Dethiosulfatibacter aminovorans gen. nov., sp. nov., a novel thiosulfate-reducing bacterium isolated from coastal marine sediment via sulfate-reducing enrichment with Casamino acids

Susumu Takii,¹ Satoshi Hanada,² Hideyuki Tamaki,² Yutaka Ueno,² Yuji Sekiguchi,² Akihiro Ibe³ and Katsumi Matsuura¹

Correspondence

Susumu Takii

takii-susumu@c.metro-u.ac.jp

¹Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Minamiohsawa 1-1, Hachioji 192-0397, Japan

²Research Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Higashi 1-1-1 Tsukuba Central 6, Tsukuba 305-8566, Japan

³Tokyo Metropolitan Institute of Public Health, Hyakunin-cho 3-24-1, Shinjuku-ku, Tokyo 163-0073, Japan

A sulfate-reducing enrichment culture originating from coastal marine sediment of the eutrophic Tokyo Bay, Japan, was successfully established with Casamino acids as a substrate. A thiosulfate reducer, strain C/G2^T, was isolated from the enrichment culture after further enrichment with glutamate. Cells of strain C/G2^T were non-motile rods (0.6–0.8 µm×2.2–4.8 µm) and were found singly or in pairs and sometimes in short chains. Spores were not formed. Cells of strain C/G2^T stained Gram-negatively, despite possessing Gram-positive cell walls. The optimum temperature for growth was 28–30 °C, the optimum pH was around 7.8 and the optimum salt concentration was 20–30 g l⁻¹. Lactate, pyruvate, serine, cysteine, threonine, glutamate, histidine, lysine, arginine, Casamino acids, peptone and yeast extract were fermented as single substrates and no sugar was used as a fermentative substrate. A Stickland reaction was observed with some pairs of amino acids. Fumarate, alanine, proline, phenylalanine, tryptophan, glutamine and aspartate were utilized only in the presence of thiosulfate. Strain C/G2^T fermented glutamate to H₂, CO₂, acetate and propionate. Thiosulfate and elemental sulfur were reduced to sulfide. Sulfate, sulfite and nitrate were not utilized as electron acceptors. The growth of strain C/G2^T on Casamino acids or glutamate was enhanced by co-culturing with *Desulfovibrio* sp. isolated from the original mixed culture enriched with Casamino acids. The DNA G + C content of strain C/G2^T was 41.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain C/G2^T formed a distinct cluster with species of the genus *Sedimentibacter*. The closest relative was *Sedimentibacter hydroxybenzoicus* (with a gene sequence similarity of 91 %). On the basis of its phylogenetic and phenotypic properties, strain C/G2^T (=JCM 13356^T=NBRC 101112^T=DSM 17477^T) is proposed as representing a new genus and novel species, *Dethiosulfatibacter aminovorans* gen. nov., sp. nov.

In eutrophic coastal seas where algal blooms frequently occur, an abundance of organic matter originates from phytoplankton rich in proteins (Parsons *et al.*, 1961; Hoshi

Abbreviation: SRB, sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain C/G2^T is AB218661.

Phase-contrast and transmission electron micrographs of cells of strain C/G2^T and tables detailing the metabolic products of the novel strain from amino acids in pure and co-cultures are available with the online version of this paper.

et al., 1994) and is deposited on bottom sediments. These sedimentary proteins have been considered to be hydrolysed to amino acids and further decomposed, mainly by fermentative bacteria such as members of the class *Clostridia* (MacInerney, 1988). Sulfate-reducing bacteria (SRB) that play a major role in the mineralization of organic matter in coastal sediments (Jørgensen, 1982) have been regarded as minor contributors to the decomposition of proteins and amino acids in such sediments (Burdige, 1989, 1991; Parkes *et al.*, 1989; Hansen & Blackburn, 1995). However, some species of SRB have been reported to use

several single amino acids, e.g. members of the genera *Desulfovibrio* (Stams *et al.*, 1985; van der Maarel *et al.*, 1996; Baena *et al.*, 1998), *Desulfobacterium* (Rees *et al.*, 1998), *Desulfobulbus* (Sass *et al.*, 2002), *Desulfomusa* (Finster *et al.*, 2001) and *Desulfotomaculum* (Stams & Hansen, 1986). The main substrates of SRB have been recognized as short chain fatty acids, especially acetate, which are produced by fermentative bacteria in marine sediments (Sørensen *et al.*, 1981; Winfrey & Ward, 1983; Christensen, 1984; Parkes *et al.*, 1989; Fukui *et al.*, 1997).

