ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 4, pp. 553–560. © Pleiades Publishing, Ltd., 2015.

EXPERIMENTAL ARTICLES

Sulfate-Reducing Bacteria of the Genus *Desulfovibrio* from South Vietnam Seacoast

A. L. Tarasov[1] and I. A. Borzenkov

Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia

Received February 25, 2015

Abstract—Nine strains of sulfate-reducing bacteria were isolated from a biofouling of corroded steel samples incubated in a marine environment near Nha Trang, South Vietnam. Sulfate-reducing bacteria were obtained from all samples with black corrosion products (in rust-filled metal cavities, beneath the *Balanus* and oyster booths, and beneath *Bryozoa* or algal colonies). Analysis of the 16S rRNA gene sequences of these strains showed that they belonged to the genus *Desulfovibrio*, with *D. salexigens*, *D. marinisediminis*, *D. alaskensis*, *D. bizertensis D. indonesiensis*, and *D. dechloracetivorans* as the closest phylogenetic relatives (98–99% similarity). According to the 16S rRNA gene sequencing, one *Desulfovibrio* isolate was related to "*D. caledoniensis*", although the similarity did not exceed 97.0%. All strains utilized hydrogen (in the presence of acetate and CO_2), lactate, pyruvate, formate, and fumarate, but not acetate. Utilization of other substrates varied from strain to strain. Some isolates were capable of slow autotrophic growth with H_2 as the sole electron donor. *D. indonesiensis* and *D. alaskensis* strains were tolerant to long-term exposure to atmospheric oxygen exposure and could grow in the presence of 0.1% O_2 in the gas phase.

Keywords: sulfate-reducing bacteria, *Desulfovibrio*, biofouling, corrosion

DOI: 10.1134/S0026261715040165

Sulfate-reducing bacteria (SRB) occur in various anaerobic ecosystems and play a major part in metal corrosion in seawater. *Desulfovibrio* species often occur in marine anaerobic ecosystems. The species recently isolated from marine sediments include *D. bizertensis* [1], *D. marinus* [2], and *D. marinisediminis* [3].

Halotolerant *Desulfovibrio* species (*D. vietnamensis* and *D. alaskensis*) were isolated from the microflora of oil-producing facilities [4, 5]. *Desulfovibrio* species were found in deep-sea sediments [6, 7].

While SRB are traditionally considered strict anaerobes, they may occur in ecosystems subject to oxygen. SRB microcolonies were found in cyanobacterial mats [8], while *Desulfovibrio* species are known components of the microflora of marine plants' rhizosphere [9]. *D. indonesiensis* was isolated from corrosion products of ship metal in seawater [10]. Biofouling of various materials with mollusks and crustaceans was shown to result in formation of anaerobic zones, where sulfate reduction may be activated [11, 12].

SRB development in strictly anaerobic microniches adjacent to oxygen-saturated water remains insufficiently studied. Investigation of biofouling and the resulting corrosion of metal constructions in seawater, the process in which SRB are involved, is of practical importance due to intensive exploitation of sea shelf gas and oil deposits.

[1] Corresponding author; e-mail: tarasov1357432@yandex.ru

The goal of the present work was to investigate SRB diversity in marine epibioses on the surface of metal constructions in tropical seawater and to investigate the properties of the SRB isolates.

MATERIALS AND METHODS

Subject of research. All bacterial strains were isolated from biofouling on steel samples placed on the racks of the Marine Research and Testing Station in the South China Sea (Nha Trang, South Vietnam) organized by the Joint Russian-Vietnam Tropical Research and Test Center (Tropical Center). The racks were located in the Dam Bai bay of Hòn Tre Island (12°11′47.97″ N, 109°17′35.49″ E) and in the area of the Nha Trang port.

Steel samples were incubated in seawater, 100 m from the shore (0.5–1.0 m depth) for 3 to 12 months. Annual variations of temperature, salinity, pH, an O_2 concentrations in the port area were 24–29°C, 11–35‰, 7.0–8.2, and 2.5–16.8 mL/L, respectively [11]. Annual variations of salinity, pH, and oxygen concentrations in the Dam Bai bay were less pronounced (30.1–34.7‰, 7.02–8.38, and 3.6–6.0 mL/L, respectively).

