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Desulfovibrio zosterae sp. nov., a new sulfate reducer isolated from surface-sterilized roots of the seagrass *Zostera marina*

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A sulfate-reducing bacterium, designated strain lac^T, was isolated from surface-sterilized roots of the benthic macrophyte *Zostera marina.* Cells were motile by means of a single polar flagellum. Strain lac^T utilized lactate, pyruvate, malate, ethanol, L-alanine, fumarate, choline and fructose with sulfate as electron acceptor. In addition, fumarate, pyruvate and fructose were also degraded without an external electron acceptor. Sulfate could be substituted with thiosulfate, sulfite and elemental sulfur. Optimal growth was observed between 32·5 and 34·5 °C, at an NaCl concentration of 0·2 M and in a pH range between 6·8 and 7·3. The G+C content of the DNA was 42·7±0·2 mol%. Desulfoviridin and catalase were present. Strain lac^T contained *c*-type cytochromes. Comparative 16S rRNA gene sequence analysis and the fatty acid pattern grouped this isolate into the genus *Desulfovibrio*. However, strain lac^T differs from all other described *Desulfovibrio* species on the bases of its 16S rRNA gene sequence, the G+C content, its cellular lipid pattern and the utilization pattern of substrates. These characteristics establish strain lac^T (= DSM 11974^T) as a novel species of the genus *Desulfovibrio*, for which the name *Desulfovibrio zosterae* sp. nov. is proposed.

Keywords: root-associated bacteria, seagrass, *Desulfovibrio zosterae* sp. nov., fructose metabolism, sulfate reducer

INTRODUCTION

Sulfate-reducing bacteria have been isolated from a large number of different habitats, which has led to the conclusion that they are widespread in nature (Widdel & Bak, 1992). Typically, sulfate-reducers are found in anoxic, marine environments where sulfate is available in excess (Widdel, 1988; Jørgensen & Bak, 1991), but they have also been isolated from freshwater environments such as lake sediment (Bak & Pfennig, 1991), from anaerobic digesters (Oude Elferink *et al*., 1995), the rumen of sheep (Howard & Hungate, 1976) and the intestines of humans and termites (Beerens & Romond, 1977; Gibson *et al*., 1988; Trinkerl *et al*., 1990).

Recently, we demonstrated that high sulfate-reducing activity was associated with sediment-free roots and rhizomes of the marine macrophyte *Zostera marina* (Blaabjerg & Finster, 1998). We also showed that sulfate-reducing activity prevailed after surface sterilization of roots and rhizomes with hypochlorite (1·05%, w/v) for 30 s. This finding led us to carry out the enrichment and isolation of root-cortex-inhabiting sulfate reducers.

In the present communication, we report on the enrichment, isolation and phenotypic and genotypic characterization of strain lac^T, a mesophilic, marine, fructose-oxidizing sulfate reducer isolated from surface-sterilized root tissue of the marine angiosperm *Zostera marina*.

METHODS

Source of organisms. Strain lac^T was isolated from a mixed culture obtained from surface-sterilized roots of the eelgrass *Zostera marina*. The plants were obtained from a dense eelgrass meadow, in Løgstør Broad, Denmark. Root-surface sterility was achieved by washing sediment-free roots in a saline, 1·05% (w/v) hypochlorite solution for 30 s (Blaabjerg & Finster, 1998).

