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EXPERIMENTAL ARTICLES

Investigation of the Sulfate-Reducing Bacterial Community in the Aerobic Water and Chemocline Zone of the Black Sea by the FISH Technique

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Abstract—Fluorescent in situ hybridization (FISH) was used to analyze the abundance and phylogenetic composition of sulfate-reducing bacteria in the aerobic waters and in the oxic/anoxic transitional zone (chemocline) of the Black Sea, where biogenic formation of reduced sulfur compounds was detected by radioisotope techniques. Numerous sulfate-reducing bacteria of the genera *Desulfotomaculum* (30.5% of detected bacterial cells), *Desulfovibrio* (29.6%), and *Desulfobacter* (6.7%) were revealed in the aerobic zone at a depth of 30 m, while *Desulfomicrobium*-related bacteria (33.5%) were prevalent in the upper chemocline waters at 150-m depth. Active cells of sulfate-reducing bacteria were much more abundant in the samples collected in summer than in the winter samples from the deep-sea zone. The presence of physiologically active sulfate reducers in oxic and chemocline waters of the Black Sea correlates with the hydrochemical data on the presence of reduced sulfur compounds in the aerobic water column.

Keywords: meromictic basin, Black Sea, aerobic waters, chemocline, sulfate-reducing bacteria, fluorescent in situ hybridization.

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The Black Sea is the world's largest meromictic basin and a great reservoir of dissolved methane, sulfide, and CO_2. In the central part of the sea, the aerobic zone is 90–100 m deep, while above the continental shelf its lower border lies somewhat deeper, at 140–175 m [1]. Below the oxic zone, the water is more saline and contains sulfide (up to 335–370 μM at 1500–2000 m). A series of publications have confirmed the major role of sulfate-reducing bacteria (SRB) of the anoxic water column in sulfide production in the Black Sea [2]. The presence of reduced sulfur compounds revealed in aerobic water by some researchers [3] was also attributed to bacterial sulfate reduction.

Sulfate-reducing bacteria (SRB) are traditionally considered strict anaerobes. Some species of sulfate reducers, however, possess efficient protective mechanisms and are able to survive and even to retain their metabolic activity in the environments regularly affected by oxygen [4, 5].

Molecular biological techniques, including fluorescent in situ hybridization (FISH) with 16S rRNA-specific oligonucleotide probes and various combinations thereof with microradioautography, are widely used in microbial ecology. The rapid and sensitive FISH technique provides information on the qualitative and quantitative composition of marine microbial communities, which is especially important for determination of the phylogenetic position of uncultured marine microorganisms [6] and makes it possible to assess the ecological role of specific community components directly in the environment [7].

Few publications exist on the application of FISH for investigation of bacterioplankton in the Black Sea water column [8]. Detailed FISH investigations of vertical distribution of the major prokaryotic groups in the Black Sea [9, 10] published in 2005–2006, concentrated mainly on the structure of the microbial community involved in methane turnover in the chemocline.

The goal of the present work was FISH investigation of abundance and taxonomic diversity of sulfate-reducing bacteria in the aerobic surface water and in the chemocline of the Black Sea and comparison of these results with the measured rates of sulfate reduction and methanogenesis.

MATERIALS AND METHODS

Water from depths of 30–600 m was collected in sterile 50-ml Falcon tubes in May 2007 and June

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Table 1. Cyanine 3-labeled 16S rRNA-specific oligonucleotide probes used in this work