Samples were collected from rust-filled metal cavities and from biofouling on the surface of metal, including steel covered with paint with antiadhesive components. The samples were obtained by scraping the lower (closest to the metal) epibiosis layer, which

was usually black in color. The sampled material was collected in 15-mL sterile test tubes, filled with seawater to capacity, and sealed with rubber stoppers.

The samples nos. 1–6 were collected in Dam Bai Bay. Sample no. 1 was obtained from the internal part of a female screw filled with black iron sulfides, which replaced the metal of the bolt. Sample no. 2 was taken from a steel plate surface below partially detached paint covered with a *Bryozoa* overgrowth not more than 1 mm thick. The 12Kh18N10T high-alloyed steel was initially covered with KhS-5226 with antiadhesive components. Sample no. 3 was collected from the surface of aluminum covered with small shellfish and a mucous biofilm less than 1 mm thick. Samples nos. 4, 5, and 6 were collected below the bases of *Saccostrea cucullata* shells (1–2 cm in size) growing on the surface of 12Kh18N10T stainless steel plate. Sample no. 7 was collected from an ST1 carbon steel surface below *Saccostrea* sp. and *Balanus* sp. cells from the experimental rack in the Nha Trang port.

Cultivation techniques. The medium used to isolate 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 in some cases $Na_2S_2O_3$, 2.0); $CaCl_2 \cdot 2H_2O$, 0.15; MES, 0.5; MOPS, 0.5; Tris, 0.6; yeast extract (Difco), 0.25; and 0.2% resazurine solution, 1 mL/L. Solutions of trace elements and vitamins were added as described previously [13], together with 1 mL of the solution of Na_2SO_3 and Na_2WO_4 (0.02 mM each). The cysteine and sulfide solution and $NaHCO_3$ solution were added after sterilization to the final concentrations of 0.2–2.0 and 10–25 mM, respectively); the final pH was 7.0. The cultures were incubated at 35°C.

Enrichment cultures were obtained in the medium with lactate (20 mM) or with acetate (5 mM), $Na_2S_2O_3$ (8 mM), and H_2/CO_2 (80 : 20) in the gas phase of at positive pressure of 0.5 atm. $FeSO_4$ (2 mM) was used as a growth indicator. Media without yeast extract and vitamins, as well as media with butyrate of ethylene glycol (20 mM) instead of lactate, were used in subsequent transfers to separate the components of the microbial population. Enrichment cultures were transferred four to five times in order to achieve preferential growth of one of the morphotypes.

SRB cultures were isolated from colonies obtained by terminal dilutions (roll tube method) in agar medium of the same composition with acetate as a carbon source under the gas phase of H_2/CO_2 or with lactate (20 mM) and N_2 in the gas phase. Purity of the isolates was confirmed by transfers into media with organic substrates under oxic and anoxic conditions, by microscopy, and by the 16S rRNA gene sequencing.

Physiological and biochemical properties. Growth at different values of temperature, pH, and salinity was determined in lactate medium without $FeSO_4$. The pH value was adjusted by adding 0.5 M solutions of HCl or Na_2CO_3. Growth rates of planktonic cultures were monitored by optical density. Growth of the cultures forming stable microcolonies was monitored by sulfide formation; sulfide concentration was determined photometrically using a test kit (Merck, Germany). To determine the spectrum of utilized organic substrates, the latter were added in 10–15 mM concentrations in two replicas. The absence of changes after 21 days was considered as the absence of growth. Substrate consumption confirmed after three transfers into fresh medium was considered as positive.

Catalase activity was determined by applying 3% H_2O_2 (50 μL) to the surface of the late-exponential phase biomass collected by centrifugation (5 min at 5000 *g*) from 1 mL of the culture.

The DNA G+C content was determined by thermal denaturation on a Unicam SP 1800 spectrophotometer (United Kingdom) at 0.5°C/min and calculated according to [14].

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

Oxygen tolerance of the strains. Late exponential-phase cultures grown in the medium with lactate were adjusted to uniform optical density by diluting with mineral medium without organic compounds and reducing agents. An aliquot (0.5 mL) was centrifuged and resuspended in 5 mL of sterile mineral medium. The cell suspension was transferred to 20-mL vials with air in the gas phase and incubated on a shaker at 30°C and 120 rpm. The vials were sampled daily, and 0.05-mL aliquots were 20-fold diluted and used to inoculate the standard lactate medium (5 mL). The test tubes were incubated in a thermostat at the optimal growth temperature for each strain. Lag phase duration depending on the time of contact with atmospheric air was determined.