However, Hansen *et al.* (1993) suggested the active participation of SRB in amino acid oxidation in incubation experiments with fish food-amended sediment collected below a marine fish farm. In addition, it has been demonstrated previously that the decomposition of amino acids is suppressed by the addition of molybdate (a specific inhibitor of sulfate reduction) to sediment slurry collected in the highly eutrophic area of Tokyo Bay, Japan, where algal blooms have often been observed in warm seasons (Takii, 2003). This finding suggested that SRB have a close connection with the decomposition of amino acids on the eutrophic sea floor.

Furthermore, we have succeeded in establishing a sulfate-reducing enrichment culture with Casamino acids using sediment from Tokyo Bay as an inoculating source. Although versatile sulfate-reducers capable of using many amino acids were not found, the following two strains were successfully isolated from the enrichment: a sulfate-reducing bacterium, *Desulfovibrio* sp. strain C/L2, and a thiosulfate-reducing bacterium, strain C/G2^T. The latter strain was able to use various amino acids in the presence of thiosulfate or elemental sulfur as an electron acceptor and its growth on amino acids was enhanced by co-culturing with *Desulfovibrio* sp. strain C/L2. The results of a phylogenetic analysis based on 16S rRNA gene sequences revealed that strain C/G2^T was not closely related to any known bacterium and suggested that this novel thiosulfate-reducing isolate might represent a new taxon.

The novel isolate, strain C/G2^T originated from anoxic black sediment. The sediment was collected by an Ekman-dredge from a site (35° 37' 19" N, 140° 00' 2" E) in the inner part of Tokyo Bay. At this site, the bottom had been dredged to about 10 m below the natural sea floor to collect sand (water depth about 20 m). At the dredged site, bottom water was apt to stagnate and to be deoxygenated in the summer and the rate of sulfate reduction was also higher at this site than found in the nearby natural sea floor (Takii *et al.*, 2002).

The basal medium used for the enrichment and isolation of thiosulfate- and sulfate-reducing strains was a marine, bicarbonate-buffered, sulfide-reduced medium containing (l⁻¹): 4 g Na₂SO₄; 0.2 g KH₂PO₄; 0.25 g NH₄Cl; 20.0 g NaCl; 3.0 g MgCl₂·6H₂O; 0.5 g KCl and 0.15 g CaCl₂·2H₂O. The following were also added to the medium (l⁻¹): 1 ml SL10 trace element solution, 1 ml selenite-tungstate solution, 1 ml vitamin mixture solution,

1 ml vitamin B₁₂ solution, 30 ml NaHCO₃ solution and 7.5 ml Na₂S·9H₂O solution (Widdel & Bak, 1992). Sterile screw-capped glass bottles (70 ml) filled with the basal medium together with Casamino acids (3.5 g l⁻¹) were inoculated with a fresh sediment sample (approx. 0.5 g wet wt.) and were incubated at 30 °C. After several transfers of sulfide-positive cultures, the culture was subjected to the agar shake dilution method (Widdel & Bak, 1992). An isolated colony was transferred into liquid medium in a small screw-capped tube. Purity was investigated by phase-contrast light microscopy. Despite the repeated isolation procedure, the culture obtained contained two types of morphologically distinct cells. This culture was then successively enriched with glutamate (10 mM) as the sole substrate and the enriched cultures were subjected to the most probable number technique using the same medium. The highest dilution growing tubes were used for inoculation by the agar shake dilution method. Strain C/G2^T was isolated and maintained with a medium supplemented with glutamate. The maintenance medium was prepared by modifying the basal medium by the addition of thiosulfate (20 mM) and dithionite (approx. 40 mg l⁻¹) instead of sulfate and sulfide, respectively. All liquid media were dispensed into screw-capped tubes that were completely filled to exclude air from the vessels, unless otherwise described.

Cell morphology was examined under a phase-contrast microscope (Optiphot; Nikon). For transmission electron microscopy, a centrifuged cell pellet was fixed with 5 % (v/v) glutaraldehyde and 1 % (v/v) osmium tetroxide. Ultra-thin sections of the sample, embedded in epoxy resin, were prepared with a Reichert ultramicrotome. The sections were post-stained with uranyl acetate and lead citrate and examined using a transmission electron microscope (H-7000; Hitachi). Gram staining was performed according to the Hucker method (Doetsch, 1981) and the KOH test was performed as described by Gregersen (1978).

Duplicate culture tubes were used in all experiments. Growth at various temperatures, pH values and NaCl concentrations was studied using the maintenance medium with added glutamate as described above. The pH was adjusted with 2 M HCl or 1 M Na₂CO₃. In NaCl tolerance tests, various amounts of NaCl (0–60 g l⁻¹) were added. The utilization of electron donors was tested in the maintenance medium and was evaluated by monitoring the growth (determination of the optical density of the culture) for longer than one month and by the production of hydrogen sulfide as detected by cupric reagent (Widdel & Bak, 1992). Fermentation was tested with the maintenance medium without thiosulfate. Thiosulfate, sulfate, sulfite, elemental sulfur and nitrate were tested as the sole electron acceptor in the maintenance media without thiosulfate.

