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Desulfovibrio aminophilus sp. nov., a Novel Amino Acid Degrading and Sulfate Reducing Bacterium from an Anaerobic Dairy Wastewater Lagoon

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Summary

A mesophilic strain of sulfate-reducing bacterium, designated ALA-3^T (T = type strain), was isolated from an anaerobic lagoon of a dairy wastewater treatment plant. The curved, Gram-negative, non-sporeforming cells (0.2 × 3.0–4.0 µm) existed singly or in chains, and were motile by single polar flagella. Optimum growth occurred at 35 °C and pH 7.5 on a medium containing lactate and sulfate. Thiosulfate or sulfite but not elemental sulfur, nitrate, or fumarate could also replace sulfate as an electron acceptor. Formate, alanine, aspartate, leucine, isoleucine, valine, and methionine, H_2/CO_2 and ethanol also served as electron donors with sulfate as an electron acceptor. Pyruvate, casamino acids, peptone, serine, glycine, cysteine and threonine were fermented. Sulfite and thiosulfate were disproportionated to sulfate and sulfide. The G+C content of the DNA was 66 mol % G+C. Phylogenetic analysis revealed that *Desulfovibrio africanus* was the nearest relative (similarity of 89%). Strain ALA-3^T is physiologically and phylogenetically different from other *Desulfovibrio* species, and is designated *Desulfovibrio aminophilus* sp. nov. (DSM 12254).

Key words: Sulfate-reducing bacteria – Amino acid utilization – Thiosulfate – Sulfite disproportionation – *Desulfovibrio aminophilus*

Introduction

The anaerobic degradation of proteins has not been studied as extensively as carbohydrate fermentation though the input of proteins into anaerobic environments is large (MCINERNEY, 1988). Because of the presence of proteins in almost any ecosystem, the turnover of amino acids is a very important microbiological process (GIRBAL et al., 1997). The degradation of amino acids by anaerobic bacteria has been investigated most comprehensively in fermentative *Clostridium* species (BAKER, 1981; ELSDEN et. al., 1979), but other anaerobes are also known to utilize amino acids especially in the presence of external electron acceptors or in mixed cultures with hydrogen scavengers (STAMS, 1994). In contrast, the use of amino acids by sulfate-reducing bacteria has been poorly studied, whereas their activities regarding amino acid degradation has been clearly established in marine sediments (HANSEN and BLACKBURN, 1995). Only a few members of the genera *Desulfobacterium, Desulfotomaculum* and *Desulfovibrio* are known to utilize single amino acid (REES et al., 1998; STAMS et al., 1985; STAMS and HANSEN, 1986; VAN DER MAAREL et al., 1996). Furthermore, recent reports have shown that the addition of thiosulfate to the growth medium enabled the utilization of amino acids by non sulfate-reducing bacteria such as *Thermoanaerobacter brockii* (FAUDON et al., 1995; FARDEAU et al., 1997a) and *Dethiosulfovibrio peptidovorans* (MAGOT et al., 1997). Thus, these data suggest an important role of sulfate and thiosulfate in the degradation of proteinaceous compounds. In our present study, we isolated and characterized a sulfate-reducing bacterium, designated strain ALA-3^T, which degraded peptides and amino acids as an energy source.

KESSLER, B., DE LORENZO, V., TIMMIS, K., N.: A general system to integrate *LacZ* fusion into the chromosome of gram negative bacteria: regulation of the Pm promoter of the TOL plasmid studied with all controlling elements in monocopy. Mol. Gen. Genet. 233, 293–301 (1992).

KUSHNER, D. J.: Life in high salt and solute concentrations: halophilic bacteria, p. 317–368. In: Microbial life in extreme environments (D. J. KUSHNER, ed.). London, Academic Press 1978.

LOUIS, P., GALINSKI, E. A.: Characterization of genes for the biosynthesis of the compatible solute ectoine from *Marinococcus halophilus* and osmoregulated expression in *Escherichia coli*. Microbiology 143, 1141–1149 (1997).

MEHTA, P. K., HALE, T. I., CHRISTEN, P.: Aminotransferase: demonstration of homology and division into evolutionary subgroups. Eur. J. Biochem. 214, 549–561 (1993).

MELLADO, E., MOORE, E. R., NIETO, J. J., VENTOSA, A.: Analysis of 16S rRNA gene sequences of *Vibrio costicola* strains: description of *Salinivibrio costicola* gen. nov., comb. nov. Int. J. Syst. Bacteriol. 46, 817–821 (1996).

MELLADO, E., MOORE, E. R., NIETO, J. J., VENTOSA, A.: Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui, Volcaniella eurihalina,* and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. Int. J. Syst. Bacteriol. 45, 712–716 (1995).

MIN-YU, L., ONO, H., TAKANO, M.: Gene cloning of ectoine synthase from *Halomonas* sp. Annu. Rep. Int. Center Cooperative Res. Biotechnol., Japan 16, 193–200 (1993).

NIETO, J. J., FERNÁNDEZ-CASTILLO, R., MÁRQUEZ, M. C., VENTOSA, A., RUIZ-BERRAQUERO, F.: Survey of metal tolerance in moderately halophilic eubacteria. Appl. Environ. Microbiol. 55, 2385–2390 (1989).