Probe	Phylogenetic specificity	Nucleotide sequence (5' ⟶ 3')	16S rRNA target fragment	T_m, °C	Formamide, %
EUB338 + EUB338 II	Most *Bacteria*	GCTGCCTCCCGTAGGAGT	338–355	55	30
		GCAGCCACCCGTAGGTGT	338–355	55	30
ARCH344 + ARCH915	*Archaea*	TTCGCGCCTGSTGCRCCCCG	344–363	58	20
		GTGCTCCCCCGCCAATTCCT	915–934	58	20
DSB129	Most *Desulfobacter*	CAGGCTTGAAGGCAGATT	129–146	48	15
DSB985	*Desulfobacter*, *Desulfobacula*, *Desulfospira*, *Desulfotignum*	CACAGGATGTCAAACCCAG	985–1003	51	20
DSV214	Most *Desulfomicrobium*	CATCCTCGGACGAATGC	214–230	49	10
DSV698	Some *Desulfovibrio*, *Bilophila wadsworthia*, *Lawsonia intracellularis*	GTTCCTCCAGATATCTACGG	698–717	52	35
DSV1292	Some *Desulfovibrio*, *Bilophila wadsworthia*	CAATCCGGACTGGGACGC	1292–1309	55	35
Dtm229	*Desulfotomaculum* (cluster I), other *Firmicutes*	AATGGGACGCGGATCCAT	229–246	50	15
SRB385	Most *Desulfovibrionales*, most δ-*Proteobacteria* sulfate reducers	CGGCGTCGCTGCGTCAGG	385–402	59	35

2008 onboard r/v *Akvanavt* at the 1300-m station (44°.458 N, 37°.882 E) located on the depth drop in the Russian sector of the Black Sea, 10–12 mi from the Golubaya Bay (continental slope near Gelendzhik). In March 2009, water samples were collected from r/v *Professor Shtokman* in the Russian sector of the deep-water zone of the Black Sea (station 20, 44°.052 N, 36°.632 E, depth 1940 m).

Enrichment cultures of SRB were obtained by inoculating the samples from the deep-water zone of the Black Sea (depths of 30, 70, 120, and 165 m, station 20) into liquid Widdel marine medium [11] supplemented with vitamins and trace elements [12]. The media were prepared using the Hungate anaerobic technique [13]. The tubes were incubated for 7–21 days at 22–23°C.

Growth was assessed by increase in sulfide content compared to the control. Sulfide was determined colorimetrically with N,N-dimethyl-*p*-phenylenediamine [14] on a Spekol 21 semiautomatic spectrophotometer (Analytic Jena, Germany) at $\lambda = 670$ nm.

For enumeration and identification of microorganisms, water samples and enrichment cultures were fixed with formaldehyde and stored at 4°C in the dark. The correct choice of a fixation protocol for the water samples was of utmost importance. Fixation with fresh 40% formaldehyde in phosphate buffer saline (PBS), pH 7.0, at a final formaldehyde concentration of 4% yielded much better results than fixation with 48% ethanol. In the latter case, both the specificity of hybridization and intensity of the hybridization signal decreased drastically, probably due to the dehydration of the samples.

Permeabilization with 0.05% lysozyme (76200 U/mg) in 0.1 M Tris-HCl buffer, pH 7.5, for 30 min at 37°C is often recommended after fixation of prokaryotic cells in order to improve the probe penetration into the cell. However, our experiments with Black Sea sulfate reducers revealed no visible difference in hybridization efficiency between lysozyme-treated and untreated samples.

Microbial cells (7–15 ml for water samples and 40–400 μl for enrichment cultures) were concentrated on GTBP 2500 black polycarbonate filters (25 mm diameter, 0.2 μm pore size, Millipore, United States) with nitrocellulose backing (25 mm diameter, 0.45-μm pore size); the pressure (50 kPa) was created with a waterjet pump. The filters were washed with PBS/ethanol (1 : 1), air-dried, and stored at –20°C.

For hybridization, sectors of the filters were placed on microscopic slides and treated with 9 μl of the freshly prepared hybridization buffer with 1 μl of a Cy3-labeled 16S rRNA-specific oligonucleotide probe (50 ng μl^{-1}). The hybridization buffer contained 0.9 M NaCl, 0.01% sodium dodecyl sulfate, 20 mM Tris–HCl (pH 7.5), and 0.2% blocking reagent (Roche Diagnostics, Switzerland) supplemented with formamide (MP Biomedicals, United States) in a concentration adjusted for each probe in order to obtain precise and specific hybridization [15–17]. Hybridization was carried out for 2 h in a moist chamber in a BD 53 thermostat (Binder, Germany) at the recommended temperature.

