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EXPERIMENTAL ARTICLES

Desulfovibrios from Marine Biofoulings at the South Vietnam Coastal Area and Description of *Desulfovibrio hontreensis* sp. nov.

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Abstract—A *Desulfovibrio* strain physiologically similar to and phylogenetically related to *"D. caledoniensis"* SEBR 7250, *D. portus* MSL79, and *D. dechloracetivorans* ATCC 700912 (96.9, 95.9, and 95.8% similarity of the 16S rRNA gene sequences, respectively) was isolated from marine biofouling in the coastal zone of the South China Sea (Nha Trang, South Vietnam). The cells of strain ME were gram-negative motile vibrios (0.4–0.6 × 1.3–2 μm) with a single flagellum. The strain grew at 20 to 39°C (growth optimum at 34–37°C), pH 5.8 to 8.5 (pH optimum at 6.8–7.5), and salinity from 0.08 to 1.1 M Na^+ (optimum at 0.2–0.3 M Na^+). In the presence of sulfate, the strain grew autotrophically with hydrogen or on lactate, formate, pyruvate, fumarate, and malate. Weak growth occurred on succinate, glycerol, and fructose. In the absence of sulfate, the strain was able to ferment pyruvate, malate (weakly), but not lactate. Sulfate, sulfite, thiosulfate, elemental sulfur, and dimethyl sulfoxide were used as electron acceptors. Vitamins and yeast extract were not required for growth. The G + C content was 52.4 mol %. Predominant fatty acids were C18:0 (13.9%), C16:0 (9.6%), *iso*-C16:0 (9.5%), C18:1ω7 (8.8%), *anteiso*-C15:0 (8.1%), and *iso*-C17:1 (7.2%). The fatty acid composition was close to that of *D. dechloracetivorans* BO and has some similarity to that of *D. portus*. Based on its genotypic and phenotypic characteristics, strain ME maybe considered as a new species, for which the name *Desulfovibrio hontreensis* sp. nov. is proposed.

Keywords: sulfate-reducing bacteria, *Desulfovibrio*, marine biofouling, corrosion, *Desulfovibrio hontreensis* sp. nov
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Investigation of the samples of biofouling on a metal surface (South Vietnam) revealed diverse sulfate-reducing microflora not only below the shells of molluscs and crustaceans, but also in thin surface biofilms (Tarasov and Borzenkov, 2015). The isolated sulfate-reducing bacteria (SRB) were closely related (with at least 99% similarity between the 16S rRNA gene sequences) to the species *D. salexigens* (strains PE and OZB), *D. marinisediminis* (strains Mol4 and Mol5), *D. alaskensis* (strain DB3), *D. bizertensis* (strain RH2), *D. indonesiensis* (strain OP12), and *D. dechloracetivorans* (strain BO). Another isolate, *Desulfovibrio* sp. strain ME, was related to *D. dechloracetivorans* ATCC 700912, *"D. caledoniensis"* SEBR 7250, and *D. portus* MSL79 with the similarity not exceeding 97%. The SRB group related to *D. dechloracetivorans* is important for both basic and applied reasons. *"D. caledoniensis"* are known to participate actively in metal corrosion (Cetin and Aksu, 2011; Mori et al., 2010) and as degraders of polychlorinated biphenyls (Wu et al., 2002). *D. dechloracetivorans* was described a degrader of chlorinated phenols (Sun et al., 2000), while some isolates were able to methylate mercury (Gilmour et al., 2011). The physiology and phylogeny of this group, which includes our isolates BO and ME, is insufficiently studied.

The goal of the present work was to investigate the physiological and biochemical properties of new *Desulfovibrio* isolates in order to determine their phylogenetic position among *Desulfovibrio* species.

MATERIALS AND METHODS

Subject of study. The strains were isolated from biofouling on metal samples placed on the racks of the Marine Research and Testing Station in the South China Sea organized by the Joint Russian-Vietnam Tropical Research and Test Center (Tropical Center). The racks were located in the Dam Bai Bay of Hón Tre Island close to Nha Trang port. The samples were collected by scraping the lower biofilm layer, closest to the metal surface (usually of black color). The material was stored in 15-mL sterile test tubes filled to capacity with sterile seawater. Strain BO was isolated from the black sediment at the inner part of a nut, where the bolt was destroyed by corrosion. Strain ME was isolated from the surface of a stainless steel plate below the shells of *Saccostrea cucullata* clams (1–2 cm) overgrowing the metal.

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Cultivation techniques. The medium for enrichment cultures and isolation of anaerobic microorganisms contained the following (g/L): NaCl, 20.0; KCl, 0.6; NH_4Cl, 0.3; K_2HPO_4, 0.05; $MgCl_2 \cdot 6H_2O$, 5.0; Na_2SO_4, 3.0 or $Na_2S_2O_3$, 2.0; $CaCl_2 \cdot 2H_2O$, 0.15; MES, 0.5; MOPS, 0.5; Tris, 0.6; yeast extract (Difco), 0.25; resazurin (0.2% solution), 1 mL. The medium was supplemented with vitamins and trace elements according to Widdel and Back (1992), as well as with Na_2SeO_3 and Na_2WO_4 (1 mL of 0.01 M solution). After sterilization, cysteine and sulfide solutions were added to the final concentration of 0.2–2.0 mM and bicarbonate solution to the final concentration of 10–25 mM and pH 7.0. The cultures were incubated at 35°C.

