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SHORT COMMUNICATIONS

A Psychrophilic Sulfate-Reducing Bacterium from the Black Sea Aerobic Water

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Although traditionally sulfate-reducing bacteria (SRB) have been considered strict anaerobes, they are capable of survival in the biotopes affected by oxygen, such as activated sludge, cyanobacterial mats, water column, bottom sediments, etc. [1, 2].

The Black Sea is a meromictic water body. Its anaerobic, sulfide-rich deep waters do not mix with the upper, oxic water layer. Fluorescent in situ hybridization revealed viable SRB cells (*Desulfotomaculum, Desulfovibrio*, and *Desulfobacter*) in both anaerobic and aerobic zones of the Black Sea [3]. We therefore attempted to obtain SRB enrichments and pure cultures from the aerobic water column of the Black Sea.

To obtain enrichment cultures, 90-mL water samples from the depth of 30 m were concentrated on nitrocellulose filters. The filters were then placed into Hungate tubes with Widdel medium for marine sulfate reducers supplemented with vitamins and yeast extract [4]. Trace element solution was prepared according to Pfennig [5]. The medium was supplemented with $MnSO_4$ (400 mg/L). The pure culture was obtained using the same medium from the colonies formed in sequential dilutions on solid medium. The colonies were transferred into liquid medium. Sulfide formation was used to assess bacterial growth. Sulfide was determined colorimetrically [6].

The taxonomic position of the SRB isolates was determined by analysis of the 16S rRNA gene sequence. Chromosome DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega, United States) according to the manufacturer's recommendations. The universal primer system [7] was used for a polymerase chain reaction and sequencing of the amplified 16S rRNA gene fragments. The PCR mixture (25 μL contained the following: 1 μM of each primer, 200 μM dNTP, 1 U Bio*Taq* DNA polymerase (Dialat, Russia), and 50 ng DNA template. PCR products were analyzed by electrophoresis in 2% agarose gel in TAE buffer at 6 V/cm and staining with ethidium bromide. Gel images were obtained using a Gel Doc XR documentation system (Bio-Rad, United States). PCR products were isolated and purified using the Wizard PCR Preps DNA Purification System (Promega, United States) according to the manufacturer's recommendations. The sequencing was carried out in the service laboratory (Syntol, Russia) on an ABI Prism 3100 automatic sequencer (Applied Biosystems, United States) using the BigDye Terminator v3.0 reagent kit and the universal bacterial primers 11F and 1492R. Analysis of the sequences was carried out using the BLAST software package (http://www.ncbi.nlm.nih.gov/BLAST).

The ultrastructure of the cells was studied under a JEM-100CX transmission electron microscope (Jeol, Japan).

From the aerobic zone of the Black Sea, a sulfate-reducing bacterium, strain SrB-30, was obtained in enrichment and pure cultures. The cells grew well only in the presence of manganese in the medium. In manganese-free medium, the culture retained viability only for several transfers. Thus, the sulfate-reducing isolate was obligately dependent on the presence of manganese in the medium.

The best growth (assessed nephelometrically and by cell counts) and sulfide production occurred within the temperature range from 6 to 23°C (Fig. 1). At 12–23°C, a significant increase in sulfide concentration (1490–1755 mg/L) occurred on the seventh day of cultivation. At 6°C, sulfide production occurred after a certain lag. At 3°C, sulfide production was low even after 15 days. No sulfide was formed at 27°C and higher temperatures. The presence of psychrophilic sulfate reducers in the Black Sea at 30-m depth was not surprising, since this is the usual location of the thermocline, where the water temperature does not exceed 10–12°C even in summer.

Electron microscopy revealed that the cells of strain SrB-30 were rods with rounded ends, 0.5–0.8 × 3.7–4.2 μm (Fig. 2). Spores were not found. The cell wall was of gram-negative type with a pronounced