The optical density of cultures was measured at 610 nm by inserting the culture tubes into the cuvette holder of a

photometer (Tokyo Photoelectric Co.). Catalase was detected by pouring 3 % H₂O₂ solution onto a cell pellet in a centrifugation tube. Indole production was tested as described by Smibert & Krieg (1981). The total DNA of the isolate was extracted as described previously (Kamagata & Mikami, 1991) and digested with P1 nuclease using a GC kit (Yamasa Shoyu Co.). The DNA G+C content was measured with an HPLC system (model LC-6A; Shimadzu) equipped with a CLC-ODS column (6 × 150 mm). For the analysis of end products, a 40 ml sample of maintenance medium in 70 ml serum vials was used. The gas phase of the vial was replaced with nitrogen gas. The optical density of the liquid cultures was measured in a 1 cm cuvette at 585 nm with a spectrophotometer (UV-1600; Shimadzu). Sulfide was determined by the method of Cline (1969). After filtration of the culture with 0.22 µm Millipore filters, dissolved organic carbon was analysed by a total organic carbon analyser (TOC-500; Shimadzu). Single amino acids present in the culture media were quantified using the cuprizone method (Janssen & Barea, 1989). Amino acids in the Casamino acids-amended culture were analysed by an amino acid analyser (L8500; Hitachi). Organic acids in the cultures were determined by HPLC as described previously (Purdy *et al.*, 1997) or GC (GC-8A; Shimadzu) equipped with a packed column (FAL-M 10%, Shincarbon A; Shinwa Chem. Ind. Ltd) and a flame ionized detector. The column and detector temperatures were 140 and 200 °C, respectively. Hydrogen in the gas phase was analysed by GC (GC-14A; Shimadzu) equipped with a Porapak Q (Waters) column and a thermal conductivity detector. Nitrogen was used as the carrier gas for both GCs.

The 16S rRNA gene fragments were amplified by PCR (Hiraishi, 1992) and sequenced directly on a DNA Sequencer (ABI 310; Applied Biosystems) using a BigDye Terminator version 1.1 cycle terminator kit (Applied Biosystems). The 16S rRNA gene sequence obtained was aligned and compared with reference sequences using the ARB program package (<http://www.arb-home.de/>). The phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with the ARB program package. Bootstrap analysis was performed for 1000 replicates (Felsenstein, 1985).

A stable, sulfate-reducing culture isolated from a sediment sample collected in the inner part of Tokyo Bay was successively enriched with Casamino acids after several transfers of sulfide-positive cultures. Strain C/G2^T was isolated from the enrichment culture after further enrichment with glutamate and was used for detailed characterization.

Strain C/G2^T showed a rod-shaped morphology (0.6–0.8 µm × 2.2–4.8 µm (see Supplementary Fig. S1 in IJSEM Online) without motility. The cells occurred as single cells or pairs and short chains were sometimes formed, especially under sub-optimum conditions. Spore formation was never observed under the microscope under any culture conditions. Although the results of Gram staining

and the KOH lysis test were negative, the cell-wall ultrastructure resembled that of Gram-positive bacteria (see Supplementary Fig. S2 with the online version of this paper).

The optimum growth temperature for strain C/G2^T on glutamate and thiosulfate was 28–30 °C. No growth was observed at 10 or 40 °C. Only faint growth occurred at 37 °C and most cells formed chains under this condition. The strain grew at an initial pH range of between 5.9 and 8.8, with an optimum pH of around 7.8. Strain C/G2^T was able to grow in media supplemented with NaCl at concentrations of 8–55 g l⁻¹ and optimal growth took place at NaCl concentrations of between 20 and 30 g l⁻¹. Some vitamins were required for growth of the novel strain on glutamate and thiosulfate as no growth was observed at the second transfer (1 % inoculation size) to the vitamin-free medium. The addition of yeast extract greatly stimulated both the growth rate and cell yield of the novel strain. Strain C/G2^T showed fermentative growth on various fatty acids (20 mM) and amino acids (10 mM) as a single substrate, e.g., lactate, pyruvate, serine, cysteine, threonine, glutamate, histidine, lysine and arginine. Complex substrates such as Casamino acids (3.5 g l⁻¹), peptone (5 g l⁻¹) and yeast extract (1 g l⁻¹) were also found to be suitable, whereas gelatin was not. Sugars (20 mM) such as glucose, mannose, maltose and sucrose did not support fermentative growth. A Stickland reaction was observed with alanine, leucine or isoleucine as an electron donor and glycine as the electron acceptor, but the strain did not ferment these substrates as a sole substrate. Indole production was positive in the absence of the electron acceptor. The novel strain was able to use thiosulfate (20 mM) and elemental sulfur (1 g l⁻¹) as an electron acceptor, but not sulfate (20 mM), sulfite (5 mM) or nitrate (10 mM). The addition of thiosulfate enabled strain C/G2^T to grow on the following unsuitable fermentative substrates: fumarate, alanine, proline, phenylalanine, tryptophan, glutamine and aspartate.

