Anaerobe

Anaerobe (1998) 4: 241–250
Article No. an980170

TAXONOMY/SYSTEMATICS

Aminobacterium colombiense gen. nov. sp. nov., an Amino Acid-degrading Anaerobe Isolated from Anaerobic Sludge

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(Received 24 June 1998, accepted 30 September 1998)

Key Words: *Aminobacterium colombiense, Methanobacterium formicicum,* amino acid degradation, interspecies hydrogen transfer

A new Gram-negative, non-sporulating, mesophilic, amino acid-fermenting bacterium, designated strain ALA-1^T (T = type strain), was isolated from an anaerobic lagoon of a dairy wastewater treatment plant. The strain is curved (3–4 μm × 0.2–0.3 μm) and occurs singly or in pairs. Optimum growth occurs at 37°C and pH 7.3. The G + C content of the DNA is 46 mol %. The strain requires yeast extract for growth, grows poorly on casamino acids, peptones, cystein, and α-ketoglutarate, but readily grows on serine, threonine, glycine and pyruvate. When cocultured with the hydrogenotrophic methanogen *Methanobacterium formicicum,* strain ALA-1^T oxidized alanine, glutamate, leucine, isoleucine, valine, aspartate, and methionine. Phylogenetic analysis revealed that it forms a distinct and independent line of descent in the vicinity of *Dethiosulfovibrio peptidovorans, Dictyoglomus thermophilum,* and *Anaerobaculum thermoterrenum* which are members of the low G + C containing Gram-positive bacteria. The phylogenetic results concur with the phenotypic and genomic data which reveal that it is a novel strain. Based on these findings, we designate strain ALA-1^T as *Aminobacterium colombiense* (DSM 12261) gen. nov., sp. nov.

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1075-9964/98/050241 + 10 $30.00/0

FICHE DESCRIPTIVE

Auteur(s) : Baena S., **Fardeau M.-L., Labat M., Ollivier B.,** Thomas P., **Garcia J.-L.**, Patel B.K.C.

Titre original : *Aminobacterium colombiense* gen nov. sp. nov., an amino acid-degrading anaerobe isolated from anaerobic sludge.

Revue : Anaerobe 1998, **4**, 241-250.

Titre en Français : *Aminobacterium colombiense* gen nov. sp. nov., une bactérie anaérobie dégradant les acides aminés et isolée d'une boue anaérobie.

Mots-clés matières : *Aminobacterium colombiense*, anaérobiose, phylogénie, taxonomie, acides aminés
(10 au plus)

Résumé en Français :
(150 mots maximum)

Plan de classement : Monde végétal et Animal - Fermentations

Introduction

In addition to carbohydrates and lipids, proteins are regarded as the main organic substrates of anaerobic sludge and waste waters from swine and poultry slaughterhouses. It has been documented that anaerobically treated protein-rich wastes produce less biogas than wastes with low nitrogen content, due in part to the production of high ammonium concentrations from protein degradation [1]. From this data it can be inferred that amino acid degradation is an important physiological event in such environments. Though protein-degrading saccharolytic microorganisms have been isolated and characterized, very little attention has been paid to obligately protein and amino acids degrading bacteria. Again most investigations have been limited to anaerobic degradation of proteinaceous compounds in the rumen ecosystem rather than other ecosystems [2–4].

Proteins are hydrolysed to peptides and amino acids which are converted to methane and CO_2 by mixed bacterial populations. Single amino acids can be degraded either through: (i) fermentation; (ii) reductive deamination; or (iii) oxidative deamination linked to proton reduction. Oxidative deamination reactions are endergonic under standard conditions and only proceed if the reducing equivalents produced are removed by a biological or a chemical electron scavenger [5–7]. This is possible via interspecies hydrogen transfer in the presence of hydrogentrophic methanogens or sulfate-reducing bacteria [8–10], but also when amino acids (Strickland reaction) [11], acetate [12] or thiosulfate [13,14] act as the final electron acceptor.

In this paper, we describe the characteristics of a mesophilic, syntrophic alaline-degrading bacterium, strain ALA-1^T isolated from an anaerobic dairy wastewater lagoon as part of our ongoing studies on protein degradation.

Materials and Methods

Sample source and strains

Sludge samples were collected from an anaerobic dairy wastewater treatment lagoon (SantaFe de Bogota, Columbia). The *in situ* temperature at the sample collection point was 24°C. Samples were transported to laboratory and maintained at 4°C until used. The pure culture isolated from the sample was designated strain ALA-1^T. *Methanobacterium formicicum* (DSM 1535) was purchased from the Deutsche Sammlung von Mikroorganismen, Germany.