The fluorochrome-labeled oligonucleotide probes (Syntol, Russia) used in the work are listed in Table 1. The probes for identification of bacteria [18] and

archaea [19] were used, as well as the more specific ones for differentiation between SRB groups [20–23]. The probeBase database (http://www.microbial-ecology.net/probebase) was used to select the relevant probes. Hybridization efficiency was tested on pure cultures of *Escherichia coli* TG-1 (Stratagene, United States), *Desulfotomaculum nigrificans* ssp. *salinus 435* (Laboratory of Microbiology of Anthropogenic Habitats, Winogradsky Institute of Microbiology), and *Desulfovibrio vulgaris* Hildenborough ATCC 29579 as positive and negative controls. Filter sections without a probe were used as controls for possible autofluorescence.

In order to remove the unbound probe, the filters were washed in the dark with sterile deionized water and incubated for 10 min at hybridization temperature in preheated washing buffer. The washing buffer contained the following: 5 mM EDTA (pH 8.0), 20 mM Tris–HCl (pH 7.5), 0.01% sodium dodecyl sulfate, and 70–440 mM NaCl, depending on the formamide concentration used. The filters were washed with water and air-dried on clean microscopic slides.

Total microbial numbers were determined by staining with 0.5 ng μl^{-1} 4',6-diamidino-2-phenylindole (DAPI), 10 μl per filter section. After 15-min incubation in the dark at room temperature, the filters were washed with water and air-dried. The preparations were mounted in a 4 : 1 mixture of Citifluor AF1 (Citifluor Ltd., United Kingdom) and Vectashield (Vector Laboratories, Canada) under a long coverslip and stored in the dark at –20°C. Microscopy was carried out at 1000× magnification under an Axio Imager.D1 epifluorescence microscope (Carl Zeiss, Germany) equipped with an Axio Cam HRc digital camera, filters (Zeiss 20 for Cy3-labeled probes and Zeiss 49 for DAPI staining), and the Axio Vision software package.

The cells were counted in 25–30 fields of view (in average, 100–120 DAPI-stained cells per field) for each filter section. Cell number (N) per 1 ml of the sample was calculated from the equation: $N = n \times (S_{filter}/S_{field})/V$, where n is the average cell number per field of view, V is the sample volume (ml), and S_{filter} and S_{field} are areas of the filter and of the field of view, respectively.

Sulfate reduction rate was determined by the radioisotope method with $^{35}S–SO_4^{2-}$ as described previously [8]. The samples in 30-ml glass vials were incubated with the substrate for 2–3 days at 7–8°C and fixed with 1 ml of 2 N NaOH. The final concentration of $^{35}S–SO_4^{2-}$ in the sample was 10 μCi. Prior to fixation, 0.2 ml of 0.05 M Na_2S was added to the samples in order to prevent the oxidation of reduced sulfur compounds.

RESULTS

Rates of sulfate reduction in the water column of the Black Sea. Depth profiles of O_2 and H_2S and the rates of sulfate reduction in the water column over the continental shelf are presented on Fig. 1. Similarly to our earlier determination of sulfate reduction rates [8], anoxic waters at the 190- to 400-m depth exhibited elevated sulfate reduction rates (Fig. 1c). Some peaks were, however, revealed in the aerobic zone, not only at a 150-m depth, where oxygen concentration was close to the limit of the Winkler method (about 3 μM), but also at the 100- to 110-m depth and in subsurface water (Fig. 1b).

Sulfate-reducing bacteria in the aerobic surface waters. At the depth of 30 m, most prokaryotic cells (about 75% of the total number of DAPI-stained cells) belonged to bacteria (probes EUB338 + EUB338 II). The number of archaea (probes ARCH915 + ARCH344) in the surface layer did not exceed 11% of the total microbial number. In shallow waters, cocci and short rods were predominant (about 90%), while filamentous forms were not revealed.

In the aerobic zone of the continental slope (300 μM O_2, 8.82×10^5 DAPI-stained cells ml^{-1}), SRBs of the genera *Desulfotomaculum, Desulfovibrio*, and *Desulfobacter* were plentiful, constituting 22.9, 21.15, and ~5% of the total microbial number, respectively (Table 2, Fig. 2). Members of these genera were also found at this depth in the central part of the Black Sea. Active *Desulfotomaculum* cells (probe Dtm229) were almost never retrieved from greater depths.