PETERS, P., GALINSKI, E. A., TRÜPER, H. G.: The biosynthesis of ectoine. FEMS Microbiol. Lett. 71, 157–162 (1990).

QUESADA, E., VENTOSA, A., RUIZ-BERRAQUERO, F., RAMOS-CORMENZANA, A.: *Deleya halophila*, a new species of moderately halophilic bacteria. Int. J. Syst. Bacteriol. 34, 287–292 (1984).

REGEV, R., PERI, I., GILBOA, H., AVI-DOR, Y.: ^{13}C NMR study of the interrelation between synthesis and uptake of compatible solutes in two moderately halophilic eubacteria. Arch. Biochem. Biophys. 278, 106–112 (1990).

SAMBROOK, J., FRITSCH, E. F., MANIATIS, T.: Molecular cloning: a laboratory manual, 2nd ed., N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor 1989.

SEVERIN, J., WOHLFARTH, A., GALINSKI, E. A.: The predominant role of recently discovered tetrahydropyrimidines of the osmoadaptation of halophilic eubacteria. J. Gen. Microbiol. 138, 1629–1638 (1992).

SPAINK, H. P., OKKER, R. J. H., WIJFFELMAN, C. A., PEES, E., LUGTENBERG, B. J. J.: Promoters in the nodulation region of the *Rhizobium leguminosarum* Sym plasmid pRL1JI. Plant Mol. Biol. 9, 27–39 (1987).

TAO, T., YASUDA, N., ONO, H., SHINMYO, A., TAKANO, M.: Purification and characterization of 2,4-diaminobutyric acid transaminase from *Halomonas* sp. Annu. Rep. Int. Center Cooperative Res. Biotechnol., Japan 15, 187–199 (1992).

UBBEN, D., SCHMITT, R.: Tn*1721* derivatives for transposon mutagenesis, restriction mapping and nucleotide sequence analysis. Gene 41, 145–152 (1986).

VARGAS, C., CORONADO, M. J., VENTOSA, A., NIETO, J. J.: Host range, stability and compatibility of broad host-range-plasmids and a shuttle vector in moderately halophilic bacteria. Evidence of intragenic and intergenic conjugation in moderate halophiles. Syst. Appl. Microbiol. 20, 173–181 (1997).

VENTOSA, A., GUTIÉRREZ, M. C., GARCÍA, M. T., RUIZ-BERRAQUERO, F.: Classification of *"Chromohalobacter marismortui"* in a new genus, *Chromohalobacter* gen. nov., as *Chromohalobacter marismortui* comb. nov., nom. rev. Int. J. Syst. Bacteriol. 39, 382–386 (1989).

VENTOSA, A., NIETO, J. J., OREN, A.: Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. 62, 504–544 (1998).

VENTOSA, A., NIETO, J. J.: Biotechnological applications and potentialities of halophilic microorganisms. World J. Microbiol. Biotechnol. 11, 85–94 (1995).

VREELAND, R. H., LITCHFIELD, C. D., MARTIN, E. L., ELLIOT, E.: *Halomonas elongata,* a new genus and species of extremely salt-tolerant bacteria. Int. J. Syst. Bacteriol. 30, 485–495 (1980).

VREELAND, R. H., MARTIN, E. L.: Growth characteristics, effects of temperature, and ion specificity o the halotolerant bacterium *Halomonas elongata*. Can. J. Microbiol. 26, 746–752 (1980).

VREELAND, R. H.: The family *Halomonadaceae*. pp 3181–3188. In: The prokaryotes (A. BALOWS, H. G. TRÜPER, M. DWORKIN, W. HARDER, K. H. SCHLEIFER, eds.), 2nd ed., N.Y., Springer-Verlag 1992.

WOHLFARTH, A., SEVERIN, J., GALINSKI, E. A.: The spectrum of compatible solutes in heterotrophic eubacteria of the family *Halomonadaceae*. J. Gen. Microbiol. 136, 705–712 (1990).

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Materials and Methods

Sample source: Sludge samples were collected from an anaerobic lagoon of a dairy wastewater treatment plant (SantaFe de Bogota, Colombia) by completely filling 100 ml bottles to the brim. The samples were transported to the laboratory and maintained at 4 °C until used. The pH and temperature from where the sample was collected was 6.8 and 24°C, respectively.

Media, Culture Conditions and Isolation: The Hungate technique (HUNGATE, 1969) was used throughout these studies. The basal medium consisted of (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 (IMHOFF-STUCKLE and PFENNING, 1983), 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 and cooled to room temperature. Aliquots of 5-ml from the medium were dispensed into Hungate tubes under a stream of O2-free N2 gas, and subsequently the gas phase was replaced with N_2-CO_2 (80:20). 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 (BALCH et al., 1979) were injected into each tube.