Enrichment cultures were obtained under H_2/CO_2 gas mixture (80 : 20) at excessive pressure of 0.5 atm (52 kPa), in the medium with acetate (5 mM) and $Na_2S_2O_3$ (8 mM). $FeSO_4$ (2 mM) was added as an additional reducing agent and an indicator of SRB growth. In subsequent transfers, the medium with lactate (20 mM) and without $FeSO_4$, yeast extract, and vitamins was used. The enrichment was transferred four to five times in order to achieve the preferential growth of specific microbial morphotypes.

Pure cultures were isolated from the colonies obtained by the roll tube method in agar medium with acetate and thiosulfate, with H_2/CO_2 mixture as the gas phase. Purity of the isolates was confirmed by transfers to media with organic substrates and incubation under oxic and anoxic conditions, as well as by microscopy and analysis of the 16S rRNA gene sequences.

Physiology and biochemistry. Growth rates at different values of temperature, pH, and salinity were determined in the medium with lactate. The pH values were adjusted with 0.5 M solutions of HCl and Na_2CO_3. Optical density (OD_{600}) and sulfide production were used as criteria of cell growth. The latter was measured photometrically using the Merck test kit (Germany). Substrate specificity was determined using the medium described above and supplemented with 10–15 mM of the studied substrate. Each experimental variant was carried out in 2–3 repeats. The absence of OD_{600} changes and sulfide accumulation after 21 day of incubation was interpreted as the absence of growth.

Fatty acid composition was determined in late-exponential stage biomass grown in lactate–sulfate medium at 34°C. Fatty acids were extracted from 5 mg of lyophilized biomass by acid methanolysis at 80°C for 1 h with 0.4 mL methanol with 1.2 M HCl. Fatty acid methyl esters were extracted twice with hexane (0.2 mL) and analyzed on an AT-5850/5973 chromatograph–mass spectrometer (Agillent Technologies, United States).

Desulfoviridin was determined spectrophotometrically by absorption of cell extracts at 630 nm (Badziong et al., 1978). To determine catalase activity, 1 mL of the late-exponential phase culture was centrifuged (5 min at 5000 *g*), and 50 μL 3% H_2O_2 was applied upon the biomass pellet.

Cell morphology was studied under a phase contrast microscope (Carl Zeiss, Germany) at ×1000.

For electron microscopy, whole cells were negatively stained with 2% phosphotungstic acid. The preparations were examined under an EC-100C electron microscope (Jeol, Japan).

The DNA G + C content was determined by thermal denaturation using a Unicam SP 1800 spectrophotometer (United Kingdom) at the heating rate of 0.5°C/min. The calculation was carried out according to Owen et al. (1969).

Identification of the isolates. Phylogenetic analysis of the 16S rRNA gene sequences was used to determine the taxonomic position of the isolates. The universal primers 11F (5'-GTTTGATCMTGGCTCAG-3'); 518R (5'-CGTATTACCGCGGCTGCTGG-3'), 1100R (5'-AGGGTTGCGCTCGTTG-3'), and 1492R (5'-TACGGÒTACCTTGTTACGACTT-3') were used for amplification and sequencing of the 16S rRNA gene fragments (Turner et al., 1999; Lane, 1991; Kane et al., 1993). Sequencing was carried out in the Syntol center (Moscow, Russia). The gaps and unidentified bases were excluded from analysis.

Initial analysis of the 16S rRNA gene sequences was carried out using the NCBI Blast database and software (http://www.ncbi.nlm.nih.gov/blast).

The sequences were aligned using CLUSTAL W implemented in the MEGA 5.2.2 software package (Tamura et al., 2011). The phylogenetic tree was constructed using the neighbor-joining algorithm implemented in Mega 5.2.2. The branching order was determined by bootstrap analysis of 1000 alternative trees.

The nucleotide sequences were deposited to GenBank under accession nos. KP682305 and KP682306.

RESULTS AND DISCUSSION

Characterization of strain BO. According to the results of the 16S rRNA gene sequencing, the isolate BO was identified as a *Desulfovibrio dechloracetivorans* strain (99.6% similarity). Comparison of its spectrum of utilized substrates with that of the type strain was difficult, since growth of the type strain *D. dechloracetivorans* SF3 = ATCC 700912T (NR_025078.1; Sun et al., 2000) was studied mainly in the presence of chlorinated organic compounds, rather than sulfate, as electron acceptors. However, the literature data indicate occurrence of this species in sulfate-containing saline aquatic environments (Mori et al., 2010; Cifuentes et al., 2003; Santana, 2008).

