Acidaminococcus intestini sp. nov., isolated from human clinical samples

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Eleven strains of a hitherto unknown, Gram-negative, anaerobic coccus were recovered from various human clinical samples of patients hospitalized in two geographically distant French hospitals. These strains displayed the morphology and growth characteristics of those related to the genus Acidaminococcus. The clinical isolates shared at least 99.9 and 99.7 % of their nucleotide positions in the 16S and 23S rRNA gene sequences, respectively. They displayed 95.6 and 88.9 % 16S and 23S rRNA gene sequence similarities, respectively, with Acidaminococcus fermentans. The 16S rRNA-based phylogeny revealed that all the clinical isolates grouped in a statistically well supported cluster separate from Acidaminococcus fermentans. Enzymic activity profiles as well as metabolic end product patterns, including propionic acid production, differentiated the novel bacteria from Acidaminococcus fermentans. Finally, phenotypic, genotypic and phylogenetic data, including large-scale chromosome structure and DNA G+C content, supported the proposal of a novel species of the genus Acidaminococcus, for which the name Acidaminococcus intestini sp. nov. is proposed. The type strain is ADV 255.99T (=AIP 283.01T=CIP 108586T=CCUG 50930T).

The genus Acidaminococcus was erected by Rogosa (1969) to group anaerobic, Gram-negative diplococci from the alimentary tract of a pig, previously reported by Fuller (1966). Amino acids, mainly glutamic acid are used as the sole energy source for growth. This genus comprises the type species Acidaminococcus fermentans. Further emendation of the description of the genus Acidaminococcus and its type species was proposed by Cook et al. (1994), which demonstrated that these bacteria can also utilize citrate as an energy source and are able to produce hydrogen and hydrogen sulfide. The genus Acidaminococcus was shown to belong to the family ‘Acidaminococcaceae’, formerly Sporomusa sub-branch, in the phylum Firmicutes (Both et al., 1992; Willems & Collins, 1995; Garrity & Holt, 2001).

The 11 clinical strains studied are presented in Table 1. They were isolated over an 8-year-period from various samples collected from 11 patients hospitalized in two geographically distant French hospitals; the University Hospital of Montpellier, South of France and the University Hospital of Nancy, East of France. All strains were recovered from mixed cultures. Strains were grown at 37 °C on Columbia sheep blood agar for 2–5 days in an anaerobic jar using the Anaerogen System (Oxoid). Among them, the isolate ADV 255.99T was previously analysed for a phylogenetic reconstruction of the family ‘Acidaminococcaceae’ (Marchandin et al., 2003a) and an almost-complete 16S rRNA gene sequence was deposited in the GenBank database under the accession number AF473835. From both phylogenetic analysis and level of sequence similarity with A. fermentans (95.8 %), this strain represents a novel species of the genus Acidaminococcus.

Abbreviations: EM, electron microscopy; ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S and 23S rRNA gene sequences of strain ADV 255.99T are AF473835 and EF060100, respectively.
PFGE migration of I-CeuI-restricted DNAs and EM of cells of strain ADV 255.99T are available as supplementary figures with the online version of this paper.
(Marchandin et al., 2003a). The 11 strains were subjected to polyphasic investigations, to compare with four A. fermentans strains, including A. fermentans type strain CIP 106432T (=DSM 20731T=ATCC 25085T=CCUG 9996T) and three clinical isolates from our collection, strains ADV 2297.03, ADV 6092.03 and ADV 1338.05. Identified as A. fermentans by sequencing 600 bp in the 5’-part of the 16S rRNA gene; these three strains displayed 16S rRNA gene sequence similarity levels above 99.5% with the A. fermentans type strain.

DNAs were rapidly extracted by a boiling–freezing method and 16S rRNA was selectively amplified by PCR using primers 271F and 1492r as described previously (Carlier et al., 2002). The 5’-part of the 23S rRNA gene was amplified using universal primers 6 and 10 as described previously (Anthony et al., 2000). The PCR products were directly sequenced with forward and reverse primers using an Applied Biosystems Automated Sequencer (Genome Express). The sequences were compared with known sequences in the GenBank and EMBL databases using the DIALIGN program (Morgenstern, 2002). An evolutionary tree based on the 16S RNA sequences was inferred using the maximum-likelihood (ML) (Olsen et al., 1994), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (NJ) (Saitou & Nei, 1987) methods from the PHYLIP suite of programs (Felsenstein, 1993). Regardless of the method used, the reconstructed trees were congruent and the strains formed a statistically well supported lineage, related but distinct from that of A. fermentans (Fig. 1). From sequence analysis and phylogeny, the strains studied can be considered to belong to a novel species of the genus Acidaminococcus, for which the name Acidaminococcus intestini is proposed.

