

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 *A. fermentans*. Enzymic activity profiles as well as metabolic end product patterns, including propionic acid production, differentiated the novel bacteria from *A. 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 255.99^T (=AIP 283.01^T=CIP 108586^T=CCUG 50930^T).

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 255.99^T 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 255.99^T are AF473835 and EF060100, respectively.

PFGE migration of I-CeuI-restricted DNAs and EM of cells of strain ADV 255.99^T are available as supplementary figures with the online version of this paper.

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.

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

**A. intestini* ADV 255.99^T (=AIP 283.01^T=CIP 108586^T=CCUG 50930^T).

(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 106432^T (=DSM 20731^T=ATCC 25085^T=CCUG 9996^T) 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 gene was selectively amplified by PCR using primers 27f and 1492r as described previously (Carrier *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 BLAST program (Altschul *et al.*, 1997) and the LALIGN software (www.expasy.org). The GenBank accession numbers for the 16S rRNA gene sequences are given in Fig. 1 and those for the 23S rRNA gene partial sequences of about 320 bp are EF060094–EF060103. Despite several attempts, 23S rRNA gene sequences could not be determined for the *A. fermentans* type strain and strain ADV 6092.03. The 11 clinical isolates formed a very tight group since they displayed at least 99.9 and 99.7% identity in the 16S and 23S rRNA gene sequences, respectively. The clinical isolates were most closely related to *A. fermentans* but 16S rRNA gene similarity level of about 95.6% indicated that the isolates did not belong to this species. Their 23S rRNA gene sequences displayed less than 88.9% similarity with those of *A. fermentans* strain ADV 2297.03 (EF060093) and ADV

1338.05 (EF060092), confirming that they might represent a novel species.

The 16S rRNA gene sequences (1365 nt) were aligned against sequences of representative strains and clones retrieved in the GenBank database using the DIALIGN program (Morgenstern, 2002). An evolutionary tree based on the 16S rRNA 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). The algorithm F84 (Kishino & Hasegawa, 1989) was used to generate evolutionary distance matrices for the NJ method. The robustness of the trees was evaluated by bootstrap analysis of 100 resamplings using the SEQBOOT and CONSENSE programs from the PHYLIP package (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* sp. nov. 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 255.99^T 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 *rrn* skeletons, as described