According to the literature (Sun et al., 2000), in the presence of sulfate the type strain *D. dechloracetivorans* ATCC 700912^T could grow on lactate and pyruvate. With sulfate, acetate was not used as the sole

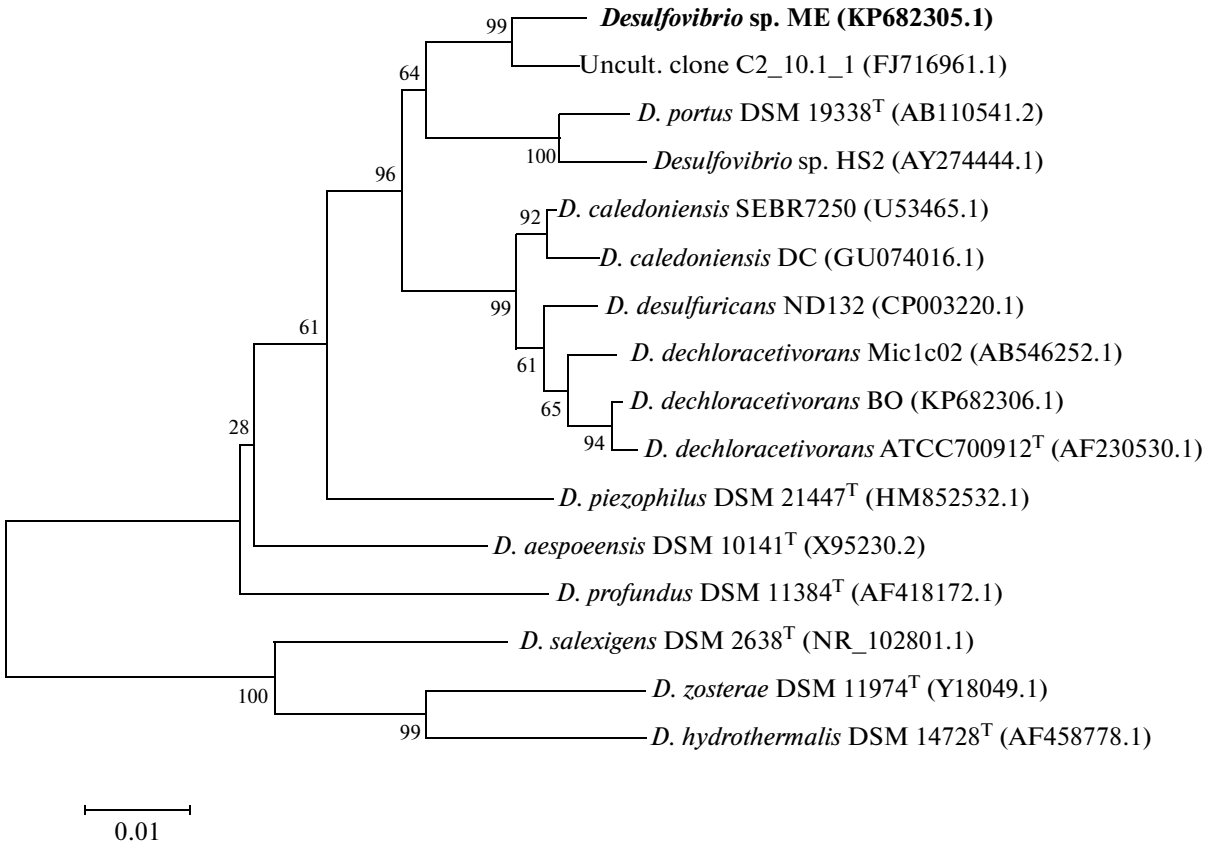

Fig. 1. Phylogenetic tree constructed using the 16S rRNA sequences of strains ME, BO, and the closely related *Desulfovibrio* strains. The unrooted phylogenetic tree was constructed using the neighbor-joining algorithm. The branching order is shown in percent, scale bar corresponds to 10 replacements per 1000 nucleotides.

organic substrate; it was consumed in the presence of chlorinated organic compounds. Utilization of lactate, pyruvate, propionate, fumarate, alanine, and ethanol could also be coupled to dechlorination. Utilization of propionate, fumarate, alanine, and ethanol by the type strain in the media with sulfate was not studied.

Similar to the test strain, our isolate, *D. dechloracetivorans* strain BO, utilized lactate and pyruvate in the presence of sulfate. Moreover, formate, fumarate, ethanol, propanol, butanol, and glycerol also supported growth in the presence of sulfate. Weak growth occurred on serine and aspartate, while no growth occurred on acetate, methanol, malate, succinate, fructose, alanine, glycine, propionate, or butyrate. Strain BO could grow autotrophically on hydrogen ($H_2/CO_2 + SO_4^{2-}$) in the absence of acetate, yeast extract, and vitamins. Growth on hydrogen in the presence of acetate was considerably more active. Unlike the type strain, strain BO was not capable of nitrate reduction. Its optimal growth temperature (34–37°C) was higher than that of the type strain (30°C), as was its salt tolerance (60 g/L vs. 25 g/L according to Sun et al., 2000).

While *D. dechloracetivorans* LS1101 isolated from a Portuguese saline bog (Santana, 2008) has the optimal temperature similar to that of strain BO (30–35°C), it did not use pyruvate and formate.

Characterization of strain ME. The position of the other isolate, strain ME, was less definite. According to the results of the 16S rRNA gene sequencing, strain ME exhibited similarity to the species *D. dechloracetivorans* (AF230530.1 = NR_025078.1), to the unvalidated species *"D. caledoniensis"* (U53465.1), and with *D. portus* DSM 19338T (AB110541.2) (Fig. 1, Table 1). Similarity to these species, however, did not exceed 97%, which makes it possible to consider strain ME as a member of a new species (Stackebrandt and Goebel, 1994).

