

Emendation of the Description of *Acidaminococcus fermentans*, a *trans*-Aconitate- and Citrate-Oxidizing Bacterium

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Ruminal fluid which was enriched with *trans*-aconitate yielded a gram-negative diplococcus (strain AO) which was identified by 16S ribosomal DNA sequence analysis as *Acidaminococcus fermentans*. In contrast to the original description, the *A. fermentans* type strain and strain AO were found to utilize citrate as an energy source and to produce hydrogen and hydrogen sulfide. The descriptions of the genus and species are emended accordingly.

Grass tetany, a potentially fatal magnesium deficiency disease of ruminants, has been correlated with the accumulation of *trans*-aconitate in plants (1, 3). Recent work has revealed that ruminal bacteria reduce *trans*-aconitate to tricarballylate (15-18). This nonmetabolizable tricarboxylic acid chelates blood magnesium and increases excretion of this element (19). Mixed-culture studies revealed that some of the *trans*-aconitate can also be converted to acetate, a nontoxic end product, but *trans*-aconitate-oxidizing bacteria were not isolated in these studies (15, 16, 18).

When ruminal contents from a cow fed grass hay were transferred successively in a medium containing 10 mM *trans*-aconitate and 100 mM sodium (4), high-pressure liquid chromatography (HPLC) revealed (7) that more than 60% of the *trans*-aconitate was oxidized. The *trans*-aconitate enrichment preparations used produced four colony types on 2% agar at 39°C in an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, Mich.), but only the large, circular, white colonies utilized *trans*-aconitate (10 mM) as an energy source for growth. Five *trans*-aconitate-utilizing strains were isolated initially, but all of these nonmotile, nonsporulating bacteria had the same cell morphology (they were diplococci) and Gram staining characteristics (they were gram negative).

Strain AO did not produce indole, and gelatin hydrolysis was virtually undetectable. Strain AO utilized citrate, glutamate, and pyruvate (Table 1). No growth was observed in the presence of glucose, maltose, sucrose, cellobiose, xylose, arabinose, ribose, lactate, oxaloacetate, malate, fumarate, or succinate. Strain AO could not grow aerobically. Growth was observed at 39 or 45°C but not at 25 or 50°C. Acetate was always the predominant fermentation end product, but butyrate was also detected, particularly when glutamate was the energy source (Table 1). Hydrogen gas (as detected with a Gow-Mac model 550 thermal conductivity gas chromatograph equipped with a Supelco S-8100 column) was also an end product of *trans*-aconitate or citrate fermentation. When glutamate was the energy source, ammonia (6) was produced. Tricarballylate was never detected. Strain AO produced hydrogen sulfide (ferrous sulfate precipitated as ferrous sulfide). Ammonia could not be utilized as a sole nitrogen source, and the bacterium grew slowly (specific growth rate, 0.05 h⁻¹)

when the Trypticase concentration was less than 1 mg/ml and no other energy source was provided.

On the basis of the results described above, it appeared that strain AO might belong to the genus *Acidaminococcus* (non-sporulating, nonmotile, anaerobic, gram-negative diplococci), but *Acidaminococcus fermentans*, the only species currently recognized, was described as a bacterium which (i) could not utilize citrate or pyruvate as an energy source, (ii) was completely suppressed by pyruvate, and (iii) could not produce hydrogen or hydrogen sulfide (13, 14). Since strain AO grew rapidly (specific growth rate, 0.3 h⁻¹) in the presence of citrate and fairly rapidly in the presence of pyruvate (specific growth rate, 0.14 h⁻¹), produced large amounts of sulfide, and produced hydrogen gas as an end product, the phylogenetic position of strain AO was in question.

Direct comparisons between strain AO and strain ATCC 25085, the type strain of *A. fermentans*, revealed (i) that both strains utilized *trans*-aconitate, citrate, and glutamate as energy sources and that the growth rates obtained with these substrates were similar, (ii) that both strains produced hydrogen gas when either citrate or *trans*-aconitate was the energy source, (iii) that both strains produced small amounts of hydrogen gas from glutamate, and (iv) that both strains produced hydrogen sulfide when cysteine was used as a reducing agent (0.6 mg of cysteine hydrochloride per ml). Strain ATCC 25085^T (T = type strain) did not readily grow on pyruvate in our studies, but other workers have found that this organism can ferment pyruvate under the correct cultural conditions (8). The G+C content of the DNA of strain AO was 54.7 mol% (as determined by the HPLC method), while the G+C content determined previously for *A. fermentans* ATCC 25085^T was 56.6 mol% (as determined by the buoyant density method). On the basis of these results, it appeared that strain AO and strain ATCC 25085^T were virtually identical.