Culture conditions

The Hungate technique [15] was used throughout these studies. The basal medium used during this study contained (per liter) 0.2 g of KH_2PO_4, 0.3 g of NH_4Cl, 1.0 g of NaCl, 0.4 g of $MgCl_2 \cdot 6H_2O$, 0.5 g of KCl, 0.15 g of $CaCl_2 \cdot 2H_2O$, 1 ml of trace element solution [16], 1.0 ml of 0.1% resazurin. The pH was adjusted to 7.2 with 10 M KOH. The medium was boiled under a stream of O_2-free N_2 gas, cooled to room temperature and 5-ml aliquots distributed in Hungate tubes under a stream of O_2-free N_2 gas. This gas phase was replaced with N_2-CO_2 (80:20) and the tubes autoclaved. Prior to inoculation, 0.15 ml of 2% $Na_2S \cdot 9H_2O$, 0.25 ml of 10% $NaHCO_3$, and 0.05 ml of Balch vitamin solution [17] were injected into each tube.

Isolation

For isolation, the sludge sample was pulverized in an anaerobic chamber using a hand held homogeniser. A ten-fold serial dilution was prepared and inoculated into Hungate tubes containing 5 ml basal medium with alanine, yeast extract and thiosulfate at a final concentration of 10 mM, 0.1% and 10 mM, respectively. Incubations were performed at 37°C for 2 weeks after which ten-fold serial dilutions were prepared from the last positive serial dilution and inoculated into roll tubes containing enrichment medium fortified with 2% agar. Isolates were purified by repeated use of the Hungate roll tube method until deemed to be axenic. Subsequent routine culturing of ALA-1^T was performed using the basal medium containing 10 mM serine and yeast extract 0.2%.

Light and electron microscopy

Light microscopy was performed using a Nikon phase contrast microscope. Negative staining, preparation of thin sections for electron microscopy and electron microscope examination was performed as described previously [18].

Determination of pH, temperature, and NaCl for growth

All experiments were conducted in duplicates unless indicated in basal medium containing 10 mM serine and 0.2% yeast extract. The isolate was subcultured at least once under the same experimental conditions. For pH studies, the medium was adjusted with anaerobically prepared stock solutions of HCl (1 M), $NaHCO_3$ (10%), or Na_2CO_3 (10%) to the desired pH. Temperature range for growth, was determined between 18°C and 55°C. For studies on NaCl requirements, NaCl was weighed directly in the tubes for concentrations higher than 1% NaCl, and

the medium dispensed into the tubes as described above. For concentrations lower than 1%, different amounts were injected from a stock solution of NaCl (10%) into predispensed medium to give the required concentration.

Substrate utilization tests

The isolate was subcultured at least once under the same experimental conditions. For substrates utilization studies, 0.2% yeast extract was added to the basal medium. L-amino acids (serine, threonine, cysteine, alanine, glutamate, valine, isoleucine, proline, methionine, aspartate, leucine, phenylalanine, histidine, asparagine, glutamine, arginine, lysine), organic acids (pyruvate, succinate, malate, fumarate, citrate, α-ketoglutarate, lactate, acetate, propionate, butyrate) and sugars (glucose, saccharose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose, mannitol) were tested at a final concentration of 10 mM. Glycerol and ethanol were added to a final concentration of 5 mM, whereas Biotrypcase, peptone, casamino acids, gelatin, and casein were tested at a final concentration of 0.5%. The electron acceptors tested were: thiosulfate (10 mM), sulfate (10 mM), elemental sulfur (2%), sulfite (2 mM), sodium fumarate (20 mM), and nitrate (10 mM).

Amino acid degradation via the Stickland reaction was tested in a basal medium containing 10 mM alanine as electron donor and 20 mM glycine, 20 mM serine, 20 mM proline or 20 mM arginine as electron acceptor.

Mixed culture experiments

Methanobacterium formicicum was grown under a H_2:CO_2 (80:20) atmosphere in basal medium containing 0.1% yeast extract, acetate and formate. Strain ALA-1^T was grown in the basal medium containing 10 mM serine and yeast extract 0.2%. Inoculum from both cultures (10%) was injected into tubes containing 5 ml basal medium, 0.2% yeast extract and selected amino acids (alanine, glutamate, valine, isoleucine, leucine, cysteine, threonine, methionine, aspartate, lysine, arginine) and organic acids (succinate, α-ketoglurate and malate) at concentrations of 10 mM and incubated for 2–3 weeks after which the concentration of amino acids utilized and the end-products formed were determined. Basal medium containing 0.2% yeast extract but lacking amino acids were used as controls.

Analytical techniques

Growth was measured by inserting growth tubes directly into a model UV-160A spectrophotometer (Shimadzu Corp., Kyoto, Japan) and measuring the optical density at 580 nm. The fermentation end-products were determined as described by Fardeau *et al.* [19]. Amino acid concentrations were determined by high pressure liquid chromatography [20]. Growth and product formation were analysed after 2 to 3 weeks of incubation at 37°C.

Determination of G + C content

The G + C content was determined by the DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmb, Braunschweig, Germany). The DNA was isolated and purified by chromatography on hydroxyapatite. The G + C content was determined by high performance liquid chromatography (HPLC) as described by Mesbah *et al.* [21].

