

**Prevotella aurantiaca** sp. nov., isolated from the human oral cavity

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Two anaerobic, pigmented, non-spore-forming, Gram-stain-negative, rod-shaped strains isolated from the human oral cavity, OMA31T and OMA130, were characterized by determining their phenotypic and biochemical features, cellular fatty acid profiles and phylogenetic positions based on 16S rRNA gene sequence analysis. 16S rRNA gene sequence analysis showed that the new isolates belonged to a single species of the genus *Prevotella*. The two isolates showed 100 % 16S rRNA gene sequence similarity with each other and were most closely related to *Prevotella intermedia* ATCC 25611T with 96.4 % 16S rRNA gene sequence similarity; the next most closely related strains to the isolates were *Prevotella pallens* AHN 10371T (96.1 %) and *Prevotella falsenii* JCM 15124T (95.3 %). Phenotypic and biochemical characteristics of the isolates were the same as those of *P. intermedia* JCM 12248T, *P. falsenii* JCM 15124T and *Prevotella nigrescens* JCM 12250T. The isolates could be differentiated from *P. pallens* JCM 11140T by mannose fermentation and α-fucosidase activity. Conventional biochemical tests were unable to differentiate the new isolates from *P. intermedia*, *P. falsenii* and *P. nigrescens*. However, *hsp60* gene sequence analysis suggested that strain OMA31T was not a representative of *P. intermedia*, *P. pallens*, *P. falsenii* or *P. nigrescens*. Based on these data, a novel species of the genus *Prevotella, Prevotella aurantiaca* sp. nov., is proposed, with OMA31T (=JCM 15754T =CCUG 57723T) as the type strain.

During studies on the human oral microbiota, pigmented *Prevotella*-like strains were isolated from a periodontal pocket of a patient with periodontitis. Phenotypically, these isolates were most closely related to *Prevotella intermedia*, *Prevotella nigrescens* and a novel black-pigmented *Prevotella* species, *Prevotella falsenii* (Sakamoto et al., 2009). In this study, preliminary analysis of the *hsp60* gene sequence was carried out in addition to 16S rRNA gene sequence analysis. The present study was designed to determine the taxonomic status of the new isolates. Based on the results presented here, it is proposed that these strains should be classified as representatives of a novel species of the genus *Prevotella*.

The strains used in the present study were maintained on Eggerth–Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood for 2 days at 37 °C in an atmosphere containing 100 % CO₂. Strains OMA31T and OMA130 were isolated from a periodontal pocket of a patient with periodontitis. Growth of the isolates on Bacteroides bile esculin agar (Shah, 1992) was also assessed.

Physiological reactions were determined in duplicate with an API 20A anaerobe test kit as recommended by the manufacturer (bioMérieux). Fatty acid methyl esters were obtained from about 40 mg wet cells by saponification, methylation and extraction using minor modifications (Kuykendall et al., 1988) of the method of Miller (1982). Cellular fatty acid profiles were determined by the MIDI microbial identification system (Microbial ID). Biochemical reactions were determined in duplicate with the Rapid ID 32A anaerobe identification kit as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated according to previously described methods (Marmur, 1961; Saito & Miura, 1963), with some modifications. The DNA G+C content was determined by the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M NH₄H₂PO₄ and acetonitrile (20:1, v/v). The 16S rRNA gene was analysed as described previously (Sakamoto et al., 2002). The partial *hsp60* gene was amplified by PCR using primers H729 (5'-
CGCAGGGTTTTCGGTACGACGACGAIICGICGGIGA-
YGGIACIACIACI3’ and H730 (5’-AGGGATAACAA-
TTACACACAGGATK1YK1TC1CCRAAICCGGIGGCTT-3’)
(Broussseau et al., 2001); inosine was used to reduce the
degeneracy of the sequences (Ohtsuka et al., 1985). These
primers were derived from the previously described H279A
and H280A primers (Goh et al., 1997) by the addition of
sequences for commercially available M13 24 bp sequencing
primers (underlined nucleotides). Primers were designed to
amplify the ‘universal target’ (UT) (Hill sequences for commercially available M13 24 bp sequencing
primers were derived from the previously described H279A
primers were derived from the previously described H279A
formed in a total volume of 50 μl containing 2.5 μl DNA
(50 ng), 1.25 U TaKaRa Ex Taq (Takara Bio), 5 μl 10 × Ex
Taq buffer, 4 μl dNTP mixture (2.5 mM each) and 5 pmol of
each primer. The hsp60 genes were amplified in a Biometra
Thermocycler TGradient using the following programme:
94 °C for 5 min, followed by 40 cycles consisting of 94 °C for
30 s, 50 °C for 30 s and 72 °C for 45 s, with a final extension
period at 72 °C for 10 min. Sequencing of purified PCR
products was performed with standard M13 sequencing
primers as indicated on the primers above. Related sequences
were aligned with the CLUSTAL_X 2.0 program (Larkin
et al., 2007) and corrected by manual inspection. Nucleotide
substitution rates (K values) were calculated (Kimura,
1980) after gaps and unknown bases were eliminated. The
phylogenetic tree was constructed by the neighbour-joining
method (Saitou & Nei, 1987). Bootstrap resampling analysis
(Felsenstein, 1985) was performed to estimate the confidence
of tree topologies.