SRB in the chemocline zone. The average total microbial number in the upper zone of the chemocline at the continental slope (149–152 m, 3–6 μM O_2) was 20% higher than in the upper oxic layers. The ratio of *Bacteria* (represented mainly by coccoid forms) decreased from 75 to 40% of the total number of microorganisms. Rod-shaped bacterial cells, often in filament-like chains, were more numerous than in the surface horizons. In the chemocline and below, the ratio of rod-shaped bacteria increased with depth, reaching 30% and more.

Desulfomicrobium species were the dominant phylogenetic group of sulfate reducers in the chemocline constituting 13.4% of the total microbial number of 1.06×10^6 cells ml^{-1} (Table 2, Fig. 3). In the central deep-water zone of the Black Sea, at the depth of 115 m (3 μM O_2), *Desulfomicrobium* spp. predominated among sulfate reducers. Almost no *Desulfomicrobium* cells were found in the oxidized surface water and in anoxic deep horizons.

In the water column of the continental slope at 157.5 m, where trace amounts of sulfide were detected, sulfate reducers of the genus *Desulfomicrobium* were still present. The results obtained with the probe DSV1292 demonstrated the presence of *Desulfovibrio* species at this depth (5–10% of the total bacterial number), while the probe DSV698 (specific

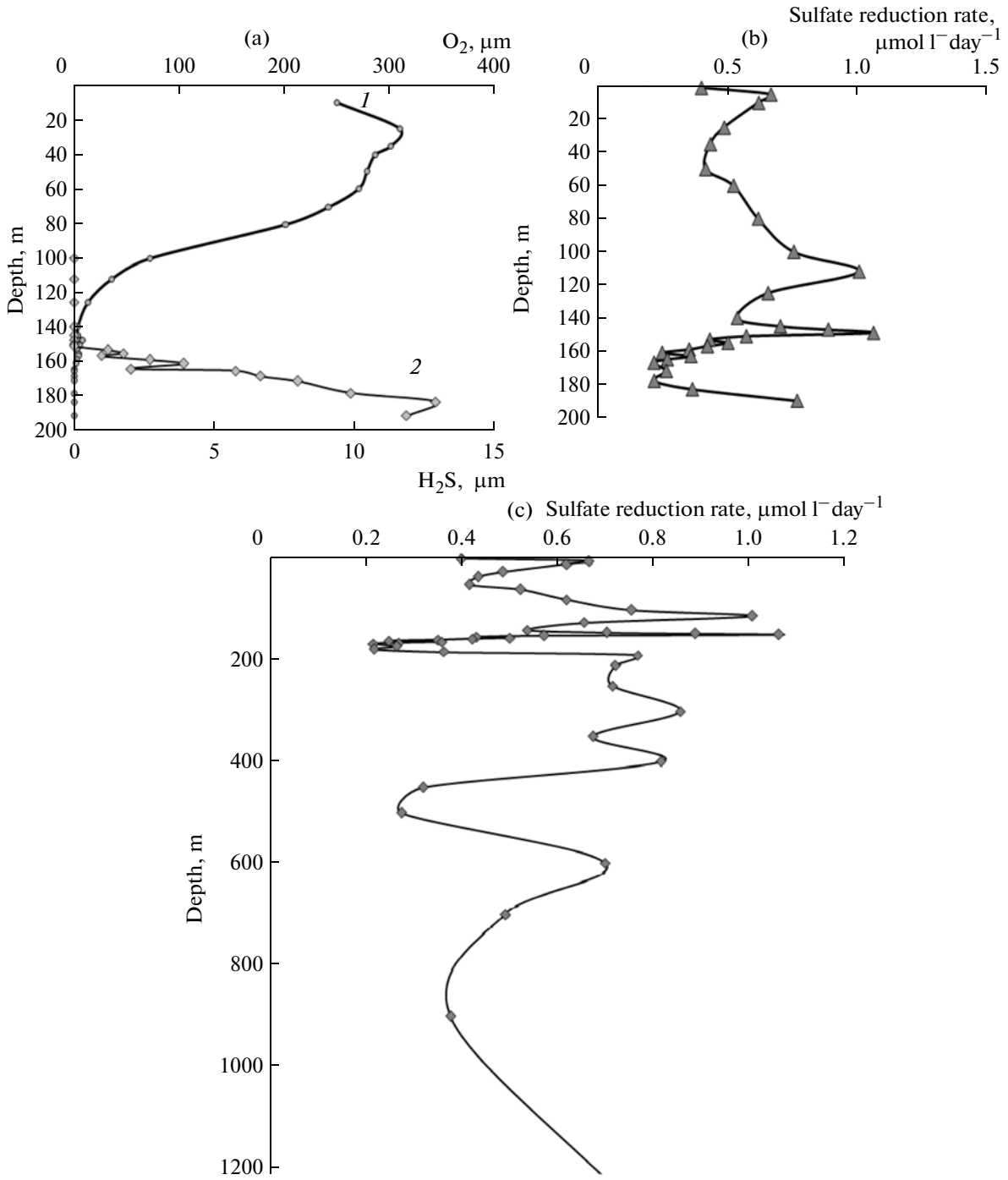

Fig. 1. Oxygen and sulfide concentrations (a) and sulfate reduction rates (b, c) in the water column of the Black Sea deep-water zone near Gelendzhik (depth, 1300 m). The data on oxygen (*1*) and sulfide (*2*) concentrations were kindly provided by E.V. Yakushev (Southern Department, Institute of Oceanology, Russian Academy of Sciences). Profiles of sulfate reduction rate in the upper water layers (0-200 m) (b) and throughout the water column, to 1280 m (c).