16S rRNA sequence studies

DNA was extracted from the isolate as described previously [22,23]. The universal primers Fd1 and Rd1 were used to obtained a PCR product of approximately 1.5 kb corresponding to base positions 8 to 1542 based on *E. coli* numbering of the 16S rDNA [24]. A 50-µl reaction contained 1–20 ng of genomic DNA, 1 µM of each primer, 5 µl of 10 × buffer, 200 µM of dNTP, 3.5 mM of $MgCl_2$, and 2.5 U of *Taq* polymerase (Promega). PCR was carried out by an initial denaturation at 94°C for 7 min, then 29 cycles of annealing at 55°C for 2 min, extension at 72°C for 4 min, denaturation at 94°C for 1 min, and finally an extension cycle of 55°C for 2 min and 72°C for 20 min.

PCR products were putified using QIAquick Kit (Qiagen). DNA concentration of purified PCR product was estimated by comparison with the Low Mass Ladder (Gibco BRL) on an agarose gel containing ethidium bromide.

PCR products were sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Kit with AmpliTaq FS DNA polymerase and ABI 373A sequencer. A 10-µl reaction contained 35 ng of PCR product, 4 µl of cycle sequencing reaction mix, 3.2 pmol of primer [22], and 2.5 µg of BSA. Thermal cycling was carried out using a RapidCycler (Idaho Technology) at a temperature transition slope of 2, an initial denaturation of 94°C for 15 s, then 25 cycles of denaturation at 94°C for 0 s, annealing at 50°C for 10 s, extension at 60°C for 3 min.

The new sequence data that was generated was aligned and checked for accuracy manually using the alignment editor, ae2 [25]. The program Blast [26] was initially used against the GenBank database to determine if any newly released closely related sequences existed in the database. For analysis, the sequences of *Anaerobaculum thermoterrenum* and

Dethiosulfovibrio peptidovorans, were extracted from the GenBank (accession numbers U50711 and U52817, respectively) and manually aligned with the pre-aligned sequences obtained from the Ribosomal Database Project [25]. Pairwise evolutionary distances based on 1151 unambiguous nucleotides were computed using dnadist (Jukes and Cantor option) and neighbor-joining programs that form part of the PHYLIP package [27]. TREECON was used extensively for bootstrap analysis (100 data sets) [28].

Nucleotide sequence accession number

The SSU rRNA gene sequence of *Aminobacterium colombinense* strain ALA-1^T has been deposited in GenBank under accession number AF069287.

Results

Isolation

A positive culture developed in the basal medium containing alanine, yeast extract and thiosulfate after 2 weeks incubation at 37°C with acetate and H_2S detected as the major end-products. Microscopic examination revealed two morphotypes, viz. a rod and a vitrio. Roll tubes inoculated with the highest serially diluted tube showed two distinct types of colonies after 2 weeks incubation at 37°C. One type of colony was round, whitish and possessed smooth edges, whereas the second type was smaller, white and lens-shaped. A rod shaped bacterium was isolated from the later colony, designated ALA-1^T and was characterized further.

Strain ALA-1^T grew very poorly in medium containing alanine and yeast extract, a medium used to initiate enrichments. Again sulfide was not detected though the initial enrichment culture had produced sulfide from thiosulfate indicating that a bacterium other than strain ALA-1^T was responsible. Subsequent experiments revealed that strain ALA-1^T grew much better with 10 mM of serine and 0.2% yeast and hence these substrates were used for subsequent routing culturing of the strain.

Morphology

Strain ALA-1^T, stained Gram-negative, was slightly curved and measured 3–4 μm × 0.2–0.3 μm when grown on a medium containing serine and yeast extract (Figure 1a). Motility and spores were not observed. Electron microscopy of negatively stained cells revealed the absence of flagella. Ultrathin sections of strain ALA-1^T revealed a thick cell wall with an external S-layer similar to that of Gram-positive type cell walls (Figure 1b,c).