Strain lac^T was isolated by repeated application of the agar-shake dilution method in an iron-rich APW medium, previously described by Coates *et al*. (1995). Agar (1%,

The EMBL/GenBank/DDBJ accession number for the 16S rDNA sequence of strain lac^T (= DSM 11974^T) reported in this paper is Y18049.

w/w) was added to solidify the medium. Lactate (10 mM) and sulfate (10 mM) served as electron donor/carbon source and electron acceptor, respectively. Colonies of sulfate reducers were identified by their black colour, resulting from ferrous sulfide precipitation. Well-separated colonies were withdrawn from agar-shake tubes of the terminal positive dilution steps by using drawn, sterile Pasteur pipettes. The colonies were transferred to glass test tubes containing APW medium supplemented with 10 ml lactate/sulfate. Six morphologically identical strains were isolated. One strain, designated strain lac^T, was studied in detail. Culture purity was examined in sulfate-free APW medium that was supplemented with fumarate (5 mM), pyruvate (5 mM), glucose (5 mM) and 0·1 % (w/w) yeast extract. In addition, cultures were regularly checked for purity by phase-contrast microscopy.

Desulfovibrio desulfuricans subsp. *desulfuricans* DSM 1926 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, and was used as a positive control in the desulfoviridin test. *Rhodococcus* sp. was obtained from our own culture collection and served as a positive control in the Gram-staining test.

Culture medium and substrates. Strain lac^T was routinely cultivated in defined medium of the following composition (l^{-1} Milli-Q water): NaCl, 20·0 g; $MgCl_2.6H_2O$, 3·0 g; $CaCl_2.2H_2O$, 0·15 g; Na_2SO_4, 3·0 g; NH_4Cl, 0·25 g; KH_2PO_4, 0·2 g; KCl, 0·5 g. The medium was sterilized by autoclaving for 25 min and cooled under oxygen-free N_2. After addition of $NaHCO_3$, the gas phase was exchanged with a mixture of oxygen-free N_2/CO_2 (90 %/10 %). The following sterile solutions were added aseptically: $NaHCO_3$ solution (1 M), 30 ml; $Na_2S.9H_2O$ solution (0·2 M), 7·5 ml; trace metal solution, 1 ml; vitamin solutions according to Widdel & Bak (1992); selenite/tungstate solution (0·02 mM), 1 ml; lactate stock solution (2 M), 5 ml. The trace metal solution was prepared according to Ingvorsen & Jørgensen (1984), all other solutions according to Widdel & Bak (1992). The medium was distributed into 50 ml bottles and closed with rubber-sealed, aluminium screw caps. Electron donors, carbon sources and acceptors were added from sterile stock solutions to give the concentrations desired. Most stock solutions were sterilized by autoclaving at 121 °C for 25 min. Elemental sulfur was heated to 114 °C for 30 min three times at 24 h intervals. Heat-labile solutions were sterilized by filtration through sterile 0·2 μm filters.

Electron donors and acceptors tested. Electron donor/acceptor tests were carried out in 50 ml screw-capped bottles. Growing cultures were transferred three times into culture bottles with the respective electron donor/acceptor combination to ensure that growth was not due to residual lactate and/or sulfate from the original stock culture. Controls contained basal medium and inoculum but no additional electron donor or acceptor.

Diazotrophic growth was tested in NH_4Cl-free medium. The tolerance of strain lac^T to oxygen was tested with cells harvested from exponentially growing cultures. The cells were washed to remove residual sulfides and inoculated into Erlenmeyer flasks containing basal sulfide-free, phosphate-buffered marine medium (composition as the standard medium, with the exception that $NaHCO_3$ was replaced by 0·04 M KH_2PO_4 and 0·06 M K_2HPO_4). The flasks were closed with sterile cotton stoppers. The medium contained lactate and sulfate. The flasks were incubated on a shaker and shaken vigorously throughout the experiment. The oxygen concentration of the medium was monitored with an oxygen electrode. Cell survival was evaluated by testing subcultures for their ability to grow in anaerobic standard medium after increasing periods of oxygen exposure. The last subsample was taken after 72 h.

Analytical procedures. Lactate, acetate, succinate, ethanol, pyruvate, fumarate and fructose were analysed by ion-exclusion chromatography by using an Aminex HPX-87H column (Bio-Rad) for compound separation. H_2SO_4 (0·05 mM) was used as eluent. The flow rate was 0·9 ml min^{-1}. The oven temperature was 65 °C. The injected sample volume was 100 μl. Fructose was quantified with a refractory index detector. All other compounds were measured with a UV-detector at 210 nm.