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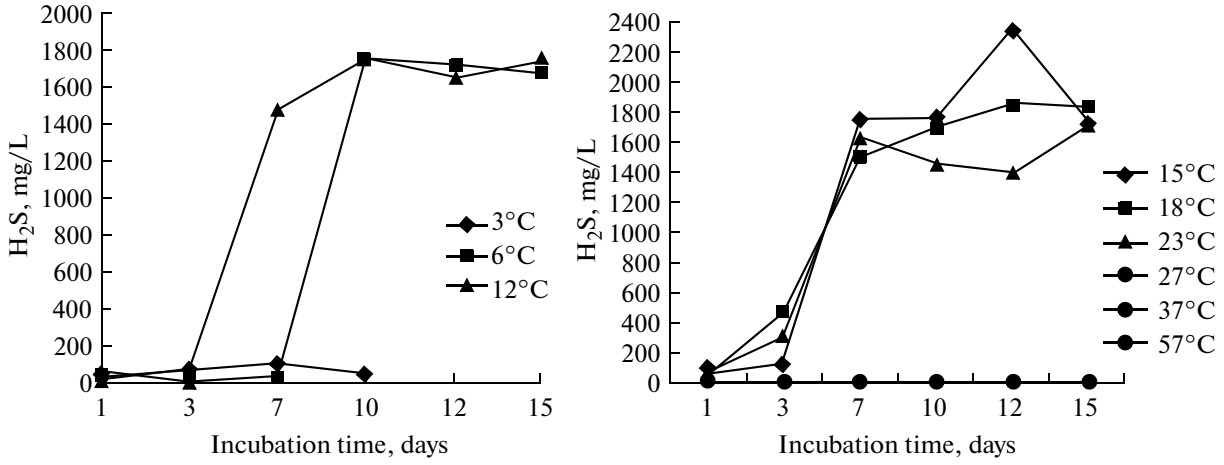

Fig. 1. Sulfide production by the sulfate-reducing bacterium from the aerobic zone of the Black Sea.

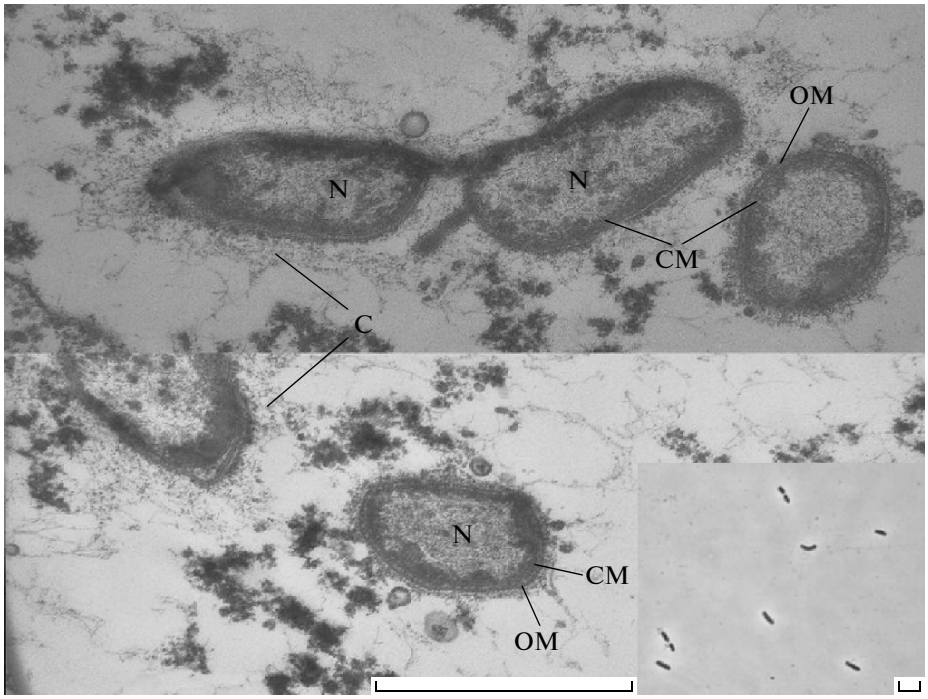

Fig. 2. Electron micrographs of the cells of strain SrB-30, ultrathin sections. Designations: N, nucleoid, CM, cytoplasmic membrane, OM, outer membrane, C, capsule. The insert shows phase microscopy of the cells. Scale bar, 1 μm.

lipoprotein layer. The three-layered cytoplasmic membrane was well visible below the cell wall. The nucleoid was large, occupying almost all the interior of the cell. Capsular material was also visible.

BLAST analysis of the sequenced 16S rRNA gene fragments (~1400 nucleotides) revealed that they probably belonged to the genus *Desulfofrigus*. The similarity to the type strain of *D. fragile*, which was isolated from Arctic marine sediments at Svalbard [8, 9], Norway, was the highest (99%). According to the rules of taxonomy [10], based on the data obtained, strain SrB-30 was identified as a new *D. fragile* strain.

Thus, a psychrophilic anaerobic sulfate-reducing bacterium was isolated from the aerobic water column of the Black Sea. Its metabolism, including obligate manganese requirement, deserves special investigation.

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