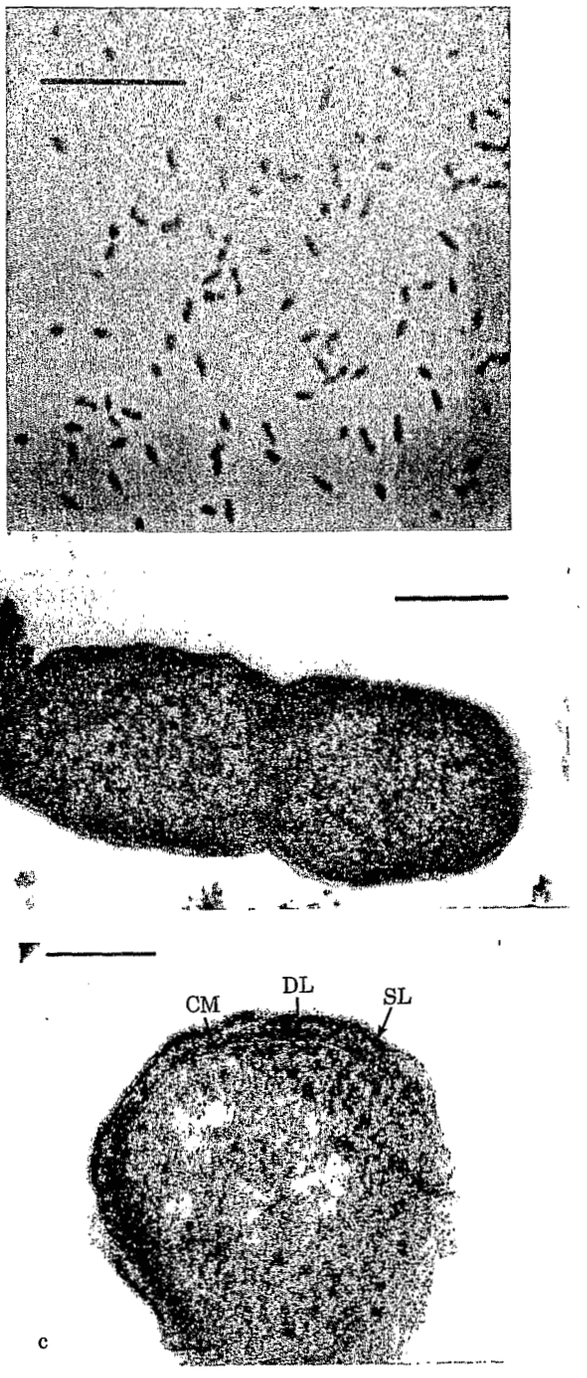

Figure 1. (a) Phase-contrast photomicrograph of cells from exponential growth phase of strain ALA-1^T. Bar, 10 μm. (b) Transmission electron micrograph of a thin section of whole cells of strain ALA-1^T. Bar, 0.2 μm. (c) Transmission electron micrograph of cell wall of strain ALA-1^T showing the presence of cytoplasmic membranes (CM), dense layer (DL) and S-layer (SL). Bar = 0.1 μm.

Growth and metabolic properties

The optimal growth temperature for strain ALA-1^T was 37°C and the temperature range for growth was

between 20 and 42°C, with no growth occurring at 18 and 45°C. Strain ALA-1^T did not require NaCl for growth but tolerated less than 1.5% NaCl. Optimum growth occurred in the presence of 0.05 to 0.5% NaCl. The pH range for growth was 6.6 to 8.5 with an optimum pH around 7.3.

Table 1 shows the substrates used for fermentation and the end-products formed. Strain ALA-1^T required yeast extract for growth and fermented serine, glycine, threonine, and pyruvate in its presence. Poor growth was obtained on casamino acids, peptone, biotrypcase, cysteine and α-ketoglutarate. The fermentation and end-products included acetate and H_2 and also propionate in the case of α-ketoglutarate fermentation. Carbohydrates, gelatin, casein, glycerol, ethanol, acetate, propionate, butyrate, lactate, citrate, fumarate, malate, succinate and the other amino acids tested were not utilized.

Table 2 shows the oxidation of substrates and end-products that formed in the mixed cultures. Alanine, glutamate, valine, isoleucine, leucine, methionine, aspartate and malate were oxidized only in the presence of the hydrogenotroph, *M. formicicum*. In addition, the utilization of cysteine, threonine and α-ketoglutarate were also improved in the presence of *M. formicicum*. The typical oxidation products from growth in mixed cultures were acetate and in addition, branch fatty acids such as isobutyrate, 2-methylbutyrate and isovalerate from the branched-chain amino acids valine, isoleucine and leucine, respectively. Propionate was also produced from glutamate oxidation and the amounts of propionate produced from α-ketoglutarate was greater than that observed for pure cultures.

Table 3 shows the effect of H_2 on the growth of strain ALA-1^T. An 80% hydrogen atmosphere (supplied as H_2–CO_2 (80:20) at 2 bar pressure) inhibited growth of strain ALA-1^T on threonine and α-ketoglutarate, whereas glycine degradation was not affected. Serine and pyruvate were partially affected by the presence of hydrogen.