Cell morphology. Gram-negative cells of strain ME were bent, slightly spirally curved rods (vibrios), usually single and sometimes in pairs. Morphologically they were similar to the cells of *D. dechloracetivorans* BO. In some cases, during growth of strain ME the culture formed spiral chains. The curvature of the cells varied considerably, so that almost straight rods were also present. The cells were 1.3–2.0 μm long and 0.4–0.6 μm in diameter, motile to a single polar flagellum (Fig. 2). On solid medium with lactate or hydrogen +

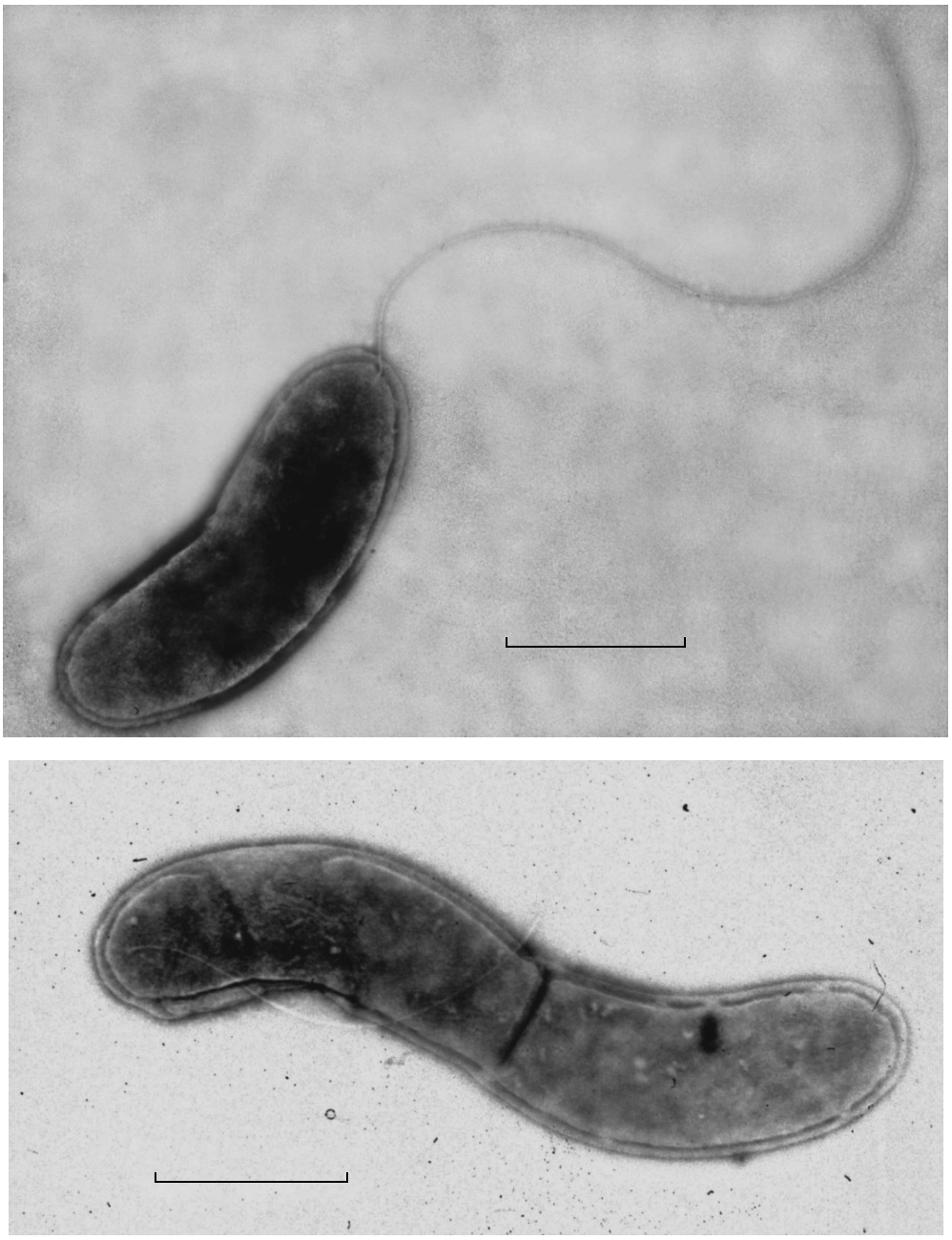

Fig. 2. Electron microscopy of strain ME, negative staining with phosphotungstic acid. Scale bar, 0.5 μm.

CO_2, the strain formed round white colonies, subsequently obtaining beige color, 0.2 to 1 mm in diameter.

Physiological properties. The strain grew within the temperature range from 20 to 39°C, with the highest growth rate at 34–37°C and no growth at 42°C. Growth occurred at pH from 5.8 to 8.5 with the optimum at pH 6.8–7.5. Growth was possible at NaCl concentrations from 0.08 to 1.1 M with the optimum at 0.2–0.32 M. The maximal growth rate of strain ME under optimal conditions was 0.23 h^{-1}.