For isolation and cultivation studies, L-alanine, yeast extract and thiosulfate were injected into Hungate tubes containing basal medium, from sterile anaerobic stock solutions (×100) to give a final concentration of 10 mM, 0.1% and 10 mM respectively. The sludge sample was homogenized using a hand held homogeniser in an anaerobic chamber and subsequently used for ten-fold serial dilutions in the medium described above. Dilution series were incubated at 37 °C for 2 weeks after which ten-fold dilutions were prepared from the last positive dilution and inoculated into roll tubes containing the medium described above, fortified with 2% agar. Single colonies that developed were picked and the procedure repeated several times. All cultures derived from this procedure had similar morphology and were unable to grow in sulfate-free basal medium supplemented with glucose (20 mM), Biotrypcase (0.2%) and yeast extract (0.2%).

Growth Characterization: Growth at various temperatures, pH and in sodium chloride was studied using basal medium containing 20 mM lactate and 20 mM thiosulfate as electron donor and acceptor respectively. For pH studies, the medium was adjusted with sterile anaerobic stock solutions of 1 M HCl, 10% $NaHCO_3$ or 8% Na_2CO_3. NaCl was either weighed directly into the tubes for concentrations higher than 1% and the medium dispensed into the tubes as described above, or was injected into the pre-reduced medium from a sterile stock solution of 10% NaCl.

For studies on electron donors, the basal medium with or without yeast extract and containing 20 mM sulfate was used. The electron donors ethanol, n-propanol, butanol and benzoate were added to a final concentration of 5 mM. Pyruvate, acetate, propionate, butyrate, valerate, fumarate, succinate, malate, alanine, glutamate, valine, serine, arginine, aspartate, leucine, threonine, glycine, cysteine, isoleucine, methionine, lysine, proline, histidine, glucose, fructose, ribose, maltose, galactose, and mannitol were added at a final concentration of 10 mM. Casein (0.1%), gelatin (0.1%), peptone (0.5 and 1.0%), casamino acids (0.5 and 1.0%) and H_2 + CO_2 (80:20) were also tested. Thiosulfate (10 mM), sulfate (10 mM), sulfite (2 mM), sodium fumarate (20 mM), elemental sulfur (2%) and nitrate (10 mM) were tested as electron acceptors in the basal medium containing lactate (20 mM). Fermentation tests with lactate, pyruvate, peptone, casamino acids, serine, cysteine, glycine, threonine, glutamate, alanine, leucine, isoleucine, valine, aspartate, methionine, arginine, lysine, proline, histidine yeast extract, glucose, fructose, malate, succinate and fumarate were conducted using the basal medium.

Strain ALA-3^T was subcultured at least once under the same experimental conditions. For disproportionation of thiosulfate and sulfite studies, basal medium containing acetate (5 mM) as a carbon source and either thiosulfate (20 mM) or sulfite (5 mM) as energy source and electron acceptor were used (BAK and PFENNING, 1987). Disproportionation was verified by measuring optical density increase and the formation of sulfate and sulfide.

Analytical Techniques: Duplicate culture tubes were used throughout this study. Growth was measured by inserting tubes directly into a model UV-160A spectrophotometer (Shimadzu Corp., Kyoto, Japan) and measuring the optical density at 580 nm. Sulfide was determined photometrically (CORD-RUWISCH, 1985). Sulfate was measured according to the method of MADSEN and AAMAND (1991) and fermentation products determined as described by FARDEAU et al., 1993. Amino acid concentrations were determined by High Pressure Liquid Chromatography (MOORE et al., 1958). Growth and product formation were analyzed after 2 to 3 weeks of incubation at 37 °C. Cytochromes and desulfoviridin were determined as described by POSTGATE (1959). Cell morphology was determined using a Nikon phase-contrast microscope. For electron microscopy, cells were negatively stained with 1% sodium phosphotungstic acid (pH 7.2) as described previously (FARDEAU et al., 1997b). The G+C content was determined at DSM – Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmb, Braunschweig, using high performance liquid chromatography (HPLC) as described by MESBAH et al., (1989).

16S rRNA sequence studies: DNA was extracted from the strain ALA-3^T as described previously (ANDREWS and PATEL, 1996; REDBURN and PATEL, 1993). 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 conditions used were as follows: initial denaturation at 94 °C for 7 min, followed by 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 a final extension cycle of 55 °C for 2 min and 72 °C for 20 min. PCR products were purified using QIAquick Kit (Qiagen), the concentration estimated by comparison with the Low Mass Ladder (Gibco BRL) on an agarose gel containing ethidium bromide and sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Kit 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 (ANDREWS and PATEL, 1996), and 2.5 µg of Bovine Serum Albumin. Thermal cycling was carried out using a Rapid Cycler (Idaho Technology) at a temperature transition slope of 2, an initial denaturation of 94 °C for 15 sec, then 25 cycles of denaturation at 94 °C for 0 sec, annealing at 50 °C for 10 sec, extension at 60 °C for 3 min. The determined sequence was aligned manually and checked for accuracy manually using the alignment editor, ae2 (MAIDAK et al., 1996). The program BLAST (ALTSCHUL et al., 1990) 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 *D. oxyclinae, D. profundus, D. fructosovorans,* and *D. alcoholovorans* were extracted from GenBank database (accession numbers U33316, U90726, AF050101 and AF053751, respectively) and manually aligned with the prealigned sequences obtained from the Ribosomal Database Project (MAIDAK et al., 1996). Bootstrap and pairwise evolutionary distances based on 892 unambiguous nucleotides were computed using TREECON (VAN DE PEER et al., 1993) and the programs DNADIST (Jukes and Cantor option), NEIGHBOR-JOINING, SEQBOOT and CONSENSE programs that form part of the PHYLIP package (FELSENSTEIN, 1993).