Capacity of the isolates for growth in the presence of oxygen was determined in 16-mL test tubes containing 5 mL of the lactate medium. Required oxygen concentrations were obtained by injecting sterile air into the gas phase. The final oxygen concentration was determined on a Kristall 5000.2 gas–liquid chromatograph (Russia) with CaA 0.2–0.4 columns, argon as a carrier gas, and a katharometer detector.

The inoculum was prepared from the biomass collected at the end of the exponential growth phase. For this purpose, 0.5 mL of the culture was centrifuged, the pellet was resuspended aseptically in 1.0 mL of the medium, and centrifuged again. The biomass was then resuspended in 5 mL of the medium, and 0.1 mL of this suspension was used for inoculation. The test tubes were incubated on a shaker (120 rpm) for 12 days at 30 or 37°C, depending on the strain.

Identification of the isolates. The taxonomic position of the strains was determined by analyzing their 16S rRNA gene sequences. The following universal primers were used for amplification and sequencing of the 16S rRNA genes: 11F (5'-GTTTGATCMTGGCTCAG-3'), 518R (5'-CGTATTACCGCGGCT-

GCTGG-3'), 1100R (5'-AGGGTTGCG-CTCGTTG-3'), and 1492R (5'-TACGGTTACCT-TGTTACGACTT-3') [15–17]. For identification of methanogenic strains, the following primers were used: Arch20F (5'-TTCCGGTTGATCAYGCCRG), Arch915R (5'-GTG-CTCCCCCGCCAATTCCT), and Arch1204R (5'-TTMGGGGCATRCIKACCT) [18–20]. Sequencing was carried out in the Syntol center (Moscow, Russia). All gaps and unidentified bases were excluded from analysis. Comparison of the sequences was carried out using the NCBI Blast software and databases (http://www.ncbi.nlm.nih.gov/blast). The sequences were deposited to GenBank under accession nos. KP682305, KP682306, KP682307, KP682308, KP682309, KP682310, and KP682311.

RESULTS AND DISCUSSION

Taxonomic position and physiological properties of the isolates. Nine SRB strains were isolated: BO, PE, RH2, Mol4, ME, Mol5, OZB, OP12, and DB3. Strains BO, PE, and RH2 were isolated from samples nos. 1, 2, and 3, respectively. Strains Mol4, ME and Mol5, and OZB and OP12 were isolated from samples nos. 4, 5, and 6, respectively. Strain DB3 was isolated from sample no. 7. Since the 16S rRNA gene sequencing revealed strains PE and OZB to be related to the same species *D. salexigens*, while strains Mol4 and Mol5 were related to *D. marinisediminis*, strains DB3, RH2, ME, BO, Mol5, OP12, and OZB were used for further study. The morphological and physiological characteristics of the isolates are listed in the table.

According to analysis of the 16S rRNA genes, strain DB3 belonged to the species *D. alaskensis* (99%, see table) [5]. The similarity between the isolate and *D. alaskensis* DSM 16109^T (NR_029338.2) was evident in their characteristics. Strain DB3 grew well in the presence of 0.14–0.6 M NaCl, while the optimum for the type strain was 25 g/L (0.43 M). Strain DB3 had, however, a higher temperature optimum than the type strain (39–42°C) [5] and could grow at temperatures up to 45°C (table). The strain used hydrogen in the presence of acetate, as well as lactate, formate, malate, and fumarate. Similar to the type strain, it did not use glycerol and ethanol, although, unlike the type strain [5], it did not grow on succinate (table). We observed slow mixotrophic growth of strain DB3 on hydrogen and acetate in the presence of sulfur. In liquid media, the strain grew forming dense microcolonies, from which motile cells were released.

D. vietnamensis DSM 10520^T (NR_026303.1) is another species closely related to strain DB3 (~99% similarity). The species *D. vietnamensis* and *D. alaskensis* have very similar 16S rRNA gene sequences (98.9%) and have been differentiated based on the results of DNA–DNA hybridization [4]. *D. vietnamensis* is capable of growth on glycerol and ethanol, which were not used by strain DB3 (table) or by the type strain of *D. alaskensis* [5].

The type strain *D. alaskensis* DSM 16109^T was isolated from a petroleum reservoir [5], while other strains were isolated in the course of investigation of pipelines and oil well equipments (CP000112.1 [21], DQ083980.1 [22]), as well as from the sediments of a Tunisian coastal zone (DQ867001.1).

