Rarimicrobium hominis gen. nov., sp. nov., representing the fifth genus in the phylum Synergistetes that includes human clinical isolates

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Five human clinical isolates of an unknown, strictly anaerobic, slow-growing, Gram-stain-negative, rod-shaped microorganism were subjected to a polyphasic taxonomic study. Comparative 16S rRNA gene sequence-based phylogeny showed that the isolates grouped in a clade that included members of the genera Pyramidobacter, Jonquetella, and Dethiosulfovibrio; the type strain of Pyramidobacter piscolens was the closest relative with 91.5–91.7 % 16S rRNA gene sequence similarity. The novel strains were mainly asaccharolytic and unreactive in most conventional biochemical tests. Major metabolic end products in trypticase/glucose/yeast extract broth were acetic acid and propionic acid and the major cellular fatty acids were C13:0 and C16:0, each of which could be used to differentiate the strains from P. piscolens. The DNA G+C content based on whole genome sequencing for the reference strain 22-5-S 12D6FAA was 57 mol%. Based on these data, a new genus, Rarimicrobium gen. nov., is proposed with one novel species, Rarimicrobium hominis sp. nov., named after the exclusive and rare finding of the taxon in human samples. Rarimicrobium is the fifth genus of the 14 currently characterized in the phylum Synergistetes and the third one in subdivision B that includes human isolates. The type strain of Rarimicrobium hominis is ADV70T (=LMG 28163T=CCUG 65426T).

The phylum Synergistetes was delineated by Jumas-Bilak et al. (2009) and, at the time of writing, encompasses 14 genera of strictly anaerobic, non-spore-forming, Gram-stain-negative rods or vibrios. In addition and despite not yet validated recognized, the species Acetomicrobium flavidum has to be reclassified as belonging to the phylum Synergistetes (Soutschek et al., 1984). Finally, Candidatus Tammella caduceiae, related to the genus Thermanaerovibrio in the phylum Synergistetes, has also been proposed for an ecotrophic, flagellated rod partially embedded in the flagellate Caducea versatilis, in the gut of the termite Cryptotermes cavifrons (Hongoh et al., 2007).

For a significant period of time, the phylum Synergistetes has been known to include environmental isolates and...
clones. Three main types of environmental habitats have been reported for members of this phylum, i.e. sludge and wastewater from anaerobic digesters, natural springs, seawater and sulfur mats, and water related to petroleum and gas production facilities. Host-associated isolates and clones have also been reported in a diverse range of animals, from insects to humans (Jumas-Bilak & Marchandin, 2014). However, Synergistetes from human origin remain rarely reported in the literature or databases despite the huge expansion of culture-independent studies of human microbiota (Marchandin et al., 2010). The names of four genera including strains of human origin have been validly published: Jonquettella, Cloacibacillus, Pyramidobacter (Jumas-Bilak et al., 2007; Ganesan et al., 2008; Downes et al., 2009; Looft et al., 2013) and Fretibacterium, accommodating previously uncultivated clones from human subgingival plaque during periodontitis (Vartoukian et al., 2009, 2010, 2013). A few human isolates corresponding to undescribed taxa in the phylum have also been reported (Horz et al., 2006; Marchandin et al., 2010). Similarly, cloned sequences representing yet-to-be cultivable microorganisms from human origin remain rare. Altogether, and as previously reported, members of the phylum Synergistetes appear to be widely distributed but rare in diverse microbial ecosystems (Godon et al., 2005).

The phylum Synergistetes was subdivided into five main robust lineages named A–E and subdivision A was described as the class Synergista while the other subdivisions had no standing in the literature regarding their phylogenetic level and nomenclature (Jumas-Bilak et al., 2009). Characterized genera including human isolates are distributed in three of these five divisions: the genus Cloacibacillus is a member of the class Synergista; Jonquettella and Pyramidobacter group in subdivision B; and Fretibacterium belongs to subdivision E (Jumas-Bilak et al., 2009). At lower phylogenetic levels, environmental and mammalian strains and clones formed independent lineages according to the origin of the sequences and human-associated clusters of sequences have been demonstrated (Marchandin et al., 2010).

In the present study, five hitherto unknown strains of strictly anaerobic, Gram-stain-negative rods belonging to subdivision B of the phylum Synergistetes were characterized. Four of these strains were previously identified as forming a new taxon in the phylum (new taxon 1 in Marchandin et al., 2010) showing 16S rRNA gene sequence similarity of less than 92% with the most closely related species, Pyramidobacter piscicola (Yarza et al., 2014). The results of the polyphasic taxonomic approach conducted herein on these four human clinical isolates and a fifth isolate recovered from human faeces in Canada supported the fact that the five isolates studied represent a novel species of a new genus and the fifth genus that includes human isolates in the phylum Synergistetes.