Nucleotide sequence accession number: The 16S rRNA gene sequence of strain ALA-3^T has been deposited in the GenBank data base under accession number AF067964.

Results

Isolation

Cultures were regarded as positive after incubation at 37 °C for 2 weeks based on growth, acetate and H_2S production. Microscopic examination of the highest dilution positive culture revealed the presence of two bacterial morphotypes, viz. rod-shaped and vibrio-shaped cells. Roll tubes inoculated with this culture also showed two distinct types of colonies. One type of colony was small, white and lense-shaped, and the other was 1 mm in diameter, round, whitish and possessed smooth edges. The latter colony type was isolated in pure culture, designated ALA-3^T and characterized further.

Morphology, physiology and G+C content

Cells of strain ALA-3^T were Gram negative non-sporulating vibrios, 3.0 to 4.0 µm long and 0.2 µm wide (Fig. 1). Strain ALA-3^T was motile by means of a single polar flagellum (data not shown).

Strain ALA-3^T was a strictly anaerobic chemoorganotrophic bacterium. The optimal growth temperature was 35°C (temperature range was between 25 and 40 °C). The optimum pH for growth was around 7.5 (pH growth range between 6.7 to 8.0). Strain ALA-3^T did not require NaCl for growth but tolerated up to 2% NaCl with optimum growth occurring in the presence of 0.05 to 0.75% NaCl. The cells contained c-type cytochromes and desulfoviridin. The G+C content of strain ALA-3^T is 66 mol %.

In the presence of thiosulfate or sulfate as electron acceptor, strain ALA-3^T grew on lactate and did not require yeast extract (though 0.2% yeast extract enhanced growth). Strain ALA-3^T grew with ethanol and H_2/CO_2 only with added acetate or yeast extract. Furthermore, the following amino acids are only used in the presence of 0.2% yeast extract: alanine, leucine, isoleucine, valine, aspartate, and methionine with sulfate as an electron acceptor (Table 1). Glutamate was converted mainly to pyroglutamate and the small amounts of acetate and propionate and sulfide produced could be a result of partial oxidative deamination.

In the absence of sulfate, strain ALA-3^T used pyruvate, peptone and casamino acids. Serine, glycine, threonine and cysteine were used in the presence of yeast extract (0.2%). Serine, glycine and cysteine were fermented to acetate. Threonine was fermented to acetate and traces of propionate. Only pyroglutamate was detected from glutamate utilization in the absence of sulfate.

Sulfate, thiosulfate and sulfite were utilized as electron acceptors but not elemental sulfur, nitrate and fumarate. Thiosulfate and sulfite were disproportionated to sulfate and sulfide.

16S rRNA sequence analysis

An almost complete 16S rRNA gene sequence comprising of 1530 nucleotides, corresponding to positions 21 to 1539 respectively [*E. coli* numbering, according to WINKER and WOESE, (1991)], was determined. Sequence alignment and subsequent comparisons with sequences of representative members of domain *Bacteria* consistently placed strain ALA-3^T as a member of the family *Desulfovibrionaceae*. Maximum likelihood analysis using fastDNAml also gave similar results. Further analysis indicated that the closest relative of strain ALA-3^T was *D. africanus* (similarity of 89%). The clustering of strain ALA-3^T with the sole lineage represented by *D. africanus* is interesting but cannot be predicted with confidence as bootstrap analysis (100 data sets) was poor (48%) indicating the possibility that other close relatives are yet to be identified.