Growth under chemoautotrophic conditions ($H_2/CO_2 + SO_4^{2-}$) without acetate, yeast extract, and vitamins was slow (Table 2). In the presence of acetate, the growth rate on hydrogen increased significantly. Strain ME fermented pyruvate and malate (weakly), but not lactate. In the presence of sulfate, the strain grew well on lactate, formate, pyruvate, fumarate, and malate; it grew weakly on succinate, glycerol, and fructose (Table 2). No growth occurred on acetate, alcohols (ethanol, methanol, propanol, or butanol), ethylene glycol, betaine, propionate, butyrate, glucose, yeast extract, or amino acids (alanine, aspartate,

Table 1. Similarity of the 16S rRNA gene sequences of strains ME and BO to those of the closely related *Desulfovibrio* species determined by bootstrap analysis

	Desulfovibrio sp. ME	Uncult. bacterium clone C2_10.1_1 (FJ716961.1)	"*D. caledoniensis*" SEBR7250 (U53465.1)	"*D. caledoniensis*" DC (GU074016.1)	*D. portus* DSM 19338^T (AB110541.2)	*Desulfovibrio* sp. HS2 (AY274444.1)	*D. dechloracetivorans* ATCC7 00912^T (AF230530.1)	*D. dechloracetivorans* BO	*D. dechloracetivorans* Mic1c02 (AB546252)	"*D. desulfuricans*" ND132 (CP003220.1)	*D. profundus* DSM 11384^T (AF418172.1)	*D. piezophilus* DSM 21447^T (HM852532.1)
Desulfovibrio sp. ME	100.0											
Uncult. bacterium clone C2_10.1_1 (FJ716961.1)	**98.62**	100.0										
"*D. caledoniensis*" SEBR7250 (U53465.1)	**96.85**	96.72	100.0									
"*D. caledoniensis*" DC (GU074016.1)	**96.72**	96.32	**99.38**	100.0								
D. portus DSM 19338^T (AB110541.2)	**95.94**	96.34	95.93	95.54	100.0							
Desulfovibrio sp. HS2 (AY274444.1)	96.72	96.85	95.67	95.27	98.51	100.0						
D. dechloracetivorans ATCC 700912^T (AF230530.1)	95.81	96.20	**98.38**	**97.74**	95.81	95.54	100.0					
D. dechloracetivorans BO	95.94	96.33	**98.76**	**98.12**	95.94	95.68	**99.63**	100.0				
D. dechloracetivorans Mic1c02 (AB546252.1)	96.21	95.81	**98.88**	**98.51**	95.54	95.28	**99.01**	98.88	100.0			
"*D. desulfuricans*" ND132 (CP003220.1)	96.46	96.59	**98.63**	**98.25**	96.59	96.33	**98.75**	98.63	98.76	100.0		
D. profundus DSM 11384^T (AF418172.1)	93.43	92.75	94.23	94.09	93.41	92.73	93.01	93.15	93.68	93.28	100.0	
D. piezophilus DSM 21447^T (HM852532.1)	94.89	95.42	95.69	95.56	95.16	94.63	94.76	95.16	94.90	95.03	95.29	100.0

Table 2. Comparison of the morphological, physiological, and biochemical characteristics of strain ME and the previously described related strains

	Desulfovibrio sp. ME	"*D. calle-doniensis*" DC	HS2	LS2001	ND132	*D. portus* DSM 19338^T	*D. dechlorace-tivorans* BO
Cell size, μm	0.4–0.6 × 1.3–2.0	0.5 ± 0.1 × 3 ± 0.4	0.5 × 2–4	0.3–0.4 × 2–5.5	0.75 × 4.0	0.7–1.0 × 1.8–2.3	0.4–0.6 × 2.5–5.0
G + C, mol %	52.4	ND	ND	ND	ND	62.1	61.9
pH optimum (range)	6.8–7.5 (5.8–8.5)	ND (ND)	7.2–7.5 (ND)	7.0–7.5 (5.5–7.5)	7.8 (6.8–8.2)	6.5 (5.5–8.5)	6.8–7.1
Temperature optimum (range), °C	34–37 (20–39)	ND (ND)	28–30 (ND)	30–35 (25–40)	32 (30–37)	35 (10–40)	34–37 (ND)
NaCl optimum (range), M	0.2–0.32 (0.08–1.1)	ND (ND)	ND (ND)	0–0.51 (ND)	0.34 (0–0.51)	0.34 (0.025–1.1)	0.33–0.43 (up to 1)
$H_2 + CO_2$ (autotroph) + acetate (mixotroph)	+ +	ND –	– +	ND (ND)	ND (ND)	ND +	+ +
Lactate	+	–	+	+	+	+	+
Pyruvate	+	–	+	±	+	+	+
Formate	+	+	ND	±	±	+	+
Fumarate	+	+	ND	ND	+	+	+
Succinate	±	–	ND	ND	–	+	–
Malate	+	–	ND	ND	–	+	–
Ethanol, propanol, butanol	–	–	ND	ND	–	+	+
Glycerol	±	ND	ND	ND	ND	–	+
Glucose	–	–	ND	–	ND	–	–
Fructose	±	–	ND	–	ND	–	–
Propionate	–	–	ND	ND	ND	–	–
Butyrate	–	+	ND	ND	ND	–	–
Serine, aspartate	–	ND	ND	ND	ND	ND	±
Alanine, glycine	–	ND	ND	ND	ND	ND	–
Methanol	–	ND	ND	ND	ND	ND	–
Ethylene glycol	–	ND	ND	ND	ND	ND	±
Betaine	–	ND	ND	ND	ND	ND	–

Good growth, poor growth (production of less than 1.5 mM sulfide), and no growth are designated by "+", "±", and "–", respectively. ND stands for no data.

serine, or glycine). Apart from sulfate, thiosulfate, sulfite, and dimethyl sulfoxide could be used as electron acceptors. Slow growth was observed in lactate medium supplemented with sulfur. Nitrate and fumarate were not used as electron acceptors. The cells were found to contain desulfoviridin.