Sulfate, thiosulfate, elemental sulfur, sulfite, nitrate

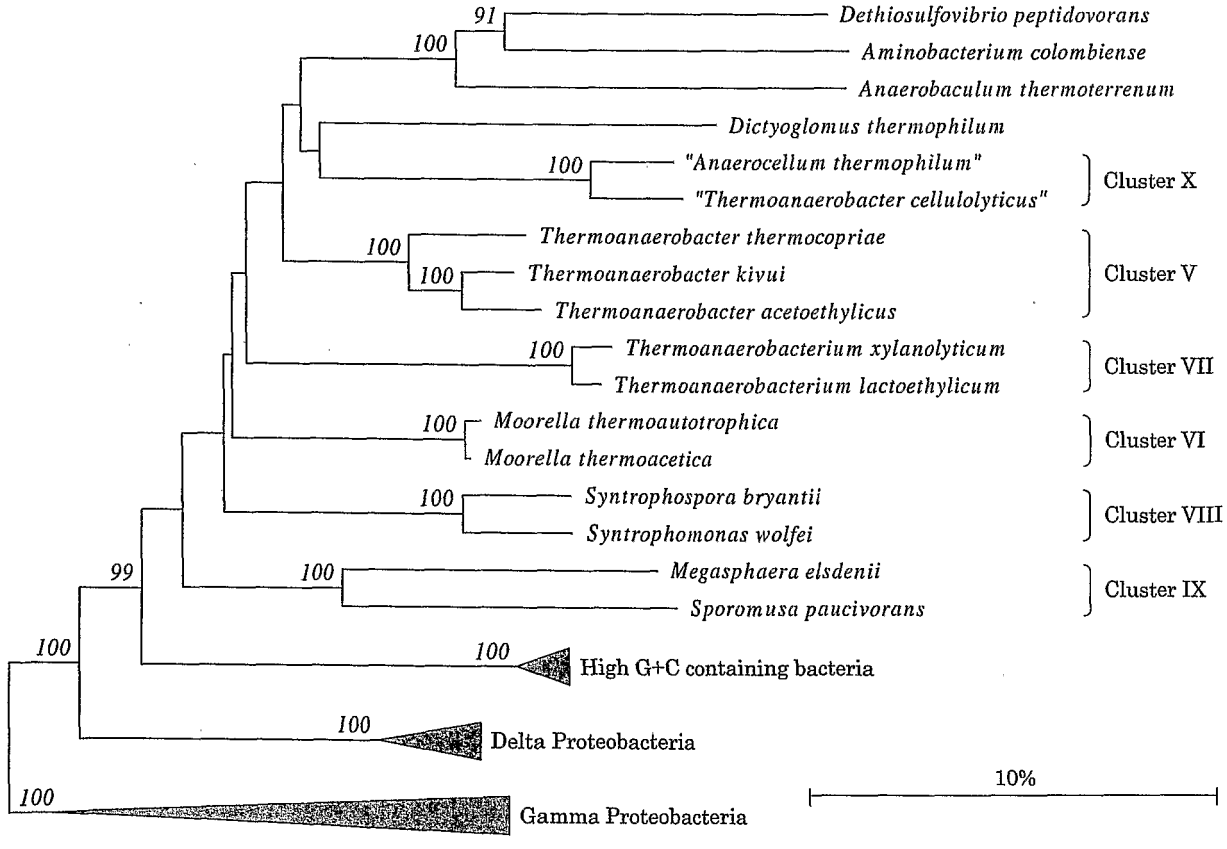

Figure 2. Phylogenetic dendroGram based on 16S rRNA sequence data indicating the position of *Aminobacterium colombiense* strain ALA-1^T within the radiation of representatives of the low G + C containing Gram-positive bacteria. All the sequences used in the analysis, with the exception of *Anaerobaculum thermoterrenum* and *Dethiosulfovibrio peptidovorans* (GenBank accession numbers U50711 and U52817, respectively), were obtained from the Ribosomal Database Project, version 5.0 (24). The assignment of the species to the various clostridial clusters has been described previously by Collins *et al.*, 1994 [28]. The shaded triangles indicate outgroups represented by *Streptomyces* species (High G + C containing bacteria), *Desulfovibrio desulfuricans* and its relatives (Delta Proteobacteria) and *Escherichia coli* and *Francisella* species (Gamma Proteobacteria). Bootstrap values, expressed as a percentage of 100 replications are shown at the branching points. Only values above 90% were considered significant and therefore reported. Scale bar indicates 10 nucleotide substitutions per 100 nucleotides.

and fumarate were not utilized as electron acceptors. Strain ALA-1^T did not perform the Stickland reaction when alanine was provided as an electron donnor and glycine, serine, arginine or proline were provided as electron acceptor.

G + C content

The G + C content of the DNA of strain ALA-1^T was determined to be 46 mol %.

16S rRNA sequence analysis

An almost complete 16S rRNA gene sequence (1519 nucleotides) of strain ALA-1^T corresponding to positions 11 to 1541 (*E. coli* numbering, according to Winker and Woese [24] was determined. Initial sequence comparisons indicated that strain ALA-1^T was a member of the low G + C Gram-positive branch. Several phylogenetic trees were generated using numerous combinations of sequences from the members of this branch and strain ALA-1^T was consistently placed as an independent branch of descent adjacent to members of cluster V [29] which currently comprises the genus *Thermoanaerobacter* (Figure 2). The closest relative of strain ALA-1^T were *Dethiosulfovibrio peptidovorans* (similarity of 85%) and *Anaerobaculum thermoterrenum* (similarity of 83%) both of which form independent lineages in the vicinity of cluster V [29]. Bootstrap analysis indicates a robust relationship amongst the three relatives (91 and 100, respectively).