Sulfate was determined by suppressed-ion chromatography as described by Isaksen & Finster (1996).

Cellular fatty acids were analysed as described by Vainshtein *et al.* (1992). Cells were harvested in the late exponential growth phase by centrifugation and freeze-dried. The cellular fatty acid analysis was carried out at the DSMZ. The fatty acid nomenclature of Vainshtein *et al.* (1992) was used.

The pH, salt and temperature tolerance of strain lac^T was studied by growth tests in which the change in optical density (at 600 nm) was monitored (using a Bausch & Lomb Spectronic 88 spectrophotometer). Experiments were carried out in duplicate. Generation times were calculated from the increase in optical density of the culture over time.

Cytochromes were analysed by using intact, washed cells suspended in phosphate buffer (1·0 g KH_2PO_4 l^{-1} and 1·0 g K_2HPO_4 l^{-1}, pH 7·0). A dithionite-reduced minus air-oxidized difference spectrum was obtained on a Hitachi U-2000 Spectrometer. The presence of desulfoviridin was tested by treating cells with 2 M NaOH and observing a red fluorescence in light of 366 nm (Widdel & Pfennig, 1984). Catalase was assayed by treating a dense cell suspension with a few drops of a 3 % H_2O_2 solution and looking for bubble formation. Intracellular polyglucose was determined with lactate-grown cells after hydrolysis in 2 M H_2SO_4 at 120 °C for 20 min, with a glucose assay kit (Sigma). The G+C content of the DNA was determined at the DSMZ according to a standard protocol, which included methods developed by Mesbah *et al.* (1989), Tamaoka & Komagata (1984) and Visuvanathan *et al.* (1989).

Comparative 16S rRNA gene sequence analysis. DNA isolation, PCR-mediated amplification of the 16S rRNA gene from positions 28 to 1491 (numbering according to the International Union of Biochemistry nomenclature for *Escherichia coli* 16S rRNA) and sequencing analysis were done as described previously by Finster *et al.* (1998). Phylogenetic placement of strain lac^T was done by use of the ARB program package (Strunk & Ludwig, 1996). The 16S rRNA gene sequence of strain lac^T was compared with a database of about 6000 complete or partial bacterial 16S rRNA gene sequences (Rodriguez-Tomé *et al.*, 1996; Maidak *et al.*, 1997; Van de Peer *et al.*, 1996) by using the automatic alignment tool of the ARB program package. This procedure revealed that strain lac^T is a member of the genus *Desulfovibrio* within the δ-subclass of the class *Proteobacteria*. The phylogenetic position of strain lac^T was determined in more detail by comparing its 16S rRNA gene sequence with reference sequences of the family *Desulfovibrionaceae* and representatives of other major lineages of the δ-subclass of *Proteobacteria*. Each of these reference sequences contained at least 1000 unambiguously determined nucleotide sequence positions. The tree topology was evaluated by using a distance matrix analysis. Only nucleo-

tide sequence positions that contained identical nucleotides in at least 50 % of 102 16S rRNA sequences comprising the major lineages of the δ-subclass of *Proteobacteria* (1369 nucleotide sequence positions) were considered for phylogenetic inference. Evolutionary distance values between pairs of micro-organisms were calculated by using the Jukes–Cantor equation (Jukes & Cantor, 1969) and only those positions present in both sequences of the respective sequence pairs. The tree was constructed by using the neighbour-joining algorithm (Saitou & Nei, 1987).