Genomic studies included DNA G+C content determination and large-scale chromosome structure analysis. The DNA G+C content, determined by HPLC at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), for strain ADV 255.99T was 49.3 mol% (Table 2). Number and size of bacterial chromosomes were analysed by PFGE of intact DNAs as described previously (Marchandin et al., 2001) and mapping experiments with the intron-encoded endonuclease I-CeuI (New England Biolabs) were undertaken to determine the rm skeletons, as described

### Table 1. Clinical strains of Acidaminococcus intestini sp. nov. used in this study

Strains labelled ‘ADV’ were from University Hospital Arnaud de Villeneuve, Montpellier, France; strains labelled ‘LBN’ were from Bacteriology Laboratory of Nancy Hospital, France.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Date of isolation (month/year)</th>
<th>Age (years)/sex of patient</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADV 255.99T</td>
<td>06/1999</td>
<td>45/M</td>
<td>Peritoneal fluid</td>
</tr>
<tr>
<td>ADV 5206.02</td>
<td>09/2002</td>
<td>88/M</td>
<td>Rectum</td>
</tr>
<tr>
<td>ADV 2290.04</td>
<td>04/2004</td>
<td>75/M</td>
<td>Abdominal fluid</td>
</tr>
<tr>
<td>ADV 5199.04</td>
<td>06/2004</td>
<td>56/M</td>
<td>Mandible necrosis</td>
</tr>
<tr>
<td>ADV 1190.04</td>
<td>12/2004</td>
<td>52/M</td>
<td>Pressure ulcer (sacrum)</td>
</tr>
<tr>
<td>LBN 316</td>
<td>01/2004</td>
<td>23/M</td>
<td>Anal abscess</td>
</tr>
<tr>
<td>LBN 317</td>
<td>07/2002</td>
<td>18/F</td>
<td>Axillary abscess</td>
</tr>
<tr>
<td>LBN 318</td>
<td>09/2001</td>
<td>58/M</td>
<td>Abdominal fluid</td>
</tr>
<tr>
<td>LBN 319</td>
<td>12/2001</td>
<td>41/M</td>
<td>Abdominal fluid</td>
</tr>
<tr>
<td>LBN 320</td>
<td>03/1996</td>
<td>67/F</td>
<td>Inguinal abscess</td>
</tr>
<tr>
<td>LBN 321</td>
<td>02/1999</td>
<td>71/M</td>
<td>Infected parietal haematoma</td>
</tr>
</tbody>
</table>

*A. intestini ADV 255.99T (=AIP 283.01T=CIP 108586T=CCUG 50936T).*
previously (Marchandin et al., 2003a, b; Teyssier et al., 2003). The rrm skeleton was previously recognized as a sensitive indicator of phylogenetic relationships between bacteria, including members of the family ‘Acidaminococcaceae’ (Liu et al., 1999; Marchandin et al., 2003a; Jumas-Bilak et al., 2005). Although all the strains studied displayed a similar genomic size of about 2.49 Mb (± 140 kb), the rrm skeleton clearly distinguished two groups of strains. Indeed, six rrm operon copies could be demonstrated on the chromosome of A. fermentans (n = 3), whereas the 11 A. intestini isolates possessed three rrm copies (Table 2) (Supplementary Fig. S1 available in IJSEM Online).

Colonies of A. intestini grew on Columbia sheep blood agar plates after 2 days incubation. The colonies were about 0.3–0.5 mm in diameter, circular, convex, whitish with a smooth surface, non-pigmented and non-haemolytic. The cells were coccoid, smaller than cells of A. fermentans type strain, usually occurring as single cells but sometimes in pairs [Supplementary Fig. S2(a) and (b) available in IJSEM Online], Gram-negative after staining, non-spore-forming and non-motile. Cells were prepared as described previously for both negative staining and ultrathin sections (Marchandin et al., 2003a; Jumas-Bilak et al., 2005) and samples were observed under a Hitachi H7100 electron microscope. Cell size was 500–600 nm in diameter [Supplementary Fig. S2(a) and (b) available in IJSEM Online]. As reported by Rogosa (1969) for the type species of the genus Acidaminococcus, an outer cell wall membrane was observed in thin sections of cells for strain ADV 255.99T by EM [Supplementary Fig. S2(c) available in IJSEM Online].