Discussion

Taxonomic consideration

Strain ALA-1^T is a mesophilic and a strictly anaerobic bacterium which ferments serine, threonine, glycine, pyruvate but not carbohydrates. These characteristics are in common with the non-saccharolytic aminolytic species *Acidaminobacter hydrogenoformans, Eubacterium acidaminophilum, Selenomonas acidaminophila* and *Peptostreptococcus anaerobius* and several species of the genus *Clostridium* which include *C. sticklandii, C. aminophilum, C. litorale, C. pascui, C. hydrobenzoicum,* and *C. acetireducens*. Phylogenetically, the non-saccharolytic aminolytic species are members of the low G + C containing Gram-positive clostridial branch which is currently comprised of 19 defined clusters and several distinct independent lineages [29]. Strain ALA-1^T is also a member of the low G + C containing Gram positive clostridial branch and is therefore related to them but only distantly. Further phylogenetic analysis revealed that strain ALA-1^T formed an independent line of descent together with *D. peptidovorans* [30], *A. thermoterrenum* [31] and *D. thermophilum* [32] in the vicinity of cluster V [29] which currently consists of members of the genus *Thermoanaerobacter*. Strain ALA-1^T is related to *D. peptidovorans* with a similarity of 85%, to *D. thermophilum* at a similarity of 83% and to *Thermoanaerobacter* species, the members of cluster V [29] at an average similarity of 84%. However, there are clear differences between strain ALA-1^T and the three distant relatives. *D. peptidovorans* [30] is a curved, non-saccharolytic amino acid fermenting, non-spore forming anaerobe, characteristics which are similar to strain ALA-1^T but the former ferments serine but not histidine and requires thiosulfate as an electron acceptor for growth on amino acids such as alanine, glutamate, isoleucine, leucine, methionine and valine. In addition, *D. peptidovorans* [30] has a G + C content of the DNA of 56% which is substantially higher than the 46% for strain ALA-1^T. *A. thermoterrenum* [31] and *D. thermophilum* [32] are obligately thermophilic and saccharolytic bacteria which do not utilize amino acids. Based on the relatively large phylogenetic distance and the numerous phenotypic differences separating the three strains from strain ALA-1^T, we propose that the new isolate be given a genus status.

Metabolic considerations

Fermentation of serine to acetate by strain ALA-1^T is similar to that reported for *S. acidaminovorans* [8] and accordingly serine is probably converted to pyruvate and ammonia via serine dehydratase. Dehydration of serine followed by hydration reactions give rise to oxo acids such as pyruvate, which is decarboxylated to acetate. This type of deamination mechanism is thermodynamically favorable and the deamination of serine could therefore occur without coupling to an electron-scavenging system [5].

Threonine was only moderately fermented to acetate similarly to that described for some *Clostridium* species which include *C. histolyticum, C. sporospheroides, C. sticklandii,* and *C. subterminale* [33]. However, most members of this genus produce propionate and *n*-butyrate and a few also produce 2-aminobutyrate. The oxidation of threonine by strain ALA-1^T in the presence of the hydrogenotrophic methanogenic, *M. formicicum* was far greater than the fermentation by strain ALA-1^T alone. In contrast, for *A. hydrogenoformans* [9], threonine was only degraded in the presence of methanogens.

The conversion of glycine to acetate by strain ALA-1^T suggest a pattern similar to that reported for *E. acidaminophilum* [10], *Peptococcus anaerobius, P. magnus, C. histolyticum* and *C. purinolyticum* [1].

Table 1. Fermentation of substrates that supported growth of strain ALA-1^T in pure culture[a]

Substrates added[b]	Products (mM)			
	Acetate	Propionate	H_2	ΔOD_{580}
Serine (8)	8.7	0	2.4	0.17
Glycine (8)	8.0	0	0.17	0.10
Threonine (8)	3.5	0	0.35	0.07
Cysteine[c] (8)	1.1	0	1.0	0.11
Pyruvate (8)	6.4	0	1.52	0.14
α-ketoglutarate[c] (5)	1.0	1	2.0	0.13
Biotrypcase[c] (0.5%)	1.0	0	0.36	0.06
Peptone[c] (0.5%)	1.1	0	0.43	0.06
Casamino acids[c] (0.5%)	2.0	0	0.24	0.07

[a] Results were recorded after 3 weeks of incubation at 37°C. The basal medium contained 0.2% yeast extract and different substrate as indicated (in mM in parentheses) unless otherwise shown. Tubes containing basal medium with 0.2% yeast extract but lacking substrates were used as control. All values were corrected for the small amounts of products formed in the controls.
[b] The following substrates were not utilized: alanine, glutamate, valine, isoleucine, leucine, proline, methionine, aspartate, histidine, asparagine, glutamine, arginine, lysine, glucose, saccharose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose, mannitol, succinate, malate, fumarate, citrate, α-ketoglutarate, lactate, acetate, propionate, butyrate, glycerol, ethanol, casein and gelatin.
[c] Growth was minor.