D. vietnamensis, isolated from a Vietnamese oil deposit, also has the upper growth limit at 45°C [23]. Occurrence of microorganisms capable of growth at high temperatures (45–50°C) in seawater with the maximal temperature not exceeding 32°C may be associated with the presence of an exploited high-temperature oil deposit Bach Ho (White Tiger) in this area [24].

Strain RH2 was closely related to *D. bizertensis* DSM 18034^T (NR_043808.1) according to both 16S rRNA gene sequencing (99% similarity) and its physiological and biochemical characteristics [1]. Similar to the type strain *D. bizertensis* DSM 18034, it had a relatively high growth temperature (39°C), was moderately halotolerant (not more than 1 M NaCl), and consumed a spectrum of substrates, including, apart from lactate and pyruvate, also dicarboxylic acids and some alcohols (table). We also observed growth of strain RH2 on glycerol and some amino acids: aspartate and alanine (table). In the absence of sulfate, growth occurred by fermentation of malate and fumarate (weak growth), but not of lactate. Sulfate, sulfite, thiosulfate, and sulfur were used as electron acceptors. Another closely related bacterium, *"D. singaporenus"* SJI1 (EF178280.1) probably belongs to the same species *D. bizertensis*.

Strain Mol5 belonging to the species *D. marinisediminis* DSM 17456^T (AB353727.1, 99% similarity) is characterized by utilization of a broad range of substrates, which included, apart from hydrogen, lactate, fumarate, and formate, also malate, ethanol, glycerol, ethylene glycol, and amino acids (table). Unlike the type strain DSM 17456^T [3], strain Mol5 was more halotolerant (up to 1.2 M NaCl), did not use succinate, and reduced elemental sulfur.

The microorganisms closely related to *D. marinisediminis* are probably widespread in marine environments. According to results of the 16S rRNA gene analysis, SRB strains closely related to this species occur in marine sediments (GU136575.1), as well as on corals damaged by pathogenic microorganisms (AY750147.1, AY750148.1, FJ202999.1, and FJ202547.1).

Strain OP12 occupied an intermediate position between the *D. indonesiensis* type strain Ind1= DSM 15121^T (NR_044916.1) and strain H1T (AJ621884.1) [10, 25]. In its physiological characteristics, strain OP12 more closely resembles strains H1T [25] and P23 [7] than strain Ind1^T [2, 10], which did not grow above 37°C and did not use ethanol or propionate

Taxonomic position, morphology, and physiological characteristics of the isolates

Feature	DB3	RH2	Mol5	OP12	OZB	BO	ME
Closest relatives	*D. alaskensis* DSM 16109^T	*D. bizertensis* DSM 18034^T	*D. marinisedi-minis* DSM 17456^T	*D. indone-siensis* DSM 15121^T	*D. salexigens* DSM 2638^T	*D. dechlorace-tivorans* ATCC700912^T	*D. caledoniensis* SEBR 7250, *D. dechlorace-tivorans* ATCC 700912^T, and Mic1c02
GenBank accession no. of the relative	NR_029338.1	NR_043808.1	AB353727.1	NR_044916.1	CP001649.1	NR_025078.1	U53465.1, NR_025078.1 and AB546252.1
Homology of the 16S rRNA genes, %	99	99	98	99	99	99	97, 96, and 96
Morphology	Vibrios	Vibrios	Vibrios	Vibrios	Rods	Rods	Rods
Size, μm	0.45–1.0 × 1.4–4.5	0.45–1.0 × 1.4–4.5	0.5–1.0 × 1.2–5.0	0.4–0.6 × 2.0–7.0	0.4–1.0 × 1.4–4.5	0.4–0.6 × 2.5–5.0	0.4–0.6 × 1.3–2.0
μ_{max}, h^{-1}	0.58	0.30	0.37	0.22	0.15	0.13	0.23
DNA G+C content of the isolate and type strain (in parenthises)	55.7 (64.1 [5])	50.6 (51 [3])	44.2 (46.2 [3])	58.3 (58.1 [2])	ND (46 [9])	61.90 (ND)	52.4 (ND)
pH optimum (interval)	7.3–7.5	6.9	6.9–7.1	7.5–8.2 (5.8–9.0)	6.9	6.8–7.1	6.8–7.5 (5.8–8.5)
Maximal temperature for glrowth (interval)	39–42 (15–45)	39	37–39	39–42 (25–52)	34–37	34–37	34–37 (20–39), no growth at 42°C
NaCl optimum (interval), M	0.14–0.60 (0.1–1.5)	0.12–0.40 (up to 1.0)	0.25–0.40 (up to 1.2)	0.16–0.46 (0.01–2.0)	0.30 (0.2–1.0)	0.33–0.43 (up to 1.0)	0.20–0.32 (up to 1.1)
Growth							
$H_2 + CO_2$ (autotrophic)	–	–	–	+ [7, 25]	± [27]	+	+
Succinate	!– [5]	+ [1]	!– [3]	± [7, 25]	± [27]	–	!± [29]
Malate	+ [5]	+ [1]	+ [3]	+ [25]	+ [6]	–	!+ [29]
Ethanol	–[5]	+ [1]	+ [3]	+ [7, 25]	+ [6, 27]	+ [28]	– [29]
Propanol	–	+ [1]	± [3]	+ [25]	± [27]	+	–
Butanol	±	+ [1]	±	+ [25]	±	+	–
Ethylene glycol (deithylene glycol)	–	–	+	+ [25] (+)	–	±	–
Glycerol	– [5]	+	+ [3]	!– [25]	+ [27]	+	!±
Fructose	±	–	–	–	–[6]	–	!± [29]
Betaine	–	–	±	– [25]	+	–	–
Propionate	±	–	– [3]	±	– [27]	!– [28]	– [29]
Butyrate	–	–	– [3]	– [7]	!+ [27]	–	!– [29]
Alanine	–	+	+ [3]	–	!– [6]	!– [28]	–
Aspartate	±	+	± [3]	–	±	±	–
Glycine	–	ND	± [3]	–	– [6]	–	–
Serine	–	+	+ [3]	–	+ [6]	±	–