The four strains ADV70T, ADV403, ADV787 and ADV897 were isolated from gynaecological surgery material, parietal abscess, sacrum and perianal abscess specimens, respectively, in four patients hospitalized at the University Hospital of Montpellier, France, between February 2003 and July 2007 (age range: 38–93 years old) (Marchandin et al., 2010). Strain 22-5-S 12D6FAA was recovered from human faeces from a healthy adult male donor in Canada. All isolates were part of mixed aero-anaerobic polymicrobial cultures.

Tiny, non-haemolytic colonies were observed on Columbia sheep blood agar plates after 3–5 days of incubation at 37 °C in an anaerobic atmosphere using AnaeroGen paper sachets (Oxoid, Unipath). After prolonged incubation (1–2 weeks), punctiform colonies were still observed. A disagreeable odour of tainted seafood was noted. No growth was obtained in microaerophilic conditions using Campygen Compact (Oxoid).

Gram staining showed small, Gram-stain-negative, rod-shaped, irregular bacilli with straight and stocky, round-tipped forms. Spores were not observed and cells were not motile. For electron microscopy, cells were stained with 0.5% uranyl acetate, loaded on carbon-coated grids, and viewed with a Philips CM10 electron microscope. Cell sizes for straight and stocky forms were 2–2.5 µm × 1 µm and 1.5 µm × 1 µm, respectively (Fig. 1).

Catalase and cytochrome oxidase activities were not detected. Susceptibility to special-potency discs (Jousimies-Somer et al., 2002) showed the five isolates to be resistant to vancomycin (5 µg) but susceptible to kanamycin (1 mg), metronidazole (4 µg) and bile (1 mg); variable susceptibility was noted for colistin (10 µg), strains ADV70T and 22-5-S 12D6FAA being susceptible to this antibiotic (Table S1, available in the online Supplementary Material).

For further phenotypic characterization, the strains were maintained anaerobically in trypticase/glucose/yeast extract (TGY) broth under anaerobic conditions at 37 °C, as described previously (Carlier et al., 2004). Biochemical reactions were performed according to the procedures of the VPI Anaerobe Laboratory Manual (Holdeman et al., 1977) by using tryptcase/yeast extract (TY) medium supplemented with 1% (w/v) of each sterilized substrate. Gas production was not detected. The strains were unreactive in most conventional biochemical tests. Urease and gelatinase activities were not detected. Indole was not produced and aesculin was not hydrolysed. Milk was not modified. Nitrate and nitrite were not reduced. None of the isolates contained desulfoviridin. Formate and fumarate were not utilized. The isolates were asaccharolytic, acid not being produced from arabinose, cellobiose, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, trehalose or xylose. Aesculin was weakly fermented by strain ADV70T. TGY broth supplemented with 1% Casamino acids increased growth slightly.

Enzyme profiles determined using the Rapid ID 32A kit (API bioMérieux) as recommended by the manufacturer showed that the five isolates shared positive glycine
arylamidase activity and, depending on the strain, leucyl-glycine arylamidase, alanine and/or proline arylamidase activities (Table 1 and Table S1).

Metabolic end products were assayed by quantitative GC as described by Carlier (1985). Acetic and propionic acids were detected for all strains, and inconsistent production of 3-phenylpropionic acid was noted, depending on the strain (Table 1 and Table S1).

Cellular fatty acid content was analysed for strains ADV70T and ADV403 by GC according to Veys et al. (1989) and as

![Fig. 1. Negative-stain electron microscopy of cells of strain 22-5-S 12D6FAA. Bars, 5 μm (a), 2 μm (b), 1 μm (c).](image)

**Table 1.** Characteristics differentiating *Rarimicrobium hominis* gen. nov., sp. nov. from closely related genera *Jonquetella* and *Pyramidobacter*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell shape</strong></td>
<td>Irregular rods (straight and stocky forms)</td>
<td>Straight rods</td>
<td>Straight rods</td>
</tr>
<tr>
<td><strong>Cell size (μm)</strong></td>
<td>2–2.5 × 1 (straight forms), 1.5 × 1 (stocky forms)</td>
<td>0.8–0.9 × 1.4–1.7</td>
<td>0.7–0.8 × 0.8–2.2</td>
</tr>
<tr>
<td><strong>Major cellular fatty acids</strong></td>
<td>C₁₃ : ₀, C₁₆ : ₀</td>
<td>C₁₅ : ₀, C₁₆ : ₀</td>
<td>C₁₃ : ₀, C₁₄ : ₀</td>
</tr>
<tr>
<td><strong>Short fatty acids produced during fermentation</strong></td>
<td>Major: acetic, propionic</td>
<td>Major: acetic</td>
<td>Major: acetic</td>
</tr>
<tr>
<td><strong>Minor: 3-phenylpropionic (some strains)</strong></td>
<td>Minor: propionic, isovaleric, lactic, succinic</td>
<td>Trace amounts: isobutyric, phenylacetic</td>
<td>Minor: isovaleric</td>
</tr>
<tr>
<td><strong>Leucyl glycine arylamidase</strong></td>
<td>V (Table S1)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>16S rRNA gene sequence similarity (%)†</strong></td>
<td>99.7–99.9</td>
<td>88.2–88.4</td>
<td>91.5–91.7</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>57*</td>
<td>59.4</td>
<td>59.0</td>
</tr>
</tbody>
</table>