Fatty acid composition of strains BO and ME. The results of fatty acid (FA) analysis confirmed the relationship between strains BO and ME established by comparison of their 16S rRNA gene sequences. The strains exhibited similarities in FA composition (Table 3) and differed from the previously described species. The content of the major component (C18:0) in strains BO and ME was 18.7 and 13.9%, respectively. C16:0 (13.5 and 9.6%, respectively), *iso*-C16:0 (5.9 and 9.5%), *anteiso*-C15:0 (9.3 and 8.1%), *iso*-C15:0 (6.8 and 5.4%), as well as C18:1ω7 (5.5 and 8.8%) were present in smaller amounts. The content of *iso*-C17:1 FA, which is common among desulfovibrios (Vainshtein et al., 1992; Suzuki et al., 2009), was 4.9% for strain BO and 7.2% for strain ME, the content of *anteiso*-C17:1 was 4.1 and 4.3%, respectively. Strains BO and ME differed in the ratio of saturated and unsaturated *n*-fatty acids, due to C14:0, C16:0, and C 18:0 in strain BO and higher levels of *iso*-saturated and unsaturated components in strain ME (*iso*-C16:0, *iso*-C17:1, C18:1ω7, and *iso*-C18:0).

Among desulfovibrios, elevated content of hexadecanoic (C16:0, 9%) and octadecanoic acids (C18:0, 15%) was found in *D. gabonensis*, in which, however, *anteiso*-C15:0 was the predominant fatty acid (up to 40%) (Tardy-Jacquenod et al., 1996). Strains BO and ME had no such dominant fatty acid, similar to *D. portus* DSM 19338^T (Suzuki et al., 2009), containing *iso*-C17:1, *iso*-C17:0, *anteiso*-C17:0, *iso*-C15:0, *anteiso*-C15:0, and C16:0 acids (Table 3). In this respect, the species group *D. portus, D. dechloracetivorans*, and *D. caledoniensis* differs from other members of this genus, which are characterized by predominance of one FA component (Suzuki et al., 2009).

Fatty acid composition of strains ME and BO differed from that of *D. portus* in higher content of C18:0) (a minor component in *D. portus*), as well as of C16:0 and *iso*-C16:0. Strains ME and BO had lower levels of *iso*-C15:0, *anteiso*-C15:0, *iso*-C17:0, *anteiso*-C17:0, and *iso*-17:1. Thus, isolates ME and BO formed a separate group according to their fatty acid composition.

Phylogenetic position. The strain *Desulfovibrio* sp. ME differed from *D. dechloracetivorans* BO in its 16S rRNA gene sequence (95.9% similarity, Table 1). The strains differed considerably in their DNA G + C content (52.4 and 61.9 mol %, respectively; the data for *D. dechloracetivorans* ATCC 700912^T and *"D. caledoniensis"* SEBR7250 are unavailable). Moreover, differences in their biochemical properties were also observed. Strain ME used succinate and malate, but did not grow in the presence of ethanol, propanol, or butanol, which were utilized by strain BO. Thus, strains BO and ME should be assigned to different species.

Analysis of the 16S rRNA gene sequences revealed strain ME to be distant from the strains representing the most closely related species: *D. dechloracetivorans* ATCC 700912^T (AF230530.1), *"D. caledoniensis"* SEBR 7250 (U53465.1), and *D. portus* DSM19338^T (AB110541.2), with 95.8, 96.9, and 95.9% similarity, respectively (Fig. 1, Table 1).

The group of *D. dechloracetivorans–D.caledoniensis* strains forms a cluster with high 16S rRNA gene similarity. The similarity between the 16S rRNA gene sequences of *D. dechloracetivorans* ATCC 700912^T and those of members of this species exceeded 99% (strain *D. dechloracetivorans* Mic1c02), while the similarity to *"D. caledoniensis"* SEBR7250 was 98.4%. Some strains, such as *"D. desulfuricans"* ND132, for which the genome was sequenced completely (CP003220.1), but the species position was not specified, occupy an intermediate position. Its similarity to *D. dechloracetivorans* ATCC 700912^T and *"D. caledoniensis"* SEBR7250 was 98.8 and 98.6%, respectively (Table 1, Fig.1).