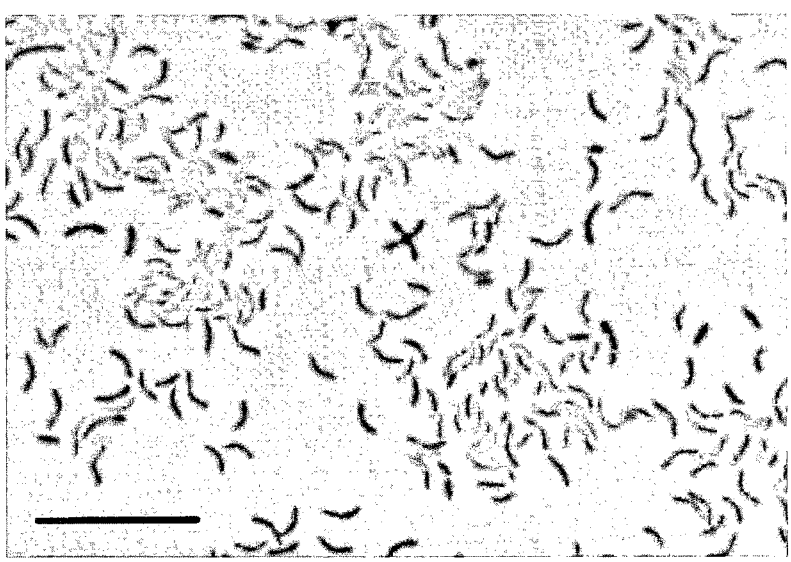
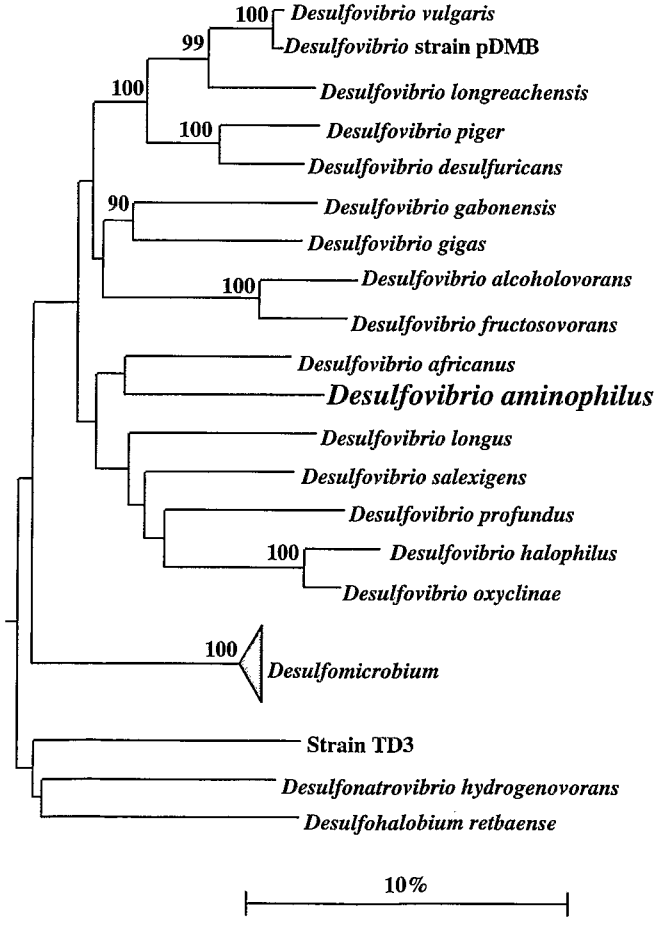


Fig 1. Phase-contrast photomicrograph of cells of strain ALA-3^T from the exponential growth phase. Bar, 10 µm.

Table 1. End-products of metabolism of strain ALA-3^T in the presence of sulfate.

Culture conditions[a]	Substrate utilized (mM)	Products formed (mM)		
		Acetate	Sulfide	Others[c]
Lactate	18	12	11.0	
Formate	15	0.0	7.0	
$H_2 + CO_2$ + Acetate[1]	nd	0	14	11 (f)
Alanine[b]	7.5	5.6	4.5	
Leucine[b]	6.5	0	2.8	6.0 (iV)
Isoleucine[b]	5.3	0	2.7	5.6 (2-mB)
Valine[b]	5.0	0.2	3.7	5.0 (iB)
Aspartate[b]	4.2	4.4	3.0	
Methionine[b, 2]	3.0	0	4.0	
Glutamate[b]	7.5	1.1	2.8	0.5 (p); 7.0 (pGlu)
Serine[b, 3]	6.0	6.0	2.0	
Cysteine[b, 3]	5.6	5.0	2.0	
Glycine[b, 3]	9.0	6.0	0.0	
Threonine[b, 3]	4.5	6.0	0.0	0.4 (p)

[a] Results were recorded after 2–3 weeks incubation at 37 °C; 10 mM sulfate was added unless otherwise indicated. All values are corrected for the small amounts of products formed in the control tubes. The following substrates were tested and were not utilized by strain ALA-3^T: argine, lysine, proline, histidine, gelatin, casein, glucose, fructose, galactose, ribose, maltose, mannitol, acetate, propionate, butyrate, valerate, fumarate, malate, succinate, *n*-propanol, butanol, glycerol or benzoate.
[b] Tested in the presence of 0.2% Y.E.
[c] Abbreviations: f, formate; p, propionate; pGlu, pyroglutamate; iv, isovalerate; 2-mB, 2-methylbutyrate; iB, isobutyrate.
[1] Tested in the presence of 20 mM sulfate.
[2] An unidentified end-product peak was detected by HPLC.
[3] These substrates are also fermented; lactate, fructose, malate, succinate and fumarate were tested but were not fermented.



Discussion

Strain ALA-3^T used a wider range of energy substrates than that reported for other *Desulfovibrio* species. It had, in particular, an important property of oxidizing or fermenting proteinaceous compounds such as peptides and amino acids (Table 1). Our results indicated that the growth of strain ALA-3^T on amino acids depended on the concentration of yeast extract. For example, growth was obtained with 0.2% but not with 0.02% yeast extract on alanine, glutamate and valine. Strain ALA-3^T reduced sulfate and thiosulfate and disproportionated thiosulfate and sulfite, suggesting that it may play a decisive role in the regulation of the electron flow in the sulfur cycle and also in protein turnover.