The cells are curved rods, slightly spiral, single or in short chains. All strains use $H_2 + CO_2$ + acetate, lactate, pyruvate, formate, and fumarate for sulfate reduction. No growth occurs on acetate, methanol, and glucose.

Designations: "+", good growth; "±", weak growth (less than 1.5 mM sulfide formed); "–", no growth. The features not agreeing with those reported in the literature (according to the relevant references) are marked by "!".

(table). The similarity between the 16S rRNA gene sequence of strain OP12 and that of strain H1T was 99.4%, while its similarity to two other strains was 99.2%. Both our isolate OP12 and strain H1T were heat-tolerant (growth at 52°C, no growth at 20 and 55°C) and halotolerant. Growth of strains OP12 and H1T occurred at NaCl concentrations up to 2.0 and 2.8 M, respectively. The similarity was further confirmed by ability to grow on dicarboxylic acids, alcohols, ethylene glycol, and ethylene glycol oligomers (table). Strain OP12, as well as *D. indonesiensis* P23 [7], was able to grow slowly under autotrophic conditions with H_2, CO_2, and sulfate (without vitamins, yeast extract, cysteine, and acetate).

Unlike strain H1T, the isolate did not use glycerol or glucose and did not reduce nitrate. It could, however, reduce elemental sulfur as the dole electron acceptor.

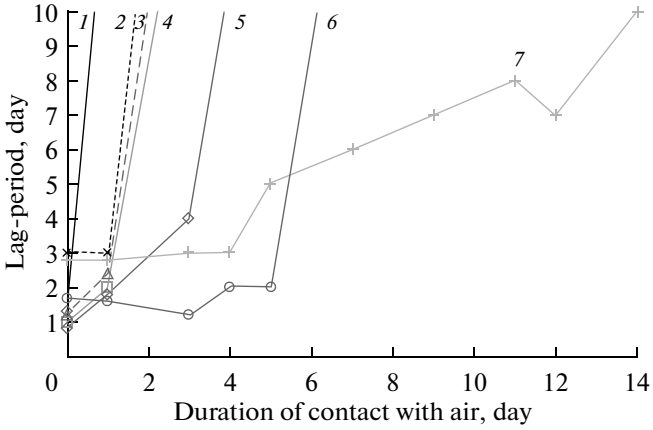
Among our *Desulfovibrio* isolates, strain OP12 exhibited pronounced thermotolerance, was halotolerant, and was moderately alkaliphilic. Its growth optimum was at pH 7.5–8.2, while the highest pH for growth was 9.0 (table). In liquid media strain OP12 grew forming microcolonies.