The metabolic products of Casamino acids and several single amino acids generated by strain C/G2^T were examined with or without thiosulfate (see Supplementary Table S1 in IJSEM Online). The best growth took place in the culture supplemented with thiosulfate and Casamino acids containing 15 amino acids (glutamate, proline, lysine, aspartate, valine, serine, alanine, leucine, threonine, glycine, arginine, isoleucine, phenylalanine, histidine and methionine, in order of molar concentration). More than 96 % of the initial concentration of all amino acids, apart from alanine, in Casamino acids was consumed after 5 days incubation in the presence of thiosulfate. In the absence of thiosulfate, more than 94 % of the initial concentration of only five amino acids (glutamate, lysine, serine, arginine and histidine) was consumed after 10 days incubation. In addition, the final concentration of alanine increased to 141 % of the initial concentration under the latter condition. Thus, the addition of thiosulfate markedly increased the cell yield and growth rate, suggesting that the

novel strain is capable of thiosulfate respiration. Acetate and propionate were the main end products from Casamino acids and glutamate, either in the presence or absence of thiosulfate. The ratio of acetate to propionate was also not affected by the addition of thiosulfate. The fermentation products from glutamate were similar to those of *Caloramator coolhaasii* (Plugge *et al.*, 2000). The only main organic product was acetate in the culture on arginine with or without thiosulfate. Acetate, propionate and an unidentified compound (presumably a fatty acid detected by HPLC) were mainly produced from histidine and proline with thiosulfate. Formate was produced from histidine in place of the unidentified compound when the novel strain was cultured without thiosulfate. Acetate and the unidentified compound were detected from cysteine. Hydrogen was produced from all substrates examined in the absence of thiosulfate, but was produced in lesser amounts in the presence of thiosulfate, as also observed in the cultures on glutamate and histidine.

Growth of strain C/G2^T on Casamino acids and glutamate was clearly enhanced by co-culturing with *Desulfovibrio* sp. strain C/L2 which was isolated from the original mixed culture enriched with Casamino acids (see Supplementary Table S2). All 15 amino acids initially detected were almost completely utilized in the co-culture, while only four (lysine, valine, arginine and histidine) were used by 5 days of incubation by strain C/G2^T alone. In the pure culture of strain C/L2, only serine, alanine, threonine and glycine were used. Although strain C/L2 could not utilize glutamate, the co-culture showed active sulfate reduction in the glutamate medium. Hydrogen was not accumulated in the co-culture with both substrates. These results clearly suggest that the fermentation of strain C/G2^T is enhanced by the consumption of H₂ by the *Desulfovibrio* strain. This syntrophic relationship, known as interspecies H₂ transfer, could play an important role in the decomposition of amino acids in sediments *in situ*, as well as in the enrichment culture (Stams & Hansen, 1984).

Neither catalase nor desulfovibridin were detected. The G + C content of the genomic DNA of the novel strain was 41.0 mol%.

Phylogenetic analysis of a nearly full length 16S rRNA gene sequence (1479 bp) from strain C/G2^T showed that the novel strain belongs to the order *Clostridiales* of the phylum *Firmicutes*, but exhibits less than 92 % sequence similarity with any known cultured bacteria (Fig. 1). The closest relatives of strain C/G2^T were *Sedimentibacter hydroxybenzoicus* (gene sequence similarity of 91 %), *Clostridium acidurici* (90 %), *Sedimentibacter saalensis* (91 %), *Soehngenia saccharolytica* (92 %), *Sporanaerobacter acetigenes* (92 %), *Clostridium purinilyticum* (91 %) and *Eubacterium angustum* (91 %). The novel strain formed a distinct cluster with members of the genus *Sedimentibacter* (Breitenstein *et al.*, 2002) with a high bootstrap value (>95 %), but was far distant from the clostridial clusters XI, XII and XIII (Collins *et al.*, 1994).