The accession numbers of the reference sequences used for the phylogenetic assignment of strain lac^T are: *Desulfovibrio africanus*, M37315; *Desulfovibrio aespoeensis*, X95230; '*Desulfovibrio bastinii*', U53462; '*Desulfovibrio caledoniensis*', U53465; *Desulfovibrio desulfuricans*, M34113; *Desulfovibrio fructosovorans*, AF050101; *Desulfovibrio gigas*, M34400; *Desulfovibrio halophilus*, U48243; *Desulfovibrio longus*, X63623; *Desulfovibrio salexigens*, M34401; *Desulfovibrio termitidis*, X87409; *Desulfomicrobium baculatum*, M37311; *Desulfuromonas acetoxidans*, M26634; *Desulfurobacter postgatei*, M26633; *Desulfocapsa thiozymogenes*, X95181; *Escherichia coli*, J01695; and *Syntrophus buswellii*, X85131.

RESULTS

Enrichment and isolation

A sulfate-reducing enrichment culture was obtained within 8 d. The culture was dominated by highly motile, *Desulfovibrio*-type cells and developed an intense smell of hydrogen sulfide.

After transfer to agar-shake tubes, the formation of black colonies was observed within 2 d. The black colour of the colonies indicated that they contained sulfate-reducing bacteria. Well-separated colonies were isolated from the highest dilutions and transferred to liquid medium. Direct microscopical examination of colonies withdrawn revealed that they developed around a nucleus of precipitated material. The cells were morphologically identical to those that were dominant in the enrichment culture. Six strains were obtained in pure culture. One strain, designated strain lac^T, was studied in detail.

Cell morphology

Cells of strain lac^T were of vibrioid to sigmoid morphology. Single cells were ~3·0 μm long, ~0·5 μm wide and motile by means of a single polar flagellum. They often appeared in pairs. Under suboptimal conditions, cells formed corkscrew-shaped chains consisting of ten or more cells. Cells stained Gram-negative. Endospores were never observed.

Physiological and biochemical characteristics

Strain lac^T grew at NaCl concentrations between 0·0 and 0·6 M. The highest growth rate (0·1 h^{-1}) was observed in the presence of 0·2 M NaCl. The highest culture density was measured in an NaCl concentration interval of 0·4–0·6 M (1·8 × final culture density measured at 0·2 M NaCl). Growth occurred in media without added NaCl.

Strain lac^T grew between 5 and 34·5 °C. The growth optimum was between 32·5 and 34·5 °C. Strain lac^T had the shortest lag phase in the temperature interval 21·5–27·5 °C and the highest final culture density at 21·5 °C. The pH range in which growth occurred was 5·5–7·5 and the optimum was between pH 6·8 and 7·3.

The isolate used sulfate (20 mM), thiosulfate (20 mM), sulfite (5 mM) and elemental sulfur as electron acceptors with lactate (10 mM) as electron donor. The culture did not grow with nitrate (1 or 10 mM), nitrite (1 or 10 mM), ferrihydrite (10 mM) or manganese oxide (10 mM) as electron acceptors. Lactate (10 mM), pyruvate (10 mM), malate (10 mM), ethanol (10 mM), L-alanine (10 mM), fumarate (10 mM), fructose (10 mM) and choline (5 mM) served as energy and carbon sources when sulfate was present as electron acceptor. Weak growth was observed with formate plus acetate. Growth with molecular hydrogen required an organic carbon source, e.g. acetate. Fumarate (10 mM), pyruvate (10 mM) and fructose (10 mM) were utilized in the absence of an external electron acceptor. Compounds tested but not utilized with or without sulfate were: acetate (10 mM), propionate (10 mM), butyrate (10 mM), pentanoate (10 mM), hexanoate (10 mM), benzoate (0·8 or 10 mM), succinate (10 mM), L-proline (10 mM), L-lysine (10 mM), L-threonine (10 mM), methanol (4 or 10 mM), isopropanol (10 mM), D-(+)-mannitol (10 mM), catechol (0·8 or 5 mM), phenol (0·8 or 5 mM), pimelate (5 mM), betaine (5 or 10 mM), glyoxylate (10 mM), nicotinate (10 mM), acetone (5 mM), citrate (10 mM), furfural (0·8 or 5 mM), D-(+)-mannose (10 mM), D-(+)-galactose (10 mM), starch (1 mg ml^{-1}), maltose (10 mM), glucose (10 mM) and saccharose (10 mM).