The strains related to *D. indonesiensis* have been isolated from both the upper sediment layers and deeper horizons. The type strain *D. indonesiensis* DSM 15121^T was isolated from a corroding ship hull used as a petroleum reservoir [10]. Strain H1T was isolated from sandstone of a subterranean gas reservoir [25]. Strain P23 was isolated from the cores of deep (200 m) marine sediments [7], while strain Mic5c10 (AB546254) was found in oil equipment [26].

Strain OZB, belonging to the species *D. salexigens* (99% similarity to the type strain DSM 2638^T, CP001649.1), possessed similar physiological and biochemical properties. Its temperature and salinity optima (34–37°C and 0.34 M, respectively) were close to those of the type strain of *D. salexigens* [6, 27]. NaCl concentration inhibiting growth of strain OZB was ~1 M. Apart from hydrogen, lactate, and pyruvate, the strain utilized formate, dicarboxylic acids, alcohols, and glycerol. Similar to the type strain, slow autotrophic growth on hydrogen was observed in the absence of acetate (table). Unlike the previously described strains, slow growth occurred on butyrate, while fructose was not used. Strain OZB grew on serine, but not on alanine. It reduced nitrate and elemental sulfur. Growth on betaine was also observed.

The organisms closely related to *D. salexigens* were found on moribund corals (AY038515.1). Although the known members of this species are not thermophiles, related organisms were detected in the vicinity of deep-water hydrotherms (AB518745.1) and as components of a thermophilic microbial community from oil equipment (FJ469370.1).

Strain BO was identified as *D. dechloracetivorans* strain SF3 = ATCC 700912^T, due to the high similarity



Lag phase duration of *Desulfovibrio* isolates after preincubation in contact with air: *D. salexigens* OZB (*1*), *Desulfovibrio* sp. ME (*2*), *D. bizertensis* RH (*3*), *D. marinisediminis* Mol5 (*4*), 5 *D. dechloracetivorans* BO (*5*), *D. alaskensis* DB3 (*6*), and *D. indonesiensis* OP12 (*7*).

of their 16S rRNA gene sequences (99% NR_025078.1). Its differences from the type strain included a higher temperature optimum (34–37 and 30°C, respectively) [28]. Since growth of the type strain was studied mostly with chlorinated organic compounds—rather than sulfate—as electron acceptors, comparison of the spectra of utilized substrates is difficult.

According to the results of the 16S rRNA gene sequencing, while the species *"D. caledoniensis"* SEBR 7250 and *D. dechloracetivorans* (strains ATCC 700912 and Mic1c02) were most closely related to strain ME, similarity levels did not exceed 97% (U53465.1, NR_025078.1, AB546252.1). The species *"D. caledoniensis"* has not been completely described and is not validated. Strain *"D. caledoniensis"* DC (GU074016), which was studied in more detail, differed from strain ME in the spectrum of utilized substrates [29]. The properties of the new isolate ME may support its classification as a new species.

Tolerance to oxygen. The data on survival of the cells of new isolates on contact with air revealed that strain OZB was the most sensitive one, with growth suppressed after 24 h of exposure to air (figure). More resistant strains RH2, Mol5, and ME survived 48 h of exposure to atmospheric air. Strains BO and DB3 were still more tolerant. They resumed growth after 4 and 6 days of exposure, respectively. Strain OP12, surviving up to 12 days of exposure to air, was the most oxygen-tolerant. It should be noted that strains DB3 and OP12 were prone to form microcolonies, inside which the cells could survive unfavorable conditions. Aggregation of SRB cells after exposure to 5% O_2 in the gas phase has been reported [8, 30]. In our isolates grown as planktonic cultures, contact with oxygen, however, did not induce cell aggregation.

These results were confirmed by experiments on the capacity of the isolates to grow in the presence of oxygen. After centrifugation, the cells were resuspended in the medium without reducing agents, with 0.01, 0.1, 0.2, or 0.5% oxygen in the gas phase. In the presence of 0.01 and 0.1% O_2, growth of strains DB3 and OP12 occurred after 2–3 and 4–5 days, respectively. Other strains did not grow under these conditions. Ability to grow at low oxygen concentrations (0.1%) was described for *D. vulgaris* Hildenborough [31] and may be a feature of a number *Desulfovibrio* species.