Table 1 shows the differential characteristics between strain C/G2^T and closely related species. Like strain C/G2^T, *Sedimentibacter hydroxybenzoicus* and *Sedimentibacter saalensis* could ferment several amino acids but could not use gelatin or sugars. Furthermore, *Sedimentibacter saalensis* reduced thiosulfate and fermented pyruvate. These features were quite similar to those of strain C/G2^T. However, these *Sedimentibacter* species were spore-forming curved rods isolated from freshwater environments and did not produce H₂, whereas strain C/G2^T was a rod-shaped marine bacterium showing no sporulation, but producing H₂. Another species of the genus *Sedimentibacter*, '*Sedimentibacter hongkongensis*', the physiological characteristics of which have not yet been described in detail, was isolated from a blood culture of a patient (Woo *et al.*, 2004). Recently, many thiosulfate- and/or sulfur-reducing bacteria belonging to the order *Clostridiales* have been found and described. These include the thiosulfate-reducing *Alkaliphilus transvaalensis* (Takai *et al.*, 2001) and *Fusibacter paucivorans* (Ravot *et al.*, 1999) in clostridial

Fig. 1. Phylogenetic tree indicating the position of strain C/G2^T among representatives of cluster XI, XII, and XIII of the clostridia (Collins *et al.*, 1994). The tree is based on a distance matrix of 16S rRNA gene sequences and was constructed using the neighbour-joining method. *Thermoanaerobacterium saccharolyticum* DSM 7060^T (GenBank accession number L09169) was used as an outgroup (not shown). Asterisks indicate bacteria able to use thiosulfate and/or elemental sulfur as an electron acceptor. Filled circles at nodes indicate branches with a bootstrap value of >95 %; open circles at nodes indicate branches with a bootstrap value of >80 %. Bar, 2 % sequence divergence.

Table 1. Characteristics used for distinguishing strain C/G2^T from related species

Taxa: 1, strain C/G2^T (data from this study); 2, *Sedimentibacter hydroxybenzoicus* (Zhang *et al.*, 1994); 3, *Sedimentibacter saalensis* (Breitenstein *et al.*, 2002); 4, '*Sedimentibacter hongkongensis*' (Woo *et al.*, 2004). +, Positive or used; –, negative or not used; (+), used only by Stickland reaction; ND, not described. The following characteristics tested negative for all strains: sulfate reduction (except taxa 3 and 4, ND), nitrate reduction, fermentation of carbohydrates and gelatin hydrolysis (except taxon 4, ND). All strains utilize lysine except for taxon 4 (ND).

Characteristic	1	2	3	4
Cell morphology	Straight rod	Slightly curved rod	Slightly curved rod	Straight or slightly curved rod
Gram stain	–	–	+	–
Spore formation	–	+	+	+
Motility	–	–	+	+
Temperature range for growth (°C)	15–37	12–41	14–40	ND
Optimum temperature for growth (°C)	28–30	33–34	37	ND
pH range for growth	5.9–8.8	6.1–10	5.8–8.0	ND
Optimum pH for growth	7.8	7.2–8.2	6.8–7.3	ND
DNA G + C content (mol%)	41.0	35.5	34	ND
Catalase production	–	–	–	+
Indole production	+	+	–	+
H ₂ production	+	–	–	ND
Thiosulfate reduction	+	–	+	ND
Substrates utilized:				
Lactate	+	–	ND	ND
Pyruvate	+	–	+	ND
Glycine	–	+	+	ND
Arginine	+	(+)	(+)	ND
Source	Marine sediment	Freshwater sediment	Freshwater sediment	Human clinical specimen

cluster XI, thiosulfate-reducing *Soehngenia saccharolytica* (Parshina *et al.*, 2003) and sulfur-reducing *Sporanaerobacter acetigenes* (Hernández-Eugenio *et al.*, 2002a) in cluster XII and *Clostridium thiosulfatireducens* (Hernández-Eugenio *et al.*, 2002b) and *Clostridium peptidivorans* (Mechichi *et al.*, 2000) in cluster I. All these thiosulfate- and/or sulfur-reducing species of the order *Clostridiales* belong to clostridial clusters I, XI or XII and are phylogenetically distant from strain C/G2^T. In addition, in contrast to strain C/G2^T, spore formation has been observed in all these species except for *Fusibacter paucivorans* and they also showed clear differences from strain C/G2^T in the following characteristics: fermentation of sugars (*Fusibacter paucivorans*, *Soehngenia saccharolytica* and *Sporanaerobacter acetigenes*) and hydrolysis of gelatin (*Clostridium thiosulfatireducens* and *Clostridium peptidivorans*). Strain C/G2^T, on the other hand, phenotypically resembled the thiosulfate-reducing bacteria *Dethiosulfatibacter peptidovorans* (Magot *et al.*, 1997), *Dethiosulfatibacter russenensis*, *Dethiosulfatibacter marinus* and *Dethiosulfatibacter acidaminovorans* (Surkov *et al.*, 2001) that belong to the other family in this order, the family *Syntrophomonadaceae*. These species are slightly halophilic, non-sporulating and utilize five to eleven amino acids, including glutamate, as a single substrate in the presence of thiosulfate as an electron acceptor, but are unable to use sugars. In spite of the phenotypic similarities, these species of the genus *Dethiosulfatibacter* were far distant from strain C/G2^T when analysed phylogenetically. On the basis of its phylogenetic and phenotypic properties, we

propose to assign strain C/G2^T as representing a novel species of a new genus in the order *Clostridiales*, with the name *Dethiosulfatibacter aminovorans* gen. nov., sp. nov.