The name *"D. caledoniensis"* for strain SEBR7250 was originally proposed in the course of annotation of the results of the 16S rRNA gene sequencing to GenBank. The information concerning the physiological and biochemical characteristics of this stain has not been published. Several isolates assigned to *"D. caledoniensis"* were subsequently obtained. Strain DC, which has been studied in most detail, is the most closely related one (Cetin and Aksu, 2011). The properties of other isolates were studied to a limited extent. The physiological properties in respect to temperature, pH, and salinity varied insignificantly within the *D. dechloracetivorans–D. Caledoniensis* group (Cetin and Aksu, 2011; Sun et al.,2000; Gilmour et al., 2011; Santana, 2008).

The 16S rRNA gene of strain ME exhibited 96.7% similarity to that of *"D. caledoniensis"* DC (GU074016.1, Table 1, Fig. 1.). The differences in biochemical characteristics were also present, since strain ME could grow on malate and succinate and did not utilize butyrate (Table 2), while strain DC grew only on butyrate, fumarate, and formate (Cetin and Aksu, 2011).

Unlike strain ME, strain HS2 from marine sediments (Hang, 2003; Hang et al., 2004), which was initially assigned to *"D. caledoniensis,"* did not grow autotrophically and had a low temperature optimum (28–30°C). Apart from hydrogen, only growth on lactate and pyruvate was studied (Table 2). According to its 16S rRNA gene sequencing, strain HS2 (AY274444.1) exhibited low similarity to strain ME (96.7%), and still lower to *"D. caledoniensis"* SEBR7250 (U53465.1) and strain DC (below 96%). Strain HS2 probably does not belong to the *"D. cale-*

Table 3. Fatty acid composition of strains BO, ME, and *D. portus* DSM 19338^T

FA	Strain BO	Strain ME	*D. portus* DSM 19338^T*
iso-C14:0	2.46	2.57	
C14:0	**5.55**	**0.91**	
***iso*-C15:0**	**6.77**	**5.37**	**12.0**
***anteiso*-C15:0**	**9.33**	**8.08**	**12.4**
C15:0	0.48	0.37	
iso-C16:1	2.18	2.64	
***iso*-C16:0**	**5.88**	**9.53**	2.1
C16:1ω7	1.75	3.25	1.4
C16:0	**13.49**	**9.63**	**7.3**
***iso*-C17:1**	**4.85**	**7.24**	**15.8**
ànteiso-C17:1	4.09	4.29	4.5
iso-3hi C15:0	0.26	0.17	2.1
***iso*-C17:0**	4.23	5.29	**11.8**
***anteiso*-C17:0**	3.88	3.62	**8.8**
cyc-C17:0	1.66	1.14	
C17:0	1.85	1.24	
iso-C18:1ω11	0.23		
iso-C18:1ω9	1.01	0.45	
iso-C18:1ω7	0.87	2.15	4.5
iso-C18:0	1.22	5.02	4.2
C18:1ω9	0.38	0.53	
C18:1ω7	5.51	8.83	
C18:0	**18.74**	**13.87**	4.2
iso-C19:1	0.79	1.05	
ànteiso-C19:1	0.19	0.60	
3hi C17:0	0.04		1.6
iso-C19:0	0.34	0.49	
ànteiso-C19:0	0.12	0.19	
cyc-C19:0	0.40	0.51	
C19:0	0.20	0.07	
iso-3hi C18	0.06	0.17	
C20:1	0.19	0.21	
3h C18	0.29	0.07	
C20:0	0.72	0.48	
% unsaturated FA	22.03	31.22	26.2
% *iso*-saturated FA	36.29	41.81	53.4

* Data from (Suzuki et al., 2009).

doniensis" cluster, but rather to the *D. portus* cluster (over 98.5% similarity, Fig. 1, Table 1).

The strain *"D. caledoniensis"* LS2001 isolated from Portuguese saline bogs (Santana, 2008) did not grow on formate, unlike strain ME. Its vibrioid cells were longer (Table 2). The annotated length of its 16S rRNA gene sequence is only 523 nucleotides (AF510405.1, EF073046.1), which is insufficient for accurate phylogenetic assessment.

Comparative analysis of the 16S rRNA gene sequences revealed strain ME to be relatively distant from *D. dechloracetivorans* strains (Fig. 1, Table 1), with 95.8% similarity to the type strain ATCC 700912 and 96.2% similarity to strain Mic1c02 (AB546252.1, Mori et al., 2010).

The strain "*D. desulfuricans*" ND132 (Gilmour et al., 2011), which occupies an intermediate position in the *D. dechloracetivorans*–*"D. caledoniensis"* cluster, also can not belong to the same species as strain ME due to the low similarity of their 16S rRNA gene sequences (96.4%, Fig. 1, Table 1). It also differs from strain ME in pH optimum, salinity range, and absence of growth on malate (Table 2).

It can be seen that strains ME and *D. portus DSM* 19338^T had similar temperature, pH, and salinity growth ranges. Strain ME, unlike *D. portus DSM* 19338^T, did not grow on alcohols (Table 3). The similarity between their 16S rRNA gene sequences was also relatively low (95.9%, Fig. 1, Table 1).

According to the results of the 16S rRNA gene sequencing, strain ME was most closely related (98.7%) to an uncultured bacterial clone from annelid worms inhabiting sea sand (FJ716961.1).