The strains were identified according to the procedures of the VPI Anaerobe Laboratory Manual (Holdeman et al., 1977). For gas formation detection, cultures were observed for areas of disruption in loosely covered TGY deep agar and for gas bubbles in TGY broth. Special potency discs were used as described by Jousimies-Somer et al. (2002). An API Rapid ID 32A kit (bioMérieux) was used for enzymic profile determination as recommended by the manufacturer. Metabolic end products were assayed by quantitative GC as described previously (Carlier, 1985). Results are listed in Table 2. Strain ADV 255.99T showed the following negative characteristics: catalase, oxidase and urease activities, indole production, nitrate reduction, lactate fermentation, gelatin liquefaction, milk modification and aesculin hydrolysis. Acid was not produced from glucose, lactose, maltose, mannose or sucrose. Gas bubbles were noted in broth cultures. Glutamate was used as an energy source. By presumptive identification tests, the

Fig. 1. NJ phylogenetic tree based on partial 16S rRNA gene sequences (1365 nt). Anaeromusa acidaminophila was used as the outgroup. Bootstrap values are indicated at corresponding nodes. GenBank sequence accession numbers are shown in parentheses. Bar, 0.01 substitutions per site.
### Table 2. Phenotypic and genotypic characteristics that differentiate Acidaminococcus intestini sp. nov. from A. fermentans

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. fermentans CIP 106432T*</th>
<th>A. intestini (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (μm)</td>
<td>0.6–1.0</td>
<td>0.5–0.6</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Coccoid, oval or kidney shaped diplococi</td>
<td>Coccoid single cells or pairs</td>
</tr>
<tr>
<td>Susceptibility to special potency discs:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin (1 mg)</td>
<td>S</td>
<td>S (10/11)</td>
</tr>
<tr>
<td>Bile (1 mg)</td>
<td>S</td>
<td>S (8/11)</td>
</tr>
<tr>
<td>Ability to ferment carbohydrates</td>
<td>–†</td>
<td>–</td>
</tr>
<tr>
<td>Indole production</td>
<td>–</td>
<td>– (9/11)</td>
</tr>
<tr>
<td>API Rapid ID 32A kit:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Code for type strain of the species§</td>
<td>0000012401</td>
<td>0000016401</td>
</tr>
<tr>
<td>Pyroglutamic acid arylamidase activity</td>
<td>–</td>
<td>+ (10/11)</td>
</tr>
<tr>
<td>Leucyl glycine arylamidase activity</td>
<td>–</td>
<td>+ (7/11)</td>
</tr>
<tr>
<td>Metabolic end products§</td>
<td>A, B</td>
<td>A, P, B, (L) (trace amounts 2-OH-B, 2-OH-V)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td></td>
<td>56 (Bd)</td>
</tr>
<tr>
<td>Chromosome size (Mb)§</td>
<td>2.35</td>
<td>2.49 (2.40–2.62)</td>
</tr>
<tr>
<td>No. rrr operons</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

*A. fermentans* CIP 106432T. Data for *A. fermentans* type strain are from Rogosa (1969, 1984) or from this study. *A. fermentans* strains ADV 2297.03, ADV 6092.03 and ADV 1338.05 were similar to the type strain tested for phenotypic characteristics (morphology, susceptibility profile to special potency discs and enzymic activities on API Rapid ID 32A kit).

†About 40% of *A. fermentans* strains catabolize glucose and the reaction is weak (Rogosa, 1969).

§A. fermentans CIP 106432T showed the following activities: arginine arylamidase, leucine arylamidase, glycine arylamidase and histidine arylamidase. *A. intestini* ADV 255.99⁷ (=AIP 283.01⁷=CIP 108586⁷=CUG 50930⁷) showed, in addition, pyroglutamic acid arylamidase (PyrA) activity. With the exception of strain LBN 321, which showed a poor profile in the API Rapid ID 32A identification panel (only two positive reactions: indole and PyrA), two activities were variable among *A. intestini* strains: leucyl glycine arylamidase and PyrA activities.

§A, Acetic acid; B, butyric acid; P, propionic acid; L, lactic acid; 2-OH-B, 2-hydroxybutyric acid; 2-OH-V, 2-hydroxyvaleric acid. Parentheses indicate that these compounds are produced in variable amounts.

¶A. fermentans, values determined for 14 strains by Rogosa (1969) ranged from 55.6 to 57.4 mol%; *A. intestini*, determined for type strain ADV 255.9⁷.

¶¶*A. fermentans* strains ADV 2297.03, ADV 6092.03 showed similar genomic structure to *A. fermentans* CIP 106432T (data available as Supplementary Fig. S2 in IJSEM Online) and their genomic sizes were estimated to be 2.49 and 2.55 Mb, respectively. Genomic size for *A. intestini* (n=11) ranged from 2.40 to 2.62 Mb (mean size, 2.49 Mb); *A. intestini* ADV 255.9⁷ chromosome size was estimated to be 2.43 Mb.