to 16S rRNA of 14 *Desulfovibrio* species) did not detect sulfate reducers (Table 2). The probe DSB985, specific to *Desulfobacter*, *Desulfobacula, Desulfospira*, and *Desulfotignum*, also gave almost no hybridization signal in the chemocline.

For the water horizons below 200 m, hybridization with most of the probes for the major groups of sulfate reducers was either absent or close to the lower limit of sensitivity for the FISH method (below 4%) both in winter and in summer (Table 2). However, active

Table 2. Distribution of sulfate-reducing bacteria in the water column of the Black Sea continental slope

Probe	Cells, ml^{-1}					
	30 m	150 m	157.5 m	167.5 m	210 m	600 m
DAPI	8.82×10^5	1.06×10^6	7.11×10^5	1.23×10^6	1.60×10^6	2.58×10^5
EUB338 + EUB338 II	6.60×10^5 (74.8%)	4.24×10^5 (40.0%)	2.72×10^5 (38.2%)	5.62×10^5 (45.7%)	5.49×10^5 (34.3%)	8.80×10^4 (34.1%)
ARCH344 + ARCH915	1.02×10^5 (11.6%)	4.77×10^5 (45.0%)	3.35×10^5 (47.1%)	4.47×10^5 (36.3%)	4.43×10^4 (2.8%)	3.87×10^3 (1.5%)
DSB129	4.40×10^4 (6.7%)	ND	ND	ND	ND	ND
DSV214	ND	1.42×10^5 (33.5%)	6.60×10^4 (24.3%)	ND	4.04×10^3 (0.7%)	2.40×10^3 (2.7%)
DSV698	1.95×10^5 (29.6%)	ND	ND	ND	ND	ND
DSV1292	1.23×10^5 (18.6%)	4.10×10^4 (9.7%)	1.91×10^4 (7.0%)	2.58×10^4 (4.6%)	4.01×10^3 (0.4%)	3.3×10^3 (3.75%)
Dtm229	2.01×10^5 (30.5%)	ND	ND	ND	ND	ND

Note: ND, not detected or individual cells. The percentage of bacteria (probes EUB338 + EUB338 II) and archaea (probes ARCH344 + ARCH915) from the total number of DAPI-stained microbial cells is presented. For sulfate reducers, the percentage of the number of bacterial cells is presented.