Description of *Dethiosulfatibacter* gen. nov.

Dethiosulfatibacter (De.thio.sul.fa.ti.bac.ter. L. pref. *de* from; N.L. n. *thiosulfas* -atis thiosulfate; N.L. masc. n. *bacter* a rod or staff; N.L. masc. n. *Dethiosulfatibacter* a rod-shaped thiosulfate-reducer).

Rod-shaped cells, singly or in pairs and sometimes forming short chains. Gram stain is negative, non-motile, no spore formation. Strictly anaerobic, mesophilic, neutrophilic and halophilic. Thiosulfate and elemental sulfur can serve as terminal electron acceptors, but sulfate, sulfite and nitrate are not reduced. Amino acids and some organic acids are incompletely oxidized by thiosulfate or sulfur reduction and are fermented to short chain fatty acids such as acetate and propionate. The genus *Dethiosulfatibacter* belongs to the order *Clostridiales* in the phylum *Firmicutes*. The type species is *Dethiosulfatibacter aminovorans*.

Description of *Dethiosulfatibacter aminovorans* sp. nov.

Dethiosulfatibacter aminovorans (a.mi.no.vo'rans. N.L. neut. n. *aminum* amine; L. part. adj. *vorans* devouring; N.L. part. adj. *aminovorans* devouring amino acids).

Cells with Gram-positive cell wall but stain Gram-negatively. Cells are non-motile, rod-shaped ($0.6\text{--}0.8\ \mu\text{m} \times 2.2\text{--}4.8\ \mu\text{m}$), occur singly or in pairs and sometimes in short chains with no spore formation. Exhibits anaerobic growth by thiosulfate or sulfur reduction or fermentation. Lactate, pyruvate, serine, cysteine, threonine, glutamate, histidine, lysine, arginine, Casamino acids, peptone and yeast extract are fermented. Fumarate, alanine, proline, phenylalanine, tryptophan, glutamine and aspartate are utilized only in the presence of thiosulfate. Acetate, propionate and other short chain fatty acids are produced as the result of fermentation or incomplete oxidation. Acetate, propionate, butyrate, malate, succinate, ethanol, glycine, valine, leucine, isoleucine, methionine, tyrosine, asparagine, gelatin and sugars (glucose, mannose, maltose and sucrose) are not utilized. Vitamins are required for the growth on glutamate and thiosulfate. Yeast extract greatly stimulates growth. Mesophilic, neutrophilic organism isolated from a marine environment. Optimum growth takes place at a pH of around 7.8, temperature of $28\text{--}30\ ^\circ\text{C}$ and salt concentration of $20\text{--}30\ \text{g l}^{-1}$. Desulfoviridin and catalase are absent. Indole production is positive. The G + C content of genomic DNA is 41.0 mol%.

The type strain, C/G2^T (=JCM 13356^T=NBRC 101112^T=DSM 17477^T), was isolated from an enrichment culture with Casamino acids that was inoculated with anoxic sediment from a highly eutrophic area of Tokyo Bay, Japan.

Acknowledgements

The authors wish to acknowledge Hideyuki Tanaka, Kunio Kohata, Yasuo Nakamura, Hisako Ogura, and Shunji Takeshita together with the crew of V *Kiyosumi* of the Department of Environment, Chiba Prefectural Government, for their valuable help during sampling. This study was supported in part by a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Fund for Special Research Projects at Tokyo Metropolitan University.