The following characteristics were common to *A. intestini* sp. nov. and *A. fermentans*: resistance to vancomycin discs (5 μg), susceptibility to metronidazole (4 μg) and colistin (10 μg) discs, gas production, glutamate fermentation and absence of lactate fermentation. +, Positive; −, negative.

**strain** was resistant to 5 μg vancomycin disc and susceptible to 1 mg kanamycin, 10 μg colistin, 4 μg metronidazole and 1 mg bile discs. The enzymic profile determined using the API Rapid ID 32A system gave the following code 0000016410 corresponding to arginine, leucine, pyroglutamic acid, glycine and histidine arylamidase activities. The metabolic end products were acetate (32.3 mmol l⁻¹), butyrate (14.3 mmol l⁻¹) and propionate (3.4 mmol l⁻¹). Some characteristics of the type strain were found to be variable among *A. intestini* strains, in particular indole production, susceptibility to special potency discs, enzymic activities and metabolic end products (Table 2). Indeed, lactic acid was produced by four of the 11 *A. intestini* strains (3.5–4.5 mmol l⁻¹) and trace amounts (≤0.5 mmol l⁻¹) of 2-hydroxybutyric acid, 2-hydroxyvaleric acid and/or iso-valeric acid were produced by five strains.

On the basis of phenotypic, genotypic and phylogenetic characteristics, we suggest that the strains studied represent a novel species of the genus Acidaminococcus. The pattern of sites of isolation of these strains (Table 1) resembles that previously observed for *A. fermentans* in humans (Moore & Holdeman, 1974; Sugihara et al., 1974; Nakashima et al., 1983; Peraino et al., 1993; Chatterjee & Chakraborti, 1995; Goldstein et al., 2000; Galán et al., 2000). However, the strains were mainly recovered from samples originating from the gastro-intestinal tract. Moreover, the isolates were related to several uncultured clones and two butyrate-producing strains, all found in human faecal microbiota and showing 99 to 99.8% 16S rRNA gene sequence similarity with strain ADV 255.9⁷ [uncultured clones O14C-G10 (DQ905693), O14C-C7 (DQ905653), O14B-E11 (DQ905592), O14B-B6 (DQ905551), O14B-E8 (DQ905589), O14B-F11 (DQ905603), O14B-B10 (DQ905555), O14C-E10 (DQ905674) and O14B-C12 (DQ905659); butyrate-producing bacteria PH05YA06 (DQ144118) and PH05YA07 (DQ144119)].

The name Acidaminococcus intestini sp. nov. is proposed for the strains analysed in this study.

The description is as emended by Cook *et al.* (1994) with the following modifications: cocci 0.5–1 μm in diameter occurring as single cells, oval or kidney-shaped diplococci. Propionate may or may not be produced. The DNA G+C content is 49.3 (T_m) or 56 mole% (Bd). Chromosome size is 4.9 Mb ± 6% and rrn copy number is three or six.

**Description of Acidaminococcus intestini** sp. nov.

*Acidaminococcus intestini* (in.tes.ti.ni. L. gen. n. intestini, of the intestine). Cells are Gram-negative after staining, non-spore-forming cocci that occur as single cells or in pairs. Individual cells are 0.5–0.6 μm in diameter. Colonies on Columbia sheep blood agar after 2 days incubation are about 0.3–0.5 mm in diameter, circular, convex, whitish with a smooth surface. Non-pigmented and non-haemolytic. Strictly anaerobic. Oxidase- and catalase-negative. Gelatinase and nitrate-reduction tests are negative. Gas bubbles are noted in broth cultures. Indole may be produced. Carbohydrates are not fermented. Lactate is not used and glutamate is fermented. The metabolic end products are acetic acid, butyric acid and propionic acid. Lactic acid may be produced. Trace amounts (≤0.5 mmol l⁻¹) of 2-hydroxybutyric acid, 2-hydroxyvaleric acid and isovaleric acid may be produced. Habitat is the gastro-intestinal tract of humans. The DNA G+C content of strain ADV 255.99T is 49.3 mole%.

Can be differentiated from *A. fermentans* by pyroglutamatic acid arylamidase activity, metabolic end products, mainly by propionic acid production, 16S and 23S rRNA gene sequencing, DNA G+C content, and rrn skeleton.

The type strain, ADV 255.99T (=ATCC 283.01T=CIP 108586T=CCUG 50930T), was isolated from human clinical specimens.

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**References**