Table 3. Numbers of sulfate-reducing bacterial cells in enrichment cultures obtained from the samples of the central zone of the Black Sea (r/v *Professor Shtokman*, station 20, March 2009)

Probe	Cells, ml^{-1}			
	30 m, aerobic zone	70 m, aerobic zone	120 m, lower chemocline	165 m, anoxic zone
DAPI	1.03×10^7	2.20×10^7	2.33×10^8	4.41×10^8
EUB338 + EUB338 II	3.80×10^6 (36.9%)	8.60×10^6 (39.1%)	1.49×10^8 (63.9%)	2.96×10^8 (67.1%)
DSB129	2.20×10^5 (5.6%)	7.90×10^4 (0.9%)	ND	ND
DSV214	2.40×10^5 (6.2%)	1.70×10^6 (20.0%)	5.65×10^6 (3.8%)	5.65×10^6 (1.9%)
DSV698	ND	ND	ND	ND
DSV1292	1.40×10^5 (3.5%)	6.80×10^4 (0.8%)	3.44×10^7 (23.1%)	3.07×10^7 (10.4%)
Dtm229	1.60×10^5 (4.3%)	2.80×10^5 (3.3%)	1.13×10^7 (7.6%)	1.32×10^7 (4.5%)
SRB385	4.30×10^5 (11.1%)	9.50×10^5 (11.0%)	1.34×10^7 (9.0%)	ND

Note: ND, not detected or individual cells. The percentage of bacteria (probes EUB338 + EUB338 II) from the total number of DAPI-stained microbial cells is presented. The percentage of sulfate reducers from the number of bacterial cells is presented.

microorganisms (up to 10^5 cells ml^{-1}) were revealed in the deep-water zone by universal bacterial and archaeal primers. The ratio of the cells hybridizing with probes EUB338 + EUB338 II and ARCH344 + ARCH915 to the number of DAPI-stained cells decreased significantly with depth (to 34–45%). This was probably due to the low rRNA content in deep-water microorganisms, which exhibited low metabolic activity at the sampling period, as well as to the possible presence of prokaryotes from other phylogenetic groups not revealed with the probes used in the present work.

Composition of the SRB community in enrichment cultures isolated from aerobic water and the chemocline zone. In enrichment cultures from the depths of 30 and 70 m (aerobic zone), the number of active bacterial cells revealed with EUB338 + EUB338 II did not exceed 39% of the total cell number (Table 3). From 120 m (lower chemocline) and 165 m (anoxic sulfide-containing water), more active SRB enrichments were obtained containing 63 and 67% of active bacterial cells, respectively.

In anaerobic SRB enrichments isolated from the aerobic zone of the Black Sea (30 and 70 m), *Desulfomicrobium*-related organisms were predominant (6.2 and 20% of total bacterial cell numbers, respectively), while the probe SRB385 for sulfate-reducers of the δ-*Proteobacteria* group revealed 11% of the cells

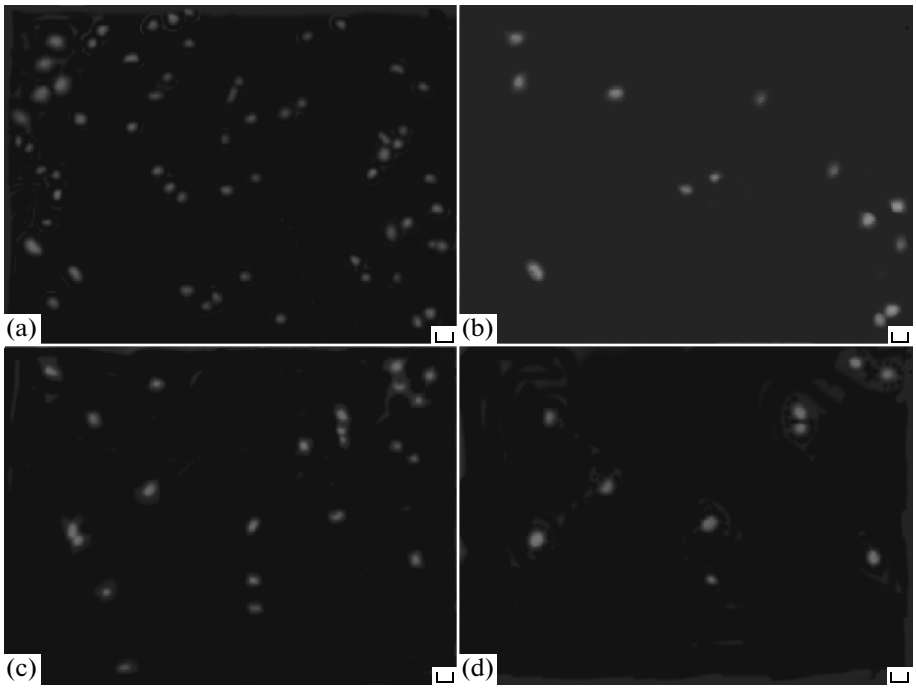

Fig. 2. Digital micrographs of the total microbial population (DAPI staining) (a, c), *Desulfotomaculum* sulfate-reducing bacteria (probe Dtm229) (b), and *Desulfovibrio* sulfate-reducing bacteria (probe DSV698) (d) from the water sample collected at the continental shelf at 30 m (aerobic waters).