References

- Baena, S., Fardesu, M.-L., Labat, M., Ollivier, B., Garcia, J.-L. & Patel, B. K. C. (1998). *Desulfovibrio aminophilus* sp. nov., a novel amino acid degrading and sulfate reducing bacterium from an anaerobic dairy wastewater lagoon. *Syst Appl Microbiol* **21**, 498–504.
- Breitenstein, A., Wiegel, J., Haertig, C., Weiss, N., Andeessen, J. R. & Lechner, U. (2002). Reclassification of *Clostridium hydroxybenzoicum* as *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., and description of *Sedimentibacter saalensis* sp. nov. *Int J Syst Evol Microbiol* **52**, 801–807.
- Burdige, D. (1989). The effects of sediment slurring on microbial processes, and the role of amino acids as substrates for sulfate reduction in anoxic marine sediments. *Biogeochemistry* **8**, 1–23.
- Burdige, D. J. (1991). Microbial processes affecting alanine and glutamic acid in anoxic marine sediments. *FEMS Microbiol Lett* **85**, 211–232.
- Christensen, D. (1984). Determination of substrates oxidized by sulfate reduction in intact cores of marine sediments. *Limnol Oceanogr* **29**, 189–192.
- Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* **14**, 454–458.
- Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.
- Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society of Microbiology.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Finster, K., Thomsen, T. R. & Ramsing, N. B. (2001). *Desulfomusa hansenii* gen. nov., sp. nov., a novel marine propionate-degrading, sulfate-reducing bacterium isolated from *Zostera marina* root. *Int J Syst Evol Microbiol* **51**, 2055–2061.
- Fukui, M., Suh, J., Yonezawa, Y. & Urushigawa, Y. (1997). Major substrates for microbial sulfate reduction in the sediments of Ise Bay, Japan. *Ecol Res* **12**, 201–209.
- Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.
- Hansen, L. S. & Blackburn, T. H. (1995). Amino acid degradation by sulfate-reducing bacteria: evaluation of four methods. *Limnol Oceanogr* **40**, 502–510.
- Hansen, L. S., Holmer, M. & Blackburn, T. H. (1993). Mineralization of organic nitrogen and carbon (fish food) added to anoxic sediment microcosms: role of sulphate reduction. *Mar Ecol Prog Ser* **102**, 199–204.
- Hernández-Eugenio, G., Fardeau, M.-L., Cayol, J.-L., Patel, B. K. C., Thomas, P., Macarie, H., Garcia, J.-L. & Ollivier, B. (2002a). *Sporanaerobacter acetigenes* gen. nov., sp. nov., a novel acetigenic, facultatively sulfur-reducing bacterium. *Int J Syst Evol Microbiol* **52**, 1217–1223.
- Hernández-Eugenio, G., Fardeau, M.-L., Cayol, J.-L., Patel, B. K. C., Macarie, H., Garcia, J.-L. & Ollivier, B. (2002b). *Clostridium thiosulfatireducens* sp. nov., a proteolytic, thiosulfate- and sulfur-reducing bacterium isolated from an upflow anaerobic sludge blanket (USAB) reactor. *Int J Syst Evol Microbiol* **52**, 1461–1468.
- Hiraishi, A. (1992). Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. *Lett Appl Microbiol* **15**, 210–213.
- Hoshi, J., Ogawa, H. & Ogura, N. (1994). The amino acid distribution and behavior of different particle sizes in surface waters of the estuarine and inner part of Tokyo Bay. *Chikyukagaku (Geochemistry)* **28**, 1–14 (in Japanese with English summary).
- Janssen, P. H. & Barea, H. (1989). Rapid determination of amino acid concentrations in microbiological media: evaluation of Borchers' cuprizon method. *J Microbiol Methods* **10**, 311–316.
- Jørgensen, B. B. (1982). Mineralization of organic matter in the sea bed – the role of sulphate reduction. *Nature* **296**, 643–645.
- Kamagata, Y. & Mikami, E. (1991). Isolation and characterization of a novel thermophilic *Methanosaeta* strain. *Int J Syst Bacteriol* **41**, 191–196.
- MacInerney, M. J. (1988). Anaerobic hydrolysis and fermentation of fats and proteins. In *Biology of Anaerobic Microorganisms*, pp. 373–415. Edited by A. J. B. Zehnder. New York: John Wiley & Sons.
- Magot, M., Ravot, G., Campaignolle, X., Ollivier, B., Patel, B. K. C., Fardeau, M.-L., Thomas, P., Crolet, J.-L. & Garcia, J.-L. (1997). *Dethiosulfatibacter 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.

Mechichi, T., Fardeau, M.-L., Labat, M., Garcia, J.-L., Verh, F. & Patel, B. K. C. (2000). *Clostridium peptidivorans* sp. nov., a peptide-fermenting bacterium from an olive mill wastewater treating digester. *Int J Syst Evol Microbiol* **50**, 1259–1264.

Parkes, R. J., Gibson, G. R., Mueller-Harvey, I., Buckingham, W. J. & Herbert, R. J. (1989). Determination of the substrates for sulphate-reducing bacteria within marine and estuarine sediments with different rates of sulphate reduction. *J Gen Microbiol* **135**, 175–187.

Parshina, S. N., Kleerebezem, R., Sanz, J. L., Lettinga, G., Nozhevnikova, A. N., Kostrikina, N. A., Lysenko, A. M. & Stams, A. J. M. (2003). *Soehngenia saccharolytica* gen. nov., sp. nov. and *Clostridium amygdalinum* sp. nov., two novel anaerobic, benzaldehyde-converting bacteria. *Int J Syst Evol Microbiol* **53**, 1791–1799.

Parsons, T. R., Stephens, K. & Strickland, J. D. H. (1961). On the chemical composition of eleven species of marine phytoplankters. *J Fish Res Board Can* **18**, 1001–1016.