Ability to utilize dimethyl sulfoxide. Among potential electron acceptors, dimethyl sulfoxide (DMSO) was found to support growth of strains BO (on lactate and ethanol) and ME (on lactate and malate). In the controls, no growth by fermentation of these substrates occurred. Increase in the optical density of cell suspensions in the presence of DMSO was accompanied by a specific smell of dimethyl sulfide (DMS) in the samples. *D. bizertensis* RH2 also exhibited weak growth on lactate and malate with DMSO, while our other isolates did not use it as an electron acceptor. While DMSO reduction was previously shown for some *D. profundus* and *D. salexigens* strains (Jonkers et al., 1996; Bale et al., 1997), our isolates OZB and PE identified as *D. salexigens* strains did not possess this feature.

In seas, DMS is formed in the course of degradation of dimethyl sulfopropionate, an osmoprotector in phytoplankton, and is the main volatile sulfur compound released to the atmosphere from the sea surface. Incomplete DMS oxidation, e.g., by purple bacteria, results in formation of DMSO (Zeyer et al., 1987), which is then reduced to DMS by SRB. Interestingly, the following phototrophic anaerobes developed in our SRB enrichment cultures with hydrogen in the gas phase under light: purple sulfur bacteria *Marichromatium purpuratum* (KR 233469) and green sulfur bacteria (Tourova et al., 2014). The presence of green sulfur bacteria together with SRB related to *D. dechloracetivorans* and "*D. caledoniensis*" was previously reported for the rhizosphere of marine plants (Cifuentes et al., 2003).

Incorporation of methylated compounds in SRB metabolism is related to an important environmental issue of formation of highly toxic mercury compounds. Mercury methylation was shown for the strain "*D. desulfuricans*" ND132, which is phylogenetically related to the *D. caledoniensis*–*D. dechloracetivorans* group (Gilmour et al., 2011).

The microorganisms exhibiting at least 96% similarity to strain ME according to the results of the 16S rRNA gene sequencing were revealed in the course of investigation of corrosion of oil equipment (U53465.1, AB546252.1, AB546253.1; Mori et al., 2010), of metal corrosion in seawater (Duan et al., 2008; Yu, et al., 2011), in the microflora of marine worms (FJ716961.1), in salt marshes (Santana, 2008), dead corals (EF123506.1), marine sediments (Gilmour et al., 2011; Cifuentes et al., 2003, AF228127.2, AY274444.1, U85475.1), and estuarine sediments (Wu et al., 2002; Wan et al., 2010). Similar bacteria probably also thrive in saline habitats of the Baltic coast (AJ289746, AJ289724), in Arabian Sea sediments (JQ042713.1), and in crude oil (GU074016.1, KJ576626.1).

Thus, the habitats of bacteria related to the *D. dechloracetivorans*–*D. caledoniensis* group are associated with marine sediments, corroded metal immersed in seawater, and oil equipment.

The isolate *Desulfovibrio* sp. ME obtained from the biofilm on stainless steel below the bases of clamshells differs from the previously described strains in its physiological, biochemical, and molecular genetic characteristics. These differences provide the basis for its classification as a new species with the proposed name *Desulfovibrio hontreEnsis* sp. nov.

Description of *Desulfovibrio hontreensis* sp. nov.

Desulfovibrio hontreensis sp. nov (hon.tre.en'sis, N.L. masc. adj. Hontreensis), from the site of isolation. The cells are gram-negative, curved, slightly curled rods and vibrios, usually single, less often in pairs or spiral chains. The cells (1.3–2.0 × 0.4–0.6 μm) are motile, with a single polar flagellum, do not form spores. Colonies on agar media are small, of brown color. The DNA G + C content is 52.4 mol %.

The organism is mesophilic, growing at 20 to 39°C with the optimum at 34–37°C; it is halotolerant (0.08 to 1.1 M NaCl with the optimum at 0.2–0.3 M); and neutrophilic (growth at pH 5.8–8.5, pH optimum at 6.8–7.5). The organism is capable of chemoautotrophic growth (H_2/CO_2 + SO_4^{2-}). Growth on

hydrogen is stimulated by acetate (H_2/CO_2 + acetate + SO_4^{2-}). In the presence of sulfate, the organism grows on lactate, formate, fumarate, pyruvate, and malate. Weak growth occurs on succinate, glycerol, and fructose. Vitamins or yeast extract are not required for growth. Pyruvate and malate (weakly), but not lactate, are fermented. No growth occurs on acetate, alcohols (methanol, ethanol, propanol, or butanol), ethylene glycol, betaine, propionate, butyrate, glucose, and amino acids (alanine, aspartate, serine, and glycine). Apart from sulfate, the following may be used as election acceptors: sulfite, thiosulfate, and dimethyl sulfoxide; nitrate or fumarate may not. Slow growth occurs with lactate and elemental sulfur.

Predominant fatty acids are C18:0 (13.9%), C16:0 (9.6%), *iso*-C16:0 (9.5%), C18:1ω7 (8.8%), *anteiso*-C15:0 (8.1%), with *iso*-C17:1 (7.2%) and *iso*-C15:0 (5.4%) as minor components. The G + C content is 55.2 mol %. Type strain is *Desulfovibrio hontreensis* ME (= VKM B-2947). The 16S rRNA gene sequence was deposited to GenBank under accession no. KP682305.

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