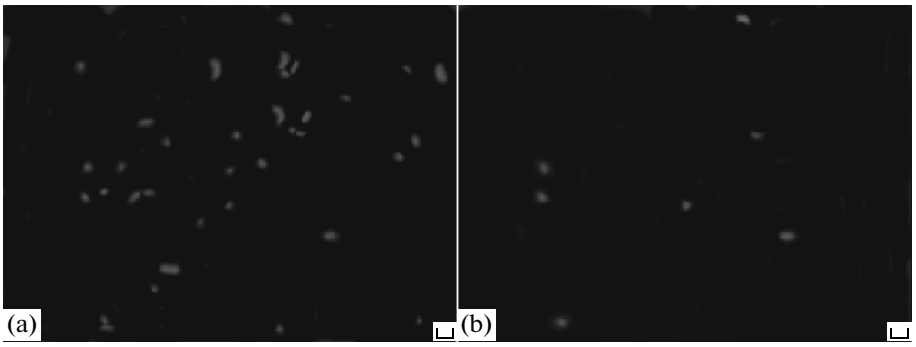

Fig. 3. Digital micrographs of the total microbial population (DAPI staining) (a) and *Desulfomicrobium* sulfate-reducing bacteria (probe DSV214) (b) from the water sample collected at the continental shelf at 150 m (chemocline zone).

(Table 3). SRB enrichments obtained from the water samples of the lower chemocline in the deep part of the Black Sea contained species of the genera *Desulfovibrio* (DSV1292, 23%), *Desulfotomaculum* (7.6%), and *Desulfomicrobium* (up to 4%). The probe SRB385 revealed 9% of the cells. No *Desulfobacter* sp. cells were detected (Table 3).

DISCUSSION

Our data on prokaryote numbers in the surface Black Sea waters correlate well with the results of previous works [8, 9], reporting elevated microbial numbers in the oxic surface waters of the Black Sea (3.04–8.48 × 10^5 cells ml^{-1}), with *Bacteria* as the dominant group (up to 65–77% of the cells).

The number of prokaryotic cells determined by FISH with universal bacterial and archaeal probes was somewhat lower than the number of microorganisms determined by staining with DAPI, a universal DNA stain. However, in some experiments in the aerobic and chemocline zones, application of these domain-specific probes resulted in total microbial numbers as high as 82–86% of DAPI-stained cells (Table 2). Thus, FISH provides sufficiently reliable data for quantitative assessment of microbial communities in the upper part of the water column. High numbers of metabolically active microorganisms in the aerobic

horizons result probably from high levels of available organic matter, both produced by photosynthesis and arriving with river flow.

Detection of viable SRBs in Black Sea aerobic waters correlates with the hydrochemical data [3], confirming the traces of reduced sulfur compounds in aerobic seawater, and with our measurements of sulfate reduction rates (Fig. 1). Thus, although sulfate reducers, being strictly anaerobic organisms, should have been expected to occur only in deep, anaerobic horizons, their active cells were present in significant numbers in upper, aerobic waters.

The previous investigation of Black Sea SRB with only one probe (SRB385) revealed a peak of (not identified more precisely) sulfate reducers immediately above the upper boundary of anaerobic, sulfide-containing waters, i.e., in the chemocline zone; these results were described as paradoxical [10]. Diversity of the antioxidant protective mechanisms in some sulfate reducers, especially in *Desulfovibrio* and *Desulfotomaculum* species, makes it possible for them to occupy the econiches and even to reduce sulfate in microaerobic zones of heterogeneous habitats (temporarily flooded soils, the photic zone of cyano–bacterial mats, biofilms, groundwater, etc.) [4, 5]. They thus gain an advantage in competition for the substrates and may survive under varying environmental conditions. Such hydrological processes as advective penetration of deep water, resulting in mixing of the water layers and of microbial populations, are another, albeit less probable, explanation for the presence of SRB in significant numbers in the upper horizons of the Black Sea water.