Strain lac^T grew diazotrophically in NH_4Cl-free medium for an unlimited number of transfers with lactate, pyruvate, ethanol, malate and fructose. Diazotrophic growth did not occur with H_2 plus acetate and fumarate in the presence of sulfate nor with fumarate in sulfate-free medium. Culturable cells of strain lac^T were retrieved after 72 h under atmospheric oxygen.

Lactate and fructose metabolism

Lactate was incompletely oxidized to acetate in the presence of sulfate. One mole of acetate was formed from 1 mol lactate. Fructose was incompletely oxidized to 2 mol acetate in the presence of sulfate. No other organic end-products were detected. A total of 20·6 g biomass was produced per mole fructose, giving a growth efficiency of approximately 11 %. The following equation for fructose oxidation is proposed:

$$C_6H_{12}O_6 + SO_4^{2-} \rightarrow 2C_2H_3O_2^- + 2CO_2 + 2H_2O + H_2S$$

Pigments

The difference spectrum of whole cells had optima at 421·5, 524·0 and 552·5 nm. These peaks are charac-

Table 1. Comparison of the properties of strain lac^T and three other sugar-utilizing sulfate reducers belonging to the genus *Desulfovibrio*

+/− indicates discrepancies in substrate utilization as reported by Widdel & Pfennig (1984) and Zellner *et al.* (1989). (+) indicates weak growth. ND, Not determined.

Character	Strain lac^T	*D. salexigens* DSM 2638^T	*D. fructosovorans*	*D. termitidis*
Growth on, in the presence of sulfate:				
Lactate	+	+	+	+
Pyruvate	+	+	+	+
$H_2 + CO_2$	−	+	−	−
H_2 + acetate + CO_2	+	+	+	−
Formate	−	+	−	+
Formate + acetate	(+)	ND	+	
Fumarate	+	+	+	ND
Malate	+	+	+	−
Alanine	+	+	−	ND
Choline	+	+/−	−	−
Methanol	−	+	(+)*	ND
Methanol + acetate	−	ND	ND	ND
Ethanol	+	+	+	ND
1-Propanol	−	+	ND	ND
Glycerol	ND	+	+	ND
Fructose	+	ND	+	+
Rhamnose	ND	ND	−	+
Mannose	−	ND	−	+
Galactose	−	ND	−	+
Xylose	ND	ND	−	+
Glucose	−	+	−	+
Succinate	−	+	−	−
Citrate	−	+	ND	ND
Fermentation of:				
Fructose	+	ND	+	ND
Pyruvate	+	−	+	+
Fumarate	+	−	+	ND
Electron acceptors:				
Sulfate	+	+	+	+
Sulfite	+	ND	+	+
Thiosulfate	+	ND	+	+
Elemental sulfur	+	ND	+	+
Nitrate	−	−	−	+
Growth temperature:				
Optimum (°C)	32·5–34·5	34–37	35	35
Maximum (°C)	34·5	42–45	42–45	45
G + C content (mol %)	43	46	64	67–68

*Growth observed after pre-incubation with pyruvate.

teristic of *c*-type cytochromes. Desulfoviridin was present. Catalase was present. Polyglucose was not detected.

Cellular fatty acid analysis

The cellular fatty acid compositions of strain lac^T and *Desulfovibrio salexigens* (Vainshtein *et al.*, 1992) are given in Table 1. Unbranched fatty acids accounted for 57% of the total fatty acids, with 16:0 (27·2%) and 16:1*cis*9 (17·3%) predominating. Forty-five per cent of the total fatty acids were monounsaturated, 16:1*cis*9 (17·3%) predominating.

G + C content

The G + C content of DNA of strain lac^T was 42·7 ± 0·2 mol% (mean value of three determinations).