Strain AO could not utilize either *trans*-aconitate or citrate as an energy source if sodium salts were omitted from the basal medium (<0.1 mM sodium), but it grew rapidly in the presence of *trans*-aconitate if 1 mM sodium was added. Rapid growth on citrate was not observed until the sodium concentration was greater than 10 mM. Because the medium used by Rogosa (13) to characterize *A. fermentans* contained more than 20 mM sodium, the contradiction regarding citrate utilization cannot be readily explained. On the basis of our results, the statement that *A. fermentans* cannot utilize citrate is not correct.

When the 16S ribosomal DNAs of strain AO and *A.*

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TABLE 1. Comparison of strains AO and ATCC 25085^T

Growth substrate	Maximum specific growth rate (h ⁻¹)		Acetate concn (mmol/mmol of substrate)		Butyrate concn (mmol/mmol of substrate)		Hydrogen gas concn (mmol/mmol of substrate)	
	Strain AO	Strain ATCC 25085 ^T	Strain AO	Strain ATCC 25085 ^T	Strain AO	Strain ATCC 25085 ^T	Strain AO	Strain ATCC 25085 ^T
<i>trans</i> -Aconitate	0.62	0.60	1.8	1.9	0.1	0.1	0.5	0.5
Citrate	0.30	0.28	1.9	2.0	0.1	0.1	0.7	0.6
Glutamate	0.31	0.35	1.1	1.1	0.3	0.4	<0.1	<0.005
Pyruvate	0.14	NG ^a	1.0	NG				

^a NG, no growth detected.

fermentans DSM 20731^T (= ATCC 25085^T) were amplified by a PCR and sequenced as described previously (11, 12), a comparison of nearly complete 16S ribosomal DNA sequences (1,507 bases) revealed that strain AO exhibited 100% homology with strain ATCC 25085^T. These 16S ribosomal DNA sequences are now available in the EMBL data base under accession numbers X77951 and X78017. On the basis of these results, it appeared that strain AO should be classified as an *A. fermentans* strain. The phylogenetic position of *A. fermentans* within the radiation of the gram-positive bacteria belonging to the *Clostridium-Bacillus* subphylum has been described previously (2).

A. fermentans was isolated previously from the intestinal tracts of pigs and humans (13, 14), but not from the rumen. When ruminal fluid was obtained from a cow fed grass hay containing 11.1% crude protein, 69.1% neutral detergent fiber, 47.1% acid detergent fiber, 0.24% magnesium, and 0.78% calcium on a dry matter basis and was diluted 10-fold ($n = 3$) in a medium containing mixed carbohydrates (4), the most probable number of carbohydrate-fermenting bacteria was 0.6×10^9 bacteria. When 10 mM *trans*-aconitate was provided as the only energy source, growth was not observed at dilutions greater than 10^2 . The paucity of *A. fermentans* was probably related to the lack of *trans*-aconitate, citrate, or pyruvate in the grass hay that was fed to the cow. The hay did contain the amino acid glutamate, another potential energy source of *A. fermentans* (Table 1), but this amino acid is rapidly deaminated by a variety of ruminal bacteria (5). The pH of the ruminal fluid was 6.7, and strain AO is a fairly acid-resistant bacterium (Fig. 1).

Since *trans*-aconitate accumulation in plants is a transient and sudden event that is observed only in grazing situations (9,

10) and commercial *trans*-aconitate is expensive, it was not possible for us to feed the cow a diet which contained *trans*-aconitate. Additional work will be needed to assess the ability of *A. fermentans* to counteract tricarballoylate accumulation. If there is insufficient time for *A. fermentans* ruminal numbers to increase, ruminal inoculation may be a way to prevent tricarballoylate-induced hypomagnesemia.

On the basis of the results obtained in this study, emended descriptions of the genus *Acidaminococcus* and the species *A. fermentans* are given below. It was necessary to emend both the genus description and the species description because of metabolic characteristics which should now be considered species characteristics. The descriptions are for the most part based on the descriptions given previously (13, 14).