Fig. 2. Dendrogram based on 16S rRNA sequence data indicating the position of *Desulfovibrio aminophilus* strain ALA-3^T within the members of *Desulfovibrio* and relataed genera. All the sequences used in the analysis, with the exception of those of *D. oxyclinae*, *D. profundus*, *D. fructosovorans* and *D. alcoholovorans* (GenBank accession numbers U33316, U90726, AF050101 and AF053751 respectively), were obtained from the Ribosomal Database Project, version 5.0 (MAIDAK et al., 1996). Bootstrap values greater than 90% (generated from 100 data sets) are shown at the branching points. The triangle indicates the cluster for the genus *Desulfomicrobium*. *Escherichia coli* was used as the outgroup member. Scale bar indicates 10 nucleotide substitutions per 100 nucleotides.

Table 2. Differentiating characteristics of strain ALA-3^T and other amino acid utilizing sulfate-reducing bacteria.

Characteristics	Strain ALA-3^T	*Desulfovibrio 20020*[a]	*Desulfovibrio 20028*[b]	*Desulfovibrio acrylicus*[c]	*Desulfobacterium vacuolatum*[d]	*Desulfococcus niacini*[e]	*Desulfotomaculum ruminis*[f]
Habitat	anaerobic waste water lagoon	marine sediments	marine sediments	anoxic marine sediments	marine sediments	marine sediments	rumen
Optimal temperature	37 °C			30–37 °C	25–30 °C	29 °C	37 °C
G+C content (mol%)	66.0	47.2	42.9	45.1	45	45.8	49
Amino acids used with SO_4^{2-}	ala, val, leu, lie, asp, met	ala, ser, gly (+) val, leu, lie, cys, thr, asp	ala, ser, gly (+) val, leu, lie, cys, thr, asp	gly, ser, ala, cys	ala, glu, gin, gly, lie, leu, pro, val, ser	glu	ala
Other substrates used with SO_4^{2-}							
formate	+	+	+	+	(+)*	+	+
lactate	+	+	+	+	+	–	+
ethanol	+ (with acetate or YE)	+	+	+	(+)	+	+
acetate	–	–	–	–	(+)	+	–
pyruvate	+	+	+	–	n.r.	+	n.r.
fatty acids C-atoms	–	–	–	–	(3)–16; isobutyrate	+	–
Amino acids used without SO_4^{2-}	ser, gly, cys, thr	ser	ser	n.r.	none	n.r.	n.r.
Casamino acids used without SO_4^{2-}	+	n.r.	n.r.	+	–	n.r.	n.r.
Peptone used without SO_4^{2-}	+	n.r.	n.r.	+	–	n.r.	n.r.

n.r. not reported.
[a, b] After STAMS et al. (1985).
[c] After VAN DER MAAREL et al. (1996).
[d] After WIDDEL (1988); REES et al. (1998).
[e] After IMMHOFF-STUCKLE and PFENNING (1983).
[f] After STAMS and HANSEN (1986).
Symbols: +, utilized; (+)*, autotrophic growth; (+), poorly utilized; –, not utilized.
n.d. not determined.

Strain ALA-3^T fermented peptone and casamino acids with production of acetate and hydrogen. Sulfate or thiosulfate was not required for casamino acids and peptone utilization, but its presence improved growth during which sulfide was produced. Acetate, isovalerate, propionate and traces of succinate were produced from the oxidation of 1% peptone or 1% casamino acids in the presence of sulfate suggesting that more than one amino acid contained in both compounds were being utilized leading to the production of mixed fatty acids.

Alanine, valine, leucine and isoleucine were degraded respectively to acetate, isobutyrate, isovalerate and 2-methyl butyrate only in the presence of sulfate as an electron acceptor. This can be explained by the fact that oxidative deamination of these single amino acids can occur if the electrons produced in this step are effectively scavenged (STAMS, 1994; ÖRLYGSSON et al., 1995).

Although HANSEN and BLACKBURN (1995) provided evidence that sulfate reducing bacteria are involved in amino acid turnover in marine sediments, most laboratory studies have been limited to occasional characterization tests with amino acids as growth substrates (REES et al., 1998; VAN DER MAAREL et al., 1996). STAMS et al., (1985) showed that members of the generus *Desulfovibrio* (strains 20020 and 20028) were able to oxidize various single amino acids such as alanine and glycine, if sulfate was present. *Desulfovibrio acrylicus* (VAN DER MAAREL et al., 1996) has also been reported to use amino acids such as serine, glycine, alanine and cysteine, as electron donors in the presence of various electron acceptors. *Desulfotomaculum ruminis* was also reported to use L-alanine as an energy source (STAMS and HANSEN, 1986). Several *Desulfobacterium* spp. are known to use glutamate (IMHOFF-STUCKLE and PFENNING 1983; BRYSCH et al., 1987; SCHNELL et al., 1989). In the addition, the complete oxidizer, *Desulfobacterium vacuolatum* (REES et al., 1998) readily used glutamate, proline, isoleucine, alanine, glutamine, glycine, valine and serine only in the presence of sulfate. However strain ALA-3^T is phenotypically, genotypically (Table 2) and phylogenetically different from all these microorganisms. Furthermore, strain ALA-3^T converts glutamate to pyroglutamate and this constitutes the first report on a sulfate-reducing bacterium with this characteristic. Pyroglutamate has so far only been reported as an end-product of glutamine metabolism by a few ammonia producing bacteria from the rumen (CHEN and RUSSELL, 1988, 1989).