Strains OP12 and BO exhibited high catalase activity. In strains ME and Mol5, catalase activity was also revealed. In other strains (RH2, DB3, and OZB) no catalase activity was detected. This finding was especially unexpected in the case of strain DB3, which exhibited relatively high tolerance to oxygen. Moreover, incubation of strain DB3 in the presence of 0.1 and 0.2% oxygen in the gas phase for 1 to 8 h did not result in emergence of catalase activity, unlike the previously described increase of catalase activity in *D. gigas* [32]. Catalase is not the only enzyme responsible for oxygen tolerance [33]. Alternative pathways of hydrogen peroxide detoxication may involve, for example, NADH peroxidase, which oxidizes H_2O_2 to water [34, 35].

It should be noticed that conditions in the microniches from which SRB strains were isolated were most probably strictly anoxic, as was indicated by the presence of methanogens in the enrichments from the material below *Balanus* and oyster shells. Apart from *D. alaskensis* DB3 and *D. salexigens* OZB, the samples contained methylotrophic methanogens, which were isolated and identified as *Methanococcoides methylutens* (99% similarity of the 16S rRNA gene with that of the type strain DSM 2657, FR733669.1).

Thus, oxygen tolerance of the new SRB isolates and their ability to grow under oxic conditions varied significantly. The strains *D. alaskensis* DB3 and *D. indonesiensis* OP12 exhibited especially high tolerance to oxygen. The tolerant strains, however, had different levels of catalase activity and probably possessed different protective mechanisms. Growth of the sensitive strains is possible in biofilms, where they are protected by other, oxygen-scavenging microorganisms.

The broad salinity range of the new isolates is in agreement with conditions of the coastal zone, where seasonal desalination occurs. For most strains, except strain OP12, the upper salinity limit did not exceed 1 M NaCl. While the temperature ranges correspond to the values for warm tropical waters, ability of some strains (DB3 and OP12) to grow at temperatures above 40°C may be associated with growth in warmer environments, such as petroleum deposits.

Cells of the isolates were curved, spirally twisted rods typical of the genus, but varied considerably in size, curvature, and proportions (table). Two strains (DB3 and OP12) formed stable microcolonies. Investigation of ageing cultures with cell deformation revealed other differences between the strains. Thus, the cells of strains DB3, RH2, and OP12 became thicker with age, while the vibrioid cells of Mol5 became spherical. The cells of OZB and BO were especially sensitive to long-term storage. These differences were probably associated with the differences in cell wall structure.

All strains were capable of mixotrophic growth on hydrogen in the presence of CO_2 and acetate, could grow on lactate, pyruvate, formate, and fumarate, but did not grow on acetate. Growth on sugars was weak or altogether absent. All strains could use sulfate, sulfite, and thiosulfate as electron acceptors.

The isolates differed in their ability to grow on C2–C4 alcohols and glycerol, as well as in capacity for autotrophic growth with hydrogen. Succinate was the least used dicarboxylic acid. Some strains utilized ethylene glycol or amino acids. *D. alaskensis* DB3 and strain ME isolated from *Blalnus*- and oyster-overgrown samples had the narrowest substrate spectra. Strain *D. dechloracetivorans* BO, isolated from a corroded steel bolt, had a limited range of substrates. Strains *D. bizertensis* RH2, *D. marinisediminis* Mol5, *D. indonesiensis* OP12, and *D. salexigens* OZB utilized the highest diversity of substrates, including amino acids, which may indicate their trophic relations with macroorganisms.

Our results revealed conditions for development of SRB belonging predominately to the genus *Desulfovibrio* to develop below epibioses of both macro and micro scale.

After oxygen consumption by biofilms and epibioses, sulfate of seawater becomes the most important electron acceptor. Formation of strictly anoxic microzones closely adjacent to oxygen-saturated seawater implies considerable electrochemical and concentrational gradients and therefore intensive matter exchange at the sites of SRB growth. Corrosion of metal, which acts as a reducer, results in specific conditions for anaerobic growth. Wide occurrence and diversity of *Desulfovibrio* strains in upper sediments and epibioses of a tropical sea may indicate their active involvement in formation of anaerobic microniches.

ACKNOWLEDGMENTS

The authors are grateful to V.A. Karpov, Yu.L. Koval'chuk, and other workers of Joint Russian-Vietnam Tropical Research and Test Center (Tropical Center) for their assistance in research. The authors thank O.P. Poltarukha for valuable consultations.

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Translated by P. Sigalevich