Strain ALA-1^T fermented amino acids such as serine, threonine and glycine in pure cultures and was also able to oxidize additional amino acids such as alanine, valine, leucine, isoleucine, glutamate, methionine, and aspartate only in the presence of *M. formicicum*, a well known H_2 scavenger (Table 2). The initial step in the degradation of alanline, valine, leucine and isoleucine is an NAD(P)-dependent deamination to the corresponding keto acid. Stams [7] showed that the $\Delta G°$ of this initial step, when coupled to hydrogen, is about +60 kJ/mol, and that hydrogen scavenging bacteria are needed to drive this reaction. The keto acid is probably converted further to the corresponding fatty acid by a ferredoxin-dependent oxidative decarboxylation, a reaction which is energetically more favorable ($\Delta G°'$ is about −50 kJ/mol). Consequently, strain ALA-1^T oxidized alanine, valine, leucine, and isoleucine in the presence of H_2 scavenging bacteria. The products formed from these amino acids suggests that oxidative deamination is followed by oxidative decarboxylation of the keto acid. Alanine was oxidized to acetate, valine to isobutyrate, leucine to isovalerate, isoleucine to 2-methylbutyrate. Metabolism of these amino acids in mixed culture resembles that reported for the mesophiles. *A. hydrogenoformans* [9], *S. acidaminovorans* [8], and *E. acidaminophilum* [10], and also the thermophile *Thermoanaerobacter brockii* [13].

Strain ALA-1^T oxidized glutamate to pripionate in mixed culture with *M. formicicum* and is similar to that reported for *A. hydrogenoformans* when cultured with the hydrogenotrophs *Methanobrevibacter arboriphilicus* or *Methanospirillum hungatei*. In *A. hydrogenoformans*, the energetic barrier is the oxidative

Table 2. Oxidation of amino acids and other substrates by strain ALA-1^T in mixed culture with *M. formicicum*[a]

Substrates tested[b]	Substrate degraded (mM)	Products formed (mM)[c]						
		Acetate	Propionate	2-methyl-butyrate	Isobutyrate	Isovalerate	CH_4	ΔOD_{580}
Alanine	9.0	8.7	0	0	0	0	3.0	0.14
Glutamate	6.2	0.2	6.5	0	0	0	2.3	0.10
Valine	5.3	0.1	0	0	5.1	0	2.4	0.15
Iso-leucine	5.5	0.1	0	5.0	0	0	3.3	0.14
Leucine	9.0	0.0	0	0	0	8.8	2.6	0.16
Cysteine	5.0	6.0	0	0	0	0	1.2	0.13
Threonine	8.0	7.0	0	0	0	0	1.0	0.14
Methionine*	6.3	0.2	0	0	0	0	1.2	0.11
Aspartate	6.0	7.5	0	0	0	0	0	0.08
α-Ketoglurate	5.0	2.5	3.7	0	0	0	1.1	0.09
Malate	4.0	4.2	0	0	0	0	1.0	0.08

[a] Results were recorded after 3 weeks incubation at 37°C. The basal medium contained yeast extract (0.2%) and substrates at a final concentration of 10 mM. Tubes containing basal medium with yeast extract (0.2%) and lacking substrates were used as control.
[b] The following substrates were not utilized in mixed culture: lysine, arginine, citrate, fumarate and succinate.
[c] The final concentrations were determined by subtracting values obtained from the control tubes (acetate 1.2 mM) from those of utilized substrates. n.d. not determined; hydrogen was not detected as the end-product.
[d] Unidentified end-product peak was detected by HPLC.

deamination of glutamate to α-ketoglutarate ($\Delta G^{\circ\prime}$ = +59.9 kJ/mol) which can be overcome by the addition of a hydrogen scavenger such as methanogens, leading to the production of α-ketoglutarate. The conversion of α-ketoglutarate to propionate is easily driven as it is exergonic ($\Delta G^{\circ\prime}$ = −65.7 kJ/mol) [34]. Pure cultures of both strains, when cultivated on α-ketoglutarate, produce propionate as end-product. The presence of an hydrogenotrophic methanogen facilitates α-ketoglutarate degradation leading to increased propionate production. Based on our results, we conclude that a similar metabolic pathway operates in both strains. However, a major difference exists in terms of the ability of the two strains to ferment glutamate; strain ALA-1^T is unable to fermente glutamate in pure culture whereas *A. hydrogenoformans* is able to do so, producing acetate, formate and traces of propionate [9].

Although we have established that methionine degradation depended on the removal of H_2 by mixed culture with the methanogen, *M. formicicum*, propionate, acetate methanethiol and butyrate, which are the usual end-products formed from methionine degradation, were not detected [5] by the HPLC techniques we used.

Aspartate was degraded to acetate in mixed culture, similarly to that reported for *E. acidaminophilum* [10]. Growth on aspartate also depends on the removal of H_2 and decarboxylation alone cannot be exploited bioenergetically to promote growth.

As demonstrated by our results, cysteine was poorly fermented to produce acetate and carbon dioxide (Table 1), but methanogenic conditions clearly improved its oxidation, and acetate, carbon dioxide and presumably ammonia, hydrogen, and sulfide were the end-products of metabolism.

The presence of hydrogen decreased growth and acetate production on serine and pyruvate (Table 3) whereas no growth occurred on threonine or α-ketoglutarate under a H_2 (H_2:CO_2) 80:20) atmosphere. Thus suggests that threonine and α-ketoglutarate were highly sensitive to hydrogen and that hydrogen removal by the methanogen facilitated its oxidation. In contrast, exposure to H_2 did not affect glycine degradation. This agrees with the fact that glucine can be reductively deaminated, hence the presence of hydrogen is not inhibitory.