The utilization of peptides and amino acids in the presence of thiosulfate has been shown for non sulfate-reducing bacteria such as *Thermoanaerobacter brockii* (FARDEAU et al., 1997a) and *Dethiosulfovibrio peptidovorans* (MAGOT et al., 1997). However, strain ALA-3^T, cannot be ascribed to any of these species due to its different physiological and phylogenetic characteristics (Fig 2). Therefore strain ALA-3^T is described as a new species of the genus *Desulfovibrio*, *Desulfovibrio aminophilus* sp. nov.

Description of *Desulfovibrio aminophilus* sp. nov.

Desulfovibrio aminophilus a.mino. phi.lus. Gr. n. aminus; Gr. adj. philus loving; M.L. adj. aminophilum amino acid loving. Vibrios (0.2 by 3.0–4.0 µm) motile by one polar flagellum, occur singly or in chains. Gram-negative, non-sporeforming. Strictly anaerobic. Colonies are round with entire edges, smooth, and white. Mesophilic. Optimum growth temperature is 35 °C (temperature range for growth is between 25 to 40 °C), optimum pH 7.5 (pH range for growth is from 6.7 to 8.0) and does not require NaCl for growth. Yeast extract is not required for growth on lactate, and pyruvate in the presence of sulfate, but stimulates growth. Not autotrophic. It requires yeast extract and sulfate for growth on alanine, valine, leucine, isoleucine, aspartate and methionine. In the presence of acetate as a carbon source, growth is obtained on ethanol or $H_2 + CO_2$ in the presence of sulfate. Sulfate, sulfite, and thiosulfate serve as electron acceptor, but not elemental sulfur, fumarate and nitrate. Thiosulfate and sulfite are disproportionated to sulfate and sulfide. Does not ferment fumarate, malate, succinate, glucose, fructose, glycerol, but is able to ferment pyruvate, peptone, casamino acids, serine, glycine, threonine and cysteine. *Desulfoviridin* present. The G+C content of the DNA is 66 mol%. The type strain is ALA-3^T (DSM12254). Isolated from anaerobic sludge of a dairy wastewater treatment plant of Santa Fe de Bogota, Colombia. Adverse effects on animals and humans are not known. Because of the ability of *Desulfovibrio aminophilus* to degrade amino acids and peptides, the possibility of harmful effects cannot be excluded. Cautious handling and autoclaving of cultures before disposal is recommended.

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References

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W., LIPMAN, D. J.: Basic local alignment search tool. J. Mol. Biol. 215, 403–410 (1990).

ANDREWS, K. T., PATEL, B. K .C.: *Fervidobacterium gondwanense* sp. nov, a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. Int. J. Syst. Bacteriol. 46, 265–269 (1996).

BAK, F., PFENNING, N.: Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionating of inorganic sulfur compounds. Arch. Microbiol. 147, 184–189 (1987).

BAKER, H. A.: Amino acid degradation by anaerobic bacteria. Ann. Rev. Biochem. 50, 23–40 (1981).

BALCH, W. E., FOX, G. E., MAGRUM, R. J., WOLFE, R. S.: Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43, 260–296 (1979).

BRYSCH, K., SCHNEIDER, C., FUCHS, G., WIDDEL, F.: Lithotrophic growth of sulfate-reducing bacteria, and description of *Desulfobacterium autotrophicum* gen. nov., sp. nov. Arch. Microbiol. 148, 264–274 (1987).

CHEN, G., RUSSELL, J.B.: Fermentation of peptides and amino acids by a monensin-sensitive ruminal *Peptostreptococcus*. Appl. Environ. Microbiol. 54, 2742–2749 (1988).

CHEN, G., RUSSELL, J.B.: More monensin-sensitive, ammonia-producing bacteria from the rumen. Appl. Environ. Microbiol. 55, 1052–1057 (1989).

CORD-RUWISCH, R.: A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. J. Microbiol. Methods. 4, 33–36 (1985).

ELSDEN, S. R., HILTON, M. G.: Amino acid utilization patterns in *Clostridial* taxonomy. Arch. Microbiol. 123, 137–141 (1979).

FARDEAU, M. L., CAYOL, J. L., MAGOT, M., OLLIVIER, B.: H_2 oxidation in the presence of thiosulfate by a *Thermoanaerobacter* strain isolated from a oil-producing well. FEMS Microbiol. Lett. 13, 327–332 (1993).

FARDEAU, M.-L., PATEL, B. K. C., MAGOT, M., OLLIVIER, B.: Utilization of serine, leucine, isoleucine, and valine by *Thermoanaerobacter brockii* in the presence of thiosulfate or *Methanobacterium* sp. as electron acceptors. Anaerobe 3, 405–410 (1997a).

FARDEAU, M.-L., OLLIVIER, B., PATEL, B.K.C., MAGOT, M., THOMAS, P., RIMBAULT, A., ROCCHICCIOLI F., GARCIA, J-L.: *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. Int. J. Syst. Bacteriol. 47, 1013–1019 (1997b).