Phylogeny

Strain lac^T is a member of the genus *Desulfovibrio* within the δ-subclass of the class *Proteobacteria* and most closely related to *Desulfovibrio salexigens*. The overall identity of their 16S rRNA gene sequences was 95·9 % (based on the comparison of 1291 nucleotide sequence positions). The next most-closely related organism is '*Desulfovibrio bastinii*' strain SEBR4225, with an overall 16S rDNA identity of 92·5 % (based on the comparison of 1450 nucleotide sequence positions).

DISCUSSION

Here we report the isolation of a sulfate-reducing bacterium from surface-sterilized roots of the submerged marine macrophyte *Zostera marina*. Due to its genotypic and phenotypic characteristics, strain lac^T was placed into the genus *Desulfovibrio*. Comparative 16S rDNA sequence analysis identified *Desulfovibrio salexigens* as its closest validly described relative (Fig. 1).

Like numerous other members of the genus *Desulfovibrio*, strain lac^T grew by the oxidation of lactate, pyruvate, ethanol, malate and fumarate. With lactate and fructose, incomplete oxidation to acetate and CO_2 was demonstrated. Lithotrophic growth with hydrogen required an organic carbon source, e.g. acetate. Interestingly, strain lac^T grew with fructose as a carbon and energy source with or without sulfate. Sugar metabolism has rarely been described among species of sulfate reducers and, apart from some *Desulfotomaculum* species (Klemps *et al.*, 1985; Daumas *et al.*, 1988), only some members of the genus *Desulfovibrio* have been shown to utilize these compounds (Hansen, 1993). *Desulfovibrio* species that have been tested positive with respect to sugar oxidation are, in addition to strain lac^T, *Desulfovibrio fructosovorans* (Ollivier *et al.*, 1988), *Desulfovibrio salexigens* (Zellner *et al.*, 1989) and *Desulfovibrio termitidis* (Trinkerl *et al.*, 1990) (for detailed comparison see Table 1).

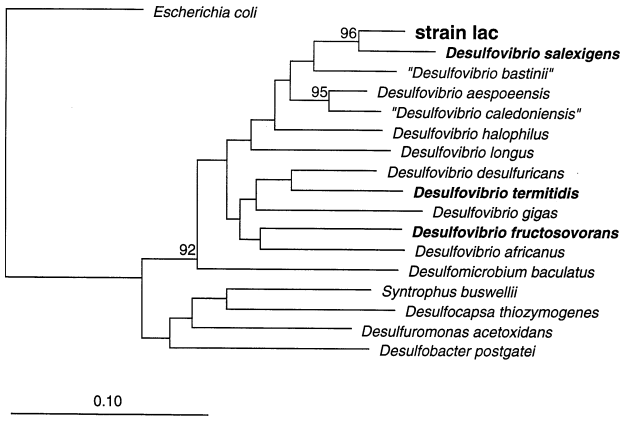

Fig. 1. Dendrogram based on 16S rRNA sequences showing the phylogenetic position of strain lac^T in relation to other members of the family *Desulfovibrionaceae* and representatives of other major lineages of the δ-subclass of *Proteobacteria*. The numbers indicate statistical significance (percentage of outcome) of the respective interior nodes in a bootstrap analysis based on 1000 neighbour-joining tests (only values above 90 % are shown). The strains highlighted in bold are compared phenotypically in Table 1. The 16S rRNA sequence of *Escherichia coli* was used as an outgroup reference. The scale bar represents 10 % estimated difference in nucleotide sequences.