Description of the genus *Acidaminococcus*. *Acidaminococcus* (Rogosa, 1969, 765) emend. Cook, Rainey, Chen, Stackebrandt, and Russell 1994 (*A. cid. a. min. o. coc' cus*. M. L. n. *acidum*, acid; M. L. adj. *amino*; Gr. n. *coccus*, a grain, berry; M.L. masc. n. *Acaminococcus*, amino acid coccus). Cells are gram-negative, nonsporulating, nonmotile, cocci that are 0.6 to 1.0 μm in diameter and often occur as oval or kidney-shaped diplococci. The cell wall contains *meso*-diaminopimelic acid; whole cells contain galactose, glucose, and ribose. Menaquinones and ubiquinones are absent. Chemoorganotrophic anaerobe. No growth occurs on the surfaces of solid media incubated in the presence of air. Temperature optimum, 30 to 37°C. Weak or no growth occurs at 25 and 45°C. Cells are not viable after heat treatment for 30 min at 60°C. The optimum pH for growth is 7.0, and growth occurs in the pH range from 6.2 to 7.5. No serological cross-reactions occur between *Acidaminococcus* strains and either *Veillonella* serovars or *Peptococcus aerogenes*. The G+C contents of the DNAs range from 54.7 to 57.4 mol%. Phylogenetically, this genus is a member of the *Spormusa* cluster (i.e., the cluster containing the genera *Spormusa*, *Megasphaera*, *Selenomonas*, *Butyrivibrio*, *Pectinatus*, and *Zymophilus*) and is a distinct lineage within the radiation of the *Clostridium-Bacillus* group. The type species is *Acidaminococcus fermentans*.

Description of *Acidaminococcus fermentans*. *Acidaminococcus fermentans* (Rogosa, 1969, 765) emend. Cook, Rainey, Chen, Stackebrandt, and Russell 1994 (*fer. men' tans*. M. L. part. adj. *fermentans*, fermenting). The characteristics are the same as those described above for the genus. Additional characteristics are as follows. Glutamate, citrate, and *trans*-aconitate are used as sole energy sources for growth as long as sodium is present, and the end products of metabolism are acetate, butyrate, and hydrogen gas. Pyruvate is utilized by some strains. About 40% of the strains weakly metabolize glucose. Extremely weak or no growth occurs with cellobiose, lactose, melibiose, rhamnose, ribose, and fucose. Adonitol, esculin, amygdalin, arabinose, dulcitol, erythritol, erythrose, fructose, fumarate, galactose, glycerol, inositol, inulin, lactate,

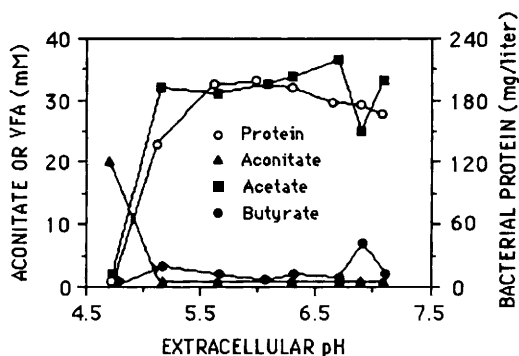


FIG. 1. Effect of extracellular pH on the growth of strain AO in a continuous culture (specific growth rate, 0.1 h^{-1}) when *trans*-aconitate was used as an energy source and on the production of volatile fatty acids (VFA). The pH was decreased by adding concentrated HCl to the medium reservoir.

malate, maltose, mannitol, mannose, melibiose, α -methyl-D-glucoside, α -methyl-D-mannoside, raffinose, salicin, sorbitol, sorbose, succinate, sucrose, trehalose, and xylose are not utilized as energy sources.

Arginine, glutamic acid, tryptophan, and valine are required for growth; in addition, cysteine and histidine are required by 93% of the strains, phenylalanine and serine are required by 50% of the strains, and tyrosine is required by 79% of the strains. Glycine sometimes stimulates growth, while alanine, aspartic acid, isoleucine, leucine, lysine, methionine, proline, and threonine are not required for growth. In amino acid-containing media, no growth occurs in the absence of biotin, pantothenate, pyridoxal, and vitamin B₁₂; *p*-aminobenzoic acid is essential or highly stimulatory for growth. Exogenous putrescine, folic acid, folinic acid, niacin, riboflavin, and thiamine are not required for growth. Benzidine, catalase, cytochrome oxidase, indole, and nitrate reduction tests are negative. H₂S is produced in cysteine-reduced media. Ammonia is produced. Sulfonthalein indicators are not reduced. Gelatin is not normally liquefied, but slow or partial liquefaction sometimes occurs. Resistant to vancomycin (7.5 μ g/ml). *Acidaminococcus fermentans* strains have been isolated from the intestinal tracts of pigs and humans and from the cow rumen. The type strain is VR4 (= ATCC 25085 = DSM 20731).

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