Strains OMA31 T and OMA130 were obligately anaerobic,
pigmented, non-spore-forming, non-motile, Gram-stain-
negative rods or coccobacilli. Cells on EG agar were 0.5–
0.8 × 1.6 μm or 0.8 × 0.9–1.0 μm and occurred singly.
Colonies on EG agar plates after 48 h incubation at
37 °C under anaerobic conditions were 1–2 mm in
diameter, light to dark brown, circular, entire, slightly
convex and smooth. Colonies exhibited orange fluo-
scence under long-wave UV light (365 nm); colonies of P.
intermedia JCM 12248 T, P. falsenii JCM 15124 T, P.
nigrescens JCM 12250 T and Prevotella pallens JCM 11140 T
exhibited red fluorescence as described previously
(Könönen et al., 1998; Slots & Reynolds, 1982; Shah &
Gharbia, 1992). On Bacteroides bile aesculin agar, growth
of the new isolates was inhibited and aesculin hydrolysis
was negative. Results of phenotypic and biochemical
characteristics are given in the species description. The
phenotypic and biochemical characteristics of the two new
isolates were the same as those of P. intermedia JCM
12248 T, P. falsenii JCM 15124 T and P. nigrescens JCM
12250 T. However, the new isolates could be differentiated from P. pallens JCM 11140 T by mannose fermentation and
α-fucosidase activity.

The cellular fatty acid composition of species of the genus
Prevotella has been determined previously (Sakamoto et al.,
2004, 2005a, b, 2007, 2009). In this study, the cellular fatty
acid compositions of the two new isolates were similar to
those of P. falsenii (n=8), P. intermedia JCM 12248 T, P.
nigrescens JCM 12250 T and P. pallens JCM 11140 T (Table
1). However, the amounts of anteiso-C15:0 present in the
novel isolates were slightly higher than those in P.
intermedia JCM 12248 T.

The DNA G+C contents of strains OMA31 T and OMA130
were 39.7 and 41.0 mol%, respectively. These values are
slightly lower than that of P. intermedia JCM 12248 T
(43.1 mol%).

Approximately 1500 bases of the 16S rRNA gene sequence
were determined for each of the novel isolates. For
phylogenetic analysis, 1359 bp (positions 56–1391; E. coli
numbering system) sequences of each species were used.
16S rRNA gene sequence analysis showed that the isolates
were representatives of a single species of the genus
Prevotella (Fig. 1 and Supplementary Fig. S1 available in
IJSEM Online). These strains shared 100 % 16S rRNA gene
sequence similarity with each other and were most closely
related to P. intermedia ATCC 25611 T with 96.4 % 16S
rRNA gene sequence similarity; the next most closely
related strains were P. pallens AHN 10371 T (96.1 %) and
P. falsenii JCM 15124 T (95.3 %). According to Stackebrandt &
Goebel (1994), strains showing 16S rRNA sequence
similarity values of less than 97 % will not show DNA–
DNA reassociation values of more than 60 % and will thus
represent different species. In addition, hsp60 gene
sequence analysis suggested that strain OMA31 T was
distinct from P. intermedia, P. pallens, P. falsenii and
P. nigrescens; hsp60 gene sequence similarities of strain
OMA31 T with the type strains of P. intermedia, P. falsenii,
P. pallens and P. nigrescens were 87.1, 85.3, 85.1 and 84.2 %,
respectively.

Based on the above-mentioned findings, a novel species of
the genus Prevotella, Prevotella aurantiaca sp. nov., is
proposed to accommodate the new isolates. Conventional
biochemical tests were unable to differentiate P. aurantiaca
sp. nov. from P. intermedia, P. falsenii and P. nigrescens. 16S
rRNA and hsp60 gene sequence analyses are recommended
for unequivocal identification of these four species.

Description of Prevotella aurantiaca sp. nov.

Prevotella aurantiaca (au.ran.ti.a’ca. N.L. fem. adj. aur-
antiaca orange-coloured).