* Determined from whole genome sequence of strain 22-5-S 12D6FAA.
† Indicated among the five *R. hominis* strains (column 1) and between the five *R. hominis* strains and *J. anthropi* (column 2) or *P. piscolens* (column 3) type strains.
Table 2. Relative fatty acid methyl ester content of strains ADV70T and ADV403 and other Synergistetes subdivision B members

Taxa: 1, strain ADV70T; 2, strain ADV403; 3, P. piscolens (n=2 strains; data from Downes et al., 2009); 4, J. anthropi (n=5; Jumas-Bilak et al., 2007); 5, Dethiosulfovibrio peptidovorans DSM 11002T (Jumas-Bilak et al., 2007). TR, Trace; –, not detected.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3*</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C11:0</td>
<td>1.6</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C12:0</td>
<td>1.0</td>
<td>0.7</td>
<td>2.0–2.5</td>
<td>0.8</td>
<td>–</td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.1–7.8</td>
<td>6.9</td>
</tr>
<tr>
<td>C13:0</td>
<td>32.6</td>
<td>41.1</td>
<td>12.1–13.9</td>
<td>1.5–4.0</td>
<td>0.8</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>–</td>
<td>–</td>
<td>TR–1.2</td>
<td>0–2.3</td>
<td>–</td>
</tr>
<tr>
<td>C14:0</td>
<td>9</td>
<td>6.2</td>
<td>16.5–19</td>
<td>4.9–9.4</td>
<td>3.9</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>–</td>
<td>–</td>
<td>TR–0.9</td>
<td>24.8–42.6</td>
<td>59.7</td>
</tr>
<tr>
<td>antiso-C15:0</td>
<td>1.7</td>
<td>1</td>
<td>TR–1.7</td>
<td>2.9–4.7</td>
<td>–</td>
</tr>
<tr>
<td>C15:0</td>
<td>5.9</td>
<td>9.6</td>
<td>1.4–2.0</td>
<td>6.6–8.8</td>
<td>2.4</td>
</tr>
<tr>
<td>C16:0</td>
<td>23.6</td>
<td>15.3</td>
<td>3.8–4.4</td>
<td>13.6–20.6</td>
<td>8.5</td>
</tr>
<tr>
<td>C16:1</td>
<td>–</td>
<td>–</td>
<td>TR–0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.9–2.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Unknown</td>
<td>9.9</td>
<td>9.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C17:0</td>
<td>2.3</td>
<td>5.1</td>
<td>TR–1.3</td>
<td>4.3–12.6</td>
<td>1.8</td>
</tr>
<tr>
<td>C18:1(9c)</td>
<td>4.3</td>
<td>3.7</td>
<td>TR–0.1</td>
<td>0–1.1</td>
<td>3.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>7.7</td>
<td>6.4</td>
<td>7.1–7.2</td>
<td>9.1–15.2</td>
<td>9.0</td>
</tr>
<tr>
<td>C19:0</td>
<td>–</td>
<td>–</td>
<td>TR</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Eight unidentifiable fatty acid methyl esters were not listed here (Downes et al., 2009) of which none corresponded to the unknown compound found in strains ADV70T and ADV403.

described by Jumas-Bilak et al. (2007). Both strains showed similar relative content of cellular fatty acids with C13:0 and C16:0 being major components, and notable differences from the profiles of phylogenetically related species (Table 2).

The 16S rRNA genes were amplified by PCR as previously described (Carlier et al., 2002). Sequences were aligned using the BioEdit program version 7.1.9 (Hall, 1999) and alignments were manually corrected to exclude gaps, ambiguous and degenerate positions, and ambiguously aligned regions. Similarity tables constructed using utilities implemented in BioEdit revealed that the five strains shared 99.7–99.9 % of their 16S rRNA gene nucleotides and that P. piscolens was the most closely related species to the group formed by the clinical isolates at 91.5–91.7 % sequence similarity (alignment length, 1273 nt) (Table 1). The unique most closely related sequence was that of uncultured clone nbw763a09c1 from human skin microbiome (98.9–99.1 % 16S rRNA gene sequence similarity) (GenBank accession number GQ014695) (Grice et al., 2009). The most closely related cultivated strain was the as yet uncharacterized Synergistes sp. RMA 14551 (GenBank accession number DQ412722) (Horz et al., 2006), which has been previously proposed to represent a new taxon in the phylum Synergistetes (Marchandin et al., 2010) and displayed 93.5–93.7 % 16S rRNA gene sequence similarity with the five isolates studied herein. It is noteworthy that no additional related sequence has appeared in the GenBank database since the deposit of clone nbw763a09c1 from cutaneous origin in May 2009, despite the increasing number of high-throughput metagenomic studies conducted on diverse human microbiota in various physiological and pathophysiological conditions.