Ecological considerations

Strain ALA-1^T ferments serine; threonine, glycine and pyruvate and not carbohydrates, suggesting that it may be involved in the turnover the amino acids in its natural ecosystems. An increase in the spectrum of amino acids used and/or the increased amounts used in coculture with the hydrogenotroph, *M. formicicum*, suggests further the important role that such microbes could play in protein rich dairy waste ecosystems. In the last decade, several anaerobic amino acid-degrading bacteria which dispose reducing equivalents formed in the oxidation of amino acids by the reduction of proton to hydrogen and/or by the reduction of bicarbonate to formate, have been described [7]. With the isolation of strain ALA-1^T, we extend our knowledge on the biodiversity of syntrophic amino acid degraders and also the ecosystem from which they could be isolated. It is obvious that further studies on the metabolism of amino acids needs to be developed especially for the non-saccharolytic amino acid degrading bacteria.

Because of the specific phenotypic and phylogenetic characteristics of strain ALA-1^T, we propose that it be designated *Aminobacterium colombiense* gen. nov., sp. nov.

Description of Aminobacterium *gen. nov.*

Aminobacterium (A.min.o.bac.té.ri.um. M.L. adj.

Table 3. Effect of hydrogen on the growth of strain ALA-1^T on different substrates[a]

Substrates	Products formed (mM)[c]			
	Acetate	Propionate	H_2	ΔOD_{580}
Serine + (H_2 + CO_2)[b]	3.8	0	n.d.	0.14
Serine	7.8	0	2.4	0.22
α-ketoglutarate + (H_2 + CO_2)[b]	0.6	0.04	n.d.	0.04
α-ketoglutarate	1.1	1.0	2.0	0.13
Glycine + (H_2 + CO_2)[b]	7.4	0	n.d.	0.10
Glycine	8.0	0	0.1	0.10
Pyruvate + (H_2 + CO_2)[b]	2.4	0	n.d.	0.03
Pyruvate	6.4	0	1.51	0.14
Threonine + (H_2 + CO_2)	0.05	0	n.d.	0.03
Threonine	3.5	0	0.35	0.10

[a] Results were recorded after 3 weeks incubation at 37°C. The basal medium contained yeast extract (0.2%) and substrates at a final concentration of 9 mM. Tubes containing basal medium with yeast extract (0.2%) and lacking substrates were used as control.
[b] H_2 + CO_2 (80:20) at 2 bar pressure.
[c] The final concentrations were determined by subtracting values obtained from the control tubes (acetate 1.2 mM) from those of utilized substrates. n.d. not determined.

Amino amino; Gr. n. *bacterion* a small rod; M.L. masc. n. *Aminobacterium* the amino acid rod).

Cells are Gram-negative slightly curved rods which occur singly or rarely as chains. No spores are formed. Non-motile. Strictly anaerobic mesophile. Growth by fermentation of a limited range of amino acids but not carbohydrates and only in the presence of yeast extract. *Aminobacterium* represents a new line of descent in the low G + C Gram-positive containing branch based on 16S rRNA sequence analysis. Habitat: Anaerobic sludge of a dairy wastewater treatment plant. The type species is *Aminobacterium colombiense*.

Description of Aminobacterium colombiense *sp. nov.*

Aminobacterium colombiense (co.lom.bi.en'se M.L. neut. adj. *colombiense* pertaining to Columbia, the origin of the isolate). Slightly curved to rod shaped bacterium with 0.2 to 0.3 by 3.0 to 4.0 μm, occurs singly or pairs. Gram-negative, non-sporeforming. Strictly anaerobic. Colonies (up to 1.0 mm) are round, smooth, lens, and white. Mesophilic. Optimal growth temperature 37°C; range for growth 20 to 42°C, pH range from 6.6 to 8.5, optimum pH 7.3 and did not require NaCl for growth but tolerated up to 1.5% with optimum growth between 0.05 and 0.5% NaCl. Yeast extract is required for growth. It ferments serine, threonine, glycine and pyruvate and uses several other amino acids in mixed culture with *M. formicicum* (alanine, glutamate, valine, leucine, isoleucine, aspartate, cysteine and methionine). No growth was observed on succinate, malate, fumarate, citrate, lactate, glucose, saccharose, ribose, xylose, cellobiose, mellobiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose, mannitol, acetate, propionate, butyrate, glycerol, ethanol, gelatin, and casein. The G + C content of the DNA is 46 mol %. The type strain is ALA-1^T (DSM 12261). Isolated from anaerobic sludge of a dairy wastewater treatment plant of Sante Fe de Bogota, Colombia.

Acknowledgements

We thank N. Zylber (BIP-CNRS) for amino acid analysis. This work was supported by grants from French Foreign Office (P.C.P) and Instituto Colombiano para el desarrollo de la Ciencia y la Tecnologia (COLCIENCIAS) (to S.B.) and in part from the Australian Research Council (to B.K.C.P.)

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