In the chemocline of the continental slope, *Desulfomicrobium* was the dominant SRB, while the number of *Desulfovibrio* was significantly lower than in aerobic waters. *Desulfobacter* and *Desulfotomaculum* cells, which were present in the aerobic layers, were not detected in the chemocline samples (Table 2).

The structure of the SRB community in enrichment cultures differed significantly from that observed in native water samples, probably because the enrichment culture conditions differed greatly from those in the natural habitats, especially in the aerobic marine environment. For example, while *Desulfotomaculum* and *Desulfovibrio* species prevailed in the samples from the surface oxic layers (Dtm229 and DSV698 or DSV1292, 30.5 and 18.6–29.6% of the total number of bacterial cells, respectively), in enrichment cultures from the aerobic zone, only 4.3 and 3.5% of the cells were revealed with the probes Dtm229 and DSV1292, respectively. For the chemocline zone, significant differences between the native samples and SRB enrichments were also observed. For example, in the samples collected from the lower chemocline, DSV214 and DSV1292 revealed ~24 and 7% of bacterial cells, respectively, while Dtm229 detected no microorganisms. For the relevant enrichments, the values obtained with these probes were 3.8, 23.1, and 7.6%, respectively (Tables 2, 3). Thus, enrichment cultures should not be relied upon in assessment of the SRB community structure in marine environments.

Importantly, in the samples collected in March in the central part of the Black Sea, the number of detected prokaryotic cells both in the surface waters and in the chemocline zone was about 1.5 times lower than in the samples collected in summer from the continental slope near Gelendzhik. The number of active SRB revealed by FISH also decreased drastically (being three to five times lower than in the summer samples), although the distribution of the phylogenetic groups of sulfate reducers resembled that obtained for the water column of the continental slope. This decrease in microbial numbers is probably associated with the sharp decrease of bacterioplankton concentration (and consequently of available organic matter) in winter.

The universal and specific probes for SRB used in this work exhibited significantly lower hybridization in the deep water of the Black Sea. These results correlate with the data of earlier investigations, also reporting low detection of prokaryotes in Black Sea anoxic waters, compared to other marine environments [24]. Moreover, application of FISH for detection of marine prokaryotes has certain limitations depending on their metabolic activity [25]. According to Lin et al. [10], in the Black Sea water column, the average number of cells detected with EUB338 + ARCH915 was 55% of the number determined by DAPI staining, while this ratio reached 82% in the meromictic marine Cariaco Basin.

The members of *Bacteria* (probe EUB338), depending on depth, constituted 21–65% of the total number of free-living microorganisms. It was impossible to determine the phylogenetic position of a significant part of the Black Sea bacteria (~57%) with probes specific for the major bacterial subdomains (α-, β-, and γ-proteobacteria, sulfate-reducing δ-proteobacteria, and the CFB group) [10].

Probes for detection of sulfate-reducing archaea were not used in the present work. While oligonucleotide probes suitable for reliable detection of *Archaeoglobus* cells by FISH do not exist presently, these hyperthermophilic archaea, according to the published data, have never been recovered from the water column of meromictic basins.

Thus, the results of both FISH and radioisotope measurement of the rates of sulfate reduction indicate active biogenic sulfate reduction in the aerobic waters of the Black Sea. The aerobic surface waters and the boundary between aerobic and anoxic waters (chemocline zone) differed in both the total microbial numbers and the phylogenetic composition of sulfate-reducing bacteria. High abundance of SRB of the genera *Desulfotomaculum* and *Desulfovibrio*, which exhibit relatively high aerotolerance, were discovered in the oxidized surface horizons. In the chemocline zone, the cells of *Desulfomicrobium* and, to a lesser degree,

Desulfovibrio were found. However, *Desulfomicrobium* and *Desulfovibrio* prevailed in anaerobic enrichment cultures obtained from the water samples of the aerobic and chemocline zone, respectively.

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