Plugge, C. M., Zoetendal, E. G. & Stams, A. J. M. (2000). *Caloramator coolhaasii* sp. nov., a glutamate-degrading, moderately thermophilic anaerobe. *Int J Syst Evol Microbiol* **50**, 1155–1162.

Purdy, K. J., Nedwell, D. B., Embley, T. M. & Takii, S. (1997). Use of 16S rRNA-targeted oligonucleotide probes to investigate the occurrence and selection of sulfate-reducing bacteria in response to nutrient addition to sediment slurry microcosms from a Japanese estuary. *FEMS Microbiol Ecol* **24**, 221–234.

Ravot, G., Magot, M., Fardeau, M.-L., Patel, B. K. C., Thomas, P., Garcia, J.-L. & Ollivier, B. (1999). *Fusibacter paucivorans* gen. nov., sp. nov., an anaerobic, thiosulfate-reducing bacterium from an oil-producing well. *Int J Syst Bacteriol* **49**, 1141–1147.

Rees, G. N., Harfoot, C. G. & Sheehy, A. J. (1998). Amino acid degradation by the mesophilic sulfate-reducing bacterium *Desulfobacterium vacuolatum*. *Arch Microbiol* **169**, 76–80.

Saitou, N. & Nei, M. (1987). The neighbor joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sass, A., Rutters, H., Cypionka, H. & Sass, H. (2002). *Desulfobulbus mediterraneus* sp. nov., a sulfate-reducing bacterium growing on mono- and disaccharides. *Arch Microbiol* **177**, 468–474.

Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society of Microbiology.

Srensen, J., Christensen, D. & Jrgensen, B. B. (1981). Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. *Appl Environ Microbiol* **42**, 5–11.

Stams, A. J. M. & Hansen, T. A. (1984). Fermentation of glutamate and other compounds by *Acidaminobacter hydrogeniformans* gen. nov., sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulfate-reducing and methanogenic bacteria. *Arch Microbiol* **137**, 329–337.

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

Stams, A. J. M., Hansen, T. A. & Skyring, G. W. (1985). Utilization of amino acids as energy substrates by two marine *Desulfovibrio* strains. *FEMS Microbiol Ecol* **31**, 11–15.

Surkov, A. V., Dubinina, G. A., Lysenko, A. M., Glckner, F. O. & Kuever, J. (2001). *Dethiosulfovibrio russensis* sp. nov., *Dethiosulfovibrio marinus* sp. nov. and *Dethiosulfovibrio acidaminovorans* sp. nov., novel anaerobic, thiosulfate- and sulfur-reducing bacteria isolated from ‘Thiodendron’ sulfur mats in different saline environments. *Int J Syst Evol Microbiol* **51**, 327–337.

Takai, K., Moser, D. P., Onstott, T. C., Spoelstra, N., Pfiffner, S. M., Dohnalkova, A. & Fredrickson, J. K. (2001). *Alkaliphilus transvaalensis* gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. *Int J Syst Evol Microbiol* **51**, 1245–1256.

Takii, S. (2003). Amino acids as main substrates for sulfate-reducing bacteria in surface sediment of a eutrophic bay. *J Gen Appl Microbiol* **49**, 329–336.

Takii, S., Tanaka, H., Kohata, K., Nakamura, Y., Ogura, H. & Takeshita, S. (2002). Seasonal changes in sulfate reduction in sediments in the inner part of Tokyo Bay. *Microbes Environ* **17**, 10–17.

van der Maarel, M. J. E. C., van Bergeijk, S., van Werkhoven, A. F., Laverman, A. M., Meijer, W. G., Stam, W. T. & Hansen, T. A. (1996). Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus* sp. nov. *Arch Microbiol* **166**, 109–115.

Widdel, F. & Bak, F. (1992). Gram-negative mesotrophic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn, vol. 4, pp. 3352–3378. Edited by A. Balows, H. G. Trper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer-Verlag.

Winfrey, M. R. & Ward, D. M. (1983). Substrates for sulfate reduction and methane production in intertidal sediments. *Appl Environ Microbiol* **45**, 193–199.

Woo, P. C. Y., Teng, J. L. L., Leung, K.-w., Lau, S. K. P., Wong, M. K. M. & Yuen, K.-y. (2004). Bacteremia in a patient with colonic carcinoma caused by a novel *Sedimentibacter hongkongensis* sp. nov. *Diagn Microbiol Infect Dis* **50**, 81–87.

Zhang, X., Mandelco, L. & Wiegel, J. (1994). *Clostridium hydroxybenzoicum* sp. nov., an amino acid-utilizing, hydroxybenzoate-decarboxylating bacterium isolated from methanogenic freshwater pond sediment. *Int J Syst Bacteriol* **44**, 214–222.