The 16S rRNA gene sequences of the five isolates were compared with those of the type strains of the 23 species currently characterized in the phylum Synergistetes. Due to low quality of the sequences, an alignment of 1088 bp was used in phylogenetic analyses to include sequences for all type strains of Synergistetes species while an alignment of 1197 bp could be generated by excluding Aminomonas paucivorans and Aminobacterium mobile sequences. The evolutionary distance was analysed by the neighbour-joining (NJ; Kimura two-parameter substitution model) and maximum-likelihood (ML) (general time-reversible model plus gamma distribution and invariant sites) methods using phylogenetic analyses available at http://www.phylogeny.fr (Dereeper et al., 2008). Bootstrap support was computed after 100 or 1000 reiterations for ML and NJ analysis, respectively. Thermodesulfatator indicus DSM 15286T (GenBank accession number AF393376) was used as the outgroup micro-organism in all phylogenetic analyses. The ML and NJ trees reconstructed from the 1197 bp alignment are shown in Fig. 2 and Fig. S1, respectively. The ML tree reconstructed from the 1088 bp alignment including sequences for all type strains of Synergistetes species is presented in Fig. S2. Phylogenetic analyses gave congruent results showing that the five isolates formed a clade supported by a phylogenetic branch clearly independent from that supporting P. piscolens (Yarza et al., 2014). The topology of the trees confirmed that the strains belong to subdivision B of the phylum Synergistetes together with members of the genera Dethiosulfovibrio, Jonquetella and Pyramidobacter. Human-associated members of this subdivision have been recovered from the digestive and the genital tracts, and in the oral cavity during health and disease (Downes et al., 2009; Marchandin et al., 2010; Belibasakis et al., 2013). The main habitat of the new taxon, despite the few available representatives, appears to be infra-diaphragmatic, the species being found in human specimens from both the gut and the vaginal tracts. The species appears to be associated with humans because no other strain or uncultured clone has yet been described from other origins. In addition, as reported previously, the rarity of the novel species is supported by the scarcity of matching sequences deposited in databases.
Despite the increase in cultivation-independent studies of environmental ecosystems and host microbiota in the past decade.

Based on phenotypic, biochemical, chemotaxonomic and phylogenetic considerations, we suggest the microorganism recovered from human sources be considered as a novel species of a new genus within the phylum Synergistetes, for which the name *Rarimicrobium hominis* gen. nov., sp. nov. is proposed. In addition to the unique 16S rRNA gene sequence, characteristics that are useful in differentiating *R. hominis* from related genera *Jonquetella* and *Pyramidobacter* are shown in Tables 1 and 2.

**Description of Rarimicrobium hominis gen. nov.**

*Rarimicrobium* (Ra.r.i.mi.cro’bi.um. L. adj. rarus rare; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Rarimicrobium* microbe rarely encountered).

Cells are Gram-stain-negative rods. Strictly anaerobic. Slow-growing and non-haemolytic on Columbia blood agar. Non-spore-forming and immobile. Mainly asaccharolytic and unreactive in most conventional biochemical tests. Major cellular fatty acids are C₁₃:₀ and C₁₆:₀. Can be differentiated from other genera in the phylum Synergistetes based on 16S rRNA gene sequence analysis, cellular fatty acid content and metabolic end products.

The type species is *Rarimicrobium hominis*. The DNA G+C content of *R. hominis* reference strain 22-5-S 12D6FAA is 57 mol%.

**Description of Rarimicrobium hominis sp. nov.**

*Rarimicrobium hominis* (ho’mi.nis. L. gen. n. *hominis* of a man, of a human being, named because the type and only species is of human origin).

The description is the same as for the genus, with the following additions. Irregular rods with straight and stocky, round-tipped forms 1.5–2.5 μm × 1 μm in size. Colonies are punctiform on Columbia sheep blood agar even after prolonged incubation. Unpleasant seafood odour. Catalase and cytochrome oxidase activities are not detected. Negative for urease and gelatinase activities, milk modification, and cytochrome oxidase activities are not detected. Negative for ornithine decarboxylase activity.

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http://ijes.microbiologyresearch.org
The type strain is ADV70T (=LMG 28163T=CCUG 65426T), isolated from gynaecological surgery material from a 69-year-old woman in Montpellier, France.

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References