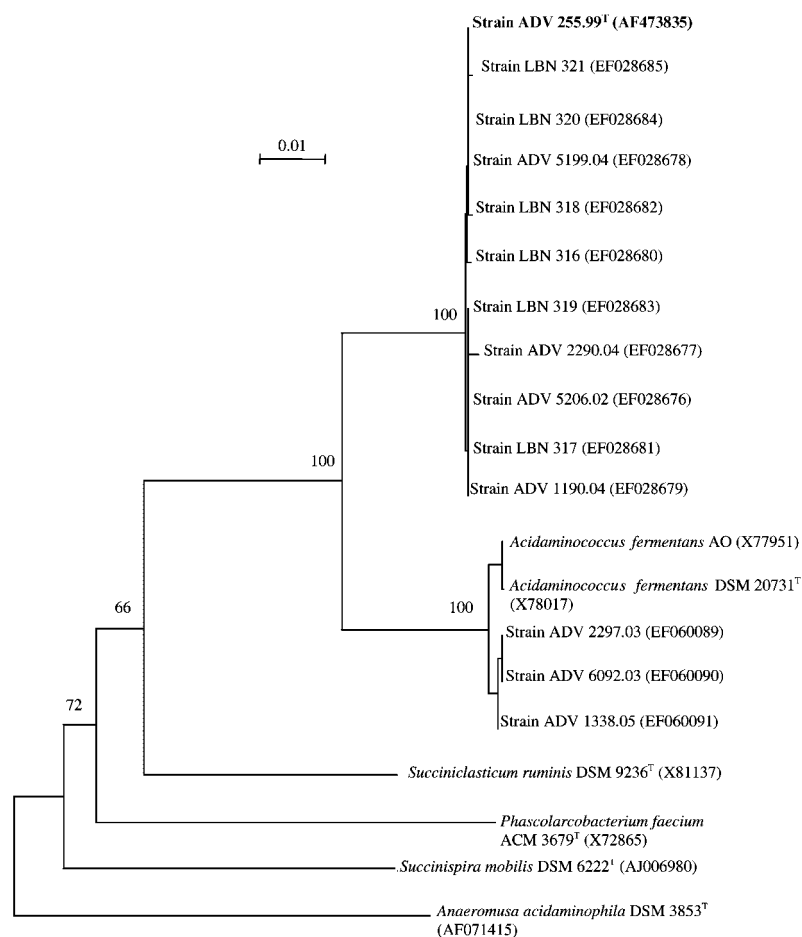


Fig. 1. NJ phylogenetic tree based on partial 16S rRNA gene sequences (1365 nt). *Anaeromusa acidaminophila* was used as the outgroup. Bootstrap values are indicated at corresponding nodes. GenBank sequence accession numbers are shown in parentheses. Bar, 0.01 substitutions per site.

previously (Marchandin *et al.*, 2003a, b; Teyssier *et al.*, 2003). The *rrn* 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 *rrn* skeleton clearly distinguished two groups of strains. Indeed, six *rrn* operon copies could be demonstrated on the chromosome of *A. fermentans* ($n=3$), whereas the 11 *A. intestini* isolates possessed three *rrn* 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 255.99^T 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 255.99^T 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

Table 2. Phenotypic and genotypic characteristics that differentiate *Acidaminococcus intestini* sp. nov. from *A. fermentans*

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.

Characteristic	<i>A. fermentans</i> CIP 106432 ^T *	<i>A. intestini</i> (n=11)
Cell size (µm)	0.6–1.0	0.5–0.6
Cell morphology	Coccoid, oval or kidney shaped diplococci	Coccoid single cells or pairs
Susceptibility to special potency discs:		
Kanamycin (1 mg)	S	S (10/11)
Bile (1 mg)	S	S (8/11)
Ability to ferment carbohydrates	–†	–
Indole production	–	– (9/11)
API Rapid ID 32A kit:		
Code for type strain of the species‡	0000012401	0000016401
Pyroglutamic acid arylamidase activity	–	+ (10/11)
Leucyl glycine arylamidase activity	–	+ (7/11)
Metabolic end products§	A, B	A, P, B, (L) (trace amounts 2-OH-B, 2-OH-V)
DNA G + C content (mol%)	56 (Bd)	49.3 (T _m)
Chromosome size (Mb)¶	2.35	2.49 (2.40–2.62)
No. <i>rrn</i> operons	6	3

**A. fermentans* CIP 106432^T. 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 106432^T showed the following activities: arginine arylamidase, leucine arylamidase, glycine arylamidase and histidine arylamidase. *A. intestini* ADV 255.99^T (=AIP 283.01^T=CIP 108586^T=CCUG 50930^T) 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 255.99^T.

¶*A. fermentans* strains ADV 2297.03, ADV 6092.03 showed similar genomic structure to *A. fermentans* CIP 106432^T (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 255.99^T chromosome size was estimated to be 2.43 Mb.

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 255.99^T [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 (DQ905569); butyrate-producing bacteria PH05YA06 (DQ144118) and PH05YA07 (DQ144119)].

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

Emended description of the genus *Acidaminococcus* Rogosa 1969 emend. Cook *et al.* 1994

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 mol% (Bd). Chromosome size is 4.9 Mb \pm 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 255.99^T is 49.3 mol%.

Can be differentiated from *A. fermentans* by pyroglutamic 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 255.99^T (=AIP 283.01^T=CIP 108586^T=CCUG 50930^T), was isolated from human clinical specimens.

Acknowledgements

We are indebted to C. Bizet and D. Clermont from the Collection de l'Institut Pasteur for providing us the type strain of *A. fermentans*. We thank I. Zornigotto, R. Devine and M. Chaon for excellent technical assistance. This work is partly supported by the association ADEREMPHA, Montpellier, France.

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