Cells are obligately anaerobic, pigmented, non-spore-
forming, non-motile, Gram-stain-negative rods or cocco-
bacilli (0.5–0.8 × 1.6 μm or 0.8 × 0.9–1.0 μm). Colonies on
EG agar plates after 48 h incubation at 37 °C under
anaerobic conditions are 1–2 mm in diameter, light to
dark brown, circular, entire, slightly convex and smooth.
Colonies exhibit orange fluorescence under long-wave
UV light (365 nm). Their growth is inhibited in the
presence of 20 % (w/v) bile. Acid is produced from glucose,
maltose, D-mannose, raffinose and sucrose, but not from

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**Table 1.** Cellular fatty acid contents (%) of *Prevotella aurantiaca* sp. nov. and related species

Taxa: 1, *P. aurantiaca* sp. nov. OMA31<sup>T</sup>; 2, *P. aurantiaca* sp. nov. OMA130; 3, *P. falsenii* (*n* = 8); 4, *P. intermedia* JCM 12248<sup>T</sup>; 5, *P. nigrescens* JCM 12250<sup>T</sup>; 6, *P. pallens* JCM 11140<sup>T</sup>; tr, Trace amount (<0.5%); –, not detected. Values are percentages of total fatty acids.

<table>
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<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
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<td>tr</td>
<td>0.6 ± 0.1</td>
<td>0.6</td>
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<td>1.1</td>
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<td>C&lt;sub&gt;15:0&lt;/sub&gt;</td>
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<td>–</td>
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<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>3.9</td>
<td>3.6</td>
<td>6.2 ± 0.9</td>
<td>5.1</td>
<td>4.1</td>
<td>5.1</td>
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<tr>
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<td>0.6</td>
<td>1.4 ± 0.4</td>
<td>1.1</td>
<td>0.5</td>
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<td>–</td>
<td>1.6 ± 0.3</td>
<td>–</td>
<td>1.0</td>
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<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt; c09c</td>
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<td>5.2 ± 0.7</td>
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<td>1.4</td>
<td>1.6</td>
<td>1.1 ± 0.3</td>
<td>1.0</td>
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<tr>
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<td>1.6</td>
<td>1.2 ± 0.5</td>
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<td>17.6</td>
<td>6.3 ± 2.7</td>
<td>16.1</td>
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<td>1.1 ± 0.1</td>
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<td>3.0 ± 1.1</td>
<td>6.2</td>
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<td>2.2 ± 0.5</td>
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<td>6.1</td>
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<td>12.5 ± 4.7</td>
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<td>10</td>
<td>1.3</td>
<td>1.6</td>
<td>2.1 ± 0.3</td>
<td>1.7</td>
<td>1.3</td>
<td>2.2</td>
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</table>

*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 contains one or more of an unknown fatty acid of ECL 13.570 and/or iso-C<sub>15:0</sub> ALDE. Summed feature 10 contains one or more of an unknown fatty acid of ECL 17.834 and/or C<sub>18:1</sub>c9/c11t fatty acid methyl ester.

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**Fig. 1.** Phylogenetic tree showing relationships between *Prevotella aurantiaca* sp. nov. and related species. The tree was constructed by the neighbour-joining method based on 16S rRNA gene sequences. Numbers at nodes indicate the bootstrap values (%) of 1000 replicates. 16S rRNA gene sequence accession numbers are given in parentheses for each strain. Bar, 0.02 substitutions per nucleotide position.
l-arabinose, cellobiose, glycerol, lactose, d-mannitol, melezitose, L-rhamnose, salicin, D-sorbitol, trehalose or d-xylene. Aesculin is not hydrolysed. Indole is produced. Gelatin is digested. Catalase and urease are not produced. Positive reactions are obtained using the Rapid ID 32A system for χ-glucosidase, χ-fucosidase, indole production, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Mannose and raffinose are fermented. All other tests performed (urease, arginine dihydrolase, χ-galactosidase, β-galactosidase, 6-phospho-β-galactosidase, β-glucosidase, χ-arabinosidase, β-glucuronidase, β-N-acetylglucosaminidase, glucosamine decarboxylase, nitrate reduction, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyrogallolglucuronic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase) are negative. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The major cellular fatty acids are anteiso-C15:0, iso-C17:0 3-OH and C19:1ω9c.

The type strain is OMA31T (=JCM 15754T=CCUG 57723T), isolated from a periodontal pocket of a patient with periodontitis. The DNA G+C content of the type strain is 39.7 mol%. An additional strain, OMA130 (=JCM 15755), is also included in this species.

Acknowledgements

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References