Table 2. Fatty acid percentage profiles of strain lac^T and *Desulfovibrio salexigens*

Fatty acid	Strain lac^T	*D. salexigens*
11:0	0·1	—
13:0 iso	—	0·1
14:0	0·7	1·0
15:1 iso	—	0·4
15:0 iso	5·6	15·8
15:0 anteiso	10·5	4·8
15:0	0·5	—
16:1 iso H	2·2	0·3
16:0 iso	1·4	0·3
16:1*cis*9	17·3	9·8
16:1*cis*11	0·3	—
16:0	27·2	21·6
15:0 iso 3-OH	—	0·1
17:1 iso C	8·7	25·5
16:0 DMA	—	0·9
17:1 anteiso C	8·3	4·0
17:0 iso	3·0	5·4
17:0 anteiso	2·1	1·7
17:0	0·5	—
17:0 DMA	0·4	—
16:0 3-OH	0·4	0·1
18:1*cis*9	0·6	0·3
18:1*cis*11/*trans*9/*trans*6	8·5	5.1
18:0	1·9	1·4
17:0 iso 3-OH	0·2	—
Sum	100·1	98·9

—, Not detected; DMA, dimethylacetal.

The cellular fatty acid pattern of strain lac^T also supported an affiliation of this strain with the genus *Desulfovibrio*. As in most species of this genus, significant amounts of iso-17:1 [which was pinpointed as a biomarker for the genus *Desulfovibrio* (Taylor & Parkes, 1985)], iso-15:0 and 16:0 were present in strain lac^T. In addition, the fatty acid pattern of strain lac^T clearly distinguished it from its closest relative, *Desulfovibrio salexigens* (Table 2). While in *Desulfovibrio salexigens* iso-15:0 predominated (15·8 % versus 5·6 % in strain lac^T), anteiso-15:0 dominated in strain lac^T (10·5 % versus 4·8 % in *Desulfovibrio salexigens*). 16:1*cis*9 accounted for 17·3 % of the cellular fatty acids in strain lac^T and for 9·8 % in *Desulfovibrio salexigens*. The biomarker iso-17:1 was three times more abundant in *Desulfovibrio salexigens* than in strain lac^T (25·5 % in *Desulfovibrio salexigens* versus 8·7 % in strain lac^T). In addition, the fatty acids 11:0 (0·1 %), 16:1*cis*11 (0·3 %), 17:0 (0·5 %), 17:0 DMA

(0·4 %) and 17:0 iso 3-OH were present in strain lac^T but not in *Desulfovibrio salexigens*.

In contrast to *Desulfovibrio salexigens*, strain lac^T oxidized choline and grew fermentatively with pyruvate and fructose and oxidized fructose with sulfate as electron acceptor.

On the basis of the physiological traits and the phylogenetic position, strain lac^T (= DSM 11974^T) may be regarded as the type strain of a new species, for which we propose the name *Desulfovibrio zosterae* sp. nov.

Description of *Desulfovibrio zosterae* sp. nov.

Desulfovibrio zosterae (zo.ste.ra′e. N.L. bot. n. *zostera*; N.L. gen. n. *zosterae* denoting that the bacterium was isolated from the plant *Zostera marina*).

Curved rods to sigmoid-shaped cells, motile by a single polar flagellum, ~ 3·0 μm long and ~ 0·5 μm wide, normally occurring singly or in pairs. Cells stain Gram-negative and no spores were observed. The optimum pH is 6·8–7·3 and the optimum temperature is between 32·5 and 34·5 °C. Optimal growth at 0·2 M NaCl. In the presence of sulfate, hydrogen plus acetate, lactate, pyruvate, fumarate, malate, ethanol, L-alanine, choline and fructose serve as growth substrates. Fermentative growth is possible on pyruvate, fumarate and fructose. Sulfate, thiosulfate, sulfite and elemental sulfur are utilized as electron acceptors. Oxidation of lactate and fructose with sulfate is incomplete with acetate as the end product. Grows diazotrophically. Desulfoviridin and *c*-type cytochrome are present. Catalase is present. Aerotolerant. The G+C content of the DNA is 42·7 mol %. Habitat is roots of the marine macrophyte *Zostera marina*. The type strain is strain lac^T (= DSM 11974^T).

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