FAUDON, C., FARDEAU, M. L., HEIM, J., PATEL, B. K. C., MAGOT, M., OLLIVIER, B.: Peptide and amino acid oxidation in the presence of thiosulfate by members of the genus *Thermoanaerobacter*. Curr. Microbiol. 31, 152–157 (1995).

FELSENSTEIN, J.: PHYLIP (Phylogenetic Inference Package) version 3.51c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA (1993).

GIRBAL, L., ÖRLYGSSON, J., REINDERS, B. J., GOTTSCHAL, J. C.: Why does *Clostridium acetireducens* not use interspecies hydrogen transfer for growth on leucine? Curr. Microbiol. 35, 155–160 (1997).

HANSEN, L. S., BLACKBURN, T. H.: Amino acid degradation by sulfate-reduction bacteria: Evaluation of four methods. Limnol. Oceanogr. 40, 502–510 (1995).

HUNGATE, R.E.: A roll tube method for the cultivation of strict anaerobes. pp 117–132. In: NORRIS J. R., RIBBONS D.W. (eds). Methods in Microbiology, Vol 3 B. Academic Press, New York.(1969).

IMHOFF-STUCKLE, D., PFENNING, N.: Isolation and characterization of an nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. Arch. Microbiol. 136, 194–198 (1983).

MADSEN, T., AAMAND, J.: Effect of sulfuroxy anions on degradation of pentachlorophenol by a methanogenic enrichment culture. Appl. Environ. Microbiol. 57, 2453–2458 (1991).

MAGOT, M., RAVOT, G., CAMPAIGNOLLE, X., OLLIVIER, B., PATEL, B. K. C., FARDEAU, M. L., THOMAS, P., CROLET, J.-L., GARCIA, J.-L.: *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. Int. J. Syst. Bacteriol. 47, 818–824 (1997).

MAIDAK B.L., OLSEN, G.J., LARSEN, N., OVERBEEK, R., MCCAUGHEY, M. J., WOESE, C. R.: The ribosomal database project (RDP). Nucleic Acids Res. 24, 82–85 (1996).

MCINERNEY, M.J.: Anaerobic hydrolysis and fermentation of fats and proteins, pp. 373–409. In: Biology of anaerobic organisms. ZEHNDER, A. J. B. (ed.). John Wiley, N.Y. (1988).

MESBAH, M., PREMACHANDRAN, U., WHITMAN, W. B.: Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int. J. Syst. Bacteriol. 39, 159–167 (1989).

MOORE, S., SPACKMAN, D. H., STEIN, W.H.: Chromatography of amino acids on sulfonated polystyrene resins: An improved systems. Anal. Chem. 30, 1158–1190 (1958).

ÖRLYGSSON, J., HOUWEN, F. P., SVENSSON, B. H.: Thermophilic anaerobic amino acid degradation: deamination rates and end-product formation. Appl. Microbiol. Biotechnol. 43, 235–241 (1995).

POSTGATE, J. R.: A diagnostic reaction of *Desulphovibrio desulphuricans*. Nature 183, 481–482 (1959).

REDBURN, A. C., PATEL, B. K .C.: Phylogenetic analysis of *Desulfotomaculum thermobenzoicum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. FEMS Microbiol. Lett. 113, 81–86 (1993).

REES, G. N., HARFOOT, C. G., SHEEHY, A. J.: Amino acid degradation by the mesophilic sulfate-reducing bacterium *Desulfobacterium vacuolatum*. Arch. Microbiol. 169, 76–80 (1998).

Schnell, S., Bak, F., Pfenning, N.: Anaerobic degradation of aniline and dihydroxybenzenes by newly isolated sulfate-reducing bacteria and description of *Desulfobacter anilini*. Arch. Microbiol.152, 556–563 (1989).

STAMS, A. J .M.: Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie van Leeuwenhoek. 66, 271–294 (1994).

STAMS, A. J. M., HANSEN, T. A., SKYRING, G. W.: Utilization of amino acids as energy substrates by two marine *Desulfovibrio* strains. FEMS Microbiol. Ecol. 31, 11–15 (1985).

STAMS, A. J .M., HANSEN, T. A.: Metabolism of L-alanine in *Desulfotomaculum ruminis* and two marine *Desulfovibrio* strains. Arch. Microbiol. 145, 277–279 (1986).

VAN DER MAAREL, M., VAN BERGEIJK, S., VAN WERKHOVEN, A. F., LAVERMAN, A. M., MEIJER, W. G., STAM, W. T., HANSEN, T. A.: Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus* sp. nov. Arch. Microbiol. 166, 109–115 (1996).

VAN DE PEER, DE WACHTER, R.: TREECON: a software package for the construction and drawing of evolutionary trees. Comput. Applic. Biosci. 9, 177–182 (1993).

WIDDEL. F.: Microbiology and ecology of sulfate- and sulfur-reducing bacteria. pp. 469–585. In: Biology of anaerobic organisms. Zehnder, A.J.B. (ed.). John Wiley, N.Y. (1988).

WINKER, S., WOESE, C. R.: A definition of the domain Archaea, Bacteria and Eucarya in terms of small subunit ribosomal RNA characteristics. System. Appl. Microbiol. 13, 161–165 (1991).

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