmented and non-haemolytic. Strictly anaerobic, Gram-positive but the cells decolour easily. Motile by two to four lateral flagella. Abundant gas is produced in TGY deep agar cultures. Indole is produced; catalase and nitrate reduction are negative. Gelatin is not liquefied and milk is not modified. Acid is produced from glucose, galactose, raffinose and sucrose. Acid is not produced from aesculin, arabinose, cellobiose, fructose, glycerol, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, trehalose or xylose. Aesculin is not hydrolysed. The metabolic end products are acetate and lactic acids. The type strain is susceptible to penicillin G, ampicillin, amoxicillin, ticarcillin, mezlocillin, imipenem, cefalotin, cefotaxime, latamoxef, chloramphenicol, clindamycin and rifampicin, moderately resistant to tetracycline and resistant to erythromycin and trimethoprim sulfamethoxazole. The G+C content is 42.4 mol%. Habitat: mouth, upper respiratory tract.

The type strain is strain AIP 354.02T (= CIP 107991T = CCUG 48084T).

References


Oribacterium sinus gen. nov., sp. nov.


